



# CLINICAL GUIDELINES

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## Lab Management Program

Effective January 1, 2020





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Dear Provider,

This document provides detailed descriptions of eviCore's basic criteria for laboratory services. These criteria are used for the certification of requests and administration of laboratory benefits for our clients for a range of laboratory tests some of which are represented by one CPT or HCPCS code and others represented by several codes. They have been carefully researched and are continually updated in order to be consistent with the most current evidence-based guidelines and recommendations for laboratory testing from national and international medical societies and evidence-based medicine research centers. In addition, the criteria are supplemented by information published in peer reviewed literature. If you believe that our criteria require modification, please send suggested changes with supporting references to the Laboratory Management Program at the address listed below.

Our health plan clients review the development and application of these criteria. Every eviCore health plan client develops a unique list of CPT codes that are part of their utilization management programs. Health Plan medical policy supersedes eviCore when there is conflict with the eviCore criteria and the health plan medical policy. If you are unsure of whether or not a specific health plan has made modifications to these basic criteria in their medical policy please contact the plan or access the plan's website for additional information.

eviCore works hard to make your clinical review experience a pleasant one. For that reason, we have peer reviewers available to assist you should you have specific questions about a procedure. For your convenience, eviCore's Customer Service support is available from 7 a.m. to 7 p.m. Our toll-free number is 1-800-918-8924.

Gregg P. Allen, M. D. FAAFP  
EVP and Chief Medical Officer

# General Information About this Guideline Manual

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## Description

The eviCore healthcare (eviCore) guideline manual contains medical and reimbursement guidelines that are created and approved by eviCore's Laboratory Management Program personnel and advisors, internal Medical Advisory Committee, and external Medical Advisory Board. eviCore's guidelines are created using evidence-based medicine including, but not limited to, professional society guidelines, consensus statements, and peer-reviewed literature. eviCore's guidelines are intended to provide a library for adoption or a basis for development of tailored coverage criteria for a Health Plan.

## Purpose

To establish evidence-based definitions, decision support, medical necessity criteria, coverage limitations, and payment rules for molecular and genetic testing.

## Organization

This manual is organized into the following sections.

### Molecular and Genetic Clinical Use Guidelines

The guidelines in this section are intended to provide general guidance for the common settings and scenarios in which genetic testing is used (e.g. prenatal, diagnostic, cancer). These guidelines address the overarching coverage principles that broadly apply based on the purpose of the test. They also address specific use situations that may apply to many different tests (e.g. predictive testing for a known familial mutation).

Clinical Use guidelines may include a test-specific guidelines section to direct users to any relevant test-specific guidelines. Because tests may be used for multiple indications, the same test-specific guideline may be referenced by more than one Clinical Use guideline. When a test specific guideline is not available, the coverage principles found in these Clinical Use Guidelines will be applied.

## **Molecular and Genetic Test Specific Guidelines**

The guidelines in this section address a test or group of tests that are used to assess some health condition. The purpose of these guidelines is to provide a framework for determining medical necessity and coverage determinations for a specific test, including where more limited testing may be supported by the medical evidence when broader testing is not. These guidelines provide background about each condition, the available tests, the scenarios in which the test may be used, and the evidence used to determine medical necessity criteria.

## **Administrative Guidelines**

If applicable for this plan, administrative guidelines are included that define coding and reimbursement criteria and requirements.

## **Glossary**

This glossary contains definitions for common genetics, medical and laboratory terminology.

## **Limitations and Restrictions**

When using this manual in electronic or printed form, the following restrictions apply:

- Evidence-based genetic testing is defined as the identification of targeted genetic sequences within the genome of an individual with clinically-identified risk factors or traits suspected of being specific to the genetic disorder, condition, or trait under investigation.
- The medical guidelines contained in this manual are the proprietary property of eviCore, for use by its clients only. These medical guidelines may not be posted, shared, altered, cited or reproduced without the express written consent of eviCore. Commercial use of these guidelines is prohibited.
- Medical guidelines are not to be considered medical advice for a specific patient. Guidelines are used in the process of determining whether a service may be medically necessary and eligible for coverage.
- Medical Guidelines are interpreted and applied at the sole discretion of the Health Plan.
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# Molecular and Genetic Clinical Use Guidelines

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# Confirmatory Genetic Testing

MOL.CU.256.A  
v1.0.2020

## Description

The Centers for Medicare and Medicaid Services (CMS) developed the Clinical Laboratory Amendments (CLIA) in order to help regulate laboratory tests. CMS intended to use this program as a way to ensure that quality laboratory testing was performed. Laboratories that receive reimbursement from Medicare or Medicaid must be CLIA certified.<sup>1</sup>

Most genetic or genomic tests are performed in a CLIA certified laboratory and used for a clear medical purpose. However, some genetic or genomic tests are performed in a research laboratory that is not CLIA certified or as part of a direct to consumer test that is not necessarily performed for a medical purpose.

When genetic testing is performed in a research laboratory or in a laboratory that is not CLIA certified, it is important to confirm any genetic change found prior to using this information to change an individual's medical treatment.

## Criteria

Confirmatory single site genetic testing in a CLIA certified laboratory will be approved when the following criteria are met:

- A disease-causing genetic mutation was identified by a laboratory that is not CLIA certified (e.g. research lab), AND
- Healthcare providers can use the test results to directly impact medical care for the individual (e.g. change in surveillance or treatment plan)

## Exclusions

- Confirmatory genetic testing is not considered medically necessary if the original testing was performed in a CLIA certified laboratory.
- Confirmatory genetic testing is not considered medically necessary if healthcare providers cannot use the test results to directly impact medical care for the individual (e.g. APOE).
- Confirmatory genetic testing is not considered medically necessary if testing is considered Investigational/Experimental per eviCore clinical guidelines (e.g. APOE).
- Confirmatory genetic testing is not considered medically necessary for variants of unknown significance (VUS).

## References

1. Clinical Laboratory Improvement Amendments (CLIA). CMS.gov website. Available at: <https://www.cms.gov/Regulations-and-Guidance/Legislation/CLIA/index.html>

# Genetic Presymptomatic and Predictive Testing for Adult-Onset Conditions in Minors

MOL.CU.298.A  
v1.0.2020

## Introduction

Genetic presymptomatic and predictive testing of minors for adult onset conditions is addressed by this guideline.

## Description

Inherited disorders display a range of symptom onset, from congenital to adult. Some adult onset conditions have surveillance or medical intervention recommendations that are initiated in childhood, while for others there is no change in medical management. The National Society of Genetic Counselors (NSGC) states that individuals should be able to make the decision to have testing for themselves, after understanding and assessing the risks, benefits, and limitations of the test. In their 2017 position statement entitled “Genetic Testing of Minors for Adult-Onset Conditions,” NSGC “encourages deferring predictive genetic testing of minors for adult-onset conditions when results will not impact childhood medical management or significantly benefit the child.”<sup>1</sup>

According to the Genetics Home Reference, presymptomatic testing “can determine whether a person will develop a genetic disorder,” while predictive testing “can identify mutations that increase a person’s risk of developing disorders with a genetic basis.”<sup>2</sup> Predictive testing should be limited to disorders for which the genetic contribution is strong. Testing of minors for genetic variants that are not causative but confer susceptibility to disease is not medically necessary; and therefore, is not reimbursable.

Certain individual medical circumstances (such as consideration of a minor for organ/tissue donation or pregnancy in a minor with a family history of adult-onset disease) may present sufficient clinical utility to outweigh the criteria presented in this guideline. Such rare cases should be carefully considered on an individual basis.

## Criteria

### Introduction

Requests for genetic presymptomatic and predictive testing for adult-onset conditions in minors are reviewed using these criteria.

## Criteria: General Coverage Guidance

Predictive molecular testing of minors (members under the age of 18 years) for X-linked or autosomal dominant disorders will be approved when the following criteria have been met:

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Testing:
  - No previous testing for the condition, and
  - A familial disease-causing mutation has been identified in a 1st or 2nd degree biological relative who is affected with an adult onset autosomal dominant or X-linked condition, AND
- Predictive Testing for Asymptomatic Individuals:
  - The minor is at risk for inheriting the familial disease-causing mutation, and
  - The condition may have onset in childhood, or
  - The condition has recommendations for surveillance that begin in childhood, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

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**Note** Testing of any minor who is symptomatic for a condition, regardless of typical circumstances of onset, is considered diagnostic testing and should be reviewed using *Genetic Testing to Diagnose Non-Cancer Conditions* or the appropriate test-specific guideline.

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## Limitations and Exclusions

Testing of minors for genetic variants that are not causative of inherited disease is not medically necessary; and therefore, is not reimbursable. Examples of mutations or variants that are not causative include:

- variants assessed by a testing laboratory to be of uncertain clinical significance
- variants that confer susceptibility for disease
- variants in genes of uncertain clinical significance.

## Criteria: Test-specific Guidelines

Test-specific guidelines are available for some tests that may be requested for minors. For tests without a specific guideline, use the General Coverage Guidance in Section 1.

## References

### Introduction

This guideline cites the following references.

1. National Society of Genetic Counselors. Genetic testing of minors for adult-onset conditions. Available at: <https://www.nsgc.org/p/bl/et/blogaid=860>.
2. Genetics Home Reference. What are the types of genetic tests? Available at: <https://ghr.nlm.nih.gov/primer/testing/uses>



# Genetic Testing by Multigene Panels

MOL.CU.116.A

v1.0.2020

## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedures addressed by this guideline | Procedure codes |
|--|-----------------|
| Genomic Sequencing Procedures          | 81410-81471     |
| Tier 1 Molecular Pathology Procedures  | 81161-81383     |
| Tier 2 Molecular Pathology Procedures  | 81400-81408     |
| Unlisted Molecular Pathology Procedure | 81479           |

## What are multi-gene panels?

### Definition

Various methodologies can be used to identify potential disease-causing gene mutations. Gene sequencing involves evaluating each DNA nucleotide along the length of a gene. Full gene sequencing is the best approach when many different mutations in the same gene can cause the disorder.

- There are two main ways to sequence a gene:
  - Until recently, most sequencing tests used the Sanger sequencing methodology that was originally developed in the 1970s. Sanger sequencing is labor intensive and did not lend itself to high-throughput applications.<sup>1</sup>
  - Next generation sequencing (NGS), also called massively parallel sequencing, has been developing since about 2005 to allow larger scale and more efficient gene sequencing. NGS relies on sequencing many copies of small pieces of DNA simultaneously and using bioinformatics to assemble the sequence.<sup>1</sup>
- The efficiency of NGS has led to an increasing number of large, multi-gene testing panels.
  - NGS panels are particularly well-suited to conditions caused by more than one gene or where there is considerable clinical overlap between conditions making it difficult to reliably narrow down likely causes.

- Panels including genes associated with a high risk of a condition are of greatest value since these mutation-positive results often lead to changes in medical management.
- Panels may also include genes believed to be associated with a particular condition, but with a more modest impact on risk. Results for such genes are of less clear value because there often are not clear management recommendation for mutation-positive individuals.
- Laboratories offer panel testing for multiple genes at the same time in an effort to increase the likelihood of finding a causative gene mutation in a more efficient manner. Such testing may be performed for diagnostic or predictive purposes.
  - Diagnostic testing is performed in patients with clinical signs or symptoms of a genetic condition. The genetic test may confirm or rule out a clinical diagnosis. However, many genetic conditions have overlapping features, which can make determining appropriate genetic testing difficult. The use of clinical and family history information may not always lead to a likely diagnosis for an individual. In some cases, many genes may be candidates for a person's symptoms. In these cases, testing one gene at a time may be time-consuming and costly. It may also lead to a situation where a mutation is missed in another gene that was not tested.<sup>2</sup>
  - Predictive genetic testing is performed in people known to be at increased risk of developing an inherited condition based on their family history. For some conditions, a positive genetic test predicts with certainty that the person will eventually develop signs and symptoms of a condition. For other conditions, a positive genetic test result indicates an increased risk (susceptibility) for a condition. Without a specific known mutation running in the family, a negative result rarely rules out a condition. Having test results may improve medical management through improved screening, preventive measures (e.g. prophylactic medication, surgery) and other means. In order to better define a person's risk, it is preferable to first test someone in the family who is affected.

## Test information

- Multi-gene panel tests, even for similar clinical scenarios, vary considerably in the genes that are included and in technical specifications (e.g. depth of coverage, extent of intron/exon boundary analysis, methodology of large deletion/duplication analysis). Therefore, technologies used in multi-gene testing may fail to identify mutations that might be identifiable through single-gene testing.
- If high clinical suspicion remains for a particular syndrome after negative multi-gene test results, consultation with the testing lab and/or additional targeted genetic testing may be warranted.
- Results may be obtained that cannot be adequately interpreted based on the current knowledgebase. When a sequence variation is identified that has not been

previously characterized or shown to cause the disorder in question, it is called a variant of uncertain significance (VUS). VUSs are relatively common findings when sequencing large amounts of DNA with NGS.<sup>3</sup>

- Since genes can be easily added or removed from multi-gene tests over time by a given lab, medical records must document which genes were included in the specific multi-gene test used from each patient, and in which labs they were performed.
- Tests should be chosen that maximize the likelihood of identifying mutations in the genes of interest.

## Guidelines and evidence

- The National Society of Genetic Counselors states the following regarding the use of multi-gene panels:<sup>4</sup>
  - “The National Society of Genetic Counselors (NSGC) endorses the use of multi-gene panel tests when clinically warranted and appropriately applied. These tests can provide a comprehensive and efficient route to identifying the genetic causes of disease. Before ordering a multi-gene panel test, providers should thoroughly evaluate the analytic and clinical validity of the test, as well as its clinical utility. Additional factors to consider include, but are not limited to: clinical and family history information, gene content of the panel, limitations of the sequencing and informatics technologies, and variant interpretation and reporting practices.”
  - “Panels magnify the complexities of genetic testing and underscore the value of experts, such as genetic counselors, who can educate stakeholders about appropriate utilization of the technology to mitigate risks of patient harm and unnecessary costs to the healthcare system. NSGC supports straightforward and transparent pricing so that patients, providers, laboratories, and health plans can easily weigh the value of genetic testing in light of its cost.”
- The American College of Medical Genetics has a policy statement that offers general guidance on the clinical application of large-scale sequencing focusing primarily on whole exome and whole genome testing. However, some of the recommendations regarding counseling around unexpected results and variants of unknown significance and minimum requirements for reporting apply to many applications of NGS sequencing applications.<sup>5</sup>

## Criteria

- This guideline applies to multi-gene panel testing, which is defined as any assay that simultaneously tests for more than one gene associated with a condition. The testing may focus on sequence variants and/or deletions/duplications of those genes. Panels vary in scope, such as:

- Panels consisting of multiple genes that are associated with one specific genetic condition (e.g. Noonan syndrome,<sup>6</sup> Stickler syndrome,<sup>7</sup> etc.)
- Panels consisting of multiple genes that are associated with a symptom or non-specific presentation (e.g. epilepsy, intellectual disability,<sup>8</sup> hearing loss, retinal disorders, etc.)
- Coverage determinations generally rely on the medical necessity of the components of a panel. A panel approach to testing is most compelling when:
  - Multiple genes are known to cause the same condition and a limited subset of genes does not account for the majority of disease-causing mutations.
  - The clinical presentation is highly suspicious for a genetic disorder, but the constellation of findings in the personal or family history does not suggest a specific diagnosis or limited set of conditions.
- Multiple policies may apply, including test-specific policies where they exist or the following clinical use policies:
  - Genetic Testing to Diagnose Non-Cancer Conditions
  - Genetic Testing to Predict Disease Risk
- Panel coding and billing should reflect the efficiency gains for the laboratory in testing multiple candidate genes simultaneously. Currently, laboratories are billing for panels in a variety of ways. When a panel approach to testing is determined to be medically necessary, the following billing guidelines will apply.
  - Panel is to be billed with a single panel-specific code (e.g., Genomic Sequencing Procedure or GSP) or single unit of the unlisted molecular pathology code 81479:
    - The billed amount should not exceed the list price of the test.
  - Panel is to be billed with multiple procedure codes representing individual genes analyzed:
    - If a more specific code exists that adequately describes the requested panel, the panel will be redirected to the more specific code (e.g., a genomic sequencing procedure code), or
    - If no more specific code exists, the panel will be redirected to a single unit of the unlisted molecular pathology code 81479, which can be used to represent a panel in total, or
    - If the laboratory will not accept redirection to a single code, the medical necessity of each billed component procedure will be assessed independently. Only the individual panel components that meet medical necessity criteria as a first tier of testing will be reimbursed. The remaining individual components will not be reimbursable, and

- The billed amount should not exceed the list price of the test.
- The following general principles apply:
  - Broad symptom-based panels (e.g. comprehensive ataxia panel) are not medically necessary when a narrower panel is available and more appropriate based on the clinical findings (e.g. autosomal dominant ataxia panel).
  - More than one multi-gene panel should not be necessary at the same time. Multi-gene panel testing should be performed in a tiered fashion with independent justification for each panel requested.
  - If more than ten units of any combination of procedure codes will be billed as part of a panel with no stated differential, the panel will be deemed excessive and not medically necessary.
  - Genetic testing is only necessary once per lifetime. Therefore, a single gene included in a panel or a multi-gene panel may not be reimbursed if testing has been performed previously. Exceptions may be considered if technical advances in testing demonstrate significant advantages that would support a medical need to retest.
- This guideline may not apply to multi-gene panel testing for indications that are addressed in test-specific guidelines.

### **Billing and reimbursement considerations**

- If a panel was previously performed and an updated, larger panel is being requested, only testing for the medically necessary, previously untested genes will be reimbursable. Therefore, only the most appropriate procedure codes for those additional genes will be considered for reimbursement.
- If the member meets medical necessity, billing of the deletion/duplication portion of the panel with a microarray code (typically billed with 81228 or 81229) is allowed when at least 3 genes are included on the panel. Panels with less than 3 genes are more appropriately billed with individual CPT codes.

### **References**

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# Genetic Testing for Cancer Susceptibility and Hereditary Cancer Syndromes

MOL.CU.109.A

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## Description

Genetic testing for cancer susceptibility and hereditary cancer syndromes is performed in people with known risk factors for an inherited form of cancer. Testing may be used in people diagnosed with cancer when there are “red flags” in the individual’s personal medical and/or family history for a hereditary form. Predictive genetic testing may also be performed for this group of conditions, in people known to be at increased risk of developing an inherited condition based on their family history. A positive genetic test result increases the risk for cancer (types vary by the gene involved) and, therefore, impacts medical management decisions around screening, prevention, and treatment.

- Tests used to screen for or make a diagnosis of cancer are covered separately as *Genetic Testing for the Screening, Diagnosis, and Monitoring of Cancer*.
- This policy does not address diagnostic or predictive testing for conditions other than hereditary cancer. Refer to *Genetic Testing to Diagnose Non-Cancer Conditions and Genetic Testing to Predict Disease Risk* for those purposes.

## Criteria

### Criteria: General Coverage Guidance

Individuals may be considered for genetic testing for hereditary cancer syndromes when **ALL** of the following conditions are met:

- **Technical and clinical validity:** The test must be accurate, sensitive and specific, based on sufficient, quality scientific evidence to support the claims of the test.
- **Clinical utility:** Healthcare providers can use the test results to provide significantly better medical care for the individual.
- **Reasonable use:** The usefulness of the test is not significantly offset by negative factors, such as expense, clinical risk, or social or ethical challenges.

Limits:

- Testing will be considered only for the number of genes or tests necessary to establish carrier status. A tiered approach to testing, with reflex to more detailed testing and/or different genes, will be required when clinically possible.

- Genetic testing is indicated once per lifetime per condition. Exceptions may be considered if technical advances in testing demonstrate significant advantages that would support a medical need to retest.

### Criteria: Special Circumstances

The following policies address a group of tests that are used for similar purposes. Because a variety of tests may be used, but the circumstances that justify testing are the same, individual test-specific policies are not necessary.

#### Predictive testing for at-risk people with known familial mutations

The genetic mutation(s) associated with a hereditary cancer syndrome can often be defined in an affected family member, allowing for testing of at-risk relatives for those specific mutations. Testing for known familial mutations is reasonable when **ALL** of the following conditions are met:

- The mutation(s) in the family have been **clearly defined** by previous genetic testing and **information about those mutations can be provided** to the testing lab.
- **Technical and clinical validity**: The test must be accurate, sensitive and specific to the familial mutation(s).
- **Clinical utility**: Healthcare providers can use the test results to provide significantly better medical care for the individual.
- **Reasonable use**: The usefulness of the test is not significantly offset by negative factors, such as expense, clinical risk, or social or ethical challenges.

Limits:

- Testing will be considered only for the known familial mutations when clinically possible.
- Predictive genetic testing is indicated once per lifetime per condition.
- Predictive genetic testing will be considered only for adult individuals (age 18 and over). Exceptions may be considered if there are medical management and/or significant psychosocial benefits to testing prior to adulthood.<sup>1,2,3</sup>

### Criteria: Test-specific Guidelines

Test-specific guidelines are available for some hereditary cancer syndrome tests. For tests without a specific guideline, use the General Coverage Guidance in Section 1.



## References

1. Ross LF, Saal HM, David KL, Anderson RR. Technical report: ethical and policy issues in genetic testing and screening of children. *Genet Med*. 2013;15:234–245. Available at: <http://www.nature.com/gim/journal/v15/n3/pdf/gim2012176a.pdf>
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# Genetic Testing for Carrier Status

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## Introduction

Carrier screening is performed to identify genetic risks that could impact reproductive decision-making for parents or prospective parents. Carriers are generally not affected but have an increased risk to have a child with a genetic condition.

## Availability of genetic testing for carrier status

Carrier screening may be available for autosomal recessive conditions, X-linked conditions, and certain chromosome abnormalities. Ideally, carrier screening is performed prior to pregnancy so that a full range of reproductive options are available to an at-risk couple. However, in practice, it is often performed early in pregnancy when prenatal care is established.

### Other applications of carrier testing

This guideline does not address prenatal or preimplantation genetic testing. Refer to guidelines on *Genetic Testing for Prenatal Screening and Diagnostic Testing and Preimplantation Genetic Screening and Diagnosis* for those purposes.

Testing that may identify carriers who have clinical signs and symptoms, such as cystic fibrosis testing for men with congenital absence of the vas deferens or fragile X genetic testing for women with premature ovarian failure, is addressed in test specific guidelines or *Genetic Testing to Diagnose Non-Cancer Conditions*.

## Criteria

### Introduction

Requests for carrier screening are reviewed using these criteria.

### Criteria for general coverage guidance

Individuals may be considered for genetic testing for carrier screening when ALL of the following conditions are met:

- **Technical and clinical validity** — The test must be accurate, sensitive and specific, based on sufficient, quality scientific evidence to support the claims of the test.
- **Clinical utility** — Healthcare providers can use the test results to provide significantly better medical care and/or assist individuals with reproductive planning.
- **Reasonable use** — The usefulness of the test is not significantly offset by negative factors, such as expense, clinical risk, or social or ethical challenges.

**Limits**

- Testing will only be considered for the number of genes or tests necessary to establish carrier status. A tiered approach to testing, with reflex to more detailed testing and/or different genes, will be required when clinically possible.
- Carrier testing will be allowed once per lifetime. Exceptions may be considered if technical advances in testing demonstrate significant advantages that would support a medical need to retest.
- Carrier testing is indicated only in adults. Carrier screening in minor children is not indicated, except in the case of a pregnancy of the minor child.

**Routine carrier screening**

Individuals may be considered for routine carrier screening when testing is supported by evidence-based guidelines from governmental organizations and/or well-recognized professional societies in the United States.<sup>1,2,3</sup>

**Carrier screening based on family history**

Individuals may be considered for carrier screening based on a family history of a genetic condition when ALL of the following conditions are met in addition to the general criteria above:

- The diagnosis of a genetic condition in a family member is known.
- The parent(s) or prospective parent(s) are at-risk to be carriers of that condition based on the pattern of inheritance.
- The genetic condition is associated with potentially severe disability or has a lethal natural history.

**Partner testing of known carrier or affected individuals**

Individuals may be considered for carrier screening if their partners are known carrier or affected individuals when all of the following conditions are met in addition to the general criteria above:

- The diagnosis of a genetic condition or carrier status in the partner is known.
- The genetic condition is associated with potentially severe disability or has a lethal natural history.

**Test-specific guidelines**

Test-specific guidelines are available for some tests designed to predict carrier status. For tests without a specific guideline, use the General Coverage Guidance in Section 1.

## References

### Introduction

This guideline cites the following references.

1. ACOG Committee Opinion 690: Carrier screening in the age of genomic medicine. March 2017, reaffirmed 2019. Available at: <https://www.acog.org/Clinical-Guidance-and-Publications/Committee-Opinions/Committee-on-Genetics/Carrier-Screening-in-the-Age-of-Genomic-Medicine>
2. ACOG Committee Opinion 691: Carrier screening for genetic conditions. March 2017, reaffirmed 2019. Available at: <https://www.acog.org/Clinical-Guidance-and-Publications/Committee-Opinions/Committee-on-Genetics/Carrier-Screening-for-Genetic-Conditions>
3. Grody WW, Thompson BH, Gregg AR, et al., ACMG position statement on prenatal/preconception expanded carrier screening. *Genet Med*. 2013;15(6):482-483.

# Genetic Testing for Known Familial Mutations

MOL.CU.291.A

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## Introduction

Genetic Testing for Known Familial Mutations is addressed by this guideline.

## Description

When genetic testing reveals the cause of an inherited disease in an affected family member, the genetic change is called a 'known familial mutation' (KFM). Relatives of the affected individual should generally have genetic testing that targets this disease-causing KFM rather than full sequencing of a gene or a multi-gene panel.

KFM testing is less expensive, less complex, and avoids finding variants of uncertain clinical significance (VUS) that have unclear medical management implications.

Presymptomatic or diagnostic testing for known familial mutations should only be offered when the variant is considered disease-causing, or classified as pathogenic or likely pathogenic per American College of Medical Genetics and Genomics (ACMG) variant classification guidelines.<sup>1</sup>

If there is a KFM in the family, testing for this mutation should be performed prior to any other genetic testing for the disease in an individual.<sup>2,3</sup>

## Criteria

### Introduction

Requests for genetic testing for KFM are reviewed using the following criteria.

### Criteria: General Coverage Guidance

- Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- No previous genetic testing of the requested gene that would have included the KFM, AND
- KFM is disease-causing (classified as pathogenic or likely pathogenic), AND
- Member is a 1st, 2nd, or 3rd degree biological relative of the family member with the KFM, AND
- If testing is being performed on an asymptomatic individual for carrier testing or presymptomatic testing for an adult onset condition, that person is 18 years of age or older, AND

- Healthcare providers can use the test results to provide significantly better medical care for the individual, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

### **Billing and Reimbursement Considerations**

- Once the mutation(s) that cause disease in the family have been identified, KFM testing is generally the only testing needed for that particular gene. As a result, if broad gene testing (for example, full gene sequencing or deletion/duplication analysis) is requested and a KFM has been identified in a family member, testing will be redirected to KFM testing.
- In rare circumstances, additional gene testing may be indicated following KFM testing, which will be assessed on a case-by-case basis.
- CPT codes specific for KFM testing (generally including language such as “known familial variant” in the code description) may not be used to bill for any other types of testing. There must be a documented KFM in the family. For example, the use of a KFM CPT code when billing part of a panel of genes, which is generally used as the initial step in identifying a disease-causing mutation in an individual, is not a correct use of these codes and is therefore not eligible for reimbursement.

### **Criteria: Test-specific Guidelines**

Test-specific guidelines are available for some tests designed to assess known familial mutations. For tests without a specific guideline, use the General Coverage Guidance in Section 1.

## **References**

### **Introduction**

This guideline cites the following references.

1. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med*. 2015; 17(5):405-24. doi: 10.1038/gim.2015.30.
2. NCCN Clinical Practice Guidelines: Genetic/Familial High-Risk Assessment: Breast and Ovarian. Version 3.2019.  
[https://www.nccn.org/professionals/physician\\_gls/pdf/genetics\\_screening.pdf](https://www.nccn.org/professionals/physician_gls/pdf/genetics_screening.pdf).
3. NCCN Clinical Practice Guidelines: Genetic/Familial High-Risk Assessment: Colorectal. Version 1.2018.  
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# Genetic Testing for Non-Medical Purposes

MOL.CU.111.A

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## Description

While most traditional genetic tests are used for clear medical purposes, advances in gene discovery and genetic testing technology allow laboratories to offer genetic testing for other uses. Testing for paternity, ancestry, and non-disease traits such as baldness and eye color may be highly accurate and interesting. However, because these kinds of tests are not useful for medical management in the vast majority of cases, they are typically excluded from consideration.

## Criteria

### Criteria: General Coverage Guidance

Any genetic test that **DOES NOT** meet the following criteria is excluded from consideration:

- **Technical and clinical validity:** The test must be accurate, sensitive and specific, based on sufficient, quality scientific evidence to support the claims of the test.
- **Clinical utility:** Healthcare providers can use the test results to provide significantly better medical care for the individual.
- **Reasonable use:** The usefulness of the test is not significantly offset by negative factors, such as expense, clinical risk, or social or ethical challenges.

### Criteria:

The following types of testing are not considered medically necessary and therefore, not eligible for reimbursement:

- Genome-wide association studies (GWAs): testing a large number of genetic variations spread across the whole genome for disease associations, generally done for information outside of a specific clinical need or context
  - Common trade names: 23andMe, Pathway Genomics
- Paternity testing: testing to establish biological relationships, often between a father and child(ren) but sometimes to determine other kinds of relationships (siblings, grandparents, etc.)
- Ancestry testing: testing that helps people discover more about the genetic make-up of their ancestors, generally used by genealogists and those interested in family history

- Common trade names: Ancestry.com, 23andMe, Pathway Genomics, Family Tree DNA
- Nutritional testing: for variations in metabolism pathways that may suggest vitamin or other nutritional supplements.
  - Common trade names: myDNA Vitamins, Vitagene
- Athletic ability: Testing to predict athletic performance types.
  - Common trade names: DNA Sport Test, Athleticcode
- Genetic testing related to dating services
  - Common trade names: Pheramor, GenePartner, DNA Romance



# Genetic Testing for Prenatal Screening and Diagnostic Testing

MOL.CU.112.A

v1.0.2020

## Description

Prenatal screening and diagnostic testing is performed during pregnancy to identify fetuses at increased risk for or affected with genetic conditions and birth defects. Screening with ultrasound and maternal serum markers is routinely offered. Prenatal diagnosis by chorionic villus sampling or amniocentesis for chromosome abnormalities is available to all women. However, it is usually offered specifically to those at higher risk because of maternal age, a positive screen result, abnormal ultrasound findings, or known risk of a genetic condition based on family history. Investigations for fetal infection and blood antigen incompatibility may also be performed in the prenatal period. Results of testing are used to guide reproductive decision-making, pregnancy management and anticipatory management of the infant at birth.

**Note** This policy does not include prenatal or preconception carrier screening or preimplantation genetic testing. Please refer to *Genetic Testing for Carrier Status* and *Preimplantation Genetic Screening and Diagnosis* for those purposes.

## Criteria

### Criteria: General Coverage Guidance

Individuals may be considered for genetic testing for prenatal screening and diagnostic testing when **ALL** of the following conditions are met:

- **Technical and clinical validity:** The test must be accurate, sensitive and specific, based on sufficient, quality scientific evidence to support the claims of the test.
- **Clinical utility:** Healthcare providers can use the test results to provide significantly better medical care and/or assist patients with reproductive planning.
- **Reasonable use:** The usefulness of the test is not significantly offset by negative factors, such as expense, clinical risk, or social or ethical challenges.

Limits:

- Testing will only be covered for the number of genes or tests necessary to establish a prenatal diagnosis. A tiered approach to testing, with reflex to more detailed testing and/or different genes, will be required when clinically possible.
- Prenatal diagnostic testing will be allowed once per pregnancy. Exceptions may be considered if ambiguous results require retesting for clarification.

- If prenatal samples are studied concurrently with a maternal DNA sample to rule out prenatal analytic errors due to maternal cell contamination, a single unit of CPT code 81265 may be approved.

### **Criteria: Special Prenatal Diagnosis Circumstances**

Each of the following policies addresses a group of tests that are used for similar purposes in pregnancy. Because a variety of tests may be used, but the circumstances that justify testing are the same, individual test-specific policies are not necessary.

#### **Prenatal diagnostic testing based on family history**

Prenatal genetic testing, generally by amniocentesis or CVS, for the diagnosis of a genetic condition is reasonable when the following conditions are met:

- The pregnancy is at an increased risk for a genetic disease because of ANY of the following:
  - At least one parent is known or suspected to be a carrier of a genetic condition based on the family history and/or previous carrier testing results; or
  - One or both parent(s) are affected with a genetic condition; or
  - A sibling is affected with a genetic condition; AND
- The genetic condition is associated with potentially severe disability or has a lethal natural history.

#### **Fetal infectious disease testing**

Genetic testing may be used for the diagnosis of an infectious disease (e.g., cytomegalovirus, toxoplasmosis, parvovirus B19, and varicella zoster) in a fetus according to current guidelines from the American College of Obstetricians and Gynecologists (ACOG). Prenatal testing, generally by amniocentesis or CVS, is reasonable when ANY of the following conditions are met:

- Clinical signs and symptoms of a current infection in the mother; OR
- Serologic evidence of a current or recent infection in the mother (with or without clinical signs); OR
- Fetal abnormalities identified on ultrasound indicating an increased risk for a congenital infection

#### **Blood antigen incompatibility testing**

Prenatal genetic testing, generally by amniocentesis, for the determination of blood antigen genotype is supported by current evidence-based recommendations from the American College of Obstetricians and Gynecologists. Fetal antigen genotyping is reasonable when the following conditions are met:

- A positive erythrocyte antibody screen in the mother; AND EITHER
  - The father's blood antigen genotype is known and indicates a risk for the fetus to be positive; OR
  - The father's blood antigen genotype is not known and unavailable

### Criteria: Test-specific Guidelines

- Test-specific guidelines are available for some prenatal screening tests and diagnostic tests. For tests without a specific guideline, use the General Coverage Guidance in Section 1.

### References

1. ACOG Practice Bulletin. Cytomegalovirus, Parvovirus B19, Varicella Zoster, and Toxoplasmosis in Pregnancy. Number 151, June 2015 (reaffirmed 2017). *Obstet Gynecol.* 2015;125(6):1510-1525.
2. ACOG Practice Bulletin, Number 192, March 2018. Management of alloimmunization during pregnancy. *Obstet Gynecol.* 2018;131:e82-90.

# Genetic Testing for the Screening, Diagnosis, and Monitoring of Cancer

MOL.CU.113.A

v1.0.2020

## Description

Genetic testing for screening, diagnosis and monitoring of cancer refers to molecular diagnostic tests whose purposes include identifying the possible presence of cancer in asymptomatic, average risk individuals; confirming the absence or presence of cancer; and monitoring the absence or presence of cancer after a prior diagnosis and treatment.

## Screening

The goal of cancer screening is to identify the possible presence of cancer before symptoms appear. Screening tests cannot diagnose cancer, but typically determine if there is an increased chance cancer is present, and triages individuals for more invasive, diagnostic testing. Most cancer screening does not include genetic testing, but instead relies on physical exam, radiological exams, or non-genetic laboratory tests. Advances in human genetics, however, have identified several molecular diagnostic tests that may provide clues for early cancer detection.

## Diagnosis

When cancer is suspected because of an abnormal screening test or symptoms, blood tests for tumor markers or molecular testing on tissue samples can aid in confirming a diagnosis of cancer. These tests may contribute information to helping the clinician understand prognosis and treatment options.

## Monitoring

During treatment, or after an apparently successful treatment, active monitoring is often recommended to identify if the cancer is responding to treatment or has returned or spread, before any symptoms appear. Monitoring may include increased surveillance or routine blood tests for tumor markers, and increasingly, molecular genetic tests.

- Tests used to determine hereditary cancer risk are covered separately as *Genetic Testing for Cancer Susceptibility and Hereditary Cancer Syndromes*.
- This policy does not address drug response to cancer, or testing to determine which therapies to use. Please refer to *Pharmacogenomic Testing for Drug Toxicity and Response* for that purpose.
- This policy does not address molecular tumor marker testing in solid tumors. Please refer to *Somatic Mutation Testing—Solid Tumors* and *Liquid Biopsy Testing – Solid Tumors* for that purpose.

- This policy does not address diagnostic or predictive testing for conditions other than non-inherited cancer. Refer to *Genetic Testing to Diagnose Non-Cancer Conditions* and *Genetic Testing to Predict Disease Risk* for those purposes.

## Criteria

### Criteria: General Coverage Guidance

Individuals may be considered for genetic testing for screening, diagnosing, or monitoring cancer when **ALL** of the following conditions are met:

- **Technical and clinical validity:** The test must be accurate, sensitive and specific, based on sufficient, quality scientific evidence to support the claims of the test.
- **Clinical utility:** Healthcare providers can use the test results to provide significantly better medical care for the individual.
- **Reasonable use:** The usefulness of the test is not significantly offset by negative factors, such as expense, clinical risk, or social or ethical challenges.

Limits:

- Testing will be considered only for the number of genes or tests necessary. A tiered approach to testing, with reflex to more detailed testing and/or different genes, will be required when clinically possible.
- For tests that look for changes in germline DNA (i.e., not tumor DNA or viral DNA), testing will be allowed once per lifetime per gene. Exceptions may be considered if technical advances in testing demonstrate significant advantages that would support a medical need to retest.

### Criteria: Test-specific Guidelines

Test-specific guidelines are available for some tests designed to screen for, diagnose, or monitor cancer. For tests without a specific guideline, use the General Coverage Guidance in Section 1.

# Genetic Testing for Variants of Uncertain Clinical Significance

MOL.CU.292.A

v1.0.2020

## Introduction

Genetic testing for variants of uncertain clinical significance is addressed by this guideline.

## Description

Genetic testing of an affected individual by gene sequencing or multi-gene panel testing can reveal genetic variants that have an unknown effect. These variants of uncertain clinical significance (VUS) may or may not cause disease in the individual; there is simply not enough known at the time of the report to call the variant disease-causing or benign.<sup>1</sup>

The accumulation of sufficient data to reclassify a VUS may take many years and require identification of the variant in multiple individuals. Pathogenicity of a variant is determined by labs through assessing:

- Disease-specific or gene-specific mutation databases
- Large population variant frequency databases
- In silico prediction tools
- Multi-species conservation assessment
- Literature searches
- Functional studies
- Family assortment studies

Family studies may be offered by the laboratory at no charge to the family, as the result may assist the lab in future classification of the variant. Testing relatives for a VUS may not always lead to reclassification of a variant to either disease-causing or benign, but it can be helpful in certain clinical scenarios, potentially contributing evidence that it is more or less likely to be disease-causing.

## Targeted VUS Testing

Testing the parents of an affected child who has a VUS may be helpful in determining the clinical significance of that variant in some situations. For instance, if the condition is dominant and the VUS is not inherited from either parent (de novo), it is more likely to be disease-causing. If it is inherited from a healthy parent, it may be more likely to be benign.

Similarly, for an autosomal recessive condition, one or both of two potential disease-causing variants in a child may be called VUS. Testing parents should confirm whether one of the variants was inherited from each parent, and therefore fits the recessive pattern of inheritance.

If a VUS is identified in apparent homozygosity (2 copies), testing parents should determine copy number. A VUS that is inherited in two copies, one from each parent, would be consistent with the expected pattern of inheritance for recessive disease. If the VUS is only inherited from one parent, other mechanisms for pathogenicity (such as gene deletion or uniparental disomy) should be investigated.

Simply testing a relative for a VUS will not determine if that variant is disease causing or benign. This is especially true for adult onset conditions (hereditary cancer syndromes) or conditions for which there is reduced or non-penetrance or highly variable expressivity. After targeted testing for a VUS, careful clinical and family history evaluation and correlation with the result is essential.

### Genes of Uncertain Clinical Significance

Broader tests, such as whole exome sequencing or whole genome sequencing, may identify variants in genes that have an unknown effect. That is, for a gene of uncertain clinical significance (GUS) there is not enough known about the gene and its function to say whether it can cause the disease in question.<sup>1</sup>

### Potential Outcomes of Targeted VUS testing

Results of testing and possible significance of testing.

| Result of VUS testing   | Possible significance                       |
|---|---|
| VUS is not inherited (de novo)  | Increased likelihood of causing disease     |
| VUS is inherited from affected parent                                       | Increased likelihood of causing disease     |
| VUS is inherited from unaffected parent                                     | Decreased likelihood of causing disease     |
| VUS is inherited with a disease-causing variant or VUS from the same parent | Decreased likelihood of causing disease     |
| VUS that is apparently homozygous is not inherited from both parents        | Alternate mechanisms should be investigated |

## Criteria

### Introduction

Requests for genetic testing for variants of uncertain clinical significance are reviewed using these criteria.

**Criteria: General Coverage Guidance**

- Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- No previous genetic testing of the requested gene, AND
- No known alternate genetic cause for the diagnosis in the family, AND
- Member is the biological parent of a child in whom a VUS was identified, AND
- VUS is in a gene that is
  - Known to be disease-associated, and
  - Consistent with the child's clinical diagnosis, AND
- Purpose of testing is to determine
  - Whether the VUS is inherited or de novo, or
  - Whether the VUS is present in homozygosity, AND
- Determination of the inheritance or copy number of the VUS will lead to treatment changes for the member or the member's child, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy

**Limitations and Exclusions**

- Testing of multiple affected and unaffected relatives to determine if a VUS assort with symptoms in the family is not considered medically necessary; therefore, it is not reimbursable.
- Testing for variants in genes of uncertain clinical significance (GUS) is not considered medically necessary; therefore, it is not reimbursable.
- Each test request for VUS testing should be reviewed based on the medical information available for the member and the clinical utility and technical and clinical validity of the service requested.

**Criteria: Test-specific Guidelines**

Test-specific guidelines may be available for tests that could target a VUS. For tests without a specific guideline, use the General Coverage Guidance in Section 1.

**References****Introduction**

This guideline cites the following references.



1. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med.* 2015; 17(5):405-24. doi: 10.1038/gim.2015.30.

# Genetic Testing to Diagnose Non-Cancer Conditions

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## Description

Diagnostic testing is performed in patients with clinical signs or symptoms of a non-cancer genetic condition. The genetic test may confirm or rule out a clinical diagnosis. In some cases, genetic testing is the gold standard for making a diagnosis based on evidence- or consensus-based guidelines. In others, it may be used to confirm a clinical diagnosis, offer prognostic information that impacts management, or rule out a diagnosis in the differential. Often, diagnostic testing of an affected individual will offer results that are relevant to the testing of other family members.

- This guideline does not include risk assessment or predictive testing for at-risk, asymptomatic individuals. Please refer to *Genetic Testing to Predict Disease Risk* for that purpose.
- Diagnostic testing of a pregnancy or an embryo is addressed by guidelines on *Genetic Testing for Prenatal Screening and Diagnostic Testing* and *Preimplantation Genetic Screening and Diagnosis*, respectively.
- In addition, testing for hereditary cancer syndromes is addressed separately under *Genetic Testing for Cancer Susceptibility and Hereditary Cancer Syndromes*.

## Criteria

### Criteria: General Coverage Guidance

Individuals may be considered for diagnostic genetic testing when **ALL** of the following conditions are met:

- **Clinical signs and symptoms** in the individual are consistent with the diagnosis in question.
- **Technical and clinical validity:** The test must be accurate, sensitive and specific, based on sufficient, quality scientific evidence to support the claims of the test.
- **Clinical utility:** Healthcare providers can use the test results to provide significantly better medical care for the individual.
- **Reasonable use:** The usefulness of the test is not significantly offset by negative factors, such as expense, clinical risk, or social or ethical challenges.

Limits:

- Testing will be considered only for the number of genes or tests necessary to establish mutation status. A tiered approach to testing, with reflex to more detailed testing and/or different genes, will be required when clinically possible.
- Diagnostic genetic testing will be allowed once per lifetime per condition. Exceptions may be considered if technical advances in testing demonstrate significant advantages that would support a medical need to retest.

### Criteria: Special Circumstances

#### ***Diagnostic testing of an individual to inform reproductive planning and testing for parents or testing for siblings.***

Diagnostic genetic testing may be requested in a symptomatic individual with a known genetic condition. While diagnostic testing may not impact management of the affected individual, the information gained from genetic testing may be needed to perform accurate carrier testing in the parent(s), genetic diagnosis in a pregnancy, or genetic diagnosis in a sibling.\*

In these circumstances, diagnostic genetic testing in a symptomatic individual may be considered when **ALL** of the following conditions are met:

- The diagnosis of the disease in the affected individual is ***certain or highly probable*** based on clinical signs and symptoms, history, imaging, and/or results of other laboratory testing.
- The results of the genetic test in the symptomatic individual must be ***required*** in order to perform accurate carrier testing in the parent(s), genetic diagnosis in a pregnancy, or genetic diagnosis in a sibling.
- ***Technical and clinical validity***: The test must be accurate, sensitive and specific, based on sufficient, quality scientific evidence to support the claims of the test.
- ***Clinical utility***: Healthcare providers can use the test results to provide informative genetic testing for the sibling, parents, or for a current or future at-risk pregnancy.
- ***Reasonable use***: The usefulness of the test is not significantly offset by negative factors, such as expense, clinical risk, or social or ethical challenges.

#### Limits:

- Testing will be indicated only for the number of genes or tests necessary to establish the familial mutation(s). A tiered approach to testing, with reflex to more detailed testing and/or different genes, will be required when clinically possible.
- Diagnostic genetic testing will be allowed once per lifetime per condition. Exceptions may be considered if technical advances in testing demonstrate significant advantages that would support a medical need to retest.

\*Parent or sibling must also be a covered member under the same health plan.

**Criteria: Test-specific Guidelines**

Test-specific guidelines are available for some tests designed to diagnosis non-cancer conditions. For tests without a specific guideline, use the General Coverage Guidance in Section 1.

# Genetic Testing to Predict Disease Risk

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## Description

Predictive genetic testing is performed in people known to be at increased risk of developing an inherited non-cancer condition (for the purposes of this guideline) based on their family history. For some conditions, a positive genetic test predicts with certainty that the person will eventually develop signs and symptoms of a condition. For other conditions, a positive genetic test result indicates an increased risk (susceptibility) for a condition. A negative result may rule out a condition, or lower the risk significantly. Having test results may improve medical management through improved screening, preventive measures, prophylactic medication, and other means.

- This guideline does not include testing of a symptomatic individual. Please refer to *Genetic Testing to Diagnose Non-Cancer Conditions* for that purpose.
- Predictive testing for hereditary cancer syndromes is addressed separately under *Genetic Testing for Cancer Susceptibility and Hereditary Cancer Syndromes*.
- Testing of minors is addressed separately under *Genetic Presymptomatic and Predictive Testing for Adult-Onset Conditions in Minors*.

## Criteria

### Criteria: General Coverage Guidance

Individuals may be considered for predictive genetic testing when **ALL** of the following conditions are met:

- The individual is **known to be at-risk** for developing inherited condition because a parent, sibling, or child is affected by or known to be a carrier of a genetic disease.
- **Technical and clinical validity:** The test must be accurate, sensitive and specific, based on sufficient, quality scientific evidence to support the claims of the test.
- **Clinical utility:** Healthcare providers can use the test results to provide significantly better medical care for the individual.
- **Reasonable use:** The usefulness of the test is not significantly offset by negative factors, such as expense, clinical risk, or social or ethical challenges.

Limits:

- Testing will be considered only for the number of genes or tests necessary to establish carrier status. A tiered approach to testing, with reflex to more detailed testing and/or different genes, will be required when clinically possible.

- Predictive genetic testing will be allowed once per lifetime per condition. Exceptions may be considered if technical advances in testing demonstrate significant advantages that would support a medical need to retest.
- Predictive testing will be considered only for adult individuals (age 18 and over). Exceptions may be considered if there are medical management and/or significant psychosocial benefits to testing prior to adulthood.<sup>1,2,3</sup>

### Criteria: Special circumstances

#### Testing for Known Familial Mutations

The genetic mutation(s) associated with a genetic disease can often be defined in an affected family member, allowing for testing of at-risk relatives for those specific mutations. Testing for known familial mutations may be considered when **ALL** of the following conditions are met:

- The mutations in the family have been **clearly defined** by previous genetic testing and **information about those mutations can be provided** to the testing lab.
- **Technical and clinical validity**: The test must be accurate, sensitive and specific to the familial mutations.
- **Clinical utility**: Healthcare providers can use the test results to provide significantly better medical care for the individual.
- **Reasonable use**: The usefulness of the test is not significantly offset by negative factors, such as expense, clinical risk, or social or ethical challenges.

#### Limits:

- Testing will be considered only for the known familial mutations when clinically possible.
- Predictive genetic testing will be allowed once per lifetime per condition.
- Predictive testing will be considered only for adult individuals (age 18 and over). Exceptions may be considered if there are medical management and/or significant psychosocial benefits to testing prior to adulthood.<sup>1,2,3</sup>

### Criteria: Test-specific Guidelines

Test-specific guidelines are available for some tests designed to predict disease risk. For tests without a specific guideline, use the General Coverage Guidance.

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# Hereditary (Germline) Testing After Tumor (Somatic) Testing

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## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedures addressed by this guideline       | Procedure codes |
|--|-----------------|
| APC Sequencing                               | 81201           |
| APC Deletion/Duplication Analysis            | 81203           |
| ATM Sequencing                               | 81408           |
| BRCA1/2 Sequencing                           | 81163           |
| BRCA1/2 Deletion/Duplication Analysis        | 81164           |
| BRCA1 Sequencing                             | 81165           |
| BRCA1 Deletion/Duplication Analysis          | 81166           |
| BRCA2 Sequencing                             | 81216           |
| BRCA2 Deletion/Duplication Analysis          | 81167           |
| BRCA1/2 185delAG, 5385insC, 617delT variants | 81212           |
| BRCA1/2 Known Familial Variants              | 81215           |
| Chromosomal Microarray [BAC], Constitutional | 81228           |
| Chromosomal Microarray [SNP], Constitutional | 81229           |
| MLH1 Sequencing                              | 81292           |
| MLH1 Deletion/Duplication Analysis           | 81294           |
| MLH1 Known Familial Variants                 | 81293           |
| MSH2 Sequencing                              | 81295           |
| MSH2 Deletion/Duplication Analysis           | 81297           |
| MSH2 Known Familial Variants                 | 81296           |
| MSH6 Sequencing                              | 81298           |
| MSH6 Deletion/Duplication Analysis           | 81300           |



| Procedures addressed by this guideline   | Procedure codes |
|--|-----------------|
| MSH6 Known Familial Variants   | 81299           |
| PMS2 Sequencing  | 81317           |
| PMS2 Deletion/Duplication Analysis   | 81319           |
| PTEN Sequencing  | 81321           |
| PTEN Deletion/Duplication Analysis   | 81323           |
| PTEN Known Familial Variants   | 81322           |
| Hereditary breast cancer-related disorders (e.g., hereditary breast cancer, hereditary ovarian cancer, hereditary endometrial cancer); genomic sequence analysis panel, must include sequencing of at least 10 genes, including BRCA1, BRCA2, CDH1, MLH1, MSH2, MSH6, PALB2, PTEN, STK11, and TP53 | 81432           |
| Hereditary breast cancer-related disorders (e.g., hereditary breast cancer, hereditary ovarian cancer, hereditary endometrial cancer); duplication/deletion analysis panel, must include analyses for BRCA1, BRCA2, MLH1, MSH2, and STK11  | 81433           |
| Hereditary colon cancer disorders (e.g., Lynch syndrome, PTEN hamartoma syndrome, Cowden syndrome, familial adenomatosis polyposis); genomic sequence analysis panel, must include sequencing of at least 10 genes, including APC, BMPR1A, CDH1, MLH1, MSH2, MSH6, MUTYH, PTEN, SMAD4, and STK11   | 81435           |
| Hereditary colon cancer disorders (e.g., Lynch syndrome, PTEN hamartoma syndrome, Cowden syndrome, familial adenomatosis polyposis); duplication/deletion analysis panel, must include analysis of at least 5 genes, including MLH1, MSH2, EPCAM, SMAD4, and STK11                                 | 81436           |

| Procedures addressed by this guideline   | Procedure codes |
|--|-----------------|
| Hereditary neuroendocrine tumor disorders (e.g., medullary thyroid carcinoma, parathyroid carcinoma, malignant pheochromocytoma or paraganglioma); genomic sequence analysis panel, must include sequencing of at least 6 genes, including MAX, SDHB, SDHC, SDHD, TMEM127, and VHL | 81437           |
| Hereditary neuroendocrine tumor disorders (e.g., medullary thyroid carcinoma, parathyroid carcinoma, malignant pheochromocytoma or paraganglioma); duplication/deletion analysis panel, must include analyses for SDHB, SDHC, SDHD, and VHL  | 81438           |
| Miscellaneous hereditary cancer syndrome gene tests  | 81400           |
| Miscellaneous hereditary cancer syndrome gene tests  | 81401           |
| Miscellaneous hereditary cancer syndrome gene tests  | 81402           |
| Miscellaneous hereditary cancer syndrome gene tests  | 81403           |
| Miscellaneous hereditary cancer syndrome gene tests  | 81404           |
| Miscellaneous hereditary cancer syndrome gene tests  | 81405           |
| Miscellaneous hereditary cancer syndrome gene tests  | 81406           |
| Miscellaneous hereditary cancer syndrome gene tests  | 81407           |
| Miscellaneous hereditary cancer syndrome gene tests  | 81408           |
| Miscellaneous hereditary cancer syndrome gene tests  | 81479           |

## What is germline hereditary cancer testing following somatic tumor testing

### Definition

Most cancer is sporadic and due to the acquisition of somatic variants. In addition, about 5-10% of cancer has a hereditary etiology due to constitutional germline variants.<sup>1</sup>

- In oncology, next generation sequencing (NGS) technology makes it feasible to catalog the DNA sequence variations within a person's cancer (i.e., somatic mutation profiling). This helps define therapeutic targets which might improve outcomes through the use of specific medications directed at those mutations.<sup>2</sup>
- Germline variants can also be identified as an ancillary finding during primary tumor profiling to identify somatic mutations. "In the course of analyzing tumor DNA (without matched normal DNA), sequencing can identify potential constitutional (germline) DNA variations that are associated with disease or susceptibility to disease as well as carrier states for Mendelian disorders. Centers may use matched tumor-normal sequencing to facilitate more accurate calling of somatic mutations by using the normal DNA to exclude germline variants from the tumor cells."<sup>3,4</sup>
  - In a study by Schrader et al, "Targeted tumor sequencing with a panel of 341 genes and matched normal DNA in 1566 individuals with advanced malignant neoplasms revealed presumed pathogenic germline variants (PPGVs) in about 16% of individuals. Most PPGVs (80.5%, 95% CI, 75.1%-85.0%) were in genes related to cancer susceptibility. The PPGVs in genes previously designated as clinically actionable cancer targets were seen in 5.0% (95% CI, 4.1%-6.2%) of individuals. Most cancer-susceptibility PPGVs were retained in the tumor (91.9%; 95% CI, 87.3%-95.0%).<sup>5</sup> This study is in line with other published studies investigating the prevalence of incidental findings with somatic tumor profiling."<sup>4,6</sup>
- The debate continues regarding whether there is an obligation to test for and report these germline findings which are secondary to the original purpose of somatic tumor profiling. In making this determination, pre-test informed consent is of utmost importance. "Honoring patient preferences requires oncology providers to communicate the potential for incidental and secondary germline information specific to the test being offered, the relevance and potential benefits of this information for patients and their relatives, and the limitations and risks of receiving incidental and secondary germline information."<sup>2</sup>

### Test information

- Testing to investigate somatic and constitutional DNA variants has become more common as sequencing technology has evolved from the more labor intensive Sanger sequencing to NGS. "NGS is a powerful technology that permits the

characterization of large amounts of DNA sequence much quicker and at lower cost than traditional Sanger sequencing.”<sup>2,7</sup>

- Laboratories performing somatic mutation profiling may include paired germline testing, not in an effort to identify hereditary etiologies but to identify variants that are genetic “drivers” of the individual’s malignancy.<sup>4</sup>
- Laboratories may also use bioinformatics to subtract the inherited variants from the somatic tumor profiling findings. Germline variants may be missed during this process without performing further analysis.<sup>8,9</sup>

## Guidelines and evidence

- The National Comprehensive Cancer Network (NCCN, 2017)<sup>10</sup> states the following regarding germline testing following somatic tumor testing for BRCA1/2 mutations.
  - BRCA1/2 germline mutation testing should be considered when a BRCA1/2 mutation is detected by tumor profiling on any tumor type.
- There have been various peer-reviewed publications that reviewed pre- and post-test considerations for germline testing following somatic tumor testing.
  - Pre-test considerations:
    - Somatic tumor-only NGS testing is used to guide treatment for an affected person. The testing is not designed to elucidate a hereditary etiology. A germline variant may not be detected (due to differences in coverage in the testing, cellularity of the sample, allelic loss of the germline mutation) or may not be reported by the somatic testing laboratory.<sup>2,3,11</sup>
    - Directed germline genetic testing can be ordered to identify a potential hereditary etiology for the person’s tumor. Referrals to oncology genetic counselors or other specialized healthcare providers should occur if the individual’s personal and/or family history meets established criteria to warrant a more detailed discussion.<sup>10,12,13</sup>
    - Ancillary findings from somatic or germline testing may include variants in genes that cause a hereditary cancer syndrome, a non-oncologic hereditary syndrome, or identify carrier status for Mendelian disease. Specific findings are dependent on specific testing performed by the laboratory.<sup>2,3,11</sup>
    - Many patients undergoing somatic tumor profiling have advanced stage disease. Centers performing somatic tumor profiling should consider obtaining a surrogate individual to receive results in the event that the proband has passed away or is otherwise unable to receive the results.<sup>2,3,11</sup>
  - Post-test considerations:

- Clinicians must determine the technical specifications of the laboratory used for somatic tumor profiling and determine if this includes paired germline testing. Some laboratories may not report germline variants.<sup>2,3,14</sup>
- Tumor profiling variant interpretation may differ from the variant interpretation process for germline mutations. For example, a laboratory profiling a somatic tumor may classify a certain variant as pathogenic whereas a laboratory testing a germline mutation may classify that same variant as a variant of uncertain significance (VUS).<sup>2,3,14</sup>
- Referrals to oncology genetic counselors or other specialized healthcare providers should occur if the individual's personal and/or family history meets established criteria to warrant a more detailed discussion, regardless of somatic tumor profiling results.<sup>10,12,13</sup>

## Criteria

- Requests for single-site or full-gene sequence germline testing following somatic tumor analysis will be considered medically necessary when at least one of the following criteria is met:
  - The individual's personal or family history is suggestive of a germline mutation, a specific germline variation is identified by somatic tumor testing, and the individual meets the published test-specific criteria to test for that variant,<sup>10</sup> OR
  - One of the identified variants is a highly-recurrent or founder mutation (i.e., BRCA1 c185delAG or the recurrent inversion of MSH2 seen in some families with Lynch syndrome),<sup>3</sup> OR
  - The tumor profile shows thousands of somatic variants, suggesting a germline mutation in a DNA mismatch repair gene or in the POLE proofreading domain,<sup>3,15</sup> OR
  - Two separate primary tumors are sequenced and both harbor the same genetic variant,<sup>6</sup> OR
  - The individual's tumor harbors a mutation in BRCA1/2,<sup>10</sup> OR
  - Patient does not meet published criteria for germline testing, but variant(s) within genes known to play a role in tumor biology and to cause an inherited cancer syndrome (including but not limited to TP53, APC, CDH1) are identified and the variant allele frequency in the tumor is at least 33%.<sup>16,17,18</sup>

## Exclusions and other considerations

- Germline testing of somatic variants of uncertain significance (VUS) is not considered medically necessary.
- Germline testing for asymptomatic individuals based solely on a family member's somatic testing result is not considered medically necessary.

- In individuals meeting criteria for germline DNA testing, analysis of the entire gene, as opposed to single site testing, is recommended.<sup>6</sup>
- Resources, such as ClinVar, should be used by the provider to determine if a pathogenic variant classification provided by germline testing laboratories is consistent with independent assessments of that variant.<sup>19</sup>

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# Investigational and Experimental Molecular and Genomic Testing

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## Introduction

Investigational and experimental (I&E) molecular and genomic testing is addressed by this guideline.

## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedures address by this guideline   | Procedure codes |
|--|-----------------|
| 5-Fluorouracil (5-FU) Toxicity and Chemotherapeutic Response                     | 81232<br>81346  |
| Accelerate PhenoTest BC kit  | 0086U           |
| AmHPR Helicobacter pylori Antibiotic Resistance Next Generation Sequencing Panel | 0008U           |
| Bacterial Typing by Whole Genome Sequencing                                      | 0010U           |
| BBDRisk Dx   | 0067U           |
| ChemoFX  | 81535<br>81536  |
| DEPArray   | 0009U           |
| EsoGuard   | 0114U           |
| ExoDx®Prostate(IntelliScore)   | 0005U           |
| INFINITI Neural Response Panel   | 0078U           |
| Lymph3Cx Lymphoma Molecular Subtyping Assay                                      | 0120U           |
| MatePair Acute Myeloid Leukemia  | 0056U           |
| MatePair Targeted Rearrangements, Hematologic                                    | 0014U           |
| MatePair Targeted Rearrangements, Oncology                                       | 0013U           |



| Procedures address by this guideline   | Procedure codes                        |
|--|--|
| MicroGenDX qPCR & NGS For Infection  | 0112U                                  |
| MiPS (Mi-Prostate Score)   | 0113U                                  |
| miR-31now  | 0069U                                  |
| Molecular Microscope MMDx—Heart  | 0087U                                  |
| Molecular Microscope MMDx—Kidney   | 0088U                                  |
| MYCODART Dual Amplification Real Time PCR Panel for 4 Aspergillus species                  | 0109U                                  |
| myPath Melanoma  | 0090U                                  |
| myTAIHEART   | 0055U                                  |
| OncoTarget/OncoTreat   | 0019U                                  |
| Prostate Cancer Risk Panel   | 0053U                                  |
| +RNAinsight for ATM  | 0136U                                  |
| +RNAinsight for BRCA1/2  | 0138U                                  |
| +RNAinsight for BreastNext   | 0131U                                  |
| +RNAinsight for CancerNext   | 0134U                                  |
| +RNAinsight for ColoNext   | 0130U                                  |
| +RNAinsight for GYNPlus  | 0135U                                  |
| +RNAinsight for OvaNext  | 0132U                                  |
| +RNAinsight for PALB2  | 0137U                                  |
| +RNAinsight for ProstateNext   | 0133U                                  |
| ROMA Risk of Ovarian Malignancy Algorithm  | 81500                                  |
| Statin Induced Myopathy Genotype (SLCO1B1)   | 81328                                  |
| ToxLok   | 0079U                                  |
| Twin Zygosity, cell free fetal DNA   | 0060U                                  |
| VectraDA   | 81490                                  |
| Viracor TRAC dd-cfDNA  | 0118U                                  |
| Investigational and experimental tests that make use of molecular and genomic technologies | 81479<br>84999<br>81599, and<br>Others |

## What is I&E molecular and genomic testing

### Definition

An investigational and experimental (I&E) procedure is the use of a service, supply, drug, or device that is not recognized as standard medical care for the condition, disease, illness, or injury. Treatment is determined by the health plan based on an independent, peer review of literature and scientific data. I&E molecular and genomic tests refer to assays involving chromosomes, DNA, RNA, or gene products that have insufficient data to determine the net health impact.

### Investigational and experimental determinations

Molecular and genomic tests are routinely released to market that make use of novel technologies or have a novel clinical application. These tests are often available on a clinical basis long before the required evidence to support clinical validity and clinical utility are established. Typically, there is insufficient data to support that the test

- accurately assesses the outcome of interest, analytical and clinical validity
- significantly improves health outcomes, clinical utility, and
- performs better than an existing standard of care medical management option.

Because these tests are often proprietary, there may be no independent test evaluation data available in the early stages to support the laboratory's claims regarding test performance and utility.

As new molecular and genomic tests become commercially available, the evidence base is reviewed. Tests determined to be I&E by the Health Plan are addressed by this guideline or a test-specific guideline and are not eligible for reimbursement.

### FDA clearance

In the case of molecular and genomic testing, FDA clearance is not a reliable standard given the number of laboratory developed tests that currently fall outside of FDA oversight. FDA clearance often does not assess clinical utility.

## Criteria

### Introduction

This section catalogues some, but not all, molecular and genomic tests that have been determined to be investigational and experimental (I&E). I&E tests may also be addressed in test-specific guidelines and the reader is referred to those documents for additional information. New I&E tests may not yet be specifically listed in this guideline, but such decisions will be made using the following criteria.

**Criteria: general coverage guidance**

Molecular and genomic tests are only eligible for reimbursement when ALL of the following conditions are met:

- Technical and clinical validity: The test must be accurate, sensitive and specific, based on sufficient, quality scientific evidence to support the claims of the test.
- Clinical utility: Healthcare providers can use the test results to provide significantly better medical care for the individual.
- Reasonable use: The usefulness of the test is not significantly offset by negative factors, such as expense, clinical risk, or social or ethical challenges.

**Novel oncology molecular and genomic tests**

The following tests used in the screening, diagnosis, prognostication, and treatment decision-making for various neoplasms do not meet the above criteria and are not eligible for reimbursement.

**Gene Expression Assays**

- BluePrint Molecular Subtyping Profile [Proprietary 80-gene expression signature to classify Basal-type, Luminal-type and ERBB2-type breast cancers from Agendia]
- ColonSentry [Proprietary 7-gene signature to detect colorectal cancer from Innovative Diagnostic Laboratory]
- DecisionDx - Cutaneous Melanoma assay [Proprietary 31-gene signature to assess melanoma metastatic risk from Castle Biosciences]
- Envisia Genomic Classifier [Proprietary gene expression assay designed to aid in the diagnosis of idiopathic pulmonary fibrosis from Veracyte]
- ExoDx<sup>®</sup> Prostate(IntelliScore) [Oncology (prostate) gene expression profile by real-time RT-PCR of 3 genes (ERG, PCA3, and SPDEF), urine, algorithm reported as risk score from Exosome Diagnostics, Inc.]
- Lymph3Cx Lymphoma Molecular Subtyping Assay, [Oncology (B-cell lymphoma classification), mRNA, gene expression profiling by fluorescent probe hybridization of 58 genes (45 content and 13 housekeeping genes), formalin-fixed paraffin-embedded tissue, algorithm reported as likelihood for primary mediastinal B-cell lymphoma (PMBCL) and diffuse large B-cell lymphoma (DLBCL) with cell of origin subtyping in the latter from Mayo Clinic]
- miR-31now [Oncology (colorectal), microRNA, RT-PCR expression profiling of miR-31-3p, formalin fixed paraffin-embedded tissue, algorithm reported as an expression score from GoPath Laboratories]
- myPath Melanoma [Proprietary 23-gene expression assay to assess the risk of malignant melanoma when a result cannot be obtained by clinical assessment and/or histopathology alone from Myriad Genetics]

- OncoDefender - CRC [Proprietary gene expression assay to predict recurrence risk in early stage colorectal cancer within 3 years after surgery from Everist Genomics]
- OncoTarget/OncoTreat [Oncology, RNA, gene expression by whole transcriptome sequencing, formalin-fixed paraffin embedded tissue or fresh frozen tissue, predictive algorithm reported as potential targets for therapeutic agents from Columbia University Department of Pathology and Cell Biology, Darwin Health]
- Percepta Bronchial Genomic Classifier [Proprietary gene expression assay designed to assess the risk of malignancy of lung nodules from Veracyte]
- Pervenio Lung NGSTest [Proprietary 25-gene expression assay for risk stratification of early stage NSCLC from Life Technologies]
- RNA-Sequencing by NGS [Oncology (solid organ neoplasia), mRNA, gene expression profiling by massively parallel sequencing for analysis of 51 genes, utilizing formalin-fixed paraffin embedded tissue, algorithm reported as a normalized percentile rank from Life Technologies]

### **Other Novel Assays**

- BBDRisk Dx [Oncology (breast), immunohistochemistry, protein expression profiling of 4 biomarkers (matrix metalloproteinase-1 [MMP-1], carcinoembryonic antigen-related cell adhesion molecule 6 [CEACAM6], hyaluronoglucosaminidase [HYAL1], highly expressed in cancer protein [HEC1]), formalin-fixed paraffin-embedded precancerous breast tissue, algorithm reported as carcinoma risk score from Silbiotech, Inc]
- ChemoFX [Proprietary test from Helomics to assess chemosensitivity]
- clonoSEQ [Proprietary test that determines measurable residual disease (MRD) in the form of residual clonal cells to monitor changes in the disease burden during and post-treatment from Adaptive Biotechnologies]
- DEPArray [Oncology (breast cancer), ERBB2 (HER2) copy number by FISH, tumor cells from formalin fixed paraffin embedded tissue isolated using image-based dielectrophoresis (DEP) sorting, reported as ERBB2 gene amplified or non-amplified from PacificDx]
- HERmark Breast Cancer Assay [Proprietary test designed to evaluate Her-2 total proteins in formalin-fixed, paraffin-embedded (FFPE) tissue specimens from LabCorp]
- Know error [Proprietary test for DNA based specimen provenance confirmation from Strand Diagnostics]
- MatePair Acute Myeloid Leukemia Panel, [Hematology (acute myelogenous leukemia), DNA, whole genome next generation sequencing to detect gene rearrangement(s), blood or bone marrow, report of specific gene rearrangement(s) from Mayo Clinic]
- MatePair Targeted Rearrangements, Oncology, [Oncology (solid organ neoplasia), gene rearrangement detection by whole genome next-generation sequencing,

- DNA, fresh or frozen tissue or cells, report of specific gene rearrangement(s) from Mayo Clinic]
- MatePair Targeted Rearrangements, Hematologic, [Hematology (hematolymphoid neoplasia), gene rearrangement detection by whole genome next generation sequencing, DNA, whole blood or bone marrow, report of specific gene rearrangement(s) from Mayo Clinic]
  - MiPS (Mi-Prostate Score), [Oncology (prostate), measurement of PCA3 and TMPRSS2-ERG in urine and PSA in serum following prostatic massage, by RNA amplification and fluorescence-based detection, algorithm reported as risk score from MLabs]
  - Mitomic Prostate Test [Proprietary test using mitochondrial DNA to detect prostate cancer not identified by standard biopsy pathology from MDNA Life Sciences]
  - OncotypeDx AR-V7 Nucleus Detect [Proprietary test designed to detect AR-V7 proteins in the nucleus of CTCs to determine response to AR-targeted therapies from Genomic Health]
  - PAULA [Proprietary panel of four proteins designed to detect lung cancer in asymptomatic individuals at high risk from Genesys Biolabs]
  - Prostate Cancer Risk Panel [FISH analysis of 4 genes (ASAP1, HDAC9, CHD1 and PTEN), needle biopsy specimen, algorithm reported as probability of higher tumor grade from Mayo Clinic]
  - ProstaVysion [Proprietary panel of two biomarkers designed to predict prostate cancer prognosis from Bostwick Laboratories]
  - +RNAinsight for ATM, [ATM (ataxia telangiectasia mutated) (eg, ataxia telangiectasia) mRNA sequence analysis from Ambry Genetics]
  - +RNAinsight for BRCA1/2, [BRCA1 (BRCA1, DNA repair associated), BRCA2 (BRCA2, DNA repair associated) (eg, hereditary breast and ovarian cancer) mRNA sequence analysis from Ambry Genetics]
  - +RNAinsight for BreastNext, [Hereditary breast cancer–related disorders (eg, hereditary breast cancer, hereditary ovarian cancer, hereditary endometrial cancer), targeted mRNA sequence analysis panel (13 genes) from Ambry Genetics]
  - +RNAinsight for CancerNext, [Hereditary pan cancer (eg, hereditary breast and ovarian cancer, hereditary endometrial cancer, hereditary colorectal cancer), targeted mRNA sequence analysis panel (18 genes) from Ambry Genetics]
  - +RNAinsight for ColoNext, [Hereditary colon cancer disorders (eg, Lynch syndrome, PTEN hamartoma syndrome, Cowden syndrome, familial adenomatous polyposis), targeted mRNA sequence analysis panel (APC, CDH1, CHEK2, MLH1, MSH2, MSH6, MUTYH, PMS2, PTEN, and TP53) from Ambry Genetics]
  - +RNAinsight for GYNPlus, [Hereditary gynecological cancer (eg, hereditary breast and ovarian cancer, hereditary endometrial cancer, hereditary colorectal cancer), targeted mRNA sequence analysis panel (12 genes) from Ambry Genetics]

- +RNAinsight for OvaNext, [Hereditary ovarian cancer–related disorders (eg, hereditary breast cancer, hereditary ovarian cancer, hereditary endometrial cancer), targeted mRNA sequence analysis panel (17 genes) from Ambry Genetics]
- +RNAinsight for PALB2, [PALB2 (partner and localizer of BRCA2) (eg, breast and pancreatic cancer) mRNA sequence analysis from Ambry Genetics]
- +RNAinsight for ProstateNext, [Hereditary prostate cancer–related disorders, targeted mRNA sequence analysis panel (11 genes) from Ambry Genetics]
- ROMA Risk of Ovarian Malignancy Algorithm [Proprietary test using the combination of CA125 + HE4 antigens to assess the likelihood of malignancy before surgery; test kit from Fujirebio Diagnostics, Inc. and offered by several reference laboratories]
- Rosetta Kidney Cancer Test [Proprietary microRNA-based assay that differentiates 4 main histological types of primary kidney tumors from Rosetta Genomics]
- Rosetta Lung Cancer Test [Proprietary microRNA-based assay that identifies four main subtypes of lung cancer from Rosetta Genomics]
- ToxLok [Comparative DNA analysis using multiple selected single-nucleotide polymorphisms (SNPs), urine and buccal DNA, for specimen identity verification from InSource Diagnostics]

### **Cardiovascular molecular and genomic tests**

The following tests used to predict cardiovascular disease and/or direct therapy do not meet the above criteria and are not eligible for reimbursement.

- 4q25-AF Risk Genotype Test (rs2200733 allele)
- 9p21 Genotype Test (rs10757278 and rs1333049 alleles)
- Apolipoprotein E Genotype (APOE)
- KIF6 Genotype Test
- LPA-Aspirin Genotype Test (4399Met allele)
- LPA-Intron 25 Genotype Test
- myTAIHEART
- PAI-1 Testing for Cardiovascular Disease Risk Assessment
- Statin Induced Myopathy Genotype (SLCO1B1)

### **Gene variant or marker risk assessment tests**

The following tests that make use of inherited genomic information to assess disease risk, prognosis, or subtyping do not meet the above criteria and are not eligible for reimbursement.

- ARISk Autism Risk Assessment Test [Proprietary test from IntegraGen]
- BREVAGen [Proprietary sporadic breast cancer risk based on genetic markers from Genomic Diagnostics]
- Cardiac DNA Insight [Proprietary test from Pathway Genomics that assesses genetic markers for cardiac-related conditions]
- Crohn's prognostic test [NOD2/CARD15 gene variant testing]
- EsoGuard, [Gastroenterology (Barrett's esophagus), VIM and CCNA1 methylation analysis, esophageal cells, algorithm reported as likelihood for Barrett's esophagus from Lucid Diagnostics]
- IBD sgi Diagnostic [Proprietary test from Prometheus with genomic components including ATG16L1, STAT3, NKX2-3, and ECM1 gene variants.]
- LactoTYPE [Proprietary test from Prometheus that assesses the hypolactasia C/T genetic variant]
- Pathway Fit [Proprietary test from Pathway Genomics that focuses on metabolism, diet, and exercise traits]
- RetnaGene AMD [Proprietary test from Sequenom CMM to predict risk of wet AMD progression]
- Twin zygoty [genomic targeted sequence analysis of chromosome 2, using circulating cell-free fetal DNA in maternal blood from Natera]
- Viracor TRAC dd-cfDNA, [Transplantation medicine, quantification of donor-derived cell-free DNA using whole genome next-generation sequencing, plasma, reported as percentage of donor-derived cell-free DNA in the total cell-free DNA from Viracor Eurofins]

### Pharmacogenomic panels

- 5-Fluorouracil (5-FU) Toxicity and Chemotherapeutic Response [Proprietary panel of DPYD and TYMS gene variants to assess risk of 5-fluorouracil toxicity from ARUP Laboratory]
- Genecept Assay [Proprietary panel of biomarker tests to predict response to different psychiatric treatments from Genomind]
- GeneSightRx ADHD [Proprietary test from AssureRx assessing three genes]
- GeneSightRx Analgesic [Proprietary test from AssureRx assessing two genes]
- Mental Health DNA Insight [Proprietary test from Pathway Genomics]
- INFINITI® Neural Response Panel [Pain management (opioid-use disorder) genotyping panel, 16 common variants (ie, ABCB1, COMT, DAT1, DBH, DOR, DRD1, DRD2, DRD4, GABA, GAL, HTR2A, HTTLPR, MTHFR, MUOR, OPRK1, OPRM1), buccal swab or other germline tissue sample, algorithm reported as

positive or negative risk of opioid-use disorder from PersonalizeDx Labs, AutoGenomics Inc]

- OneOme RightMed , [Drug metabolism (adverse drug reactions), DNA, 22 drug metabolism and transporter genes, real-time PCR, blood or buccal swab, genotype and metabolizer status for therapeutic decision support from OneOme, LLC]
- Pain Medication DNA Insight [Proprietary test from Pathway Genomics]

### **Non-cancer gene expression assays**

- Molecular Microscope MMDx—Heart, [Transplantation medicine (kidney allograft rejection), microarray gene expression profiling of 1494 genes, utilizing transplant biopsy tissue, algorithm reported as a probability score for rejection from Kashi Clinical Laboratories]
- Molecular Microscope MMDx—Kidney, [Transplantation medicine (kidney allograft rejection), microarray gene expression profiling of 1494 genes, utilizing transplant biopsy tissue, algorithm reported as a probability score for rejection from Kashi Clinical Laboratories]
- Renal Transplant Monitoring (FOXP3, Granzyme B, Perforin, IP10)[Gene expression panel that is an indirect indicator of immune response designed to detect or monitor renal transplant rejection from Quest Diagnostics]
- VectraDA [Proprietary panel of 12 biomarkers that yields a rheumatoid arthritis disease activity score from Crescendo Bioscience]

### **Infectious disease assays**

- Accelerate PhenoTest BC kit, [Infectious disease (bacterial and fungal), organism identification, blood culture, using rRNA FISH, 6 or more organism targets, reported as positive or negative with phenotypic minimum inhibitory concentration (MIC)-based antimicrobial susceptibility from Accelerate Diagnostics, Inc]
- AmHPR Helicobacter pylori Antibiotic Resistance Next Generation Sequencing Panel, [Helicobacter pylori detection and antibiotic resistance, DNA, 16S and 23S rRNA, gyrA, pbp1, rdxA and rpoB, next generation sequencing, formalin-fixed paraffin embedded or fresh tissue, predictive, reported as positive or negative for resistance to clarithromycin, fluoroquinolones, metronidazole, amoxicillin, tetracycline and rifabutin from American Molecular Laboratories, Inc.]
- Bacterial Typing by Whole Genome Sequencing, [Infectious disease (bacterial), strain typing by whole genome sequencing, phylogenetic-based report of strain relatedness, per submitted isolate from Mayo Clinic]
- MicroGenDX qPCR & NGS For Infection, [Infectious agent detection and identification, targeted sequence analysis (16S and 18S rRNA genes) with drug-resistance gene from MicroGenDX]



- MYCODART Dual Amplification Real Time PCR Panel for 4 Aspergillus species, [ Infectious disease (Aspergillus species), real-time PCR for detection of DNA from 4 species (A. fumigatus, A. terreus, A. niger, and A. flavus), blood, lavage fluid, or tissue, qualitative reporting of presence or absence of each species from RealTime Laboratories, Inc/MycoDART, Inc]
- PCR Fungal Screen for Onychomycosis [Proprietary PCR test to identify genus and species of fungus causing onychomycosis from Bako]
- SmartGut [Proprietary test designed to sequence the 16S rRNA gene to identify 33 species and 32 genera of the gastrointestinal (GI) microbiome related microorganisms, including 5 pathogenic organisms from uBiome]

# Pharmacogenomic Testing for Drug Toxicity and Response

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For the purposes of this guideline, pharmacogenomic tests are those germline tests performed to predict or assess an individual's response to therapy as well as the risk of toxicity from drug treatment. Testing may be performed prior to treatment in order to determine if the individual has genetic mutations that could affect drug response and/or increase the risk for adverse drug reactions. Testing may also be performed during treatment to assess whether an individual is having an adequate response or investigate the cause of an unexpected or adverse reaction.

Companion diagnostics are assays that help determine whether a drug may be safe or effective for a particular patient. Companion assays are evaluated as part of the Food & Drug Administration's (FDA's) development and approval process for the new drug. According to the FDA, "A companion diagnostic is a medical device, often an in vitro device, which provides information that is essential for the safe and effective use of a corresponding drug or biological product. The test helps a health care professional determine whether a particular therapeutic product's benefits to patients will outweigh any potential serious side effects or risks."<sup>1</sup> Although specific companion diagnostic tests may be identified in the FDA label for a new drug approval, similar laboratory-developed tests (LDTs) performed by a CLIA-certified laboratory are generally accepted as alternatives that can typically provide the required information.

Complementary diagnostics are assays that were developed and in use prior to the FDA's approval of a new drug. They are not evaluated through the FDA's development and approval process for new drugs. Complementary diagnostics are used to help provide additional information about how a drug might be used, or whether someone should receive a certain class of drugs. These tests are not specifically required for the safe and effective use of a drug, which is part of what differentiates them from companion diagnostics. As with companion diagnostics, LDTs that are similar to the defined complementary diagnostic, when performed by a CLIA-certified laboratory, are able to provide the same information.<sup>2</sup>

## Criteria

### Criteria: General Coverage Guidance

Pharmacogenomic tests may be indicated when ALL of the following conditions are met:

- The individual is currently taking or considering treatment with a drug potentially affected by a known mutation that can be detected by a corresponding test.
- Technical and clinical validity: The test must be accurate, sensitive, and specific, based on sufficient, quality scientific evidence to support the claims of the test.

- Clinical utility: Healthcare providers can use the test results to guide changes in drug therapy management that will improve patient outcomes.
- Reasonable use: The usefulness of the test is not significantly offset by negative factors, such as expense, clinical risk, or social, or ethical challenges.

### **Criteria: Companion or Complementary Diagnostic Testing**

Single gene testing for purposes of medication usage will be approved when the following criteria are met:

- Testing is being performed in a CLIA-certified laboratory, AND
- Testing of the requested gene has not previously been performed, AND
- A medication's FDA label requires results from the genetic test to effectively or safely use the therapy in question, AND
- Healthcare providers can use the test results to directly impact medical care for the individual, OR
- The member meets all criteria in a test-specific guideline, if available

### **Criteria: Limits**

Testing will be covered only for the number of genes or tests necessary to establish drug response. When available and cost-efficient, a tiered approach to testing, with reflex to more detailed testing and/or different genes, is recommended.

For pharmacogenomic tests that look for changes in germline DNA (i.e., not tumor DNA or viral DNA), testing will be allowed once per lifetime per gene. Exceptions may be considered if technical advances in testing or the discovery of novel genetic variants demonstrate significant advantages that would support a medical need to retest.

Testing performed in a CLIA-certified laboratory will be considered for coverage. The use of a specific FDA approved companion diagnostic is not necessary for coverage to be considered.

### **Criteria: Exclusions**

Coverage for some tests may be excluded from the plan's benefit. These tests may be considered investigational or are not supported by existing evidence, professional guidelines and/or the FDA, or their use in medical management is deemed to be still evolving.

The following pharmacogenomic tests are typically not a covered benefit.<sup>3-10</sup> This list is not intended to be all inclusive

- 5HT2C (Serotonin Receptor) gene variants
- Ankyrin G gene variants

- COMT (Catechol Methyl Transferase) gene variants
- Catechol-O-Methyltransferase (COMT) Genotype from Mayo Clinic (CPT 0032U)
- CYP450 gene variants (including, but not limited to CYP1A2, CYP2D6, CYP2C9, CYP2C19, CYP3A4 [CPT 81230], CYP3A5 [CPT 81231]) for psychotherapeutic, cardiovascular, or general drug response
- Cytochrome P450 1A2 Genotype from Mayo Clinic (CPT 0031U)
- CYP2C19 testing for the management of H. pylori
- DRD2 (Dopamine Receptor) gene variants
- Focused Pharmacogenomics Panel from Mayo Clinic (CPT 0029U)
- IFNL3 rs12979860 gene variant (CPT 81283)
- KIF6 gene variants
- MTHFR gene variants
- NAT2 gene variants
- OPRM1 gene variants
- Serotonin Receptor Genotype (HTR2A and HTR2C) from Mayo Clinic (CPT 0033U)
- SLC6A4 (5-HTTLPR) serotonin transporter variants
- Thiopurine Methyltransferase (TPMT) and Nudix Hydrolase (NUDT15) Genotyping from Mayo Clinic (CPT 0034U)
- Warfarin Response Genotype from Mayo Clinic (CPT 0030U)

### Criteria: Test-specific Guidelines

Test-specific guidelines are available for some pharmacogenomic tests. Please see the guidelines manual for a list of test-specific guidelines. For tests without a specific guideline, use the General Coverage Guidance.

For somatic mutation testing in solid tumor tissue, see the guideline *Somatic Mutation Testing - Solid Tumors*.

### References

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3. Sachse C, Brockmöller J, Bauer S, Roots I. Cytochrome P450 2D6 variants in a Caucasian population: allele frequencies and phenotypic consequences. *Am J Hum Genetics*. 1997;60:284-95.
4. Schroth W, Antoniadou L, Fritz P, et al. Breast cancer treatment outcome with adjuvant tamoxifen relative to patient CYP2D6 and CYP2C19 genotypes. *J Clin Oncol*. 2007;25(33):5187-93.
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# Preimplantation Genetic Screening and Diagnosis

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## Description

Preimplantation Genetic Diagnosis (PGD) and Preimplantation Genetic Screening (PGS) are used to detect genetic conditions, chromosome abnormalities, and fetal sex during assisted reproduction with in vitro fertilization (IVF). PGD refers to embryo testing that is performed when one or both parents have a known genetic abnormality. This includes single-gene mutations and chromosome rearrangements. PGS refers to screening an embryo for aneuploidy when both parents are chromosomally normal. Genetic testing is performed on cells from the developing embryo prior to implantation. Only those embryos not affected with a genetic condition are implanted. PGD may allow at-risk couples to avoid a pregnancy affected with a genetic condition. The Society for Assisted Reproductive Technology and the American Society for Reproductive Medicine have published joint practice committee opinions to address the safety, accuracy, and overall efficacy of PGD and PGS.<sup>1,2</sup>

- This guideline does not include prenatal or preconception carrier screening. Please refer to *Genetic Testing for Carrier Status* for that purpose.
- This guideline does not include prenatal genetic testing. Please see *Genetic Testing for Prenatal Screening and Diagnostic Testing* for genetic testing done during pregnancy.

## Criteria

### Criteria: General coverage guidance

Preimplantation genetic diagnosis may be considered when **ALL** of the following conditions are met:

- **Technical and clinical validity:** The test must be accurate, sensitive and specific, based on sufficient, quality scientific evidence to support the claims of the test. In the case of PGD, the mutation(s) or translocation(s) to be tested in the embryo should first be well-characterized in the parent(s) AND the embryonic test results must be demonstrated to be highly accurate.
- **Clinical utility:** Healthcare providers can use the test results to provide significantly better medical care and/or assist patients with reproductive planning.
- **Reasonable use:** The usefulness of the test is not significantly offset by negative factors, such as expense, clinical risk, or social or ethical challenges.

AND THE FOLLOWING APPLY:

- The couple is known to be at-risk to have child with a genetic condition because of ANY of the following:
  - Both parents are known carriers of a recessive genetic condition and the specific gene mutation has been identified in each parent; OR
  - One parent is affected by or known to be a carrier of a dominant condition and the specific gene mutation has been identified; OR
  - The female contributing the egg is known to be a carrier of an X-linked condition and the specific gene mutation has been identified; OR
  - One or both parents are carriers of a structural chromosome rearrangement (e.g., translocation or inversion); OR
  - One or both parents have a known chromosome microdeletion (e.g. 22q11 deletion – DiGeorge syndrome, 7q11.23 deletion – Williams syndrome);

AND

- The genetic condition is associated with potentially severe disability or has a lethal natural history.

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**Note** This guideline ONLY addresses the genetic testing component of PGS or PGD. Coverage of any procedures, services, or tests related to assisted reproduction is subject to any applicable plan benefit limitations.

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### **Criteria: Special circumstances**

#### ***Sex determination***

- PGD for sex (X and Y chromosome testing) is considered medically necessary only for identification of potentially affected embryos for gender-related conditions.

#### ***HLA typing***

- PGD for human leukocyte antigen (HLA) typing for transplant donation is considered medically necessary only if:
  - A couple has child with a bone marrow disorder needing a stem cell transplant;  
AND
  - The only potential source of a compatible donor is an HLA-matched sibling

#### ***Chromosome abnormality screening***

- PGS for de novo chromosome abnormalities is not considered medically necessary. This includes the following indications:<sup>1</sup>
  - Maternal age alone
  - To improve in vitro success rates

- For recurrent unexplained miscarriage and/or recurrent implantation failures

### ***Variants of Unknown Significance (VUS)***

- PGD for variants of unknown significance is not considered medically necessary.

### **References**

1. American Society for Reproductive Medicine and Society for Assisted Reproductive Technology. Preimplantation genetic testing: a practice committee opinion. *Fertil Steril*. 2008;90(5 Suppl):S136-143.
2. American Society for Reproductive Medicine and Society for Assisted Reproductive Technology. The use of preimplantation genetic testing for aneuploidy (PGT-A): a committee opinion. *Fertil Steril*. 2018;109(3):429-436.



# Molecular and Genetic Test Specific Guidelines

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# 4Kscore for Prostate Cancer Risk Assessment

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## Introduction

4kscore testing is addressed by this guideline.

## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedure addressed by this guideline | Procedure code |
|---------------------------------------|----------------|
| 4Kscore for Prostate Cancer           | 81539          |

## What is prostate cancer

### Definition

Prostate cancer is the most common cancer among men, with over 164,000 new cases identified each year in the United States.<sup>1,2,3</sup>

### Prevalence

The median age at diagnosis is 66 years.<sup>3</sup> Older men are more likely to be affected than younger men, and African American men have higher rates compared to men of other ethnic backgrounds.<sup>3</sup> It is more likely to occur in men with a family history of prostate cancer.<sup>3,4</sup>

### Diagnosis

Screening programs for prostate cancer allow for its early detection. Screening is typically performed by prostate-specific antigen (PSA) test and digital rectal examination (DRE).<sup>2</sup>

Diagnosis is confirmed by prostate biopsy.<sup>4-6</sup> Biopsy is typically performed by a collection of approximately 12 needle biopsy cores.<sup>6</sup>

#### Poor detection with biopsies

Initial biopsies only detect 65-77% of prostate cancers and repeat biopsies are frequently performed.<sup>7,8</sup> The false negative rate of biopsy may be as high as 25%.<sup>9</sup>

## Test information

### Introduction

The 4Kscore Test (OPKO Lab) is an assay that determines an individual's risk of aggressive prostate cancer.<sup>10</sup>

### 4Kscore test

4Kscore uses a blood sample to measure total PSA, free PSA, intact PSA, and Human Kallikrein<sup>2</sup>. These measurements in combination with patient age, digital rectal exam, and negative previous biopsy status are used to come up with a risk score.<sup>10</sup>

### Results

The 4Kscore test is reported as a percent between <1% to >95%. This corresponds to the chance of having aggressive cancer in a prospective biopsy.<sup>10</sup>

## Guidelines and evidence

### Introduction

This section includes relevant guidelines and evidence pertaining to 4Kscore testing.

### National Comprehensive Cancer Network (NCCN)

The National Comprehensive Cancer Network (2019) Clinical Practice Guidelines for Prostate Cancer Early Detection state the following:<sup>6</sup>

- “Those patients with negative prostate biopsies should be followed with DRE and PSA. Tests that improve specificity in the post-biopsy setting - including percent-free PSA, 4Kscore, PHI, PCA3, and ConfirmMDx - should be considered in patients thought to be higher risk despite a negative prostate biopsy.”
- “Biomarkers that improve the specificity of detection are not, as yet, mandated as first-line tests in conjunction with serum PSA. However there may be some patients who meet PSA standards for consideration of prostate biopsy, but for whom the patient and/or physician wish to further define the probability of high-grade cancer. A percent-free PSA <10%, PHI >35, EPI score greater than 15.6, or 4Kscore (which provides an estimate of the probability of high-grade prostate cancer) are potentially informative in patients who have never undergone a biopsy or after negative biopsy; a PCA3 score >35 is potentially informative after a negative biopsy. The predictive value of the serum biomarkers discussed above has not been correlated with that of MRI. Therefore it is not known how such tests could be applied in optimal combination.”
- “The panel consensus is that [4Kscore] can be considered for patients prior to biopsy and for those with prior negative biopsy who are thought to be at higher risk for clinically significant prostate cancer. It is important for patients and urologists to

understand, however, that no optimal cut-off threshold has been established for the 4Kscore.”

### **American Urological Association**

The American Urological Association issued a Guideline Statement: Early Detection of Prostate Cancer (Reviewed and confirmed in 2018) stating:<sup>11</sup>

- “Multiple approaches subsequent to a PSA test (e.g., urinary and serum biomarkers, imaging, risk calculators) are available for identifying men more likely to harbor a prostate cancer and/or one with an aggressive phenotype. The use of such tools can be considered in men with a suspicious PSA level to inform prostate biopsy decisions.”

### **American Joint Committee on Cancer**

The American Joint Committee on Cancer (2017) states:<sup>12</sup>

- “The AJCC will continue to critically analyze emerging prostate cancer biomarkers and tools for their ability to prognosticate and guide treatment decision making with the highest level of accuracy and confidence for patients and physicians.”

### **Peer-Reviewed Literature**

A number of peer-reviewed expert-authored studies that evaluate the clinical validity and utility of the 4Kscore test for detection of aggressive prostate cancer are available.<sup>13-25</sup> Most of these studies demonstrate the potential for the assay to help urologists accurately discriminate between indolent and aggressive prostate cancer, reduce overtreatment, and reduce the burden of cost on patients with suspicion of aggressive prostate cancer. Limitations were noted across the studies and include retrospective study design, small sample sizes, and lack of randomization and blinding.

## **Criteria**

### **Introduction**

Requests for 4Kscore testing are reviewed using these criteria.

### **Criteria**

- No previous 4Kscore testing performed after the most recent negative biopsy when a result was successfully obtained, AND
- No previous ConfirmMDx testing on the most recent negative biopsy when a result was successfully obtained, AND
- Member is not under active surveillance for low stage prostate cancer, AND

- Negative prostate biopsy within the past 24 months, AND
- Member is considered at higher risk for prostate cancer by one or more of the following:
  - Family history of 1<sup>st</sup> degree relative with prostate cancer diagnosed younger than age 65 years,<sup>6,26-28</sup> and/or
  - Family history of two or more first-degree relatives with prostate cancer diagnosed at any age,<sup>27</sup> and/or
  - African American race,<sup>6,26-28</sup> and/or
  - Known mutation in a gene associated with increased risk of prostate cancer (e.g. BRCA1/2, HOXB13 (G84E mutation carriers), MLH1, MSH2, MSH6, PMS2, EPCAM),<sup>6,26,29</sup> and/or
  - PSA level of greater than 10 ng/ml,<sup>30</sup> and/or
  - PSA level increase of greater than 0.35 ng/ml/year if PSA level less than or equal to 10 ng/ml,<sup>6,31</sup> and/or
  - PSA doubling time of less than 3 years, when initial PSA level is greater than or equal to 4 ng/ml and other causes of rising PSA (i.e., infection, inflammation) have been ruled out for individuals whose PSA doubling occurred in less than 2 years<sup>32, 33</sup>

## References

### Introduction

These references are cited in this guideline.

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# ABL Tyrosine Kinase Sequencing for Chronic Myeloid Leukemia

MOL.TS.121.A

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## Introduction

ABL tyrosine kinase sequencing for chronic myeloid leukemia is addressed by this guideline.

## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedures addressed by this guideline | Procedure codes |
|--|-----------------|
| ABL1 Targeted Mutation Analysis        | 81401           |
| ABL1 Tyrosine Kinase Domain Sequencing | 81170           |

## What are CML and BCR-ABL

### Definition

Chronic myelogenous leukemia (CML) is a hematopoietic stem cell disease that results in overgrowth of white blood cells in the bone marrow. It is defined by the presence of the Philadelphia chromosome (Ph), a translocation between chromosomes 9 and 22 that results in the fusion of two genes known as BCR and ABL.<sup>1,2</sup> Acute lymphoblastic leukemia (ALL) is a different form of leukemia, but may also be positive for the Philadelphia chromosome (Ph+). About 3% of pediatric ALL and 25% of adult ALL is Ph+.<sup>3</sup>

### Diagnosis

Detection of the BCR-ABL fusion gene is diagnostic for CML and Ph+ ALL and can be established by fluorescent in situ hybridization (FISH) or quantitative real-time polymerase chain reaction (qPCR).<sup>2</sup>

### Symptoms

The three phases of CML are chronic, accelerated and blastic. In the chronic phase, there are few symptoms and most people are diagnosed after a routine blood test reveals the characteristic blood count and differential. If not treated, the disease will

progress to the accelerated and blastic phases, symptoms of which include fever, bone pain, splenomegaly, fatigue and weakness.<sup>1</sup>

## Treatment

First-line treatment for CML and some Ph+ ALL is with a class of drugs called tyrosine kinase inhibitors (TKIs), which block the activity of the BCR-ABL fusion gene protein product. Three TKI therapies are available as first-line therapies: imatinib (Gleevec®), nilotinib (Tasigna®), and dasatinib (Sprycel®). These TKI therapies have all demonstrated proven benefit, and median survival is expected to approach normal life expectancy for most patients with CML.<sup>1,2</sup>

## Monitoring

Monitoring of patients for treatment response to TKIs includes routine measurement of the BCR-ABL fusion gene protein product via qPCR prior to initiation of treatment and during treatment every 3 months. After BCR-ABL1 (IS) less than or equal to 1% has been achieved, measurement of the BCR-ABL fusion gene product is recommended every 3 months for 2 years and every 3 to 6 months thereafter.<sup>2</sup>

## Treatment resistance

For individuals who display apparent treatment resistance, consideration of alternative treatment options (or enrollment in a clinical trial) may be appropriate.<sup>2</sup> Treatment resistance in both CML and ALL can be caused by mutations in the BCR-ABL kinase domain.<sup>2,3</sup>

## Test information

### Introduction

Testing for CML ABL1 tyrosine kinase domain may include targeted mutation analysis or sequence analysis.

### Genetic testing

ABL1 tyrosine kinase domain mutation analysis is performed on a blood or bone marrow aspirate sample. Testing is performed by either

- targeted mutation analysis for specific resistance variants, such as T315I, or
- sequencing of the entire ABL1 tyrosine kinase domain.

## Guidelines and evidence

### Introduction

This section includes relevant guidelines and evidence pertaining to when BCR-ABL kinase domain analysis should be performed.

### National Comprehensive Cancer Network

The National Comprehensive Cancer Network (NCCN, 2019)<sup>2</sup> for CML states:

- BCR-ABL kinase domain analysis should be performed when:
  - "Chronic phase:
    - Failure to reach response milestones
    - Any sign of loss of response (defined as hematologic or cytogenetic relapse)
    - 1-log increase in BCR-ABL1 transcript levels and loss of MMR
  - Disease progression to accelerated or blast phase."
- "Mutational analysis is helpful in the selection of subsequent TKI therapy for patients with inadequate initial response to first-line or second-line TKI therapy. Mutational analysis would also be helpful to identify a subgroup of patients who demand careful monitoring (as these patients are at a higher risk of progression) and the subset of patients who will be eligible for allogeneic HSCT."
- These recommendations are category 2A: "based on lower-level evidence and there is non-uniform NCCN consensus (but no major disagreement)"

The National Comprehensive Cancer Network (NCCN, 2018)<sup>3</sup> for ALL states:

- ABL gene mutation testing should be considered for all Ph+ ALL in adolescents, young adults, and adults (AYA).
- These recommendations are category 2A: "based on lower-level evidence and there is non-uniform NCCN consensus (but no major disagreement)"

## Criteria

### Introduction

Requests for ABL Tyrosine Kinase analysis will be reviewed using these criteria.

### Criteria

BCR-ABL kinase domain mutation analysis is indicated in:

- Individuals with CML who have:

- Inadequate initial response to TKI therapy (lack of PCyR or BCR-ABL1 > 10% (IS) at 3 and 6 months or less than a CCyR or BCR-ABL1 > 1% (IS) at 12 months), or
  - Any sign of loss of response (hematologic or cytogenetic relapse), or
  - A 1-log increase in BCR-ABL1 transcript levels and loss of MMR, or
  - Disease progression to accelerated or blast phase, OR
- Individuals with Ph+ ALL.

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**Note** BCR-ABL kinase domain mutation analysis is not indicated in other cancer types for which tyrosine kinase inhibitor therapy may be considered.

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## References

### Introduction

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# Acute Myeloid Leukemia (AML) Genetic Testing

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## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedures addressed by this guideline                                | Procedure codes |
|---|-----------------|
| NPM1 Mutation Analysis  | 81479           |
| NPM1 MRD- Invivoscribe  | 0049U           |
| FLT3 Mutation Analysis (internal tandem duplication variants)         | 81245           |
| FLT3 internal tandem duplication MRD- Invivoscribe                    | 0046U           |
| FLT3 Mutation Analysis (tyrosine kinase domain variants)              | 81246           |
| CEBPA Mutation Analysis   | 81479           |
| IDH1 Mutation Analysis  | 81120           |
| IDH2 Mutation Analysis  | 81121           |
| KIT Mutation Analysis (targeted)                                      | 81272           |
| KIT Mutation Analysis (D816 variants)                                 | 81273           |
| DNMT3A Mutation Analysis  | 81403           |
| Hematolymphoid Neoplasm Molecular Profiling                           | 81450           |
| Solid Organ or Hematolymphoid Neoplasm Molecular Profiling - Expanded | 81455           |
| MyAML NGS- Invivoscribe   | 0050U           |
| Miscellaneous Molecular Tumor Marker Test                             | 81400           |
| Miscellaneous Molecular Tumor Marker Test                             | 81401           |
| Miscellaneous Molecular Tumor Marker Test                             | 81402           |

| Procedures addressed by this guideline    | Procedure codes |
|---|-----------------|
| Miscellaneous Molecular Tumor Marker Test | 81403           |
| Miscellaneous Molecular Tumor Marker Test | 81404           |
| Miscellaneous Molecular Tumor Marker Test | 81405           |
| Miscellaneous Molecular Tumor Marker Test | 81406           |
| Miscellaneous Molecular Tumor Marker Test | 81407           |
| Miscellaneous Molecular Tumor Marker Test | 81408           |
| Miscellaneous Molecular Tumor Marker Test | 81479           |
| Miscellaneous Molecular Tumor Marker Test | 88271           |

## What is Acute Myeloid Leukemia

### Definition

Acute myeloid leukemia (AML) is a neoplasm resulting from the clonal expansion of myeloid blasts in the peripheral blood (PB), bone marrow (BM), or other tissues. It is a heterogeneous disease clinically, morphologically and genetically.

- The required blast percentage for a diagnosis of AML is  $\geq 20\%$  myeloblasts and/or monoblasts/promonocytes and /or megakaryoblasts in the PB or BM. The diagnosis of AML can also be made when the blasts percentage is  $<20\%$  if the increase blast count is associated with: t(8;21)(q22;q22.1), inv(16)(p13.1;q22) or t(16;16)(p13.1;q22), or t(15;17)(q24.1;q21.2).
- A large number of recurrent cytogenetic abnormalities and mutated genes are recognized in AML. Some of these genetic abnormalities are associated with unique phenotype and prognostic features and are classified under acute myeloid leukemia with recurrent genetic abnormalities. The AMLs with no recurrent genetic abnormalities are classified under acute myeloid leukemia, not otherwise specified.
- In AML, the pretreatment cytogenetic abnormalities represent the single most important prognostic factor for predicting remission rates, relapse risk, and overall survival rate.<sup>1</sup> Even in de novo AMLs with no chromosomal abnormalities the clinical outcome is heterogeneous.

AML

- Studies have shown that molecular abnormalities including NPM1, FLT3-ITD, CEBPA, IDH1/2, DNMT3A, KIT and other mutations are important for prognostication not only in AMLs with a normal karyotype but also in other AML subsets.<sup>2-5</sup> Some of these molecular abnormalities also affect the choice of treatment for patients with AML.
- After treatment is selected and initiated, treatment response can be monitored by assessing the blast cell percentage in bone marrow using morphology and immunophenotyping. Individuals who have chromosomal abnormalities at initial diagnosis are monitored for disappearance (indicates remission) and re-emergence (indicates relapse) of these abnormalities.

## Test information

- The various diagnostic modalities that are utilized for front-end diagnostics are specified by the National Comprehensive Cancer Network (NCCN, 2018).<sup>6</sup>
  - These components include standard morphologic examinations on the blood and bone marrow, immunohistochemical staining, cytogenetics, flow cytometry, etc. However, this policy only pertains to those molecular techniques, which have either prognostic and/or predictive (that is, influencing chemotherapy selection with improved outcomes) implications.
- The specific methodology used to identify molecular markers is dependent upon the type of marker being investigated.
  - DNA mutations are generally detected through targeted mutation analysis of hotspots, sequencing parts of a single gene or the whole gene, or sequencing panels of multiple genes via next-generation sequencing (NGS).
  - Chromosome abnormalities, such as translocations or deletions, may be detected through direct visualization of the chromosomes (karyotyping), in situ hybridization of probes (e.g., FISH) to detect deletions or duplications that are too small to see directly, or DNA-based methods that identify deletions or translocation breakpoints.
  - Gene expression profiling simultaneously measures the amount of RNA or protein being made by many genes. Expression patterns may be used to predict the type of cancer present, tumor aggressiveness, and therapy needs.

## Guidelines and evidence

- The National Comprehensive Cancer Network (NCCN, 2018) states the following in regards to genetic testing in individuals with AML:<sup>6</sup>
  - “A variety of gene mutations are associated with specific prognoses (category 2A) and may guide medical decision making (category 2B) (See AML-A).

Currently, c-KIT, FLT3-ITD, FLT3-TKD, NPM1, CEBPA, IDH1/IDH2, and TP53 are included in this group; however, this field is evolving rapidly. While the above mutations should be tested in all patients, multiplex gene panels and next-generation sequencing analysis may be used to obtain a more comprehensive prognostic assessment (Papaemmanuil E, et al. Genomic classification and prognosis in acute myeloid leukemia. N Engl J Med 2016;374:2209-2221). The information obtained may have prognostic impact in AML, may influence medical decision making regarding consolidation with chemotherapy versus an allogeneic hematopoietic stem cell transplant, or determination for eligibility for clinical trial participation.”

- Some FDA labels require results from molecular marker tests to effectively or safely use the therapy for a specific cancer type.<sup>7</sup> A list of all Pharmacogenomic Biomarkers included in FDA labeling and associated implications can be found [here](#).
- Whereas the above mentioned biomarkers constitute established evaluation pathways for AML, there are many emerging mutations that might have clinical relevance to various types of AML that are not yet considered standard of care.

## Criteria

### Introduction

Medical necessity criteria differ based on the type of testing being performed (i.e., individual tumor markers separately chosen based on the cancer type versus pre-defined panels of tumor markers).

### Single gene testing for AML

- The member has AML and will benefit from information provided by the requested molecular marker test based on at least one of the following:
  - An oncology therapy FDA label requires results from the marker test to effectively or safely use the therapy for the member’s AML, or
  - NCCN guidelines include the tumor marker test in the management algorithm for AML and all other requirements are met (specific pathology findings, staging, etc.); however, the tumor marker must be explicitly included in the guidelines and not simply included in a footnote as an intervention that may be considered, or
  - The NCCN Biomarker Compendium has a level of evidence of at least 2A for the tumor marker’s application to AML

AML



## Panel testing for AML

Gene panels that are specific to hematological cancers and include the following genes will be eligible for reimbursement according to the criteria outlined in this policy: NPM1, FLT3, CEBPA, IDH1, IDH2, DNMT3A, KIT and TP53. This sequencing panel will only be considered for reimbursement when billed with the appropriate panel CPT code: 81450.

- Genetic Counseling
  - Pre and post-test counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - No previous panel testing for AML, AND
- Diagnostic Testing for Symptomatic Individuals:
  - Member has a diagnosis of AML, and
  - The results of the test will directly impact the diagnostic and treatment options that are recommended for the patient, AND
- Rendering laboratory is a qualified provider for service per Health Plan policy

## Billing and reimbursement considerations

- When multiple CPT codes are billed for components of a panel and there is a more appropriate CPT code representing the panel, the laboratory will be redirected to the appropriate panel code(s).
- Panels of over 50 genes billed with CPT code 81455 are considered excessive in individuals with AML and will not be reimbursed.

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# Afirma Thyroid Cancer Classifier Tests

**MOL.TS.122.A**  
v1.0.2020

## Introduction

Afirma thyroid cancer classifier tests are addressed by this guideline.

## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedures addressed by this guideline | Procedure codes |
|--|-----------------|
| Afirma Genomic Sequencing Classifier   | 81599           |
| Afirma Gene Expression Classifier      | 81545           |
| BRAF V600 Targeted Mutation Analysis   | 81210           |
| Afirma Xpression Atlas                 | 81479           |

## What are thyroid nodules

### Definition

Thyroid nodules are a common occurrence, especially in an aging population. Fine-needle aspiration (FNA) with accompanying cytology examination is the standard method for distinguishing between benign and malignant nodules and subsequent removal of tumors. Approximately 15 to 30% of thyroid nodules examined using FNA and traditional cytology examination are considered indeterminate. Clinicians are then faced with the decision to either remove the nodule unnecessarily or leave a potentially malignant nodule in place.<sup>1</sup>

Additional diagnostic procedures have been developed to help further classify indeterminate nodules as either benign or malignant. These procedures usually involve assessment of known genetic point mutations or through the expression activity of microRNA.<sup>1</sup>

## Test information

### Introduction

Afirma testing may include a combination of cytopathology and molecular testing.<sup>2</sup> This guideline addresses only the molecular testing component.

The Afirma Genomic Sequencing Classifier (GSC) is intended for<sup>2</sup>

- cytologically indeterminate FNA biopsy samples including atypia of undetermined significance/follicular lesion of undetermined significance (AUS/FLUS), and
- follicular or Hürthle Cell Neoplasms.<sup>3</sup> When indicated, the Afirma tests must be used in conjunction with cytopathology, ultrasound assessment, and other clinical factors to determine an individual's risk of thyroid cancer and the necessity of thyroid surgery.<sup>3</sup>

### When Afirma testing is performed

A FNA sample can be submitted for cytopathology assessment.

| If the cytopathology assessment is ... | Then ...                  |
|--|---------------------------|
| benign or malignant                    | the analysis is complete. |
| indeterminate                          | the GSC is performed.     |

### Afirma GSC

The Afirma Genomic Sequencing Classifier (GSC) is a second generation test that has replaced the original Gene Expression Classifier (GEC). The GSC test assesses more than 1000 core genes via next generation RNA sequencing, allowing for assessment of expression levels as well as analysis of copy number and loss of heterozygosity.<sup>4</sup> The purpose of the GSC is to further differentiate indeterminate FNA. The positive predictive value of the GSC is 47.1%.<sup>4</sup>

### Results

Afirma GSC results may help guide surgical decision making in patients with thyroid nodules. "Operation rates in patients classified as benign with Afirma testing are comparable to those with benign results on cytopathology."<sup>2</sup>

Based on the results of the Afirma GSC, additional testing may be requested or performed, such as the Afirma Malignancy Classifiers, BRAF and MTC.

### Afirma Malignancy Classifiers

The Afirma Malignancy Classifiers, BRAF and MTC, are intended to help guide surgical decisions when the cytopathology or Afirma GSC result suggests the individual should be considered for surgery.<sup>5,6</sup>

Afirma

| Test                                  | Detection                                  | When test is performed   |
|---------------------------------------|--|--|
| Afirma BRAF                           | BRAF V600E mutation                        | This test is performed when there is <ul style="list-style-type: none"> <li>• malignant or suspicious for malignancy cytopathology, or</li> <li>• a suspicious GSC result.<sup>2</sup></li> </ul>  |
| Afirma Medullary Thyroid Cancer (MTC) | Medullary thyroid carcinoma before surgery | This test is performed <ul style="list-style-type: none"> <li>• when there is malignant or suspicious for malignancy cytopathology, or</li> <li>• in conjunction with the GSC at the time of an indeterminate result.<sup>2</sup></li> </ul> |

### Afirma Xpression Atlas

The Afirma Xpression Atlas is an RNA sequencing-based test. The test is designed to analyze 761 variants and 130 fusions that have been linked to thyroid cancer. This testing is performed on nodules that are suspicious for malignancy.<sup>2</sup>

## Guidelines and evidence

### Introduction

This section includes relevant guidelines and evidence pertaining to Afirma GSC testing.

### National Comprehensive Cancer Network

The National Comprehensive Cancer Network (NCCN, 2018) Thyroid Carcinoma Guidelines state the following:<sup>7</sup>

- “The diagnosis of follicular carcinoma or Hürthle cell carcinoma requires evidence of either vascular or capsular invasion, which cannot be determined by FNA. Molecular diagnostics may be useful to allow reclassification of follicular lesions (i.e. follicular neoplasm, atypia of undetermined significance (AUS), follicular lesions of undetermined significance (FLUS)) as either more or less likely to be benign or malignant based on the genetic profile...If molecular testing, in conjunction with clinical and ultrasound features, predicts a risk of malignancy comparable to the risk of malignancy seen with a benign FNA cytology (approximately 5% or less), consider active surveillance. Molecular markers should be interpreted with caution and in the context of clinical, radiographic, and cytologic features of each individual patient.”

Afirma

## American Thyroid Association

The American Thyroid Association (2016) makes the following statement regarding molecular testing and FNA-indeterminate thyroid nodules:<sup>8</sup>

- “For nodules with AUS/FLUS cytology, after consideration of worrisome clinical and sonographic features, investigations such as repeat FNA or molecular testing may be used to supplement malignancy risk assessment in lieu of proceeding directly with a strategy of either surveillance or diagnostic surgery. Informed patient preference and feasibility should be considered in clinical decision-making. (Weak recommendation, Moderate-quality evidence)”
- “If repeat FNA cytology, molecular testing, or both are not performed or inconclusive, either surveillance or diagnostic surgical excision may be performed for an AUS/FLUS thyroid nodule, depending on clinical risk factors, sonographic pattern, and patient preference. (Strong recommendation, Low-quality evidence)”

## American Association of Clinical Endocrinologists, American College of Endocrinology, and Associazione Medici Endocrinologi (AACE/ACE/AME) Guidelines

The AACE/ACE/AME 2016 Clinical Practice Guidelines for the Diagnosis and Management of Thyroid Nodules state the following:<sup>9</sup>

- In nodules with indeterminate cytologic results, no single cytochemical or genetic marker is specific or sensitive enough to rule out malignancy with certainty. However the use of immunohistochemical and molecular markers may be considered together with the cytologic subcategories and data from US (ultrasound), elastography, or other imaging techniques to obtain additional information for management of these patients.
- When molecular testing should be considered
  - To complement not replace cytologic evaluation (BEL 2, GRADE A)
  - The results are expected to influence clinical management (BEL 2, GRADE A)
  - As a general rule, not recommended in nodules with established benign or malignant cytologic characteristics (BEL 2, GRADE A)
- Molecular testing for cytologically indeterminate nodules
  - Cytopathology expertise, patient characteristics, and prevalence of malignancy within the population being tested impact the NPV and PPV for molecular testing (BEL 3, GRADE B)
  - Consider detection of BRAF and RET/PTC and, possibly PAX8/PPARG and RAS mutations if such detection is available (BEL 2, GRADE B)

- Because of the insufficient evidence and limited follow-up, we do not recommend either in favor of or against the use of gene expression classifiers (GECs) for cytologically indeterminate nodules (BEL 2 GRADE B)
- Role of molecular testing for deciding the extent of surgery
  - Currently, with the exception of mutations such as BRAFV600E that have a PPV approaching 100% for papillary thyroid carcinoma (PTC), the evidence is insufficient to recommend in favor of or against the use of mutation testing as a guide to determine the extent of surgery (BEL 2, GRADE)
- How should patient with nodules that are negative at mutation testing be monitored?
  - Since the false-negative rate for indeterminate nodules is 5 to 6% and the experience and follow-up for mutation negative nodules or nodules classified as benign by a GEC are still insufficient, close follow-up is recommended (BEL 3, GRADE B)

## Literature Review

Patel et al. (2018) examined the performance of the Afirma GSC test:<sup>4</sup>

- They used 191 of the 210 FNA samples used to validate the GEC test.
- GSC demonstrated 91.1% sensitivity (identified 41 of 45 malignant samples) with a 68.3% specificity (identified 99 of 145 non-malignant samples) in patients with indeterminate Bethesda III or IV cytology. Prevalence of malignancy in the study population was 22.4%. The NPV was 96.1% and the PPV was 47.1%.
- The GEC test had 90% sensitivity (malignancy) and 52% specificity (benign) on samples with indeterminate Bethesda III or IV cytology. Prevalence of malignancy in study population was 24%.

No peer-reviewed clinical studies evaluating the clinical validity or clinical utility of the Xpression Atlas test were identified. Given the lack of peer-reviewed publications, no conclusions can be made regarding the clinical validity, clinical utility, or the overall value of the Xpression Atlas as an add-on to the Afirma GSC test.

## Criteria

### Introduction

Requests for Afirma GSC testing are reviewed using these criteria.

### Afirma Genomic Sequencing Classifier (GSC)

- Testing Multiple Samples:

- The Afirma GSC is reimbursed only once per date of service regardless of the number of nodules submitted for testing, and
- The Afirma GSC is indicated only once per thyroid nodule per lifetime.
- Required Clinical Characteristics:
  - Afirma GSC is indicated for thyroid nodules with indeterminate FNA results that are included in the following cytopathology categories:
    - Atypia of undetermined significance/follicular lesion of undetermined significance (AUS/FLUS), or
    - Follicular or Hürthle cell neoplasm, and
  - The patient is not undergoing thyroid surgery for diagnostic confirmation.
- Required Testing Process:
  - If FNA of a nodule is indicated to evaluate for malignancy, and the sample is sent to Veracyte for cytopathology, the classifier is only indicated when the result is indeterminate, and
  - Supporting documentation of an appropriate indeterminate cytology result will be required for reimbursement.

### **Afirma Malignancy Classifiers**

- Afirma MTC
  - Afirma MTC testing will be reimbursed if it is performed as part of the GSC as outlined above, and
  - The Afirma MTC testing must be billed as part of the Afirma GSC. The Afirma MTC may not be billed separately using an additional unit or procedure code.
- Afirma BRAF V600E
  - Afirma BRAF testing may be considered for either GSC or FNA suspicious or malignant results. See Somatic Mutation Testing – Solid Tumors guideline for criteria.
  - Afirma BRAF testing in conjunction with a GSC indeterminate result will not be reimbursed.

### **Afirma Xpression Atlas**

This test is considered investigational and/or experimental.

- Investigational and experimental (I&E) molecular and genomic (MolGen) tests refer to assays involving chromosomes, DNA, RNA, or gene products that have



insufficient data to determine the net health impact, which typically means there is insufficient data to support that a test accurately assesses the outcome of interest (analytical and clinical validity), significantly improves health outcomes (clinical utility), and/or performs better than an existing standard of care medical management option. Such tests are also not generally accepted as standard of care in the evaluation or management of a particular condition.

- In the case of MolGen testing, FDA clearance is not a reliable standard given the number of laboratory developed tests that currently fall outside of FDA oversight and FDA clearance often does not assess clinical utility.

## Billing and Reimbursement Considerations

CPT code 81545 is specific to and therefore intended to be used for Afirma's Gene Expression Classifier test, not the Genomic Sequencing Classifier. Requests for this CPT code when the GSC is being performed will be redirected to a more appropriate CPT code.

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# AlloMap Gene Expression Profiling for Heart Transplant Rejection

MOL.TS.123.A

v1.0.2020

## Introduction

AlloMap Gene Expression Profiling is addressed by this guideline.

## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedure addressed by this guideline | Procedure code |
|---------------------------------------|----------------|
| AlloMap                               | 81595          |

## What is AlloMap

### Definition

AlloMap is a non-invasive blood test that is designed to help identify heart transplant recipients with stable allograft function who have a low probability of moderate/severe acute cellular rejection at the time of testing.<sup>1</sup>

### Current uses

AlloMap is designed to help providers obtain this information without the use of endomyocardial biopsy. While endomyocardial biopsy is currently the standard of care for heart transplant recipients, it is an invasive procedure with associated risks.

### Description

AlloMap is a panel of 20 genes. The assay uses gene expression of RNA isolated from peripheral blood mononuclear cells.<sup>1</sup>

### Results

Using data from the gene expression of these genes, an AlloMap score is calculated. The lower the score, the lower the probability of acute cellular rejection at the time of testing.<sup>1</sup>

## Intended use

AlloMap is intended for use in heart transplant recipients 15 years of age or older who are at least 2 months post heart transplant.<sup>1</sup>

## Test information

### Introduction

The AlloMap assay measures the gene expression of RNA of 20 genes. 11 of these genes are thought to be informative for the assay, while the remaining 9 are used for quality control.<sup>1</sup>

### Risk score

The data collected from these genes is translated into a risk score. Scores range from 0-40 and are compared to post-transplant patients in the same post-transplant period. The lower the score, the lower the probability of acute cellular rejection at the time of testing.<sup>1</sup>

## Guidelines and evidence

### Introduction

This section includes relevant guidelines and evidence pertaining to AlloMap testing.

### International Society of Heart and Lung Transplantation

The International Society of Heart and Lung Transplantation (2010)<sup>2</sup> stated the following:

“Gene Expression Profiling (AlloMap) can be used to rule out of the presence of acute cellular rejection (ACR) of grade 2R or greater in appropriate low risk patients, between 6 months and 5 years after HT.”

#### Class IIa

Class IIa: Weight of evidence/opinion is in favor of usefulness/efficacy.

Level of evidence: B – data derived from a single randomized clinical trial or large non-randomized studies.

### U.S. Food and Drug Administration (FDA)

In 2008, the U.S. Food and Drug Administration (FDA) cleared AlloMap as a Class II Medical Device.<sup>3</sup>

### **EIMAGE (Early Invasive Monitoring Attenuation through Gene Expression)**

The EIMAGE (Early Invasive Monitoring Attenuation through Gene Expression) study (2015)<sup>4</sup> was conducted as a single-center randomized parallel 2-arm interventional study (n=60). This study compared AlloMap with heart biopsy in the first year post transplant.

- Study population consisted of 60 patients aged 18 years or older and at least 55 days post-transplant.
- Incidence of composite primary outcome in both groups was not statistically significant.
- The need for biopsy was reduced in the AlloMap monitoring group: 42 biopsies were performed in the AlloMap group vs. 253 in the biopsy group. 29 out of 42 of the biopsies performed in the AlloMap group were a direct result of the elevated AlloMap score.

### **IMAGE (Invasive Monitoring Attenuation through Gene Expression) study**

The IMAGE (Invasive Monitoring Attenuation through Gene Expression) study (2010)<sup>5</sup> serves as the first randomized, prospective trial (n=602) comparing AlloMap head-to-head with rejection monitoring by endomyocardial biopsy, the current standard of care. The study included patients who were clinically stable, 18 years of age or older, and at least 6 months post-transplant. Results of this study indicated:

- Rates of adverse events (primary outcome: rejection, graft dysfunction, death) were the same in low-risk patients monitored with AlloMap vs. traditional graft biopsy.
- The need for biopsy was reduced in the AlloMap monitoring group (since those with low scores did not get biopsies): 409 biopsies were performed in the AlloMap group vs. 1249 in the biopsy group.

However, limitations of this study were acknowledged by the authors. These limitations include:<sup>5</sup>

- a study population that was likely significantly skewed toward patients at lower risk of rejection, since only patients who had received a cardiac transplant more than 6 months previously were eligible for enrollment
- wide statistical margins for comparing AlloMap vs. biopsy, and
- primary endpoint measures that included events that may not have been due to rejection.

The authors conclude that “gene expression profiling of peripheral blood specimens may offer a reasonable alternative to routine biopsies, for monitoring cardiac-transplant recipients for rejection if the interval since transplantation is at least 6 months and the patient is considered to be low risk for rejection.”<sup>5</sup>

## Crespo-Leiro et al

Crespo-Leiro et al (2015)<sup>6</sup> conducted a study to examine the ability of AlloMap score variability to predict future events. They found that at a score variability of 0.6, the negative predictive value increased to 97% and the positive predictive value decreased to 23.3%. The authors concluded that “GEP score variability may be helpful in estimating probability of future events of death, re-transplantation or graft failure in heart transplant recipients.”

## Deng et al

Deng et al (2014)<sup>7</sup> conducted a study to examine the use of AlloMap score variability to predict clinical events in heart transplant recipients. They found that AlloMap score variability had a predictive accuracy of 0.69. They concluded, “the variability of gene expression profiling scores from an individual may help predict the risk of clinically defined future allograft dysfunction or death in the individual.”

## Limitations of the Crespo-Leiro and Deng studies

These studies attempt to demonstrate the value of AlloMap scores for prognostic purposes. However, the studies are retrospective analyses of prospective studies and have notable limitations which marginalize their ability to effectively demonstrate clinical utility.

## Criteria

### Introduction

Requests for AlloMap Gene Expression Profiling are reviewed using these criteria.

### Criteria

AlloMap is considered medically necessary when ALL of the following criteria are met:

- Medical records indicate that member has been under the care of the ordering provider within the past 30 days, and
- Member is not acutely symptomatic,<sup>2</sup> and
- Member does not have recurrent rejection,<sup>2</sup> (defined as having a documented prior rejection and currently having signs/symptoms of rejection), and
- Member is not currently receiving 20 mg or more of daily oral prednisone,<sup>2</sup> and
- Member has not received high-dose intravenous corticosteroids or myeloablative therapy in the past 21 days,<sup>2</sup> and
- Member has not received blood products or hematopoietic growth factors in the past 30 days,<sup>2</sup> and

- Member is not pregnant,<sup>2</sup> and
- Member is at least 2 months post-transplant,<sup>2,4</sup> and
- Member is less than 5 years post-transplant,<sup>2</sup> and
- Member is at least 15 years of age<sup>2</sup>

### Recommended frequency of AlloMap testing

This table describes the recommended frequency of AlloMap testing.

| Months post-transplant | Frequency of AlloMap testing |
|------------------------|------------------------------|
| 2 to 6 months          | every 2 to 4 weeks           |
| 6 to 12 months         | every 2 months               |
| 12 to 24 months        | every 3 months               |
| 24 months to 60 months | every 6 months               |
| greater than 60 months | every 12 months              |

### Exceptions to testing frequency

AlloMap may be used as a substitute for endomyocardial biopsy in surveillance of stable patients. Exceptions to the above testing frequencies may be considered as warranted by an individual patient's clinical presentation.<sup>2,4,6</sup> AlloMap testing is not routinely covered in individuals greater than 5 years post-transplant. Requests for exceptions to this criteria will be evaluated on a case by case basis.

### Exclusions

Coverage for AlloMap testing has some exclusions.

#### Exclusion for prognostic purposes

The use of AlloMap for prognostic purposes is specifically excluded by this guideline. Studies on the ability of the test to predict future clinical events do not provide enough evidence to warrant coverage at this time.

### References

#### Introduction

These references are cited in this guideline.

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# AlloSure for Kidney Transplant Rejection

MOL.TS.307.A

v1.0.2020

## Introduction

AlloSure for kidney transplant rejection is addressed by this guideline.

## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedure addressed by this guideline | Procedure code |
|---------------------------------------|----------------|
| AlloSure                              | 81479          |

## What Is Kidney Transplant Rejection

### Definition

Kidney disease is a loss of renal function which, without treatment, leads to eventual build-up of waste and other toxic substances in the blood.<sup>1</sup> Treatment of advanced kidney disease, called end-stage kidney disease, consists of dialysis or renal transplant. Transplant rejection can be acute or chronic.

### Incidence and Prevalence

According to the National Kidney Foundation, 97% of kidney transplants are functioning 1 month after transplant, and 80% are functioning after 3 years.<sup>2</sup> Approximately 20% of kidney transplants performed each year are repeat transplants.<sup>2</sup>

### Symptoms

Kidney transplant rejection can be acute (occurring suddenly and progressing quickly) or chronic (occurring slowly over time), and is typically immune system mediated.<sup>2</sup> Symptoms of transplant rejection include fever and flu-like symptoms, decreased urinary output, weight gain, fatigue, and pain over the transplanted organ.<sup>3</sup>

Acute rejection of the donated kidney is thought to lead to tissue injury, including increased cell death in the allograft, which then leads to increased donor-derived cell free DNA (dd-cfDNA) in the bloodstream.

## Cause

Transplanted kidneys can fail for multiple reasons:<sup>2</sup>

- Blood clot in the vessels leading to the kidney
- Infection
- Medication side effects
- Non-compliance with post-transplant medications and other post-surgical care
- Recurrence of the original medical problem that caused the kidney transplant
- Acute or chronic rejection caused by immune-mediated donor kidney damage

## Diagnosis

Rise in creatinine levels is currently used to initially diagnose graft rejection, and the gold standard for initial diagnosis is histological analysis based on needle biopsy of the organ.<sup>4-5</sup> However, organ biopsy is invasive and often associated with complications, patient discomfort, and inconvenience. Serum creatinine is one of the main markers used to monitor allograft functioning, but has been shown to lack sensitivity and specificity for graft injury.<sup>4-5</sup>

Alternatively, donor-derived cell-free DNA (dd-cfDNA) (as a fraction of the total cell-free DNA [cfDNA]) has been proposed as a noninvasive marker for detecting graft rejection and measuring allograft damage among recent kidney transplant patients.

## Treatment

Renal transplantation has been shown to increase the survival and quality of life (QOL) of patients with end stage renal disease (ESRD), and is often considered the preferred treatment option for these patients.<sup>6</sup> When a transplanted kidney is rejected, dialysis is performed until another organ can be procured for transplant.

## Survival

If the kidneys fail completely, survival is a few months without treatment.<sup>1</sup> After transplant, long-term survival is still limited, and acute rejection is a frequent complication and associated with reduced graft survival.<sup>1</sup>

## Test Information

### Introduction

AlloSure is an assay designed to detect allograft rejection in kidney transplant recipients.

## Description and Purpose

According to the manufacturer of AlloSure (Care Dx, Inc), the test is intended to non-invasively measure donor DNA in the blood for kidney transplant surveillance of active donor graft rejection.<sup>7</sup> Active rejection as defined by the manufacturer includes “T cell-mediated rejection [TCMR], “acute/active” antibody-mediated rejection [ABMR], and “chronic, active” ABMR”.<sup>7</sup> The test is intended for patients 18 years of age or older who are at least 2 weeks post-transplant.

## Test Targets

AlloSure is a targeted next-generation sequencing assay that uses 266 single-nucleotide polymorphisms (SNPs) to quantify dd-cfDNA in transplant patients.<sup>7</sup>

## Result

The test reports the percent of donor derived DNA in the patient’s blood sample along with quality control cut-off values.<sup>7</sup>

Interpretation of test results:<sup>8</sup>

- “Greater than 1% dd-cfDNA is associated with active rejection.”
- “0.21% dd-cfDNA is the median observed in a reference population of stable recipients.”
- “Greater than 61% increase in dd-cfDNA from a prior sample exceeds the biological and analytical variability observed in the reference population.”

## Guidelines and evidence

### Introduction

The following section includes relevant guidelines and evidence pertaining to AlloSure for Kidney Transplant Rejection.

### The Transplantation Society

The Transplantation Society, via the Kidney Disease: Improving Global Outcomes (KDIGO) Transplant Work Group, states the following regarding acute rejection, renal allograft function, and renal allograft biopsy:<sup>9</sup>

#### Treatment of Acute Rejection

- “6.1: We recommend biopsy before treating acute rejection, unless the biopsy will substantially delay treatment. (1C)”
- “6.2: We suggest treating subclinical and borderline acute rejection. (2D)”

- “6.3: We recommend corticosteroids for the initial treatment of acute cellular rejection. (1D)”
- “6.3.1: We suggest adding or restoring maintenance prednisone in patients not on steroids who have a rejection episode. (2D)”
- “6.3.2: We suggest using lymphocyte-depleting antibodies or OKT3 for acute cellular rejections that do not respond to corticosteroids, and for recurrent acute cellular rejections. (2C)”
- “6.4: We suggest treating antibody-mediated acute rejection with one or more of the following alternatives, with or without corticosteroids (2C)”
  - “plasma exchange”
  - “intravenous immunoglobulin”
  - “anti-CD20 antibody”
  - “lymphocyte-depleting antibody”
- “6.5: For patients who have a rejection episode, we suggest adding mycophenolate if the patient is not receiving mycophenolate or azathioprine, or switching azathioprine to mycophenolate. (2D)”

#### Kidney Allograft Biopsy

- “9.1: We recommend kidney allograft biopsy when there is a persistent, unexplained increase in serum creatinine. (1C)”
- “9.2: We suggest kidney allograft biopsy when serum creatinine has not returned to baseline after treatment of acute rejection. (2D)”
- “9.3: We suggest kidney allograft biopsy every 7–10 days during delayed function. (2C)”
- “9.4: We suggest kidney allograft biopsy if expected kidney function is not achieved within the first 1–2 months after transplantation. (2D)”
- “9.5: We suggest kidney allograft biopsy when there is”
  - “new onset of proteinuria (2C)”
  - “unexplained proteinuria  $\geq 3.0$  g/g creatinine or  $\geq 3.0$  proteinuria  $> 3.0$ g/g creatinine or  $> 3.0$ g per 24 hours. (2C)”

#### The Renal Association

The Renal Association clinical practice guideline for renal transplant post-operative care states the following regarding transplant rejection:<sup>10</sup>

- “Guideline 4.1 – KTR: diagnosis of acute rejection”

“We recommend that a transplant renal biopsy should be carried out before treating an acute rejection episode unless this will substantially delay treatment or pose a significant risk to the patient (1C)”

- “Guideline 5.2 – KTR: detection of chronic allograft injury”

“ We suggest that renal function should be monitored at each clinic visit by assessment of serum creatinine and qualitative evaluation of urine protein excretion by dipstick, supplemented by spot protein:creatinine ratio (PCR) or albumin:creatinine ratio (ACR) if positive (2C)”

- “Guideline 5.3 – KTR: diagnosis of chronic allograft injury”

“We suggest that renal biopsy is the optimal investigation for parenchymal causes of graft dysfunction where the cause is uncertain (2C)”

## Literature Review

There is a limited evidence base for the validity of AlloSure which includes one analytical validity study and one clinical validity study, both of moderate quality.<sup>11-14</sup> These studies are hampered by small numbers of affected individuals. In addition, the studies were non-blinded, possibly introducing assessment bias.

Additional well-designed studies are needed to establish the clinical validity and clinical utility of the AlloSure test, including assessment of AlloSure’s impact on clinically relevant health outcome measures, including morbidity and mortality. Additional research is needed to clarify the need for ongoing surveillance of dd-cfDNA post-transplant and how clinicians should approach cases in which the clinical presentation and dd-cfDNA results are in disagreement.

## Ongoing Clinical Trial

- NCT Number: NCT03326076
- Title: Evaluation of Patient Outcomes From the Kidney Allograft Outcomes AlloSure Registry<sup>15</sup>

## Criteria

### Introduction

Requests for AlloSure testing for allograft kidney transplant rejection are reviewed using the following criteria.

This test is considered investigational and/or experimental.

- Investigational and experimental (I&E) molecular and genomic (MolGen) tests refer to assays involving chromosomes, DNA, RNA, or gene products that have insufficient data to determine the net health impact, which typically means there is

insufficient data to support that a test accurately assesses the outcome of interest (analytical and clinical validity), significantly improves health outcomes (clinical utility), and/or performs better than an existing standard of care medical management option. Such tests are also not generally accepted as standard of care in the evaluation or management of a particular condition.

- In the case of MolGen testing, FDA clearance is not a reliable standard given the number of laboratory developed tests that currently fall outside of FDA oversight and FDA clearance often does not assess clinical utility.

## References

### Introduction

This guideline cites the following references.

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# Alpha-1 Antitrypsin Deficiency Testing

MOL.TS.124.A  
v1.0.2020

## Introduction

Alpha-1 antitrypsin deficiency (AATD) testing is addressed by this guideline.

## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedures addressed by this guideline | Procedure codes |
|--|-----------------|
| Protease Inhibitor (PI) Typing         | 82104           |
| SERPINA1 Targeted Mutation Analysis    | 81332           |
| SERPINA1 Sequencing                    | 81479           |

## What is alpha-1 antitrypsin deficiency

### Definition

Alpha-1 antitrypsin deficiency (AATD) results from mutations in the SERPINA1 gene, which codes for the enzyme alpha-1 antitrypsin (AAT).<sup>1</sup> This condition is also referred to as AAT Deficiency and A1AT Deficiency.

### Prevalence

It is estimated that 1 in 5000 to 1 in 7000 people in North America have AATD. AATD commonly afflicts individuals of Northern European heritage. This disorder is most common in Scandinavia, occurring in approximately 1 in 1500 to 1 in 3000 individuals there.<sup>1</sup> However, AATD is an under-recognized condition, with estimates that only 10% of those affected are actually diagnosed.<sup>2</sup>

### Symptoms

The most common clinical manifestation is chronic obstructive pulmonary disease (COPD), particularly emphysema.<sup>1-3</sup> Smoking is a major environmental risk factor for lung disease in AATD, increasing the risk for emphysema by 1000-fold.<sup>3</sup>

AATD also increases the risk for neonatal or childhood liver disease, manifested by obstructive jaundice and hyperbilirubinemia, and early onset adult liver disease, usually cirrhosis and fibrosis.<sup>1</sup>



## Inheritance

Alpha-1 antitrypsin deficiency (AATD) is inherited in an autosomal recessive manner.<sup>1</sup>

## Diagnosis

AATD may first be suspected based on reduced serum levels of alpha-1 antitrypsin. Confirmatory testing includes either protease inhibitor typing or genetic testing for common mutations.<sup>1</sup>

## Test information

### Introduction

Testing for alpha-1 antitrypsin deficiency may include protease inhibitor typing, SERPINA1 targeted mutation analysis, or SERPINA1 sequencing.

### Protease Inhibitor typing

Protease Inhibitor (PI) typing by isoelectric focusing to determine phenotype (PI\*Z, PI\*S).<sup>1</sup> PI typing is considered the gold standard for diagnosing AATD, as it can detect normal as well as variant alleles, but cannot detect null alleles.<sup>1,2</sup> Mutation testing should be performed “when serum AAT levels are not measured, PI typing is not performed, or results from serum AAT levels or PI typing are discordant”.<sup>1</sup>

### SERPINA1 targeted mutation analysis

SERPINA1 targeted mutation analysis tests for the two common mutations in the gene (Z and S), which make up greater than 95% of the mutations.<sup>1</sup> The Z allele is by far the most common and more severe variant.<sup>3</sup>

### SERPINA1 sequencing

SERPINA1 sequencing is available, but only appropriate in limited situations. The proportion of individuals with AATD that have a mutation identified by sequencing is unknown.<sup>1</sup>

## Guidelines and evidence

### Introduction

This section includes relevant guidelines and evidence pertaining to alpha-1 antitrypsin deficiency testing .

## American Thoracic Society and the European Respiratory Society recommendations

The American Thoracic Society and the European Respiratory Society states that testing for AATD is recommended for the following indications (quoted directly):<sup>3</sup>

- symptomatic adults with emphysema, chronic obstructive pulmonary disease (COPD), or asthma with airflow obstruction that is incompletely reversible after aggressive treatment with bronchodilators
- individuals with unexplained liver disease, including neonates, children, and adults, particularly the elderly
- asymptomatic individuals with persistent obstruction on pulmonary function tests with identifiable risk factors, examples include cigarette smoking and occupational exposure
- adults with necrotizing panniculitis, and
- siblings of an individual with AATD.

## Other recommendations

The following sections outline recommendations from other authorities. However, these sources do not specifically comment on the use of SERPINA1 sequencing in the diagnostic work-up. When ambiguous results are obtained between quantification, genotype or phenotype assays, gene sequencing can identify rare variants or null alleles that would otherwise be missed.

### Sandhaus et al. (2016)<sup>4</sup>

Sandhaus et al. (2016) provided recommendations for the diagnosis of AATD based on systematic review and expert scientist and clinician appraisal. For diagnostic testing of symptomatic individuals, the authors recommend “genotyping for at least the S and Z alleles. Advanced or confirmatory testing should include Pi-typing, AAT level testing, and/or expanded genotyping”. The authors also recommend that the following groups be tested for AATD.

- “All individuals with COPD, regardless of age or ethnicity”
- “All individuals with unexplained chronic liver disease”
- “All individuals with necrotizing panniculitis, granulomatosis with polyangiitis (GPA, formerly Wegener’s granulomatosis), or unexplained bronchiectasis”

In addition the authors recommend that “adult siblings of individuals identified with an abnormal gene for AAT, whether heterozygote or homozygote, should be provided with genetic counseling and offered testing for AATD”.

**Graham et al. (2015)<sup>5</sup>**

Graham et al. (2015) found pathogenic variants with sequencing after PI and targeted mutation analysis were performed. They support full gene sequencing when there is discrepancies between clinical presentation and genotyping after PI and targeted mutation analysis.

**Prins et al. (2008)<sup>6</sup>**

Prins et al. (2008) sequenced exons 2, 3, and 5 of the SERPINA1 gene from 66 patients with AAT concentration less than or equal to 1.0 g/L. They predicted that up to 22% of the disease-associated AAT deficiency alleles could be missed by S and Z genotyping or by phenotyping. They also identified rare alleles M<sub>procida</sub>, M<sub>palermo</sub>, M6<sub>passau</sub>, M<sub>wurzburg</sub>, M<sub>heerlen</sub> and the previously undescribed null alleles Q0<sub>soest</sub> and Q0<sub>amersfoort</sub>.

They found pathogenic variants in 22% of those who had negative PI and targeted mutation testing. The authors recommend direct sequencing of the coding regions of the SERPINA1 gene for patients with suspected AATD based on a serum AAT concentration  $\leq 1.0$  g/L.

**Ferrarotti et al. (2007)<sup>7</sup>**

Ferrarotti et al. (2007) described a protocol they developed to optimize AAT deficiency diagnosis from dried blood spot samples. The protocol has an initial screen using quantification of AAT and genotyping for the S and Z deficiency alleles. Discordant samples are then reflexed to PI typing.

Sequencing is used for any samples in which the plasma AAT level is low ( $<70$  mg/dL), and the genotype/phenotype results are PI\*MS or PI\*MZ. Specific testing for the Q0<sub>isola di procida</sub> allele is also performed, which results from a deletion and therefore cannot be detected by sequencing. While this report described the protocol used, it did not comment on the sensitivity or specificity of this approach.

**Criteria****Introduction**

Requests for alpha-1 antitrypsin deficiency (AATD) testing are reviewed using these criteria.

**Criteria**

Protease inhibitor (PI) typing or SERPINA1 common mutation analysis (S, Z) may be considered in individuals who meet the following criteria:<sup>1,3</sup>

- Abnormally low (less than 120mg/dL) or borderline (90-140mg/dL) alpha-1 antitrypsin (AAT) levels; AND

- At least one of the following:
  - Symptomatic adults with emphysema, chronic obstructive pulmonary disease (COPD), or asthma with airflow obstruction that is incompletely reversible after aggressive treatment with bronchodilators; or
  - Individuals of any age with unexplained liver disease (including obstructive liver disease in infancy); or
  - Asymptomatic individuals with persistent obstruction on pulmonary function tests who have identifiable risk factors (e.g., cigarette smoking, occupational exposure); or
  - C-ANCA positive vasculitis; or
  - Adults with necrotizing panniculitis; or
  - Siblings of an individual with AATD

Sequencing of the SERPINA1 gene may be considered in individuals who meet the following criteria:<sup>1</sup>

- There are discrepancies between clinical presentation, serum alpha-1 antitrypsin quantification, targeted mutation analysis, and/or PI typing; OR
- The presence of rare variants or null alleles (which cannot be identified by other methods) is suspected.

## References

### Introduction

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# Amyotrophic Lateral Sclerosis (ALS) Genetic Testing

MOL.TS.125.A

v1.0.2020

## Introduction

Amyotrophic lateral sclerosis (ALS) genetic testing is addressed by this guideline.

## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedures addressed by this guideline | Procedure codes |
|--|-----------------|
| ALS Known Familial Mutation Analysis   | 81403           |
| Genetic Testing for ALS                | S3800           |
| Miscellaneous ALS Gene Analysis        | 81400-81408     |
| ALS Gene Analysis                      | 81479           |

## What is amyotrophic lateral sclerosis

### Definition

Amyotrophic lateral sclerosis (ALS) is a disease caused by the progressive degradation of motor neurons (nerve cells that control muscle movement).<sup>1</sup> ALS may initially present with muscle weakness, twitching, cramping, or slurred speech.<sup>1</sup> Symptoms worsen over time and include muscle atrophy and difficulty swallowing.<sup>1</sup>

### Diagnosis

Most cases of suspected ALS are diagnosed based on a unique combination of symptoms and the exclusion of similar disorders. The Escorial Criteria were developed in 2000 to standardize the clinical diagnosis of ALS.<sup>2</sup> These criteria include:

- the presence of upper and lower motor neuron deterioration
- the progressive spread of symptoms, and
- no clinical evidence of other diseases with similar symptoms.

## Causes of ALS

There are more than 25 genes known to cause Familial Amyotrophic Lateral Sclerosis (FALS), and the condition demonstrates genetic overlap with frontotemporal dementia (FTD). Genetic testing for many of the genes is clinically available.<sup>1,3-6</sup> FALS subtypes are named based on the causative gene. For example, ALS1 subtype is caused by SOD1 gene mutations.

A pathogenic mutation can be identified in 70% of cases of FALS<sup>7</sup>. Mutations in SOD1, TARDBP, FUS, VCP, C9orf72, and TBK1 account for the greatest number of cases, while the remaining genes are relatively rare causes of the disorder.<sup>1,3-9</sup> The majority of combined ALS/FTD cases with a family history of either disorder are caused by C9orf72 repeat expansions, particularly in Caucasian populations, while the percentage of cases attributed to this genesis somewhat lower in China.<sup>4,9</sup> Many other candidate genes have been identified and are still pending further validation studies.<sup>6</sup>

## Genes commonly associated with FALS

Some of the most common genetic causes of ALS are summarized below. The remaining genes are relatively rare causes of the disorder. Genetic testing for many of the genes is available clinically.<sup>1,3-8</sup>

| Gene symbol  | FALS subtype | % of individuals with FALS | Inheritance        |
|--------------|--------------|----------------------------|--------------------|
| SOD1         | ALS1         | 20%                        | Autosomal dominant |
| C9orf72      | ALS/FTD      | 23%-30%                    | Autosomal dominant |
| FUS/TLS      | ALS6         | ~4%                        | Autosomal dominant |
| TARDBP/TDP43 | ALS10        | 1%-4%                      | Autosomal dominant |
| VCP          | ALS14        | 1-2%                       | Autosomal dominant |
| TBK1         | ALS/FTD      | 1-3%                       | Autosomal dominant |

## Inheritance

Most people with FALS have an autosomal dominant form, meaning only one mutation is required to cause disease. In this case, children of an affected person have a 50% chance of inheriting the disease-causing mutation.

There are rare autosomal recessive forms of ALS as well as one X-linked form. Two mutations are required to cause autosomal recessive types, usually only siblings are affected, and there is no parent-to-child transmission.

## Onset

The average age of ALS onset is 56 years if the affected individual has no family history, and 46 years old if there is a family history of ALS.<sup>1,2</sup> However, there are

infantile and juvenile onset forms that should also prompt consideration of a genetic etiology.<sup>1</sup>

## Survival

ALS is fatal. The average survival after diagnosis is 3 years, but can vary widely. Treatment focuses on slowing progression with medication and therapy.<sup>1</sup>

## Prevalence

Between 4 and 8 out every 100,000 people develop ALS. About 90% of ALS cases are sporadic, and the remaining 10% of individuals have familial ALS (FALS).<sup>1</sup>

## Test information

### Introduction

Testing for Familial Amyotrophic Lateral Sclerosis (FALS) may include targeted expansion analysis of C9orf72, single gene sequencing, multigene panels, or known familial mutation analysis.

### Targeted expansion analysis of C9orf72

Expansions of the hexanucleotide repeat non-coding region of the open reading frame C9orf72 (a protein as yet uncharacterized) is the most frequent cause of familial ALS and are assessed through targeted analysis.<sup>1,7</sup> Although estimation of the repeat size is typically accurate, there is disagreement as to the normal and pathogenic repeat size ranges.<sup>10</sup>

### Sequence analysis

Genetic testing of other genes associated with FALS is usually done by gene sequencing. Sequencing is generally >99% accurate for identifying mutations in the coding region of a gene.

Laboratories may offer individual gene sequencing or multi-gene panels for FALS.

### Sequential genetic testing approach

An expert-authored review makes the following suggestions when pursuing a sequential individual gene approach:<sup>1</sup>

- “SOD1 testing is appropriate in any individual with ALS who has another affected family member or an incomplete family history, including the early death of a close relative from any cause. Approximately 20% of individuals with FALS have ALS1 with an identified pathogenic variant in SOD1. Interpretation of the significance of a SOD1 variant regarding disease severity and progression depends on the specific variant identified because of wide variability in genotype/phenotype correlations.



Failure to detect a SOD1 variant does not rule out FALS. Up to 3% of individuals with ALS with no family history of ALS have SOD1 variants. Because data on penetrance of many variants are limited, establishing the risk to other family members of developing clinical symptoms can be difficult.”

- “SETX testing is appropriate in kindreds with adolescent-onset spinal muscular atrophy with pyramidal features. ”
- “VAPB testing should be pursued in the context of clinical symptoms of primarily adult-onset spinal muscular atrophy. ”
- “FUS/TLS, TARDBP, and ANG testing should be considered for SOD1-negative individuals with FALS. ”
- “ALS2 testing is appropriate for those with childhood-onset UMN-predominant ALS. ”
- “VCP testing should be considered for individuals with a family history of ALS with or without symptoms of inclusion body myopathy, Paget disease and/or frontotemporal dementia. ”
- “OPTN testing may be considered for individuals with a family history consistent with autosomal dominant or autosomal recessive inheritance, including simplex cases who do not have a variant in more common ALS-related genes.”

### **Known familial mutation analysis**

Known familial mutation analysis can provide predictive information about the risk to develop ALS. It can also be used to diagnose ALS when the patient does not yet meet the full ALS diagnostic criteria.<sup>11</sup>

Once a pathogenic mutation is identified in a family member, the known familial mutation can be specifically identified in asymptomatic or symptomatic family members.

## **Guidelines and evidence**

### **Introduction**

This section includes relevant guidelines and evidence pertaining to ALS genetic testing.

### **World Federation of Neurology Research Group on Motor Neuron Diseases**

The World Federation of Neurology Research Group on Motor Neuron Diseases (2015) revised the El Escorial criteria:<sup>12</sup>

- These revised criteria still do not specify when genetic testing should be done, but they do state “If a pathogenic mutation in a disease-causing gene is found in the patient and segregates with the disease the term hereditary or primary genetic ALS

(HALS/GALS) should be used. The finding of a pathogenic mutation in a known gene can substitute for either lower or upper motor neuron signs, so that diagnosis of ALS can be made on the basis of UMN or LMN signs in one body region, associated with a positive genetic test.”

- “ALS can be defined as Mendelian inheritance if a disease-causing gene variant can be shown to segregate within a family. In such cases the genetic variant can serve as a substitute for upper motor neuron deficits or a second limb or region (rule of two).”

### **Expert-authored review**

A 2015 expert-authored review states: “Presymptomatic testing for a TARDBP mutation is complicated because the penetrance is unknown, the age of onset is not predictable, and preventative measures do not exist. Because of the individualized nature of predictive testing, consultation with a genetic counselor and a psychologist to obtain informed consent is recommended. At this time, no established testing protocol (e.g., as in Huntington disease) exists, although establishment of such protocols has been suggested. However, to err on the side of caution, testing centers often follow a similar protocol.”<sup>13</sup>

Identifying a SOD1 mutation in a pre-symptomatic individual can impact future management and overall prognosis of ALS. However, it is considered controversial because of reduced penetrance, which means that not everyone with a mutation will necessarily develop symptoms. It also lacks overall intervention or prevention strategies and has an inability to predict the age of onset.<sup>1,3</sup>

### **European Federation of Neurological Societies**

A European Federation of Neurological Societies Task Force (EFNS, 2012) addressed presymptomatic testing in its diagnosis and management guidelines: “Presymptomatic genetic testing should only be performed in first-degree adult blood relatives of patients with a known gene mutation. Testing should only be performed on a strictly voluntary basis as outlined (see Table 7 in the original guideline document) and should follow accepted ethical principles.”<sup>14</sup>

### **European Federation of Neurological Societies**

Guidelines from the European Federation of Neurological Societies (EFNS, 2012) address molecular testing of ALS:<sup>14</sup>

“Clinical analysis for gene mutations should only be performed in cases with a known family history of ALS, and in sporadic ALS cases with the characteristic phenotype of the recessive D90A mutation.”

“Clinical DNA analysis for gene mutations should not be performed in cases with sporadic ALS with a typical classical ALS phenotype.”

“In familial or sporadic cases where the diagnosis is uncertain, SMN, androgen receptor, or TARDBP, FUS, ANG, or SOD1 DNA analysis may accelerate the diagnostic process.”

“Before blood is drawn for DNA analysis, the patient should receive genetic counseling. Give the patient time for consideration. DNA analysis should be performed only with the patient’s informed consent.”

### **European Federation of Neurological Societies**

Guidelines from the European Federation of Neurological Societies (EFNS, 2011) address the molecular diagnosis of ALS and other neurogenetic disorders. They state:<sup>15</sup>

“Currently, molecular diagnosis mainly has implications for genetic counseling rather than for therapy. However, when more directed causal therapies become available in the future, establishing a correct genetic diagnosis in a given patient will be essential. Despite the rather low prevalence sequencing of the small SOD1 gene should be considered in patients with ALS with dominant inheritance to offer presymptomatic or prenatal diagnosis, if this is requested by the family (Level B).”

### **World Federation of Neurology Research Group on Motor Neuron Diseases**

Consensus guidelines from the World Federation of Neurology Research Group on Motor Neuron Diseases (2000) revised the El Escorial criteria to improve ALS diagnostic sensitivity.<sup>2</sup>

This group doesn’t specify when genetic testing should be done, but they do state “The demonstration of the presence of a pathogenetically relevant gene mutation can assist in the diagnosis of ALS (such as SOD1).”

These criteria set a lower threshold for diagnosis when an ALS-causing mutation is known in the family. For example, a patient may be diagnosed as “Clinically Definite Familial ALS — Laboratory-supported” with evidence of only upper or lower motor neuron disease in one region; whereas a definite diagnosis without genetic test results requires upper and lower motor neuron disease in three regions.

## **Criteria**

### **Introduction**

Requests for familial ALS genetic testing are reviewed using these criteria.

### **Known familial mutation testing**

- Genetic Counseling

- Pre- and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - No previous genetic testing for FALS inclusive of the known family mutation, AND
- Diagnostic Testing for Symptomatic or Presymptomatic Individuals:
  - FALS known familial mutation identified in a 1st, 2nd, or 3rd degree relative(s), and
  - Age 18 years or older, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

### Other considerations

- Genetic testing for ALS, in the absence of a known familial mutation, is considered investigational and experimental and, therefore, not eligible for reimbursement

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### Introduction

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# Angelman Syndrome Testing

**MOL.TS.126.A**  
v1.0.2020

## Introduction

Angelman syndrome testing is addressed by this guideline.

## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedures addressed by this guideline             | Procedure codes |
|--|-----------------|
| Chromosome 15 Uniparental Disomy                   | 81402           |
| Chromosomal Microarray [BAC], Constitutional       | 81228           |
| Chromosomal Microarray [SNP], Constitutional       | 81229           |
| Chromosomal Microarray [CGH], Constitutional       | S3870           |
| FISH Analysis for 15q11-q13 Deletion               | 88271           |
| Imprinting Center Defect Analysis                  | 81479           |
| Imprinting Center Known Familial Mutation Analysis | 81403           |
| SNRPN/UBE3A Methylation Analysis                   | 81331           |
| UBE3A Sequencing                                   | 81406           |
| UBE3A Deletion/Duplication Analysis                | 81479           |
| UBE3A Known Familial Mutation Analysis             | 81403           |

## What is Angelman syndrome

### Definition

Angelman syndrome (AS) is a genetic disorder that can cause intellectual disability, severe speech impairment, tremors, seizures, microcephaly, and decreased need for sleep.

## Symptoms

Angelman syndrome (AS) is characterized by:<sup>1</sup>

- Severe developmental delay or intellectual disability by age 6-12 months
- Severe speech impairment — usually with minimal or no word use
- Gait ataxia and/or limb tremors
- Seizures and microcephaly
- Happy demeanor with hand flapping, and
- Decreased need for sleep.

## Causes

Features of Angelman syndrome are caused by a missing or defective UBE3A gene inherited from the individual's mother.<sup>2</sup>

A missing or defective UBE3A gene can be caused by a gene deletion, gene mutation, uniparental disomy (two copies of paternal chromosome), imprinting defect, or a chromosome rearrangement.<sup>2,3</sup>

## Test information

### Introduction

Testing for Angelman syndrome may include SNRPN/UBE3A methylation analysis, chromosomal microarray, FISH analysis for 15q11-q13 deletion, chromosome 15 uniparental disomy (UPD), imprinting center defect analysis, UBE3A sequencing and deletion testing, or known familial mutation analysis.

### SNRPN/UBE3A methylation analysis

This test is typically the first test in the evaluation of both Angelman syndrome (AS) and Prader-Willi syndrome (PWS). It will detect about 80% of patients with AS and >99% of patients with PWS. However, DNA methylation analysis does not identify the underlying cause, which is important for determining the risk to future siblings. This risk ranges from less than 1% to up to 50%, depending on the genetic mechanism. Follow-up testing for these causes may be appropriate.

### Chromosomal microarray or FISH analysis for 15q11-q13 deletion

If DNA methylation analysis for Angelman (AS) or Prader-Willi syndrome (PWS) is abnormal, deletion analysis is typically the next step. Approximately 70% of cases of both AS and PWS have a deletion in one copy of chromosome 15 involving the 15q11.2-q13 region. When looking specifically for this deletion, FISH (fluorescence in situ hybridization) analysis is most commonly performed. However, chromosomal

microarray can also detect such deletions. If chromosomal microarray (CMA, array CGH) has already been done, FISH is not likely to be necessary.

### **Chromosome 15 uniparental disomy (UPD)**

If DNA methylation analysis is abnormal but deletion analysis is normal, UPD analysis may be an appropriate next step for evaluation of both Angelman (AS) and Prader-Willi syndrome (PWS). About 28% of PWS cases are due to maternal UPD (both chromosome 15s are inherited from the mother). About 7% of cases of AS are due to paternal UPD (both chromosome 15s are inherited from the father). Both parents must be tested to diagnose UPD.

### **Imprinting center defect analysis**

This test may be considered in the evaluation of Angelman syndrome (AS) and Prader-Willi syndrome (PWS) when methylation is abnormal, but FISH (or array CGH) and UPD studies are normal. Individuals with such results are presumed to have an imprinting defect. An abnormality in the imprinting process has been described in a minority of cases (about 3%). However, imprinting center deletions may be familial, and if familial, the recurrence risk can be up to 50%.

### **UBE3A sequencing**

If DNA methylation analysis is normal, UBE3A gene mutations should be suspected. Such mutations are found in 11% of Angelman syndrome patients and can only be detected by sequencing the entire gene.<sup>1</sup> These mutations can be carried by the mother of an affected individual and pose up to a 50% risk of recurrence in her other children, and an increased risk to other family members.

### **Known familial mutation analysis**

If a UBE3A gene mutation has been identified in an affected individual through sequencing, testing for just the known familial mutation in UBE3A can be performed for at-risk relatives, including at-risk pregnancies.

If a mutation in the imprinting center has been identified in an affected family member, testing for just the known familial mutation in the imprinting center can be performed for at-risk relatives, including at-risk pregnancies.

## **Guidelines and evidence**

### **Introduction**

This section includes relevant guidelines and evidence pertaining to Angelman syndrome testing.



## The Angelman Syndrome Foundation

The Angelman Syndrome Foundation (2015) recommends the following test strategy to diagnose Angelman syndrome:<sup>3</sup>

- UBE3A methylation analysis
  - If abnormal (only paternal alleles are present), a diagnosis is confirmed.
  - Consider the following to identify the underlying cause for recurrence risk counseling.
- Deletion analysis (chromosomal microarray or FISH for 15q11-q13)
  - If deletion testing is abnormal, FISH testing on the mother should be done to rule out an inherited chromosome abnormality (rare).
  - If deletion testing is normal, consider UPD analysis.
- Uniparental Disomy (UPD) analysis of chromosome 15 to determine whether the proband inherited both copies of chromosome 15 from the father.
- If deletion analysis and UPD analysis are normal, an imprinting center mutation is a likely cause and should be evaluated (which may carry a higher recurrence risk than other causes).

### Expert-authored review

An expert-authored review (2011) comments on the utility of familial mutation analysis:<sup>1</sup>

- “Individuals with an imprinting center (IC) deletion can have a phenotypically normal mother who also has an IC deletion. If a proband's mother has a known IC deletion, the risk to the sibs is 50%.”
- “UBE3A mutations can be inherited or de novo. In addition, several cases of mosaicism for a UBE3A mutation have been noted. If a proband's mother has a UBE3A mutation, the risk to the sibs is 50%.”
- “If a proband's mother carries a known IC deletion or UBE3A mutation, the mother's sisters are also at risk of carrying the IC deletion or the mutation. Each child of the unaffected sisters who are carriers is at a 50% risk of having AS. Unaffected maternal uncles of the proband who are carriers are not at risk of having affected children, but are at risk of having affected grandchildren through their unaffected daughters who have inherited the IC deletion or UBE3A mutation from them.”

## Criteria

### Introduction

Requests for Angelman syndrome testing are reviewed using these criteria.

**SNRPN/UBE3A Methylation Analysis**

- Genetic Counseling:
  - Pre- and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Testing:
  - No previous SNRPN/UBE3A methylation analysis, AND
- Diagnostic Testing for Symptomatic Individuals:
  - Developmental delay by age 6-12 months, typically severe to profound, without loss of milestones, and
  - Some combination of the following:
    - Movement or balance disorder, typically with ataxia, or
    - Frequent laughter/smiling, apparent happy demeanor; easily excitable personality (often with uplifted hand-flapping, or waving movements), or hypermotoric behavior, or
    - Speech impairment with no or minimal number of words, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

**Deletion Analysis (FISH for 15q11-q13 Deletion or chromosomal microarray)**

- Genetic Counseling:
  - Pre- and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Testing:
  - No previous chromosomal microarray, and
  - No previous 15q11-q13 deletion analysis, AND
- Diagnostic Testing for Symptomatic Individuals:
  - Developmental delay by age 6-12 months, typically severe to profound, without loss of milestones, and
  - Some combination of the following:
    - Movement or balance disorder, typically with ataxia, or
    - Frequent laughter/smiling, apparent happy demeanor; easily excitable personality (often with uplifted hand-flapping, or waving movements), or hypermotoric behavior, or

- Speech impairment with no or minimal number of words, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

### **Chromosome 15 Uniparental Disomy**

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Testing:
  - SNRPN/UBE3A methylation analysis results are abnormal, and
  - 15q11-q13 deletion analysis is negative, and
  - No previous chromosome 15 UPD studies, AND
- Diagnostic Testing for Symptomatic Individuals:
  - Developmental delay by age 6-12 months, typically severe to profound, without loss of milestones, and
  - Some combination of the following:
    - Movement or balance disorder, typically with ataxia, or
    - Frequent laughter/smiling, apparent happy demeanor; easily excitable personality (often with uplifted hand-flapping, or waving movements), or hypermotoric behavior, or
    - Speech impairment with no or minimal number of words, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

### **Imprinting Center Defect Analysis**

- Genetic Counseling:
  - Pre- and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Testing:
  - SNRPN/UBE3A methylation analysis results are abnormal, and
  - 15q11-q13 deletion analysis is negative, and
  - Previous chromosome 15 UPD testing is negative, and
  - No previous imprinting center (IC) analysis, AND

- Diagnostic Testing for Symptomatic Individuals:
  - Developmental delay by age 6-12 months, typically severe to profound, without loss of milestones, and
  - Some combination of the following:
    - Movement or balance disorder, typically with ataxia, or
    - Frequent laughter/smiling, apparent happy demeanor; easily excitable personality (often with uplifted hand-flapping, or waving movements), or hypermotoric behavior, or
    - Speech impairment with no or minimal number of words, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

### **UBE3A Sequencing**

- Genetic Counseling:
  - Pre- and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Testing:
  - SNRPN/UBE3A methylation analysis results are normal, and
  - No previous sequencing of UBE3A, AND
- Diagnostic Testing for Symptomatic Individuals:
  - Developmental delay by age 6-12 months, typically severe to profound, without loss of milestones, and
  - Movement or balance disorder, typically with ataxia, and
  - Frequent laughter/smiling, apparent happy demeanor; easily excitable personality (often with uplifted hand-flapping, or waving movements), or hypermotoric behavior, and
  - Speech impairment with no or minimal number of words, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

### **UBE3A Deletion/Duplication Analysis**

- Genetic Counseling:
  - Pre- and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Testing:

- SNRPN/UBE3A methylation analysis results are normal, and
- Normal UBE3A sequencing, AND
- Diagnostic Testing for Symptomatic Individuals:
  - Developmental delay by age 6-12 months, typically severe to profound, without loss of milestones, and
  - Movement or balance disorder, typically with ataxia, and
  - Frequent laughter/smiling, apparent happy demeanor; easily excitable personality (often with uplifted hand-flapping, or waving movements), or hypermotoric behavior, and
  - Speech impairment with no or minimal number of words, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

### Known Familial Mutation Analysis

- Genetic Counseling:
  - Pre- and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Testing:
  - No previous UBE3A sequencing or imprinting center defect analysis testing, AND
- Family History:
  - Known familial UBE3A mutation in a blood relative, or
  - Known familial imprinting center defect mutation in a blood relative, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

### References

#### Introduction

These references are cited in this guideline.

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# Anser Testing

**MOL.TS.127.A**  
v1.0.2020

## Introduction

Anser ADA, Anser IFX, Anser UST, and Anser VDZ testing are addressed by this guideline.

## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedures addressed by this guideline | Procedure codes |
|--|-----------------|
| Anser ADA                              | 84999           |
| Anser IFX                              | 84999           |
| Anser UST                              | 84999           |
| Anser VDZ                              | 84999           |

## What are Anser ADA, IFX, UST, and VDZ

### Definition

The suite of Anser tests includes Anser ADA, Anser IFX, Anser UST, and Anser VDZ. All 4 tests measure serum concentrations of anti-drug antibodies in patients with diminished or suboptimal response to medications used to treat various inflammatory diseases.<sup>1</sup>

### Medications and Indications

Adalimumab (ADA), Infliximab (IFX), Ustenkinumab (UST), and Vedolizumab (VDZ) are monoclonal antibodies approved by the U.S. Food and Drug Administration (FDA) for use in various conditions:<sup>1</sup>

- ADA: Crohn's disease, ulcerative colitis, and rheumatoid arthritis
- IFX: Crohn's disease, ulcerative colitis, psoriatic arthritis, ankylosing spondylitis, plaque psoriasis, and rheumatoid arthritis
- UST: active psoriatic arthritis
- VDZ: moderate to severe active ulcerative colitis or Crohn's disease

## Loss of response

At the beginning of treatment, some patients exhibit an initial response to IFX, ADA, UST, and VDZ administration, yet experience loss of treatment response over time (secondary nonresponse).<sup>2</sup> For example, the loss of clinical effect for infliximab for patients who have an initial therapeutic response is relatively common (loss of response [LOR], ranging from 3% to 13% per patient-year).

While the reasons for nonresponse among patients varies, research shows that anti-drug antibodies neutralize or increase during drug metabolism. It has also been hypothesized that it could be due to low serum levels of the medication, use of the drug in response to higher inflammatory disease burden, and development of immunogenicity.<sup>3</sup>

Management options to loss of response include higher dosage of the drug, shorter intervals between drug doses, switching drugs, or any combination of the above. It has also been shown that the production of antibodies to either adalimumab or infliximab is associated with an increased rate of infusion reaction.<sup>1,4</sup>

## Anti-drug Antibodies

With the use of adalimumab, the development of anti-drug antibodies is correlated with reactions at the initial injection site, and infliximab-related anti-drug antibodies are correlated with acute infusion reactions and delayed hypersensitivity reactions. Infusion reactions have not been thoroughly evaluated with the use of vedolizumab-related or ustekinumab-related anti-drug antibodies.<sup>5</sup>

Several assays are available for detection and measurement of circulating anti-drug antibody levels, including enzyme-linked immunoabsorbant assay (ELISA) (earlier generation technique), radioimmunoassay (RIA), and more recently, the homogeneous mobility shift assay (HMSA) offered by Prometheus (Prometheus Laboratories, Inc.) or the electrochemiluminescence immunosassay (ECLIA).<sup>6</sup>

## Test information

### Introduction

Anser IFX, ADA, UST, VDZ are non-radiolabeled fluid-phase homogeneous mobility shift assays (HMSA) that measure the formation and serum concentrations of anti-drug antibodies in patients with diminished or suboptimal response to the inflammatory disease medications used to treat various inflammatory diseases: Infliximab (IFX; Remicade®, Janssen Biotech); Adalimumab (ADA; Humira®, AbbVie); Ustenkinumab (UST, Stelara®, Janssen Biotech, Inc.); and vedolizumab (VDZ; ENTYVIO®, Takeda Pharmaceuticals USA, Inc.).<sup>1</sup> The formation of these ADAs may lead to patients who become nonresponsive to these various medications.



## Guidelines and evidence

### Introduction

This section includes relevant guidelines and evidence pertaining to Anser ADA, Anser IFX, Anser UST and Anser VDZ testing.

### American Gastroenterological Association (AGA)

AGA Institute Guideline on Therapeutic Drug Monitoring (TDM) in Inflammatory Bowel Disease (2017) offered the following recommendation for measurement of anti-drug antibodies utilizing the Grading of Recommendations Assessment, Development and Evaluation (GRADE) tool:<sup>7</sup>

- “In adults with active IBD treated with anti-TNF agents, the AGA suggests reactive therapeutic drug monitoring to guide treatment changes. GRADE: Conditional recommendation, very low quality of evidence. The true effect is likely to be substantially different from the estimate of effect.”
- “In adult patients with quiescent IBD treated with anti-TNF agents, the AGA makes no recommendation regarding the use of routine proactive therapeutic drug monitoring. GRADE: No recommendation, knowledge gap.”

### Literature review

The evidence is insufficient to support the use of the therapeutic drug monitoring with the Anser ADA, Anser IFX, Anser UST and Anser VDZ assays in patients with inflammatory conditions to guide treatment optimization with monoclonal antibodies. The overall benefit of therapeutic drug monitoring with Prometheus Anser assays has not been established.<sup>6,8-28</sup>

There is an absence of clinical utility studies evaluating if TDM-guided dosing adjustments leads to clinically meaningful changes in patient health outcomes, and how those outcomes compare with adjustments based on patient symptoms, clinical assessment, and conventional laboratory evaluation.

Well-designed studies are needed to expand the existing evidence base to confirm if TDM with ADA, UST, and VDZ leads to changes in therapeutic interventions or other changes in disease management that improve patient health outcomes over the long term. Studies should also report how these outcomes compare with adjustments based on patient symptoms, clinical assessment, and conventional laboratory evaluation.

## Criteria

### Introduction

Requests for Anser ADA, IFX, UST, and VDZ testing are reviewed using these criteria.

## Criteria

- This test is considered investigational and/or experimental.
  - Investigational and experimental (I&E) molecular and genomic (MolGen) tests refer to assays involving chromosomes, DNA, RNA, or gene products that have insufficient data to determine the net health impact, which typically means there is insufficient data to support that a test accurately assesses the outcome of interest (analytical and clinical validity), significantly improves health outcomes (clinical utility), and/or performs better than an existing standard of care medical management option. Such tests are also not generally accepted as standard of care in the evaluation or management of a particular condition.
  - In the case of MolGen testing, FDA clearance is not a reliable standard given the number of laboratory developed tests that currently fall outside of FDA oversight and FDA clearance often does not assess clinical utility.

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# APOE Variant Analysis for Alzheimer Disease Testing

MOL.TS.128.A

v1.0.2020

## Introduction

APOE variant analysis for Alzheimer disease testing is addressed by this guideline.

## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedure addressed by this guideline | Procedure codes |
|---------------------------------------|-----------------|
| APOE Genotyping                       | 81401<br>S3852  |

## What is Alzheimer disease

### Definition

Alzheimer disease (AD) is characterized by an adult-onset, progressive dementia with cerebral cortical atrophy, beta amyloid plaque formation, and intraneuronal neurofibrillary tangles.<sup>1</sup>

### Prevalence

The general population lifetime risk of AD is about 10%. First-degree relatives, siblings or offspring of a single person in the family with AD have a 20-25% lifetime risk.<sup>2</sup>

#### Familial AD

Of all people with AD, about 25% have at least two affected people in the family. This is referred to as familial AD.<sup>2</sup>

Approximately 95% of people with familial AD develop symptoms after 65.<sup>2</sup> This is called late-onset familial AD. Late-onset familial AD is believed to have complex inheritance with multiple susceptibility genes and environmental factors playing a role.<sup>2</sup>

In about 5% of familial cases, symptoms consistently start before 65.<sup>2</sup> This is called early onset familial Alzheimer disease (EOFAD). EOFAD is an autosomal dominant inherited disorder caused by different genes than those that may predispose to late-onset AD.<sup>1</sup>

## Symptoms

Common findings include memory loss, confusion, speech issues, hallucinations, and personality and behavioral changes such as poor judgment, agitation, and withdrawal.<sup>2,3</sup>

## Onset

Symptoms of AD usually start after 60-65 years old.<sup>2</sup>

## APOE variants

There are three major allelic variants of APOE: e2, e3, and e4.

### APOE e4 allele

When present in the heterozygous state (APOE e3/e4) or the homozygous state (APOE e4/e4), the APOE e4 allele increases the risk for late-onset AD, but is not sufficient to cause disease.<sup>2</sup>

APOE e4 is not necessary to develop AD and having no copies of e4 does not rule out the disease.<sup>2,4</sup> APOE e4 appears to cause susceptibility to AD, but the reason is unclear.<sup>2,5</sup>

## Test information

### Introduction

Testing for APOE alleles is available clinically.

### APOE allele clinical testing

Many laboratories in the U.S. directly test for the three major allelic variants (e2, e3, e4) to assist diagnosis or predict risk of Alzheimer disease.

## Guidelines and evidence

### Introduction

This section includes relevant guidelines and evidence pertaining to APOE allele analysis for AD.

### Choosing Wisely/American College of Medical Genetics and Genomics (2015)

“Don’t order APOE genetic testing as a predictive test for Alzheimer disease. APOE is a susceptibility gene for later-onset Alzheimer disease (AD), the most common cause of dementia. The presence of an ε4 allele is neither necessary nor sufficient to cause AD.

The relative risk conferred by the  $\epsilon 4$  allele is confounded by the presence of other risk alleles, gender, environment and possibly ethnicity. APOE genotyping for AD risk prediction has limited clinical utility and poor predictive value.”<sup>6</sup>

### **The American College of Medical Genetics and The National Society of Genetic Counselors (2011)**

“Genetic testing for susceptibility loci (e.g., APOE) is not clinically recommended due to limited clinical utility and poor predictive value.”<sup>5</sup>

“Because the  $\epsilon 4$  allele is neither necessary nor sufficient to cause AD, there have been numerous consensus statements and articles that have recommended against using APOE genotyping for predicting AD risk.”<sup>5</sup>

### **European Federation of Neurological Societies (2010)**

“The ApoE e4 allele is the only genetic factor consistently implicated in late-onset AD, but it is neither necessary nor sufficient for development of the disease. Hence, there is no evidence to suggest ApoE testing is useful in a diagnostic setting.”<sup>4</sup>

### **National Institute of Aging/Alzheimer's Association Working Group (1997)**

“Insofar as patients with AD are more likely to have an APOE-e4 allele than are patients with other forms of dementia or individuals without dementia, physicians may choose to use APOE genotyping as an adjunct to other diagnostic tests for AD.”<sup>6</sup>

“Since genotyping cannot provide certainty about the presence or absence of AD, it should not be used as the sole diagnostic test.”<sup>7</sup>

“The use of APOE genotyping to predict future risk of AD in symptom-free individuals is not recommended at this time.”<sup>7</sup>

## **Criteria**

### **Introduction**

Requests for APOE allele analysis for AD are reviewed using these criteria.

### **Criteria**

- This test is considered investigational and/or experimental
  - Investigational and experimental (I&E) molecular and genomic (MolGen) tests refer to assays involving chromosomes, DNA, RNA, or gene products that have insufficient data to determine the net health impact, which typically means there is insufficient data to support that a test accurately assesses the outcome of interest (analytical and clinical validity), significantly improves health outcomes (clinical utility), and/or performs better than an existing standard of care medical

management option. Such tests are also not generally accepted as standard of care in the evaluation or management of a particular condition.

- In the case of MolGen testing, FDA clearance is not a reliable standard given the number of laboratory developed tests that currently fall outside of FDA oversight and FDA clearance often does not assess clinical utility

## References

### Introduction

These references are cited in this guideline.

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# Genetic Testing for Arrhythmogenic Right Ventricular Cardiomyopathy

MOL.TS.281.A

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## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedures addressed by this guideline            | Procedure codes |
|---|-----------------|
| DSC2 Mutation Analysis                            | 81406           |
| DSG2 Mutation Analysis                            | 81406           |
| DSP Mutation Analysis                             | 81406           |
| JUP Mutation Analysis                             | 81406           |
| PKP2 Mutation Analysis                            | 81406           |
| TMEM43 Mutation Analysis                          | 81406           |
| Known Familial Mutation Analysis                  | 81403           |
| Hereditary Cardiomyopathy Panel (5 or more genes) | 81439           |

## What Is Arrhythmogenic Right Ventricular Cardiomyopathy

### Definition

Arrhythmogenic Right Ventricular Cardiomyopathy (ARVC, formerly called Arrhythmogenic Right Ventricular Dysplasia, or ARVD) is a form of heart disease characterized by fibrofatty tissue replacement of the myocardium over time. This typically leads to right sided heart dysfunction.

### Incidence and Prevalence

ARVC occurs in 1/1000 to 1/5000 people.<sup>1</sup> This condition is more common in the Italian population (1/200). It may be underdiagnosed, as symptoms can be mild and some individuals are asymptomatic.<sup>2,3</sup>

### Symptoms

ARVC most commonly presents as a cardiac arrhythmia manifested by syncope or palpitations. Sudden death can be a presenting symptom, especially in young

athletes.<sup>1,4</sup> The ECG and cardiac imaging are abnormal. Although the right ventricle is most commonly involved, left ventricular abnormalities have been reported. Individuals may progress to cardiomyopathy and heart failure, with approximately 5% requiring heart transplant.<sup>2</sup> The average age at diagnosis is 31 years; however, symptoms can begin in the second decade of life.

### Cause

ARVC is caused by replacement of myocardium by fibrofatty tissue. Approximately 40% of ARVC has a genetic cause.<sup>5</sup> Non-genetic causes include sarcoidosis and myocarditis.

### Inheritance

Most cases of ARVC are inherited in an autosomal dominant pattern. Digenic inheritance (pathogenic mutations in two separate genes) has been reported in 4-47% of individuals.<sup>2</sup> These individuals are reported to have more severe arrhythmia. Several autosomal recessive syndromes caused by ARVC genes have also been described. These individuals typically have ARVC with skin and hair findings. Some genotype-phenotype correlation exists, with DSP mutations more commonly causing left ventricular involvement and PKP2 mutations more frequently associated with ventricular tachycardia.<sup>5</sup>

Variable expressivity and reduced penetrance have been reported.

### Diagnosis

Diagnostic criteria for ARVC have been established and are based on major and minor criteria broken down by image modality.<sup>4</sup>

#### Major criteria include:<sup>4</sup>

##### 2D echo

- Right ventricular akinesia, dyskinesia, or aneurysm AND
- Parasternal long axis right ventricular outflow tract (RVOT) greater than 31mm; corrected for body surface area OR
- Parasternal short axis RVOT greater than 35mm corrected for body surface area OR
- Fractional area change less than 34%

##### MRI

- Regional RV akinesia or dyskinesia or dyssynchronous RV contraction; AND
- Ratio of RV end-diastolic volume to BSA greater than or equal to 110mL/m<sup>2</sup> (male) or greater than or equal to 100 mL/m<sup>2</sup> (female) OR
- RV ejection fraction less than or equal to 40%

### Right ventricular angiography

- Regional RV akinesia, dyskinesia, or aneurysm

### Minor criteria include:<sup>4</sup>

#### 2D echo

- Regional right ventricular akinesia or dyskinesia; AND
- PLAX RVOT greater than or equal to 29 to less than 32 mm; corrected for BSA  
OR
- PSAX RVOT greater than or equal to 32 to less than 36 mm; corrected for BSA  
OR
- Fractional area change greater than 33% to less than or equal to 40%

#### MRI

- Regional RV akinesia or dyskinesia or dyssynchronous RV contraction; AND
- Ratio of RV end-diastolic volume to BSA greater than or equal to 100 to less than 110 mL/m<sup>2</sup> (male) or greater than or equal to 90 to less than 100 mL/m<sup>2</sup> (female) OR
- RV ejection fraction greater than 40% to less than or equal to 45%

### Other diagnostic criteria, which may include both major and minor criteria:<sup>2</sup>

- Electrocardiogram abnormalities
- Endomyocardial biopsy (or autopsy) finding of residual myocytes below 60% and fibrous replacement of the right ventricle in at least one sample
- Family history
- Presence of a pathogenic gene mutation (considered a major criterion)<sup>2</sup>
- Non genetic causes need to be excluded

## Clinical Diagnosis

The following table lists criteria needed to determine a clinical diagnosis and the strength of each diagnosis.<sup>4</sup>

| Strength of the Diagnosis | Made by the presence of:   |
|---------------------------|--|
| Definitive Diagnosis      | 2 major criteria, or<br>1 major and 2 minor criteria (from different categories), or<br>4 minor criteria (from different categories) |

| Strength of the Diagnosis | Made by the presence of:   |
|---------------------------|--|
| Borderline diagnosis      | 1 major and 1 minor criteria, or<br>3 minor criteria (from different categories) |
| Possible diagnosis        | 1 major criterion, or<br>2 minor criteria (from different categories)            |

## Treatment

ARVC treatment is based on presentation and focuses on avoidance of syncope, cardiac arrest, and sudden death through medication or cardioverter-defibrillator implantation. Heart transplant is occasionally required. Affected individuals are counseled to avoid rigorous physical activity, including competitive sports.<sup>2</sup> Additionally, evidence exists to suggest testing symptomatic minors or testing minors for a known familial disease-causing mutation can change their management and prevent sudden cardiac death.<sup>1,5</sup>

## Survival

The survival range for ARVC is broad. Sudden death due to ventricular arrhythmia can be a presenting symptom. Other individuals can be mildly affected, falling short of meeting diagnostic criteria. Overall, cardiac mortality and need for transplant is 5% or less.<sup>2</sup>

## Test information

### Introduction

Testing for ARVC may include known familial mutation analysis, single gene sequence analysis, single gene deletion/duplication analysis, or multi-gene panel testing.

### Sequence analysis

Until recently, most sequencing tests used the Sanger sequencing methodology that was originally developed in the 1970s. Sanger sequencing is labor intensive and did not lend itself to high-throughput applications.

Next generation sequencing (NGS), which is also sometimes called massively parallel sequencing, was developed in 2005 to allow larger scale and more efficient gene sequencing. NGS relies on sequencing many copies of small pieces of DNA simultaneously and using bioinformatics to assemble the sequence. NGS may not perform as well as Sanger sequencing in some applications.

NGS tests vary in technical specifications (e.g., depth of coverage, extent of intron/exon boundary analysis, methodology of large deletion/duplication analysis).

Sequence analysis detects single nucleotide substitutions and small (several nucleotide) deletions and insertions. Regions analyzed typically include the coding sequence and intron/exon boundaries. Promoter regions and intronic sequences may also be sequenced if disease-causing mutations are known to occur in these regions of a gene.

The efficiency of NGS has led to an increasing number of large, multi-gene testing panels. NGS panels that test several genes at once are particularly well-suited to conditions caused by more than one gene or where there is considerable clinical overlap between conditions.

Results may be obtained that cannot be adequately interpreted based on the current knowledge base. When a sequence variation is identified that has not been previously characterized or shown to cause the disorder in question, it is called a variant of uncertain significance (VUS). VUSs are relatively common findings when sequencing large amounts of DNA with NGS.

Under certain circumstances, technologies used in multi-gene testing may fail to identify mutations that might be identifiable through single-gene testing. If high clinical suspicion exists for a particular syndrome testing for that syndrome should be performed instead of a broad multi-gene panel.

Since genes can be easily added or removed from multi-gene tests over time by a given lab, medical records must document which genes were included in the specific multi-gene test used and in which labs they were performed.

Additionally, tests should be chosen to

- maximize the likelihood of identifying mutations in the genes of interest
- contribute to alterations in patient management
- minimize the chance of finding variants of uncertain clinical significance

## ARVC Sequencing

ARVC multi-gene panels should include a minimum of 6 genes: DSC2, DSG2, DSP, JUP, PKP2, and TMEM43. PKP2 mutation is the most common cause of inherited ARVC. Additional genes (RYR2, CTNNA3, DES, LMNA, PLN, TGFB3, and TTN) are included in some larger panels with limited diagnostic yield.<sup>6-9</sup>

Due to reported digenic inheritance (pathogenic mutations in two separate genes) in 4-47% of individuals, panel testing is strongly recommended for ARVC over sequential single gene testing.<sup>2</sup>

Multi-gene panels should be focused on the genes known to be associated with ARVC. No evidence has been found to suggest larger combined cardiac panels have a higher yield rate for ARVC patients.

Test yield has not been demonstrably higher when large scale testing is used versus disease specific panels.<sup>1,6,8</sup>

Predisposition testing for asymptomatic individuals by multi-gene panel testing is not recommended.<sup>2</sup>

### **Deletion/duplication analysis**

Analysis for deletions and duplications can be performed using a variety of technical platforms including exon array, MLPA, and NGS data analysis.

These assays detect gains and losses too large to be identified through sequencing technology, often single or multiple exons or whole genes.

### **Known familial mutation analysis**

Analysis for known familial mutations is typically performed by Sanger sequencing, but if available, a targeted mutation panel that includes the familial mutation may be performed.

Known familial mutations analysis is performed when a causative mutation has been identified in a close relative of the individual requesting testing.

## **Guidelines and evidence**

### **Introduction**

This section includes relevant guidelines and evidence pertaining to ARVC testing.

### **Heart Failure Society**

The Heart Failure Society (2018) states:<sup>10</sup>

- “Guideline 4: Genetic testing is recommended for patients with cardiomyopathy (Level of evidence A)”
  - “4a: Genetic testing is recommended for the most clearly affected family member.”
  - “4b: Cascade genetic testing of at-risk family members if recommended for pathogenic and likely pathogenic variants.”
- “Genetic testing is recommended to determine if a pathogenic variant can be identified to facilitate patient management and family screening.”
- “Testing should ideally be initiated on the person in a family with the most definitive diagnosis and most severe manifestations. This approach would maximize the likelihood of obtaining diagnostic results and detecting whether multiple pathogenic variants may be present and contributing to variable disease expression or severity.”
- “Molecular genetic testing for multiple genes with the use of a multigene panel is now the standard of practice for cardio-vascular genetic medicine. Furthermore,

multigene panel genetic testing is recommended over a serial single-gene testing approach owing to the genetically heterogeneous nature of cardiomyopathy. Genetic testing and cascade screening have been shown to be cost-effective.”

- “In ARVC, ICD placement for primary prevention in asymptomatic male carriers of a malignant pathogenic variant showed a significant effect on long-term clinical outcome.”

### **American College of Medical Genetics and Genomics**

The American College of Medical Genetics and Genomics (ACMG, 2018) published a practice resource on genetic testing for cardiomyopathies. This practice resource is an abbreviated version of the Heart Failure Society Guidelines above, on which ACMG collaborated. They state the following:<sup>11,12</sup>

- “Recommendation 1. Genetic testing is recommended for patients with cardiomyopathy.”
- “(a) Genetic testing is recommended for the most clearly affected family member.”
- “(b) Cascade genetic testing of at-risk family members is recommended for pathogenic and likely pathogenic variants.”
- “(c) In addition to routine newborn screening tests, specialized evaluation of infants with cardiomyopathy is recommended, and genetic testing should be considered.”

### **Heart Rhythm Society and European Society of Cardiology**

The Heart Rhythm Society and European Society of Cardiology (2011) states:<sup>6</sup>

- “Comprehensive or targeted (DSC2, DSG2, DSP, JUP, PKP2, and TMEM43) ACM/ARVC genetic testing can be useful for patients satisfying task force diagnostic criteria for ACM/ARVC.”
- “Genetic testing may be considered for patients with possible ACM/ARVC (1 major or 2 minor criteria) according to the 2010 task force criteria.”
- “Genetic testing is not recommended in patients with only a single minor criterion according to the 2010 task force criteria.”
- Mutation-specific genetic testing is recommended for family members and appropriate relatives following the identification of the ACM/ARVC- causative mutation in the index case”

### **European Society for Cardiology**

The European Society for Cardiology (2015) has the following guidelines for management of patients with ARVC:<sup>1</sup>

- “Targeted post-mortem genetic analysis of potentially disease causing genes should be considered in all sudden death victims in whom a specific inheritable channelopathy or cardiomyopathy is suspected.”
- “Genetic screening of a large panel of genes should not be performed in SUDS or SADS relatives without clinical clues for a specific disease after clinical evaluation.”

### **American College of Cardiology**

The American College of Cardiology (2013) does not have an official position statement. However, they have published an article on the genetics of ARVC as a guide to physicians which includes the following:<sup>7</sup>

- Testing for a known mutation in close relatives of an affected patient is beneficial.
- Periodic examination for persons who test positive for an ARVC genetic abnormality but do not have evidence of disease is recommended. Specifically, cardiac exam starting at 10 years of age every 2 years until age 20 and then every 5 years until age 60.
- Genetic counseling is recommended for all patients with a genetically transmitted heart disease.

## **Criteria**

### **Introduction**

Requests for ARVC testing are reviewed using these criteria.

### **Known Familial Mutation Analysis**

- Pre- and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - No previous full sequence testing or deletion/duplication analysis, and
  - Known disease-causing familial mutation in ARVC gene identified in 1<sup>st</sup> or 2<sup>nd</sup> degree relative(s), AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy

### **Multi-Gene Panel Testing**

- Genetic counseling:
  - Pre- and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND



- Previous Genetic Testing:
  - No previous full sequencing of requested genes, and
  - No known mutation identified by previous analysis, AND
- Diagnostic Testing for Symptomatic Individuals:
  - Personal History
    - Confirmed diagnosis of ARVC by electrocardiogram, MRI, or angiogram meeting the task force criteria for at least possible ARVC (defined as having one major or two minor criteria), and
    - No evidence of other syndromes with cardiac findings such as Marfan Syndrome or Thoracic Aortic Aneurysms and Dissection (TAAD), in patient or family, and
    - Non-genetic causes such as infection, toxin exposure, and metabolic/autoimmune disease have been ruled out, OR
  - Personal & Family History Combination
    - A diagnosis of ARVC or possible ARVC with one or more 1<sup>st</sup> or 2<sup>nd</sup> degree relatives with a diagnosis of ARVC, or
    - A diagnosis of ARVC or possible ARVC with a suspicious family history including a 1<sup>st</sup> or 2<sup>nd</sup> degree relative with sudden adult death or young cardiac event, AND
  - Documentation from ordering provider indicating how test results will be used to directly impact medical care for the individual (e.g. change in surveillance or treatment plan), AND
  - Rendering laboratory is a qualified provider of service per the Health Plan policy

### **Deletion/Duplication Analysis**

- Genetic Counseling:
  - Pre- and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - Member does not have a known mutation in an ARVC gene, and
  - No previous deletion/duplication analysis for ARVC genes, and
  - Member meets criteria for full sequence analysis of ARVC genes, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy

ARVC

## Billing and Reimbursement Considerations

- When multiple CPT codes are billed for components of a panel and there is a more appropriate CPT code representing the panel, eviCore will redirect to the panel code(s).
- If the laboratory will not accept redirection to a panel code, the medical necessity of each billed component procedure will be assessed independently.
  - In general, only a limited number of panel components that are most likely to explain the member's presentation will be reimbursable. The remaining panel components will not be reimbursable.
  - When the test is billed with multiple stacked codes, only the following genes may be considered for reimbursement:
    - DSC2
    - DSG2
    - DSP
    - JUP
    - PKP2
    - TMEM43

## References

### Introduction

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# Ashkenazi Jewish Carrier Screening

**MOL.TS.129.A**  
**v1.0.2020**

## Introduction

Ashkenazi Jewish carrier screening is addressed by this guideline.

## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedures addressed by this guideline        | Procedure codes |
|---|-----------------|
| ASPA Targeted Mutation Analysis               | 81200           |
| BCKDHB Targeted Mutation Analysis             | 81205           |
| BLM Targeted Mutation Analysis                | 81209           |
| CFTR Targeted Mutation Analysis               | 81220           |
| FANCC Targeted Mutation Analysis              | 81242           |
| G6PC Targeted Mutation Analysis               | 81250           |
| GBA Targeted Mutation Analysis                | 81251           |
| HEXA Targeted Mutation Analysis               | 81255           |
| IKBKAP Targeted Mutation Analysis             | 81260           |
| MCOLN1 Targeted Mutation Analysis             | 81290           |
| SMPD1 Targeted Mutation Analysis              | 81330           |
| Molecular pathology procedure, Level 1        | 81400           |
| Molecular pathology procedure, Level 2        | 81401           |
| Molecular pathology procedure, Level 3        | 81402           |
| Molecular pathology procedure, Level 4        | 81403           |
| Molecular pathology procedure, Level 5        | 81404           |
| Molecular pathology procedure, Level 6        | 81405           |
| Molecular pathology procedure, Level 7        | 81406           |
| Molecular pathology procedure, Level 8        | 81407           |
| Molecular pathology procedure, Level 9        | 81408           |
| Ashkenazi Jewish Genetic Disorders Sequencing | 81412           |

| Procedures addressed by this guideline | Procedure codes |
|--|-----------------|
| Unlisted molecular pathology procedure | 81479           |

## What is Ashkenazi Jewish carrier screening

### Definition

Ashkenazi Jewish carrier screening is available for certain genetic conditions that are either more common or for which there are higher mutation detection rates in the Ashkenazi Jewish population. “Ashkenazi” refers to someone whose Jewish ancestors originally came from Central or Eastern Europe, such as Russia, Poland, Germany, Hungary, Lithuania. Most Jewish people in the US are of Ashkenazi descent. There are regional differences in the number and types of tests commonly offered. Individuals and providers may choose all or a subset of these conditions.<sup>1-3</sup>

### Inheritance

These Jewish genetic diseases are inherited in an autosomal recessive manner. An affected individual must inherit a gene mutation from both parents.<sup>1,2</sup>

- Individuals who inherit only one mutation are called carriers. Carriers do not show symptoms of the disease, but have a 50% chance, with each pregnancy, of passing on the mutation to their children.
- Two carriers of the same disease have a 25% chance, with each pregnancy, of having a child with the disorder.

### Prevalence

While these genetic diseases are individually rare, the overall chance for an individual of Ashkenazi Jewish descent to be a carrier for one of these genetic diseases is 1 in 4 to 1 in 5.<sup>2,3</sup> An individual can also be a carrier of more than one condition.

People from other ethnic backgrounds can be carriers of these conditions, but it is generally less common. The test is typically not as effective at identifying carrier status in individuals of non-Ashkenazi Jewish descent.

## Test information

### Introduction

Ashkenazi Jewish carrier screening can be offered to couples or individuals of Ashkenazi Jewish descent when they are planning a pregnancy (preconceptional) or during a pregnancy (prenatal).<sup>1-3</sup>

## One member of couple is Jewish

If only one member of the couple is Ashkenazi Jewish, carrier screening should start with the Ashkenazi Jewish partner. Both parents must be carriers to have an affected child, so reproductive partners of known carriers should also be offered testing even if not Jewish. In some cases, full gene sequencing would be most appropriate for testing of a non-Jewish partner.

### Purpose of test

Carrier screening generally looks for a small number of gene mutations that are particularly common in the Ashkenazi Jewish population, although an increasing number of full gene sequencing panels are becoming available.

In addition, enzyme analysis is particularly effective for Tay-Sachs disease and is generally preferred to mutation testing.

### Detection rate

The carrier detection rate is greater than 95% in the Ashkenazi Jewish population for most diseases.<sup>3</sup>

The detection rate for these tests in the non-Ashkenazi population is unknown for most conditions, but generally low. Exceptions include cystic fibrosis and Tay-Sachs enzyme analysis, which each have good detection rates in non-Jewish populations.

A negative test result in one or both partners significantly lowers the chance of an affected child, but does not eliminate it.<sup>2</sup>

### Commonly tested conditions

The genes included in carrier screening panels vary widely between laboratories. The following table includes the most commonly tested conditions.

| Ashkenazi Jewish genetic disease | Ashkenazi carrier frequency | What the test looks for                           | Chance of correctly finding an Ashkenazi Jewish carrier |
|----------------------------------|-----------------------------|---|---|
| Bloom syndrome <sup>3</sup>      | 1/107                       | 1 mutation (2281del6ins7)                         | Greater than 99%  |
| Canavan disease <sup>3</sup>     | 1/41                        | 2 mutations (E285A, Y231X)                        | 97.4%   |
| Cystic fibrosis <sup>2</sup>     | 1/29                        | 23 most common mutations in several ethnic groups | 97%   |

| Ashkenazi Jewish genetic disease                       | Ashkenazi carrier frequency | What the test looks for  | Chance of correctly finding an Ashkenazi Jewish carrier |
|--|-----------------------------|--|---|
| Dihydrolipoamide dehydrogenase deficiency <sup>4</sup> | 1/107                       | 2 mutations (G229C and Y35X)                                       | Greater than 95%  |
| Familial dysautonomia <sup>3</sup>                     | 1/31                        | 2 mutations (2507+6TtoC, R696P)                                    | Greater than 99%  |
| Familial hyperinsulinism <sup>4</sup>                  | 1/68                        | 2 mutations (c.3989-9G>A and Phe11387del)                          | 90%   |
| Fanconi anemia group C <sup>3</sup>                    | 1/89                        | 1 mutation (IVS4+4AtoT)  | Greater than 99%  |
| Gaucher disease <sup>3</sup>                           | 1/18                        | 4 mutations (N370S, 84GG, L444P, IVS2+1GtoA)                       | Up to 94.6%   |
| Glycogen storage disease type 1A (GSD1A) <sup>5</sup>  | 1/71                        | 1 mutation (R83C)  | 93% to 100%   |
| Joubert syndrome 2 <sup>6</sup>                        | 1/92                        | 1 mutation (R12L)  | 99%   |
| Maple syrup urine disease (MSUD) <sup>7</sup>          | 1/80                        | 3 mutations (R183P, G278S, E372X)                                  | About 99%   |
| Mucopolipidosis IV <sup>3</sup>                        | 1/127                       | 2 mutations (IVS3-2AtoG, Del6.4kb)                                 | 95%   |
| Nemaline myopathy <sup>4</sup>                         | 1/168                       | 1 mutation (R2478_D2512del)  | Greater than 95%  |
| Niemann-Pick disease type A <sup>3</sup>               | 1/90                        | 3 mutations (R496L, L302P, fsP330)                                 | 97%   |
| Tay-Sachs disease <sup>3</sup>                         | 1/90                        | Mutation analysis: 3 mutations (1278insTATC, 1421+1GtoC, G269S) OR | 92-94%  |
|  |                             | Hexosaminidase A enzyme analysis                                   | About 98%   |
| Usher syndrome III <sup>4</sup>                        | 1/120                       | 1 mutation (N48K)  | Greater than 95%  |

## Guidelines and evidence

### Introduction

This section includes relevant guidelines and evidence pertaining to Ashkenazi Jewish carrier screening.

### American College of Obstetrics and Gynecology

The American College of Obstetrics and Gynecology (ACOG, 2017; reaffirmed 2019)<sup>8</sup> Committee on Genetics issued an opinion that "ethnic-specific (e.g. Ashkenazi Jewish), panethnic, and expanded carrier screening are acceptable strategies for pre-pregnancy and prenatal carrier screening."

If providers choose to offer ethnic-specific screening to patients of Ashkenazi Jewish ancestry, ACOG recommends<sup>9</sup> that screening include Canavan disease, cystic fibrosis, familial dysautonomia, Tay-Sachs disease, Bloom syndrome, familial hyperinsulinism, Fanconi anemia, Gaucher disease, glycogen storage disease type I, Joubert syndrome, maple syrup urine disease, mucopolidosis type IV, Niemann-Pick disease, and Usher syndrome.

Regardless of screening strategy chosen by the provider and regardless of patient ethnicity, ACOG recommends<sup>8</sup> that all patients who are considering pregnancy or are already pregnant be "...offered carrier screening for cystic fibrosis and spinal muscular atrophy, as well as a complete blood count and screening for thalassemias and hemoglobinopathies. Fragile X permutation carrier screening is recommended for women with a family history of fragile X-related disorders or intellectual disability suggestive of fragile X syndrome, or women with a personal history of ovarian insufficiency."

### ACMG guidance for adding disorders to carrier screening

The 2008 American College of Medical Genetics guidelines outline criteria for adding disorders to carrier screening in the Ashkenazi Jewish population:<sup>3</sup>

- the natural history must be well understood
- people affected with the disorder must have significant morbidity and/or mortality, and
- the test must have greater than 90% detection OR the allele frequency must be at least 1%.

#### Conditions that meet these criteria

The following conditions meet these criteria:

- cystic fibrosis
- Canavan disease
- familial dysautonomia



- Tay-Sachs disease
- Fanconi anemia (group C)
- Niemann-Pick (type A)
- Bloom syndrome
- mucopolipidosis IV
- Gaucher disease
- dilipoamide dehydrogenase deficiency<sup>4</sup>
- familial hyperinsulinism<sup>4</sup>
- glycogen storage disease type 1a<sup>5</sup>
- Joubert syndrome<sup>2</sup>
- maple syrup urine disease<sup>7,10</sup>
- nemaline myopathy,<sup>4</sup> and
- Usher syndrome type III.<sup>4</sup>

## Criteria

### Introduction

Requests for Ashkenazi Jewish Carrier Screening are reviewed using these criteria.

### Ashkenazi Jewish Genetic Diseases Carrier Screening Panels

- Carrier screening may be considered for all or any desired subset of the Ashkenazi Jewish genetic diseases eligible for coverage per the Coverage Guidance table when the following criteria are met:
  - The individual is planning a pregnancy or currently pregnant; and
  - At least one partner of a couple is Ashkenazi Jewish (NOTE: Detection rates for testing are higher in people with Ashkenazi Jewish ancestry. If only one partner of a couple is Ashkenazi Jewish, testing should start in that person when possible.); AND
- Testing will be billed using the procedure code 81412 that will represent all tests performed for the assessment of carrier status based on Ashkenazi Jewish ancestry and no additional tests for this purpose will be separately billed by the same lab for the same test date; or
- Testing for separate genes will be billed because the panel code is not more appropriate (e.g., fewer than the 9 stated genes will be assessed or a different

methodology is used), in which case individual gene test coverage will be assessed based on the guidance provided in the Coverage Guidance table.

### Single Ashkenazi Jewish Genetic Diseases Carrier Screening Tests

Carrier Screening may be considered for a single Ashkenazi Jewish disease, if any of the following are met:

- The individual is of Ashkenazi Jewish ancestry, OR
- The individual has a family history of one of these conditions, OR
- The individual's partner is a known carrier or affected with any of these conditions

### Other considerations

If an Ashkenazi Jewish carrier screening panel was previously performed and an updated, larger panel is being requested, only testing for the medically necessary, previously untested genes will be reimbursable. Therefore, only the most appropriate procedure codes for those additional genes will be considered for reimbursement.

### Coverage Guidance for Genes Included in Ashkenazi Jewish Carrier Screening Tests

| Condition                                 | Gene   | CPT   | Required claim code | Coverage   |
|---|--------|-------|---------------------|------------|
| Bloom syndrome                            | BLM    | 81209 | NONE                | MOL.TS.129 |
| Canavan disease                           | ASPA   | 81200 | NONE                | MOL.TS.129 |
| Cystic fibrosis                           | CFTR   | 81220 | NONE                | MOL.TS.129 |
| Dihydrolipoamide dehydrogenase deficiency | DLD    | 81479 | DLD                 | MOL.TS.129 |
| Familial dysautonomia                     | IKBKAP | 81260 | NONE                | MOL.TS.129 |
| Familial hyperinsulinism                  | ABCC8  | 81401 | ABCC8               | MOL.TS.129 |
| Fanconi anemia, type C                    | FANCC  | 81242 | NONE                | MOL.TS.129 |
| Gaucher disease, type 1                   | GBA    | 81251 | NONE                | MOL.TS.129 |

| Condition                          | Gene    | CPT   | Required claim code | Coverage   |
|------------------------------------|---------|-------|---------------------|------------|
| Glycogen storage disease, type 1A  | G6PC    | 81250 | NONE                | MOL.TS.129 |
| Joubert syndrome, type 2           | TMEM216 | 81479 | TMEM216             | MOL.TS.129 |
| Maple syrup urine disease, type 1b | BCKDHB  | 81205 | NONE                | MOL.TS.129 |
| Mucopolidposis, type IV            | MCOLN1  | 81290 | NONE                | MOL.TS.129 |
| Nemaline myopathy, type 2          | NEB     | 81400 | NEB                 | MOL.TS.129 |
| Niemann-Pick disease, type A       | SMPD1   | 81330 | NONE                | MOL.TS.129 |
| Tay-Sachs disease                  | HEXA    | 81255 | NONE                | MOL.TS.129 |
| Usher syndrome, type 1F            | PCDH15  | 81400 | PCDH15              | MOL.TS.129 |
| Usher syndrome, type 3             | CLRN1   | 81400 | CLRN1               | MOL.TS.129 |

**Note** Other tests may be eligible for coverage under the above criteria if the condition is associated with significant morbidity and mortality, the allele frequency is >1% in the Ashkenazi Jewish population, and the selected test method has >90% detection rate for disease-causing mutations.

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### Introduction

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# AssureMDx Testing for Bladder Cancer

MOL.TS.305.A

v1.0.2020

## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedures addressed by this guideline | Procedure codes |
|--|-----------------|
| AssureMDx                              | 81479           |

## What is bladder cancer

### Definition

Bladder cancer is one of the most common types of cancer in the U.S., especially among men. It is estimated that there will be more than 80,000 new cases of bladder cancer diagnosed in 2019 (61,700 in men and 18,770 in women).<sup>1</sup> Approximately 90% of patients are older than 55 years and the average age of diagnosis is 73 years.<sup>1</sup>

Bladder cancer is categorized as non-muscle invasive disease (NMID) or muscle invasive disease (MID).<sup>2</sup> Approximately 80% of bladder cancers are NMID, and of those, most are urothelial carcinoma (UC), also called transitional cell carcinoma.<sup>2-5</sup> Most cases of UC are low-grade and easily treated; however, UC has a high risk of recurrence (70%), and patients must be monitored for several years after treatment.<sup>4</sup> Diagnostic monitoring usually consists of regular testing of cells in the urine (cytology).<sup>2,6</sup>

Although the general survival rate is 77%, patients living with bladder cancer experience a sharp decline in quality of life associated with multiple procedures, declining health, and diminished well-being.<sup>7</sup>

The most common symptom of bladder cancer is hematuria. The current standard of care for detection of bladder cancer is cystoscopy, but this procedure is expensive and invasive, and fails to diagnose bladder cancer in up to 20% of cases.<sup>8,9</sup> In the United States, 11 million hematuria patients are referred to urologists in the United States each year. Only 33% result in an office visit, and only 6% of these undergo cystoscopy. A bladder cancer diagnosis is made in only 3%-23% of those undergoing cystoscopy.<sup>10</sup>

Due to the invasiveness of cystoscopy, there is an estimated 20,000 missed cancer cases among moderate-risk and/or high-risk hematuria patients.<sup>11</sup> In addition, there is excessive use of this invasive procedure in low-risk patients. As a result, there is a clinical need to better select patients for cystoscopies and decrease inappropriate testing.<sup>12</sup>

There is no definitive standard of care for classifying bladder cancer risk. Non-invasive procedures include urinalysis (assessing for blood in urine), urine cytology (assessing for cancer cells in urine), and urine tests for tumor markers (assessing for chromosomal changes or biomarkers).<sup>13</sup>

## Test information

### Introduction

AssureMDx is a non-invasive method to analyze tumor markers in the urine of patients with hematuria to identify patients at low risk and high risk for bladder cancer.

The test is intended to assist in deciding who is at low risk for bladder cancer and can avoid cystoscopy, and who may benefit from a cystoscopy.<sup>14</sup>

AssureMDx is a urine assay that analyzes three mutation markers or chromosomal changes (FGFR3, TERT, and HRAS) and the genetic methylation status of three biomarkers (OTX1, ONECUT2, AND TWIST1). These results, in conjunction with patient age, yield a patient's risk of bladder cancer (risk profile) and guide the recommendations for cystoscopy. Patients identified at low risk for bladder cancer can avoid an unnecessary cystoscopy. Patients at medium- to high-risk of bladder cancer will proceed with cystoscopy as the gold standard for the diagnosis of bladder cancer.<sup>14</sup>

## Guidelines and evidence

### Introduction

The following section includes relevant guidelines and evidence pertaining to AssureMDx for bladder cancer.

#### American Urological Association (AUA)

The current guidelines set forth by the American Urological Association (AUA) recommend cystoscopy for all adults age 35 and older presenting with asymptomatic microhematuria (after ruling out benign causes), and any individual presenting with gross hematuria.<sup>9</sup>

#### American College of Physicians (ACP)

Guidelines suggested by the American College of Physicians (ACP) posit that any patient presenting with gross hematuria should receive a referral to a urologist, and any patient presenting with asymptomatic microhematuria should be referred to a urologist, if benign causes are ruled out. Only 9%-25% of patients presenting with hematuria undergo cystoscopy within six months of their initial diagnosis, and this is observed across different patient populations (academic health practice, community health clinic, Medicare-eligible patients).<sup>11,12</sup>

## Literature review

There is insufficient evidence to support the use of the AssureMDx to accurately predict the risk of bladder cancer in patients with hematuria, and thus identify patients for whom cystoscopy is necessary.<sup>10,11,15</sup> Analytical validity studies are lacking. Clinical validity studies are limited by small study populations and the lack of long-term follow-up regarding the development of bladder cancer at a later date. Although one prospective clinical utility study suggests that recurrence of bladder cancer can be detected by follow-up urine assays, it is unclear if the use of AssureMDx versus conventional cystoscopy leads to changes in health care decision-making and improvement in patient survival.

### Additional studies

Additional well-designed studies are needed to add to the evidence base, corroborate the early findings in larger patient populations, and include additional clinical factors such as age, gender, smoking history, and presence of gross versus microscopic hematuria.

## Ongoing Clinical Trials

A study with the identifier: NCT03122964 is still in progress and is currently undergoing patient recruitment (updated August 17, 2018).<sup>16</sup> The primary objective of this study is to evaluate the performance of AssureMDx for the detection of bladder cancer in patients with gross or microscopic hematuria. The secondary objective is to compare the predictive accuracy of a risk model including clinical factors (age, gender, smoking history, gross versus microscopic hematuria) with and without AssureMDx testing.

## Criteria

### Introduction

Requests for AssureMDx are reviewed using the following criteria.

This test is considered investigational and/or experimental.

- Investigational and experimental (I&E) molecular and genomic (MolGen) tests refer to assays involving chromosomes, DNA, RNA, or gene products that have insufficient data to determine the net health impact, which typically means there is insufficient data to support that a test accurately assesses the outcome of interest (analytical and clinical validity), significantly improves health outcomes (clinical utility), and/or performs better than an existing standard of care medical management option. Such tests are also not generally accepted as standard of care in the evaluation or management of a particular condition.
- In the case of MolGen testing, FDA clearance is not a reliable standard given the number of laboratory developed tests that currently fall outside of FDA oversight and FDA clearance often does not assess clinical utility.

## References

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# Ataxia-Telangiectasia Testing

**MOL.TS.130.A**  
**v1.0.2020**

## Introduction

Ataxia-telangiectasia (A-T) testing is addressed by this guideline.

## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedures addressed by this guideline | Procedure codes |
|--|-----------------|
| ATM known familial mutation analysis   | 81403           |
| ATM sequencing                         | 81408           |
| ATM deletion and duplication analysis  | 81479           |

## What is Ataxia-telangiectasia

### Definition

Ataxia-telangiectasia (A-T) is a progressive neurological disorder that is caused by mutations in the ATM gene.

### Prevalence

The prevalence of A-T is approximately 1 in 40,000 to 1 in 100,000 live US births.<sup>1,2</sup> It is the most common cause of childhood progressive cerebellar ataxia in most countries.<sup>3</sup>

### Symptoms

Signs and symptoms of A-T include<sup>1</sup>

- truncal and gait ataxia
- ocular apraxia
- slurred speech
- head tilting, after the age of 6 months
- conjunctival telangiectasias
- immunodeficiencies

- malignancies, especially leukemias and lymphomas, and
- radiation sensitivity.

### **Onset**

The onset for A-T is typically between the ages of 1 and 4 years.

### **Related conditions**

ATM heterozygotes (carriers) may be at an increased risk for breast cancer, especially women with a strong family history of breast cancer.<sup>4-7</sup> Epidemiological data has also suggested an increased risk for cardiovascular disease in carriers.<sup>6,7</sup> Therefore, carriers of ATM mutant alleles may need to be screened for breast cancer and cardiovascular disease.

### **Inheritance**

A-T is inherited in an autosomal recessive inheritance pattern. Males and females are equally likely to be affected. If both parents are carriers of A-T, the risk for a pregnancy to be affected is 1 in 4, or 25%. Preimplantation and prenatal diagnosis are available for couples known to be at-risk.

### **Prognosis**

Although individuals with A-T live to adulthood, they are at an increased risk for early death. Currently, most individuals live beyond 25 years, with some surviving into their 50s. Cause of death is associated with A-T associated cancers, infection, and pulmonary failure.<sup>1</sup>

## **Test information**

### **Introduction**

Testing for Ataxia-telangiectasia may include sequence analysis, deletion/duplication analysis, or known familial mutation analysis.

### **Sequence analysis**

Sequence analysis of the ATM gene can identify 90-95% of A-T mutations in affected individuals.<sup>1</sup>

### **Deletion and duplication analysis**

Deletion and duplication analysis of the ATM gene can identify another 1-2% of mutations.<sup>1</sup>

## Known familial mutation analysis

Once a deleterious mutation has been identified, relatives of affected individuals can undergo tests. Detection of carriers impacts medical management in the case of breast cancer screening and cardiovascular disease screening.

Prenatal testing is available to individuals with a known family mutation. Genetic testing can be performed on amniocytes obtained through amniocentesis or chorionic villi obtained through a chorionic villus sampling.

## Guidelines and evidence

### Introduction

This section includes relevant guidelines and evidence pertaining to Ataxia-telangiectasia testing.

### International Workshop on A-T

The Eighth International Workshop on Ataxia-telangiectasia (A-T) was convened in 1999. The workshop described ATM mutations and cancer risk in heterozygotes, and potential therapeutic approaches. Genetic testing strategies were not described.<sup>8</sup>

## Criteria

### Introduction

Requests for Ataxia-telangiectasia testing are reviewed using these criteria.

### ATM known familial mutation analysis

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - No previous genetic testing of ATM, AND
- Carrier Screening Individuals:
  - Known family mutation in ATM in 1st, 2nd, or 3rd degree biologic relative(s), OR
- Prenatal Testing for At-Risk Pregnancies:
  - ATM mutations identified in both biologic parents.

**ATM sequencing**

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - No previous ATM gene sequencing, and
  - No known ATM mutation in family, AND
- Diagnostic Testing for Symptomatic Individuals:
  - Elevated Alpha-fetoprotein (AFP) levels, or
  - Decreased ATM protein detected by immunoblotting, and
  - Progressive cerebellar ataxia, or
  - Truncal and gait ataxia, or
  - Oculomotor apraxia, OR
- Diagnostic Testing for Carriers:
  - One mutation detected by targeted mutation analysis, and
  - Elevated Alpha-fetoprotein (AFP) levels, or
  - Decreased ATM protein detected by immunoblotting, OR
- Testing for Individuals with Family History or Partners of Carriers:
  - 1<sup>st</sup>, 2<sup>nd</sup>, or 3<sup>rd</sup>, degree relative diagnosed with Ataxia-Telangiectasia clinical diagnosis, family mutation unknown, and testing unavailable, or
  - Partner is monoallelic or biallelic for ATM mutation, and
  - Has living children with this partner, or
  - Has the potential and intention to reproduce

**ATM duplication and deletion analysis**

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - No previous deletion/duplication analysis of ATM, and

- No mutations detected in full sequencing, or
- Heterozygous for mutation and individual is expected to be affected (eg, elevated alpha-fetoprotein levels, decreased ATM protein detected by immunoblotting (if performed), other features of disorder are present).

## References

### Introduction

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# BCR-ABL Negative Myeloproliferative Neoplasm Testing

MOL.TS.240.A  
v1.0.2020

## Introduction

BCR-ABL negative myeloproliferative neoplasm (MPN) testing is addressed by this guideline.

## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedures addressed by this guideline  | Procedure codes |
|---|-----------------|
| JAK2 V617F Mutation Analysis            | 81270           |
| JAK2 Exon 12 Mutation Analysis          | 81403           |
| CALR Exon 9 Mutation Analysis           | 81219           |
| MPL Mutation Analysis                   | 81403           |
| ASXL1 Mutation Analysis                 | 81175           |
| EZH2 Common Variant(s) (e.g. codon 646) | 81237           |
| EZH2 Full Gene Sequencing               | 81236           |
| TET2 Mutation Analysis                  | 81479           |
| IDH1 Mutation Analysis                  | 81120           |
| IDH2 Mutation Analysis                  | 81121           |
| SRSF2 Mutation Analysis                 | 81479           |
| SF3B1 Mutation Analysis                 | 81479           |

## What are BCR-ABL negative myeloproliferative neoplasms

### Definition

Primary myelofibrosis (PMF), polycythemia vera (PV) and essential thrombocythemia (ET) are a group of heterogeneous disorders of the hematopoietic system collectively known as Philadelphia chromosome-negative MPN.

## Prevalence

The following table describes the prevalence of Philadelphia chromosome-negative MPNs in the U.S.<sup>1</sup>

| Disorder | Prevalence in the U.S. |
|----------|------------------------|
| PMF      | 13,000                 |
| ET       | 134,000                |
| PV       | 148,000                |

## Symptoms

Symptoms vary among the subtypes, but generally include

- constitutional symptoms
- fatigue
- pruritus
- weight loss
- symptoms of splenomegaly, and
- variable lab abnormalities, including
  - erythrocytosis
  - thrombocytosis, and
  - leukocytosis.<sup>1</sup>

## Risks

Individuals with MPNs are at risk of the condition transforming into acute myeloid leukemia (AML), which is associated with a poor response to therapy and short survival. These disorders are also associated with an increased risk of major bleeding and thrombosis or thromboembolism compared to the general population.<sup>1</sup>

## Diagnosis

The diagnosis and management of patients with MPN has evolved since the identification of mutations that activate the JAK pathway, including JAK2, CALR, and MPL. The development of targeted therapies has resulted in significant improvements in disease-related symptoms and quality of life.<sup>1</sup>

- **JAK2 V617F mutations** — JAK2 V617F mutations account for the majority of patients with PV (greater than 90%), ET or PMF (60%). Most of the mutations occur in exon 14 with rare insertions/deletions in exon 12.<sup>1</sup>



- **JAK2 exon 12 mutations** — JAK2 exon 12 mutations have been seen in approximately 2-3% of patients with PV.<sup>1</sup>
- **MPL mutations** — MPL mutations have been reported in 5-8% of patients with PMF and 1-4% of patients with ET. MPL mutations are associated with lower hemoglobin levels at diagnosis and increased risk of transfusion dependence in patients with PMF.<sup>1</sup>
- **CALR mutations** — CALR mutations are reported in approximately 20-35% of patients with ET and PMF, accounting for approximately 60-80% of patients with JAK2/MPL-negative ET and PMF. CALR deletion mutations are more commonly seen in patients with PMF and are associated with a significantly higher risk of myelofibrosis transformation in ET. CALR insertion mutations are associated with ET, low risk of thrombosis and an indolent course. CALR mutations are associated with a lower hemoglobin level, lower WBC count, higher platelet count and lower incidence of thrombosis than the JAK2 V617F mutation.<sup>1</sup>

## Test information

### Introduction

Testing for BCR-ABL negative MPN may include cytogenetic testing, single gene mutation analysis, or panel testing.

### Types of tests

There are various methods used to test for the cytogenetic and molecular abnormalities associated with MPN.<sup>1,2</sup> Tests for the cytogenetic and molecular abnormalities include:

- bone marrow (BM) cytogenetics: karyotype, with or without FISH
- single gene mutation analysis for JAK2, MPL, and CALR, and
- panel testing using next generation sequencing for somatic mutations in genes associated with MPN.

## Guidelines and evidence

### Introduction

This section includes relevant guidelines and evidence pertaining to BCR-ABL negative MPN testing.

**World Health Organization: PMF**

The World Health Organization (WHO, 2016) has established diagnostic criteria for PMF.<sup>2</sup>

| Pre Primary Myelofibrosis (prePMF)   | Overt Primary Myelofibrosis (overt PMF)   |
|--|---|
| <b>[Diagnosis requires meeting all 3 major criteria, and at least 1 minor criterion]</b>   | <b>[Diagnosis requires meeting all 3 major criteria, and at least 1 minor criterion]</b>  |
| <p>Major criteria:</p> <ul style="list-style-type: none"> <li>• Megakaryocytic proliferation and atypia, without reticulin fibrosis &gt;grade 1, accompanied by increased age-adjusted BM cellularity, granulocytic proliferation, and often decreased erythropoiesis</li> <li>• Not meeting WHO criteria for BCR-ABL1+ CML, PV, ET, myelodysplastic syndromes, or other myeloid neoplasms</li> <li>• Presence of JAK2, CALR, or MPL mutation or in the absence of these mutations, presence of another clonal marker, or absence of minor reactive BM reticulin fibrosis</li> </ul> | <p>Major criteria:</p> <ul style="list-style-type: none"> <li>• Megakaryocytic proliferation and atypia, accompanied by either reticulin and/or collagen fibrosis grades 2 or 3</li> <li>• Not meeting WHO criteria for BCR-ABL1+ CML, PV, ET, myelodysplastic syndromes, or other myeloid neoplasms</li> <li>• Presence of JAK2, CALR, or MPL mutation or in the absence of these mutations, presence of another clonal marker, or absence of reactive BM myelofibrosis</li> </ul> |
| <p>Minor criteria:<br/>Presence of at least one of the following, confirmed in 2 consecutive determinations:</p> <ul style="list-style-type: none"> <li>• Anemia not attributed to a comorbid condition</li> <li>• Leukocytosis <math>\geq 11 \times 10^9/L</math></li> <li>• Palpable splenomegaly</li> <li>• LDH increased to above upper normal limit of institutional reference range</li> </ul>   | <p>Minor criteria:<br/>Presence of at least one of the following, confirmed in 2 consecutive determinations:</p> <ul style="list-style-type: none"> <li>• Anemia not attributed to a comorbid condition</li> <li>• Leukocytosis <math>\geq 11 \times 10^9/L</math></li> <li>• Palpable splenomegaly</li> <li>• LDH increased to above upper normal limit of institutional reference range</li> <li>• Leukoerythroblastosis</li> </ul>   |

**Absence of 3 major clonal mutations**

In the absence of any of the 3 major clonal mutations, the search for the most frequent accompanying mutations help determine the clonal nature of the disease. Examples of the most frequent accompanying mutations include:

- ASXL1
- EZH2
- TET2
- IDH1
- IDH2
- SRSF2
- SF3B1

**World Health Organization: PV**

The World Health Organization (WHO, 2016) has established diagnostic criteria for PV.<sup>2</sup>

**Polycythemia Vera (PV)**

**[Diagnosis requires meeting either all 3 major criteria, or the first 2 major criteria and the minor criterion]**

**Major criteria:**

- Hemoglobin > 16.5 g/dL in men, > 16.0 g/dL in women OR Hematocrit >49% in men, >48% in women OR Increased red cell mass (RCM), defined as >25% above the mean normal predicted value
- Bone marrow biopsy showing hypercellularity for age with trilineage growth (panmyelosis) including prominent erythroid, granulocytic, and megakaryocytic proliferation with pleomorphic, mature megakaryocytes (difference in size)
- Presence of JAK2 V617F or JAK2 exon 12 mutation

**Minor criteria:**

- Subnormal serum EPO level

**Bone marrow biopsy not required in some cases**

A bone marrow biopsy may not be required in cases with sustained absolute erythrocytosis; hemoglobin levels >18.5 g/dL in men (hematocrit, 55.5%) or >16.5 g/dL in women (hematocrit, 49.5%) if 3 major criterion and the minor criterion are present. However, initial myelofibrosis (present in up to 20% of patients) can only be detected by performing a BM biopsy; this finding may predict a more rapid progression to overt myelofibrosis (post-PV PMF).

## World Health Organization: ET

The World Health Organization (WHO, 2016) has established diagnostic criteria for ET.<sup>2</sup>

### Essential Thrombocythemia (ET)

[Diagnosis requires meeting all 4 major criteria or the first 3 major criteria and the minor criterion]

Major criteria:

- Platelet count  $\geq 450 \times 10^9/L$
- Bone marrow biopsy showing proliferation mainly of the megakaryocyte lineage with increased numbers of enlarged, mature megakaryocytes with hyperlobulated nuclei. No significant increase or left shift in neutrophil granulopoiesis or erythropoiesis and very rarely minor (grade 1) increase in reticulin fibers
- Not meeting WHO criteria for BCR-ABL1+ CML, PV, PMF, myelodysplastic syndromes, or other myeloid neoplasms
- Presence of JAK2, CALR, or MPL mutation

Minor criteria:

- Presence of a clonal marker or absence of evidence for reactive thrombocytosis

## National Comprehensive Cancer Network

The National Comprehensive Cancer Network (NCCN, 2019) evidence and consensus-based guidelines recommend the following initial laboratory evaluations for individuals suspected to have MPN:<sup>1</sup>

- “Laboratory evaluations should include complete blood count (CBC), microscopic examination of the peripheral smear, comprehensive metabolic panel with serum uric acid, serum LDH, liver function tests, serum EPO level and serum iron studies.”
- “Fluorescence in situ hybridization (FISH) or a multiplex reverse transcriptase polymerase chain reaction (RT-PCR) on a peripheral blood specimen to detect BCR-ABL1 transcripts and exclude the diagnosis of CML is especially recommended for patients with left-shifted leukocytosis and/or thrombocytosis with basophilia.”
- “Molecular testing for JAK2 V617F mutations is recommended as part of the initial workup for all patients. If JAK2 V617F mutation testing is negative, molecular testing for MPL and CALR mutations should be performed for patients with MF and ET; molecular testing for the JAK2 exon12 mutation should be done for those with suspected PV and negative for the JAK2 V617F mutation.”
- “Alternatively, molecular testing using the multi-gene NGS panel that includes JAK2, CALR, and MPL can be used as part of initial workup for all patients.”

- “The application of an NGS-based 28-gene panel in patients with MPN identified significantly more mutated splicing genes (SF3B1, SRSF2, and U2AF1) in patients with PMF compared to those with ET, and no mutations in splicing genes were found in patients with PV.”
- “Bone marrow aspirate and biopsy with trichrome and reticulin stain and bone marrow cytogenetics (karyotype, with or without FISH; blood, if bone marrow is inaspirable) are necessary to accurately distinguish the bone marrow morphological features between the disease subtypes (early or prefibrotic PMF, ET and masked PV).”

## Criteria

### JAK2 V617F Mutation Analysis

- Member does not meet WHO criteria for BCR-ABL1+ CML, myelodysplastic syndromes, or other myeloid neoplasms, AND
- Member meets at least ONE of the following diagnostic criteria for MPN:
  - Bone marrow biopsy results that are consistent with WHO diagnostic criteria for prePMF, overt PMF, ET, or PV, or
  - Platelet count  $\geq 450 \times 10^9/L$ , or
  - Hemoglobin  $> 16.5$  g/dL in men,  $> 16.0$  g/dL in women, or
  - Hematocrit  $>49\%$  in men,  $>48\%$  in women, or
  - Increased red cell mass (RCM), defined as  $>25\%$  above the mean normal predicted value, or
  - A combination of two of the following symptoms:
    - Anemia not attributed to a comorbid condition, or
    - Leukocytosis  $\geq 11 \times 10^9/L$ , or
    - Palpable splenomegaly, or
    - LDH increased to above upper normal limit of institutional reference range, or
    - Leukoerythroblastosis, OR
- MPN is being considered in the differential diagnosis with the member meeting both of the following:
  - Variable lab abnormalities, including erythrocytosis, thrombocytosis and leukocytosis, which are not otherwise assigned an etiology, and

- Constitutional symptoms, including fatigue, pruritus, weight loss and symptoms of splenomegaly, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

### **JAK2 Exon 12 Analysis**

- Member does not meet WHO criteria for BCR-ABL1+ CML, myelodysplastic syndromes, or other myeloid neoplasms, AND
- JAK2 V617F mutation analysis is negative, AND
- Member meets at least ONE of the following diagnostic criteria for PV:
  - Bone marrow biopsy results that are consistent with WHO diagnostic criteria for PV, or
  - Hemoglobin > 16.5 g/dL in men, > 16.0 g/dL in women, or
  - Hematocrit >49% in men, >48% in women, or
  - Increased red cell mass (RCM), defined as >25% above the mean normal predicted value, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

### **CALR Exon 9 and MPL Mutation Analysis**

- Member does not meet WHO criteria for BCR-ABL1+ CML, PV, myelodysplastic syndromes, or other myeloid neoplasms, AND
- JAK2 V617F mutation analysis is negative, AND
- Member meets at least ONE of the following diagnostic criteria for ET or PMF:
  - Bone marrow biopsy results that are consistent with WHO diagnostic criteria for prePMF, overt PMF, or ET, or
  - Platelet count  $\geq 450 \times 10^9/L$ , or
  - A combination of two of the following symptoms:
    - Anemia not attributed to a comorbid condition, or
    - Leukocytosis  $\geq 11 \times 10^9/L$ , or
    - Palpable splenomegaly, or
    - LDH increased to above upper normal limit of institutional reference range, or
    - Leukoerythroblastosis, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

## Analysis of ASXL1, EZH2, TET2, IDH1, IDH2, SRSF2, or SF3B1

- Member does not meet WHO criteria for BCR-ABL1+ CML, PV, ET, myelodysplastic syndromes, or other myeloid neoplasms, AND
- JAK2, CALR, and MPL mutation analyses are all negative, AND
- Member meets at least ONE of the following diagnostic criteria for PMF:
  - Bone marrow biopsy results that are consistent with WHO diagnostic criteria for prePMF or overt PMF, or
  - A combination of two of the following symptoms:
    - Anemia not attributed to a comorbid condition, or
    - Leukocytosis  $\geq 11 \times 10^9/L$ , or
    - Palpable splenomegaly, or
    - LDH increased to above upper normal limit of institutional reference range, or
    - Leukoerythroblastosis, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

## References

### Introduction

These references are cited in this guideline.

1. National Comprehensive Cancer Network. NCCN Clinical Practice Guidelines in Oncology: Myeloproliferative Neoplasms. V.2.2019. Available at: [https://www.nccn.org/professionals/physician\\_gls/pdf/mpn.pdf](https://www.nccn.org/professionals/physician_gls/pdf/mpn.pdf)
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# BCR-ABL1 Testing for Chronic Myeloid Leukemia

MOL.TS.131.A

v1.0.2020

## Introduction

BCR-ABL1 chronic myeloid leukemia (CML) testing is addressed by this guideline.

## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedures addressed by this guideline | Procedure codes |
|--|-----------------|
| BCR-ABL1 detection, major breakpoint   | 81206           |
| BCR-ABL1 detection, minor breakpoint   | 81207           |
| BCR-ABL1 detection, other breakpoint   | 81208           |
| BCR-ABL1 kinase domain sequencing      | 81170           |
| FISH Analysis for t(9;22) BCR-ABL1     | 88270           |
| MRDx® BCR-ABL Test                     | 0040U           |

## What is chronic myeloid leukemia

### Definition

Chronic myeloid leukemia (CML) is a hematopoietic stem cell disease that results in overgrowth of white blood cells in the bone marrow. It is defined by the presence of the Philadelphia chromosome (Ph), a reciprocal translocation between chromosomes 9 and 22 that results in fusion of BCR and ABL1 genes.<sup>1,2</sup> There are two major protein forms of the fusion gene, p210 (major breakpoint) which is the most common in CML and p190 (minor breakpoint) which is more common in acute lymphoblastic leukemia.<sup>2</sup>

### CML phases

The three phases of CML are chronic, accelerated, and blast. In the chronic phase, there are few symptoms other than splenomegaly, and most people are diagnosed after a routine blood test reveals the characteristic blood count and differential. If not treated, the disease will progress to the accelerated and blast phases, signs and symptoms of which include:<sup>1</sup>



- fever
- bone pain
- splenomegaly

## Diagnosis

Diagnosis of CML is usually made with peripheral blood and bone marrow evaluations. Detection of the BCR-ABL1 fusion gene is diagnostic for CML and Ph+ acute lymphoblastic leukemia (ALL) and can be established by metaphase karyotype (cytogenetics), fluorescent in situ hybridization (FISH), or quantitative real-time reverse transcriptase polymerase chain reaction (qPCR).<sup>2</sup> At diagnosis, a broader qPCR may help distinguish which fusion product (p210 vs p190 vs other) is present in the patient to narrow the testing for future follow-up qPCRs.

Acute lymphoblastic leukemia (ALL) is a different form of leukemia, but may also be positive for the chromosome (Ph+), accounting for 2-4% of pediatric ALL and 25% of adult ALL.<sup>3</sup>

## Treatment

First-line treatment for CML is with a class of drugs called tyrosine kinase inhibitors (TKIs), which block the production of the BCR-ABL1 fusion gene protein product. Several TKI therapies are available as first-line therapies, including:

- imatinib (Gleevec® )
- nilotinib (Tasigna® )
- dasatinib (Sprycel® )
- bosutinib (BOSULIF®)

TKI therapies have all demonstrated proven benefit, with decreased rates of progression of disease, and increased rates of major molecular responses.<sup>1-3</sup> With treatment, median survival is expected to approach normal life expectancy for most patients with CML.<sup>1,2</sup>

### Treatment response

Monitoring of patients for treatment response to TKIs includes routine measurement of the BCR-ABL1 fusion gene protein product via qPCR prior to initiation of treatment and during treatment every 3 months.<sup>2</sup> Once the BCR-ABL1 transcript is <1%, monitoring occurs every 3 months for 2 years, and then every 3-6 months thereafter.<sup>2</sup> If there is a 1-log increase in BCR-ABL1 transcript with the major molecular response (MMR), qPCR should be repeated in 1-3 months.<sup>2</sup>

### **Treatment resistance**

Treatment resistance is defined as the failure to reach response milestones, loss of TKI response, 1-log increase in BCR-ABL1 transcript levels and loss of MMR, or those with disease progression to accelerated phase or blast phase.<sup>2</sup> For individuals who display apparent treatment resistance, consideration of alternative treatment options may be appropriate.<sup>2</sup> Treatment resistance in both CML and ALL can be caused by point mutations in the BCR-ABL1 kinase domain.<sup>3</sup> Some tyrosine kinase inhibitors are still active with certain mutations that may cause resistance to other TKIs.

### **Treatment discontinuation**

Discontinuation of TKI therapy in carefully selected patients who have maintained deep molecular responses for more than 2 years has been evaluated in studies.<sup>2</sup> These patients still need to be carefully monitored by qPCR due to risk of recurrence. Recommendations are for molecular monitoring monthly for the first year, then every 6 weeks for the second year, and then every 12 weeks thereafter (indefinitely).<sup>2</sup> Prompt resumption of TKI is recommended if there is loss of the major molecular response.

## **Test information**

### **Introduction**

Testing for chronic myeloid leukemia (CML) may include qPCR for BCR-ABL1 transcript levels or FISH for t(9;22) BCR-ABL1.

### **qPCR for BCR-ABL1 transcript levels**

Bone marrow cytogenetics and measurement of BCR-ABL1 transcript levels by quantitative polymerase chain reaction (qPCR) is recommended before initiation of treatment as well as for assessing response to therapy.<sup>2</sup>

### **FISH for t(9;22) BCR-ABL1**

If collection of bone marrow is not feasible, fluorescence in situ hybridization (FISH) on peripheral blood specimen using dual probes for the BCR and ABL1 genes is an acceptable method of confirming the diagnosis of CML.<sup>4</sup>

### **BCR-ABL1 kinase domain sequencing**

Sequencing of BCR-ABL1 kinase domain is recommended when there is treatment resistance or progression of disease on therapy.<sup>2,4</sup> Identification of such mutations can help guide in selection of subsequent tyrosine kinase inhibitor therapy.<sup>2</sup> For this assay, PCR followed by Sanger DNA sequencing of peripheral blood or bone marrow is employed to identify resistant mutations in the kinase domain.<sup>4</sup>

## Guidelines and evidence

### Introduction

This section includes relevant guidelines and evidence pertaining to BCR-ABL1 testing for chronic myeloid leukemia.

### National Comprehensive Cancer Network

The National Comprehensive Cancer Network (NCCN, 2019) recommends bone marrow cytogenetics to confirm a diagnosis of CML. If bone marrow is not available, FISH on a peripheral blood specimen using dual probes for BCR and ABL1 genes can confirm the diagnosis.<sup>2</sup>

The NCCN recommends BCR-ABL1 transcript levels be obtained by quantitative RT-PCR (qPCR) in the following scenarios:<sup>2</sup>

- At diagnosis
- Every three months after initiating treatment. After a patient reaches BCR-ABL1 transcript levels <1% IS (international scale), every 3 months for two years, and every 3-6 months thereafter
- If a patient has a rising level of BCR-ABL1 transcripts (1 log increase), repeat testing in 1–3 months

The NCCN also recommended BCR-ABL1 kinase domain sequencing in the following scenarios:<sup>2</sup>

- Failure to reach response milestones as defined by the NCCN guidelines
- Any sign of loss of response
- 1-log increase in BCR-ABL1 transcript levels and loss of major molecular response
- Disease progression to accelerated or blast phase

These recommendations are category 2A which is “Based upon lower-level evidence, there is uniform NCCN consensus that the intervention is appropriate.”

## Criteria

### BCR-ABL1 transcript level testing

BCR-ABL1 transcript level testing is indicated in individuals at the initiation of treatment and at regular intervals (ranges from every month to once every 3-6 months) during treatment with ANY of the following drug therapies:

- Imatinib (Gleevec® )
- Nilotinib (Tasigna® )

- Dasatinib (Sprycel® )
- Bosutinib (BOSULIF®)

### BCR-ABL1 kinase domain targeted sequencing

BCR-ABL1 kinase domain targeted sequencing is indicated in individuals with chronic myeloid leukemia who:

- Are on TKI inhibitor therapy, AND
- Are in chronic phase of the disease, and
  - Have failed to reach treatment milestones, or
  - Experience loss of response to TKI inhibitor therapy (hematologic or cytogenetic relapse), or
  - Experience a 1-log increase in BCR-ABL1 transcript levels and loss of major molecular response, OR
- Experience progression of disease from chronic phase to accelerated phase or blast phase, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

### References

#### Introduction

These references are cited in this guideline.

1. National Cancer Institute. Chronic Myelogenous Leukemia Treatment (PDQ® ). Last modified February 8, 2019 at <https://www.cancer.gov/types/leukemia/hp/cml-treatment-pdq>.
2. Radich JP, Deininger M, Abboud CN, et al. Chronic Myeloid Leukemia, Version 1.2019, NCCN Clinical Practice Guidelines in Oncology. *J Natl Compr Canc Netw*. 2018 Sep;16(9):1108-1135. [https://www.nccn.org/professionals/physician\\_gls/pdf/cml.pdf](https://www.nccn.org/professionals/physician_gls/pdf/cml.pdf)
3. National Comprehensive Cancer Network. NCCN Clinical Practice Guidelines in Oncology: Acute Lymphoblastic Leukemia. V.1.2019 available at [https://www.nccn.org/professionals/physician\\_glas/pdf/all/pdf](https://www.nccn.org/professionals/physician_glas/pdf/all/pdf).
4. Jones D, Kamel-reid S, Bahler Dong H, et al. (ABL Mutation Working Group of the Association for Molecular Pathology, Clinical Practice Committee). Laboratory practice guidelines for detecting and reporting BCR-ABL drug resistance mutations in chronic myelogenous leukemia and acute lymphoblastic leukemia: a report of the Association for Molecular Pathology. *J Mol Diagn*. 2009;11(1):4-11.

# Bloom Syndrome Testing

MOL.TS.132.A

v1.0.2020

## Introduction

Bloom syndrome testing is addressed by this guideline.

## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedures addressed by this guideline | Procedure codes |
|--|-----------------|
| Sister Chromatid Exchange              | 88245           |
| BLM Known Familial Mutation Analysis   | 81403           |
| BLM Targeted Mutation Analysis         | 81209           |
| BLM Sequencing                         | 81479           |
| BLM Deletion/Duplication Analysis      | 81479           |

## What is Bloom syndrome

### Definition

Bloom syndrome is a genetic disorder in which an individual's chromosomes contain large breaks, gaps, or rearrangements.<sup>1</sup>

### Symptoms

Affected individuals are usually smaller than average and suffer from a variety of symptoms.<sup>1-2</sup>

- Pre- and post-natal growth deficiency
- Short stature
- Long, narrow face, small lower jaw, and prominent nose and ears
- Sensitivity to sunlight: Exposure to sunlight causes a characteristic butterfly-shaped rash on the face.
- Chronic lung problems, diabetes, and immune deficiencies
- Gastroesophageal reflux

- Infertility
- Cancer predisposition
- Learning disabilities

### **Prevalence**

Fewer than 300 cases of Bloom syndrome have been reported since the disease was first described over 50 years ago.<sup>2,3</sup>

About 1 in 48,000 Ashkenazi Jewish individuals have Bloom syndrome, and 25% of all affected individuals have Ashkenazi Jewish ancestry.<sup>2</sup>

### **Prognosis**

There is no cure for Bloom syndrome. Treatment involves continuous monitoring by multiple physicians and specialists.<sup>1,3</sup>

### **Cause**

Bloom syndrome is caused by a genetic mutation in the BLM gene.<sup>1,3,4</sup>

BLM is essential to maintaining the stability of chromosomes during DNA replication and cell division.<sup>3,4</sup>

Mutations in BLM lead to mistakes during cellular replication.<sup>3,4</sup>

Individuals with Bloom syndrome have multiple breaks, gaps, and genetic rearrangements in their chromosomes, leading to a unique combination of signs and symptoms.<sup>3,4</sup>

### **Diagnosis**

A diagnosis of Bloom syndrome is suspected when the patient presents with the characteristic suite of signs and symptoms. This diagnosis can be confirmed by genetic testing and may be needed to differentiate between other disorders with overlapping symptoms. There are several types of tests available for diagnostic purposes.

### **Inheritance**

Bloom syndrome is an autosomal recessive disorder, meaning that an affected individual must inherit BLM gene mutations from each parent.<sup>1,4</sup>

Individuals who inherit only one mutation are called carriers. Carriers do not show symptoms of Bloom syndrome, but have a 50% chance of passing on the mutation to their children.

Two carriers of Bloom syndrome have a 1 in 4 (25%) chance for each pregnancy to be affected with Bloom syndrome.

## Test information

### Introduction

Testing for Bloom syndrome may include sister chromatid exchange, known familial mutation analysis, targeted mutation analysis, sequence analysis, or deletion/duplication analysis.

### Sister Chromatid Exchange

SCE is the standard analysis for diagnosis of Bloom syndrome. The method involves exposing an individual's cells to bromodeoxyuridine (BrdU), a compound that helps identify which cells contain chromosomes with unusually large numbers of rearrangements, or "exchanges." Individuals with Bloom syndrome will have a substantially higher number of these exchanges compared with unaffected individuals.<sup>5</sup> This test can be used for prenatal diagnosis of at-risk pregnancies on chorionic villi or amniocytes.<sup>4</sup>

### BLM Known Familial Mutation Analysis

Once a deleterious mutation has been identified in an affected person, relatives and at-risk pregnancies can be tested.

### BLM Targeted Mutation Analysis

This test looks for the BLM gene mutation most often found in Ashkenazi Jewish individuals, called  $blm^{Ash}$ .<sup>4</sup> The detection rate of this mutation in Ashkenazi Jewish individuals is greater than 97%.<sup>4</sup>

### BLM Sequencing

This test looks for mutations across the entire gene, and can identify at least 87% of disease-causing mutations in individuals with non-Jewish Ancestry and greater than 99% of disease-causing mutations in Ashkenazi Jewish individuals.<sup>4</sup> It is typically used only for diagnosis of an affected individual or carrier testing of a non-Ashkenazi Jewish individual when the partner is a known carrier.

### BLM Deletion/Duplication Analysis

This test looks for deletions and duplication in the gene that would not be detected by sequencing analysis.<sup>4</sup> It is typically performed in reflex to sequencing analysis when there is a high suspicion for disease.<sup>4</sup>

## Guidelines and evidence

### Introduction

This section includes relevant guidelines and evidence pertaining to Bloom syndrome

testing.

### **Diagnostic testing strategy**

A 2016 expert-authored review suggests the following diagnostic testing strategy:<sup>4</sup>

“The diagnosis of Bloom Syndrome (Bsyn) is established in a proband with identification of biallelic pathogenic variants in *BLM* on molecular genetic testing or, if molecular genetic testing is inconclusive, with identification of increased frequency of sister-chromatid exchanges (SCEs) on specialized cytogenetic studies.”

### **Carrier testing strategy**

The American College of Medical Genetics (ACMG, 2008)<sup>6</sup> and the American College of Obstetrics and Gynecologists (ACOG, 2009)<sup>7</sup> support offering carrier testing for Bloom syndrome to individuals of Ashkenazi Jewish descent for the common *blm*<sup>Ash</sup> mutation.

- Guidelines support the testing of individuals of Ashkenazi Jewish descent, even when their partner is non-Ashkenazi Jewish. In this situation, testing would start with the individual who is Jewish and if *blm*<sup>Ash</sup> mutation is detected, sequencing of *BLM* in the non-Ashkenazi Jewish partner would follow.<sup>6</sup> If the woman is pregnant, testing may need to be conducted on both partners simultaneously in order to receive results in a timely fashion.<sup>7</sup>
- If one or both partners are found to be carriers of Bloom syndrome, genetic counseling should be provided and prenatal testing offered, if appropriate.

### **Prenatal testing strategy**

A 2016 expert-authored review states:<sup>4</sup>

- “Prenatal diagnosis for pregnancies at increased risk is possible by sister-chromatid exchange (SCE) analysis of fetal cells obtained by amniocentesis usually performed at about 15 to 18 weeks’ gestation or chorionic villus sampling (CVS) at approximately ten to 12 weeks’ gestation.”
- “If the *BLM* pathogenic variants have been identified in an affected family member, prenatal testing for pregnancies at increased risk may be available from a clinical laboratory that offers either testing of this gene or custom prenatal testing.”
- “Preimplantation genetic diagnosis (PGD) has been successfully utilized for one couple [Bloom's Syndrome Registry, unpublished data], and may be an option for some families in which the *BLM* pathogenic variants have been identified.”



## Criteria

### Introduction

Requests for Bloom syndrome testing are reviewed using these criteria.

### **Sister Chromatid Exchange (Chromosome Analysis for Breakage Syndromes)**

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - No previous sister chromatid exchange analysis performed, and
  - No previous BLM full sequencing, or BLM sequencing performed and only one mutation identified, and
  - No known BLM mutation in biologic relative, and
  - If Ashkenazi Jewish, targeted mutation analysis performed and no mutation detected or one mutation detected, AND
- Diagnostic Testing for Symptomatic Individuals:
  - Unexplained severe intrauterine growth retardation that persists throughout infancy and childhood (less than 5<sup>th</sup> percentile), or
  - An unusually small individual (less than 5<sup>th</sup> percentile) who develops erythematous skin lesions in the “butterfly area” of the face after sun exposure, or
  - An unusually small individual (less than 5<sup>th</sup> percentile) who develops a malignancy OR
- Prenatal Testing for At-Risk Pregnancies:
  - Known increased risk due to affected first-degree relative, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

### **BLM Known Familial Mutation Analysis**

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing
  - No previous genetic testing of BLM, AND

- Carrier Screening:
  - Known family mutation in BLM identified in 1<sup>st</sup>, 2<sup>nd</sup>, or 3<sup>rd</sup> degree biologic relative(s), OR
- Prenatal Testing for At-Risk Pregnancies:
  - BLM mutation identified in both biologic parents, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

### **BLM Targeted Mutation Analysis**

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - No previous BLM genetic testing, including Ashkenazi Jewish screening panels containing targeted mutation analysis for blm<sup>Ash</sup>, AND
- Carrier Screening:
  - Ashkenazi Jewish descent, and
  - Have the potential and intention to reproduce, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

### **BLM Sequencing**

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - No previous BLM full sequencing, and
  - No known BLM mutation in biologic relative, and
  - If Ashkenazi Jewish, targeted mutation analysis performed and no mutation detected or one mutation detected, AND
- Diagnostic Testing for Symptomatic Individuals:
  - Unexplained severe intrauterine growth retardation that persists throughout infancy and childhood (less than 5<sup>th</sup> percentile), or

- An unusually small individual (less than 5<sup>th</sup> percentile) who develops erythematous skin lesions in the “butterfly area” of the face after sun exposure, or
- An unusually small individual (less than 5<sup>th</sup> percentile) who develops a malignancy, OR
- Testing for Individuals with Family History or Partners of Carriers:
  - 1<sup>st</sup>, 2<sup>nd</sup>, or 3<sup>rd</sup> degree biologic relative with Bloom syndrome clinical diagnosis, family mutation unknown, and testing unavailable, or
  - Partner is monoallelic or biallelic for BLM mutation, and
  - Have the potential and intention to reproduce, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

### **BLM Deletion/Duplication Analysis**

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - Previous BLM full sequencing, and no mutations or only one mutation detected, AND
- Diagnostic Testing for Symptomatic Individuals:
  - Unexplained severe intrauterine growth retardation that persists throughout infancy and childhood (less than 5<sup>th</sup> percentile), or
  - An unusually small individual (less than 5<sup>th</sup> percentile) who develops erythematous skin lesions in the “butterfly area” of the face after sun exposure, or
  - An unusually small individual (less than 5<sup>th</sup> percentile) who develops a malignancy, OR
- Testing for Individuals with Family History or Partners of Carriers:
  - 1<sup>st</sup>, 2<sup>nd</sup>, or 3<sup>rd</sup> degree biologic relative with Bloom syndrome clinical diagnosis, family mutation unknown, and testing unavailable, or
  - Partner is monoallelic or biallelic for BLM mutation, and
  - Have the potential and intention to reproduce, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

## References

### Introduction

These references are cited in this guideline.

1. Bloom Syndrome. (Updated 2015). In: Genetics Home References: Your Guide to Understanding Genetic Conditions (database online). A Service of the US National Library of Medicine. Available at <https://ghr.nlm.nih.gov/condition/bloom-syndrome#inheritance>
2. Bloom D. Congenital telangiectatic erythema resembling lupus erythematosus in dwarfs; probably a syndrome entity. *AMA Am J Dis Child*. 1954 Dec;88(6):754-8.
3. Hickson ID, Davies SL, Li JL, et al. Role of the Bloom's syndrome helicase in maintenance of genome stability. *Biochem Soc Trans*. 2001 May;29(Pt 2):201-4.
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5. Howell RT, Davies T. Diagnosis of Bloom's syndrome by sister chromatid exchange evaluation in chorionic villus cultures. *Prenat Diagn*. 1994 Nov;14(11):1071-3.
6. ACOG Committee Opinion No. 442: Preconception and Prenatal Carrier Screening for Genetic Diseases in Individuals of Eastern European Jewish Descent. *Obstet Gynecol*. 2009;114(4):950-910.1097/AOG.1090b1013e3181bd1012f1094.
7. Gross SJ, Pletcher BA, Monaghan KG. Carrier screening in individuals of Ashkenazi Jewish descent. *Genet Med*. Jan 2008;10(1):54-6.

# BRAF Testing for Colorectal Cancer

MOL.TS.133.A  
v1.0.2020

## Introduction

BRAF testing for colorectal cancer is addressed by this guideline.

## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedures addressed by this guideline | Procedure codes |
|--|-----------------|
| BRAF V600 targeted mutation analysis   | 81210           |
| BRAF sequencing                        | 81406           |

## What is BRAF

### Definition

BRAF is a gene that forms a part of a cell-signaling pathway to help control cell growth. Changes or mutations in the BRAF gene can cause out of control cell growth, which may lead to cancer.<sup>1</sup> The most common BRAF mutation is called V600E which was previously known as V599E.

### Prevalence

About 5-9% of colorectal cancer tumors have a V600E BRAF mutation.<sup>1</sup>

### Prognosis

Patients with a V600E BRAF mutation in their tumor appear to have a poorer prognosis. Tumors with BRAF mutations may have less response to anti-EGFR therapies like cetuximab (Erbix<sup>®</sup>) and panitumumab (Vectibix<sup>®</sup>).<sup>1</sup>

## Test information

### Introduction

Testing for a BRAF mutation may include targeted mutation analysis or sequencing.

## Available tests

The following BRAF tests are available to identify mutations.

---

**Note** BRAF mutation analysis has several other test applications with different criteria (such as melanoma therapeutic response, Lynch syndrome tumor screening, or Noonan syndrome diagnosis). Ensure you are reviewing the correct use of the test.

---

### Targeted mutation analysis

Laboratories most commonly test for the BRAF V600E mutation, which accounts for about 90% of activating BRAF mutations.<sup>2</sup> Mutation analysis requires relatively little tumor material for testing and has high sensitivity. It is also relatively inexpensive.<sup>3,4</sup>

BRAF mutation analysis is done on fresh, frozen, or paraffin-embedded tissue from either a primary tumor or metastasis.<sup>1,3,4</sup> Some molecular diagnostic laboratories perform BRAF mutation analysis by laboratory-developed methods, while others use FDA-approved test kits. Laboratory-developed tests may vary in the specimen type required, methodology used, mutations tested, sensitivity, and other test-specific data.

BRAF testing may sometimes be performed as part of a multi-mutation/multi-gene targeted panel to detect mutations in the *KRAS*, *NRAS*, and *BRAF* genes that are relevant for predictive and/or prognostic purposes in patients with colon cancer.

### Sequencing

Some laboratories sequence all or part of the BRAF gene, which will find a broader spectrum of mutations than targeted mutation analysis. Laboratories that offer sequencing generally do so for a subset of exons where most BRAF activating mutations have been identified.

## Guidelines and evidence

### Introduction

This section includes relevant guidelines and evidence pertaining to BRAF testing for colorectal cancer.

### National Comprehensive Cancer Network

The National Comprehensive Cancer Network (NCCN, 2019) states the following.<sup>1</sup>

"All patients with metastatic colorectal cancer should have tumor tissue genotyped for *RAS* (*KRAS* and *NRAS*) and *BRAF* mutations individually or as part of a next-generation sequencing (NGS) panel. Patients with any known *KRAS* mutation (exon 2, 3, 4) or *NRAS* mutation (exon 2,3,4) should not be treated with either cetuximab or

panitumumab.44-46 *BRAF* V600E mutation makes response to panitumumab or cetuximab highly unlikely unless given with a *BRAF* inhibitor."

"Testing for *KRAS*, *NRAS*, and *BRAF* mutations should be performed only in laboratories that are certified under the clinical laboratory improvement amendments of 1988 (CLIA-88) as qualifies to perform high complexity clinical laboratory (molecular pathology) testing. No specific methodology is recommended (eg, sequencing, hybridization)."

## Criteria

Testing may be considered in individuals who meet the following criteria:

- Individual has been diagnosed with stage IV, metastatic colorectal cancer, AND
- *BRAF* mutation testing is needed for prognostic or predictive purposes.

## References

### Introduction

These references are cited in this guideline.

1. National Comprehensive Cancer Network. NCCN Clinical Practice Guidelines in Oncology: Colon Cancer. Version 1.2019. Available at [http://www.nccn.org/professionals/physician\\_gls/PDF/colon.pdf](http://www.nccn.org/professionals/physician_gls/PDF/colon.pdf).
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# BRAF Testing for Melanoma Kinase Inhibitor Response

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v1.0.2020

## Introduction

BRAF testing for melanoma kinase inhibitor response is addressed by this guideline.

## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedure addressed by this guideline | Procedure code |
|---------------------------------------|----------------|
| BRAF V600 Targeted Mutation Analysis  | 81210          |

## What is BRAF

### Definition

BRAF is a gene that forms a part of a cell-signaling pathway to help control cell growth. Changes or mutations in the BRAF gene can cause out of control cell growth, which may lead to cancer.<sup>1,2</sup>

### BRAF V600 Mutations

About 40-60% of cutaneous melanomas have a BRAF mutation.<sup>1</sup> The most common BRAF mutation is called V600E, present in about 80-90% of melanomas. The next most common mutation is V600K, present in about 5-20% of melanomas. Rare mutations in codon 600 occur at frequencies of less than 1%, including V600R, V600M, and V600D.<sup>3</sup>

### Treatment options

Several FDA approved monotherapy or combination therapy melanoma treatments (e.g., vemurafenib (Zelboraf®), dabrafenib (Tafinlar®), and trametinib (Mekinist®)) are orally-administered kinase inhibitors that are able to block the function of the mutated BRAF protein.<sup>1,2,4-6</sup> They are specifically indicated for the treatment of patients with metastatic or unresectable melanoma whose tumors have a BRAF V600 activating mutation.<sup>1,2,4-6</sup> They are not recommended for use in patients with wild type BRAF melanoma.<sup>2,4-6</sup>



## Test information

### Introduction

Laboratories most commonly perform targeted mutation analysis for BRAF V600 mutations, which account for greater than 95% of activating BRAF mutations. Mutation analysis requires relatively little tumor material for testing and has high sensitivity. It is also relatively inexpensive.<sup>3</sup>

### Targeted mutation analysis

BRAF mutation analysis is done on fresh, frozen, or paraffin-embedded tissue from either a primary tumor or metastasis.<sup>1-4</sup> Some molecular diagnostic laboratories perform BRAF mutation analysis by laboratory-developed methods, while others use FDA-approved test kits. Laboratory-developed tests may vary in the specimen type required, methodology used, mutations tested, sensitivity, and other test-specific data.

## Guidelines and evidence

### Introduction

This section includes relevant guidelines and evidence pertaining to BRAF testing for melanoma kinase inhibitor response.

### National Comprehensive Cancer Network

The National Comprehensive Cancer Network (NCCN, 2019) lists several targeted combination therapies as preferred treatments in patients with BRAF V600- activating mutation positive metastatic melanoma.<sup>4</sup> These therapies require companion diagnostic testing.

### FDA

The FDA has approved multiple drugs for individuals with metastatic melanoma and BRAF mutations.<sup>2,5-7</sup> Examples of FDA approved monotherapy and/or combination therapy medications with companion diagnostic tests include:

- Zelboraf (vemurafenib)
- Tafinlar (dabrafenib)
- Mekinist (trametinib)
- Cotellic (cobimetinib)
- Braftovi (encorafenib)/ Mektovi (binimetinib)

## Criteria

Testing may be considered in individuals who meet the following criteria:

- Individual has been diagnosed with metastatic or unresectable melanoma, AND
- BRAF V600 testing has not been performed previously

## References

### Introduction

These references are cited in this guideline.

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# BRCA Analysis

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## Introduction

BRCA analysis is addressed by this guideline.

## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedures addressed by this guideline                               | Procedure codes |
|--|-----------------|
| BRCA1 Known Familial Mutation Analysis                               | 81215           |
| BRCA2 Known Familial Mutation Analysis                               | 81217           |
| BRCA1 Full Sequencing  | 81165           |
| BRCA1 Full Duplication/Deletion Analysis                             | 81166           |
| BRCA2 Full Sequencing  | 81216           |
| BRCA2 Full Duplication/Deletion Analysis                             | 81167           |
| BRCA1/2 Full Sequencing and Deletion/Duplication Analysis (Combined) | 81162           |
| BRCA1/2 Full Sequence Analysis                                       | 81163           |
| BRCA1/2 Full Duplication/Deletion Analysis                           | 81164           |

## What is hereditary breast and ovarian cancer

### Definition

Hereditary breast and ovarian cancer (HBOC) is an inherited form of cancer.

### Characteristics of HBOC

HBOC is characterized by any of the following:<sup>1,2</sup>

- personal history of
  - breast cancer at a young age, typically under age 50
  - two primary breast cancers

- both breast and ovarian cancer
- triple negative breast cancer (ER-, PR-, HER2-)
- ovarian, fallopian tube, or primary peritoneal cancer, or
- metastatic prostate cancer
- multiple cases of breast or ovarian cancer in a family
- personal or family history of
  - male breast cancer
  - pancreatic cancer with breast or ovarian cancer, or
  - prostate cancer with a Gleason score of at least 7 and a family history of ovarian, breast, prostate, or pancreatic cancer
- previously identified BRCA1 or BRCA2 mutation in the family, or
- any of the above with Ashkenazi Jewish ancestry.

### **Inheritance**

Up to 10% of all breast cancer and 15% of all ovarian cancer is associated with an inherited gene mutation, with BRCA1 and BRCA2 accounting for about 20-25% of all hereditary cases.<sup>1,3-5</sup>

BRCA mutations are inherited in an autosomal dominant manner. When a parent has a BRCA mutation, each offspring has a 50% risk of inheriting the mutation.<sup>1</sup>

### **Prevalence**

About 1 in 400 people in the general population has a BRCA1 or BRCA2 mutation. The prevalence of mutations is higher in people of Norwegian, Dutch, or Icelandic ethnicity.<sup>1,3</sup>

The prevalence of BRCA mutations varies among African Americans, Hispanics, Asian Americans, and non-Hispanic whites.<sup>3</sup>

#### **Ashkenazi Jewish ancestry**

About 1 in 40 people of Ashkenazi Jewish ancestry has a BRCA1 or BRCA2 mutation. The majority of the risk in the Ashkenazi Jewish population is associated with three common founder mutations, two of which are in the BRCA1 gene and one in the BRCA2 gene.<sup>1,6,7</sup> These three mutations account for 99% of identified mutations in the Ashkenazi Jewish population.<sup>1</sup>

### **Cancer risks**

People with a BRCA mutation have an increased risk of various types of cancer.<sup>1</sup>

| Type of cancer     | Risk                      |
|--------------------|---------------------------|
| Breast cancer      | 38-87%                    |
| Ovarian cancer     | 16.5-63%                  |
| Male breast cancer | 1-9%                      |
| Prostate cancer    | up to 20%                 |
| Pancreatic cancer  | 1-7%                      |
| Melanoma           | Increased risk with BRCA2 |

**Note** The risk for breast and ovarian cancer varies among family members and between families.

### Screening and prevention

Screening and prevention options are available to specifically address the increased risk of these cancers in a person with a BRCA mutation.<sup>1</sup>

### Breast cancer risk and other genes

Other inherited cancer syndromes that can include breast cancer are Li-Fraumeni syndrome (TP53 gene), Cowden syndrome (PTEN), Hereditary Diffuse Gastric Cancer syndrome (CDH1), and Peutz Jeghers syndrome (STK11). Additionally, other genes that can increase the risk for breast cancer are ATM, CHEK2, NBN, NF1, and PALB2.<sup>1,6,8,9</sup>

## Test information

### Introduction

BRCA testing may include full gene sequencing, deletion/duplication analysis, known familial mutation analysis, or multigene panel testing.

### Sequence analysis

Full sequence analysis of BRCA1/2 genes looks at all of the coding regions of the BRCA1/2 genes, and often includes analysis of five common BRCA1/2 gene duplications and deletions.

Full sequence testing is typically appropriate as an initial test for people who meet criteria and do NOT have Ashkenazi Jewish ancestry.<sup>1,6</sup>

### Deletion/duplication analysis

Deletion/duplication analysis looks for large rearrangements, duplications, and deletions in the BRCA1/2 genes. Both BRCA1/2 sequencing and large rearrangement

analysis are often performed concurrently as routine laboratory practice when BRCA1/2 analysis is requested.

### **Known familial mutation testing**

Known familial mutation testing looks for a specific mutation in either the BRCA1/2 gene previously identified in a family member.

This test is appropriate for those who have a known BRCA mutation in the family and are not Ashkenazi Jewish.

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**Note** Founder mutation testing may be appropriate for those with Ashkenazi Jewish ancestry, even with a known familial mutation, since these mutations are common enough that multiple mutations can be found in the same Ashkenazi Jewish individual or family. If the familial mutation is not one of the three Ashkenazi Jewish mutations, then known familial mutation analysis for that mutation should be performed in addition to the founder mutation panel.<sup>1,6</sup>

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### **Ashkenazi Jewish founder mutation testing**

Ashkenazi Jewish founder mutation testing includes the three mutations most commonly found in the Ashkenazi Jewish population: 187delAG and 5385insC in BRCA1 and 6174delT in BRCA2.<sup>1</sup>

### **Cancer multigene panels**

BRCA1/2 gene testing is also available in the form of multigene panels for individuals with a personal or family history of cancer suggestive of more than one hereditary cancer syndrome.

## **Guidelines and evidence**

### **Introduction**

This section includes relevant guidelines and evidence pertaining to BRCA analysis.

### **National Comprehensive Cancer Network**

The National Comprehensive Cancer Network (NCCN, 2019)<sup>6</sup> evidence and consensus-based guidelines address test indications for BRCA testing. These recommendations are Category 2A, defined as “lower-level evidence with uniform NCCN consensus” and are frequently updated.

NCCN recommends BRCA analysis in individuals with a personal and/or family history of HBOC-related cancers such as breast cancer (male or female), ovarian cancer, prostate cancer, and pancreatic cancer. Testing recommendations take into

consideration age of diagnosis, tumor pathology, degree of relationship, and Ashkenazi Jewish ancestry.

### **Testing unaffected individuals**

NCCN states “Testing of unaffected individuals should only be considered when an appropriate affected family member is unavailable for testing.” They caution that the significant limitations in interpreting results from unaffected relatives must be discussed.

### **American Society of Breast Surgeons**

The American Society of Breast Surgeons (2019) published a consensus guideline on genetic testing for hereditary breast cancer. They state the following:<sup>10</sup>

- "Breast surgeons, genetic counselors, and other medical professionals knowledgeable in genetic testing can provide patient education and counseling and make recommendations to their patients regarding genetic testing and arrange testing. When the patient's history and/or test results are complex, referral to a certified genetic counselor or genetics professional may be useful. Genetic testing is increasingly provided through multi-gene panels. There are a wide variety of panels available, with different genes on different panels. There is a lack of consensus among experts regarding which genes should be tested in different clinical scenarios. There is also variation in the degree of consensus regarding the understanding of risk and appropriate clinical management of mutations in some genes."
- "Genetic testing should be made available to all patients with a personal history of breast cancer. Recent data support that genetic testing should be offered to each patient with breast cancer (newly diagnosed or with a personal history). If genetic testing is performed, such testing should include BRCA1/BRCA2 and PALB2, with other genes as appropriate for the clinical scenario and family history. For patients with newly diagnosed breast cancer, identification of a mutation may impact local treatment recommendations (surgery and potentially radiation) and systemic therapy. Additionally, family members may subsequently be offered testing and tailored risk reduction strategies."
- "Genetic testing should be made available to all patients with a personal history of breast cancer. Every patient being seen by a breast surgeon, who had genetic testing in the past and no pathogenic variant was identified, should be re-evaluated and updated testing considered. In particular, a patient who had negative germline BRCA1 and 2 testing, who is from a family with no pathogenic variants, should be considered for additional testing.<sup>1</sup> Genetic testing performed prior to 2014 most likely would not have had PALB2 or other potentially relevant genes included and may not have included testing for large genomic rearrangements in BRCA1 or BRCA2."
- "Genetic testing should be made available to patients without a history of breast cancer who meet NCCN guidelines. Unaffected patients should be informed that

testing an affected relative first, whenever possible, is more informative than undergoing testing themselves. When it is not feasible to test the affected relative first, then the unaffected family member should be considered for testing if they are interested, with careful pre-test counseling to explain the limited value of “uninformative negative” results. It is also reasonable to order a multi-gene panel if the family history is incomplete (i.e., a case of adoption, patient is uncertain of exact type of cancer affecting family members, among others) or other cancers are found in the family history, as described above.”

### **National Society of Genetic Counselors**

The National Society of Genetic Counselors (2013)<sup>8</sup> guidelines also state that: “[For patients with negative sequencing results], it may be appropriate to request additional analysis to detect large genomic rearrangements in both BRCA1 and BRCA2 genes.” In non-Ashkenazi Jewish individuals: If no mutation or inconclusive results are reported after sequence analysis, testing for large deletions/duplications in BRCA1/2 should be considered.<sup>1,7,8</sup>

### **U.S. Preventive Services Task Force**

The U.S. Preventive Services Task Force (USPSTF, 2013) recommendations address women who do not have a personal history of breast and/or ovarian cancer, but rather have a family history of these cancer types.<sup>11</sup> The USPSTF guideline recommends:

- “That primary care providers screen women who have family members with breast, ovarian, tubal (fallopian tube), or peritoneal cancer with one of several screening tools designed to identify a family history that may be associated with an increased risk for potentially harmful mutations in breast cancer susceptibility genes (BRCA1/2). Women with positive screening results should receive genetic counseling and, if indicated after counseling, BRCA testing.”

#### **Grade B recommendation**

The USPSTF considers this a Grade B recommendation: “The USPSTF found at least fair evidence that [the service] improves important health outcomes and concludes that benefits outweigh harms.”

#### **Recommendations for genetic counseling**

The USPSTF guidelines no longer make explicit recommendations as to who should have BRCA1/2 gene testing; they only make recommendations for genetic counseling. In general, women identified as high risk by these screening tools have one or more of the following characteristics:<sup>12</sup>

- a first or second degree relative with breast cancer before 50 years old
- a first or second degree relative with ovarian cancer
- a first or second degree relative with bilateral/multifocal breast cancer



- a first or second degree male relative with breast cancer
- a first or second degree relative with both breast and ovarian cancers
- two or more relatives, first, second, third degree, with breast or ovarian cancer
- two or more relatives, first, second, third degree, with breast or prostate/pancreatic cancer, or
- presence of Ashkenazi Jewish ancestry with any of the above.

## Criteria

### Introduction

Requests for BRCA analysis are reviewed using these criteria.

### Scope

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**Note** This guideline does not address BRCA analysis for individuals of Ashkenazi Jewish ancestry. For information on this testing, please see the guideline ***BRCA Ashkenazi Jewish Founder Mutation Testing***. This guideline also does not address BRCA Analysis as part of multigene panels. For information on this testing, please see the guideline ***Hereditary Cancer Syndrome Multigene Panels***.

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### Known Familial Mutation Analysis

- Genetic Counseling:
  - Pre- and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy),<sup>6,8,11,13</sup> AND
- Previous Genetic Testing:
  - No previous full sequence testing or deletion/duplication analysis, and
  - Known family mutation in BRCA1/2 identified in 1st, 2nd, or 3rd degree relative(s), AND
- Age 18 years or older<sup>14</sup>, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

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**Note** If the familial mutation is not one of the three Ashkenazi Jewish mutations, then known familial mutation analysis for that mutation should be performed in addition to the founder mutation panel.<sup>1,6</sup>

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## Full Sequence Analysis

- Genetic Counseling:
  - Pre- and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy)<sup>6,8,11,13</sup>, AND
- Previous Genetic Testing:
  - No previous full sequencing of BRCA1/2, and
  - No known mutation identified by previous BRCA analysis, AND
- Age 18 years or older<sup>14</sup>, AND
- Diagnostic Testing for Symptomatic Individuals:<sup>6</sup>
  - Non-Ashkenazi Jewish descent, AND:<sup>1,6</sup>
    - Female with breast cancer diagnosis ≤45 years of age, and/or
    - Two breast primary tumors with first diagnosis ≤50 years of age and second diagnosis at any age (ipsilateral or bilateral), and/or
    - Diagnosed ≤60 years of age with estrogen receptor negative, progesterone receptor negative, and HER2 negative (triple negative) breast cancer, and/or
    - Diagnosed ≤50 years of age with a limited family history (NCCN provides this guidance regarding limited family history: “individuals with limited family history, such as fewer than two first- or second- degree female relatives having lived beyond 45 in either lineage, may have an underestimated probability of a familial mutation”), and/or
    - Male with breast cancer at any age, and/or
    - Epithelial ovarian, fallopian tube, or primary peritoneal cancer diagnosis at any age, and/or
    - Metastatic prostate cancer (radiographic evidence of or biopsy-proven disease), and/or
    - Pancreatic cancer, and/or
    - Diagnosed with three primary breast cancers at any age, OR
  - Personal & Family History Combination:<sup>6</sup>
    - Diagnosed ≤50 years of age with at least one close blood relative with breast cancer diagnosed at any age, and/or

- Diagnosed  $\leq 50$  years of age with at least one close blood relative with high grade prostate cancer (Gleason score at least 7) diagnosed at any age, and/or
- Initial breast cancer diagnosis at any age and one or more of the following:
  - Breast cancer in at least 1 close blood relative (first-, second-, or third-degree)  $\leq 50$  years of age, and/or
  - Epithelial ovarian, fallopian tube, or primary peritoneal cancer in at least 1 close blood relative (first-, second-, or third- degree) at any age, and/or
  - At least 2 close blood relatives (first-, second-, or third- degree on same side of family) with breast cancer at any age, and/or
  - Male close blood relative (first-, second-, or third- degree) with breast cancer, and/or
  - Metastatic prostate cancer (radiographic evidence of or biopsy proven disease) in at least 1 close blood relative (first-, second-, or third-degree), and/or
  - Pancreatic cancer in at least 1 close blood relative (first-, second-, or third- degree), and/or
  - A close blood relative (first-, second-, or third- degree) with a triple negative breast cancer (ER-, PR-, Her2-) occurring at age 60 or younger, and/or
- Personal history of high-grade prostate cancer (Gleason score at least 7) at any age with  $\geq 1$  close blood relatives (on the same side of the family) with ovarian cancer at any age, pancreatic cancer at any age, metastatic prostate cancer (radiographic evidence of or biopsy proven disease) at any age, breast cancer  $< 50$  years, or male breast cancer, and/or
- Personal history of high-grade prostate cancer (Gleason score at least 7) at any age with two or more close blood relatives (on the same side of the family) with breast or prostate cancer (any grade) at any age, OR
- Predisposition Testing for Presymptomatic/Asymptomatic Individuals
  - Non-Ashkenazi Jewish descent<sup>6</sup>, and
  - The member has a first or second degree relative who meets any of the “Personal History” or “Personal & Family History Combination” criteria above, and
  - Unaffected member is the most informative person to test. All affected family members are deceased, or all affected family members have been contacted and are unwilling to be tested, OR

- Ashkenazi Jewish individual who is negative for founder mutation testing, and has a high pre-test probability of carrying a BRCA mutation<sup>1,6,8</sup> OR
- BRCA1/2 mutation detected by tumor profiling in the absence of germline mutation analysis, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

\*\* First-degree relatives (parents, siblings, children); second-degree relatives (aunts, uncles, grandparents, grandchildren, nieces, nephews and half-siblings); and third-degree relatives (great-grandparents, great-aunts, great-uncles, and first cousins) on the same side of the family.

#### **Billing and reimbursement considerations**

- These criteria may only be applied to a single BRCA sequencing CPT code as defined in the table at the beginning of this guideline.
- If BRCA gene testing will be performed as part of an expanded hereditary cancer syndrome panel, please also see that guideline for guidance.

#### **Deletion/Duplication Analysis**

- Genetic Counseling:
  - Pre- and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy),<sup>6,8,11,13</sup> AND
- Previous Genetic Testing:
  - No previous BRCA deletion/duplication analysis, and
  - Meets criteria for full sequence analysis of BRCA1/2, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

#### **Billing and reimbursement considerations**

If BRCA1/2 deletion/duplication analysis will be performed concurrently with BRCA1/2 gene sequencing, CPT code 81162 is likely most appropriate.

If BRCA gene testing will be performed as part of an expanded hereditary cancer syndrome panel, please also see that guideline for guidance.

#### **Other Considerations**

BRCA genetic testing to determine eligibility for targeted treatment (e.g., PARP inhibitors for ovarian cancer or metastatic HER2-negative breast cancer) is addressed in either the *Pharmacogenomic Testing for Drug Toxicity and Response* guideline or the *Somatic Mutation Testing-Solid Tumors* guideline.

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### Introduction

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# BRCA Ashkenazi Jewish Founder Mutation Testing

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## Introduction

BRCA Ashkenazi Jewish founder mutation testing is addressed by this guideline.

## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedure addressed by this guideline                | Procedure code |
|--|----------------|
| BRCA 1 and BRCA 2 Ashkenazi Jewish Founder Mutations | 81212          |

## What is hereditary breast and ovarian cancer

### Definition

Hereditary breast and ovarian cancer (HBOC) is an inherited form of cancer.

### Characteristics of HBOC

HBOC is characterized by any of the following:<sup>1,2</sup>

- personal history of
  - breast cancer at a young age, typically under age 50
  - two primary breast cancers
  - both breast and ovarian cancer
  - triple negative breast cancer (ER-, PR-, HER2-)
  - ovarian, fallopian tube, or primary peritoneal cancer, or
  - metastatic prostate cancer
- multiple cases of breast or ovarian cancer in a family
- personal or family history of
  - male breast cancer

- pancreatic cancer with breast or ovarian cancer, or
- prostate cancer with a Gleason score of at least 7 and a family history of ovarian, breast, prostate, or pancreatic cancer
- previously identified BRCA1 or BRCA2 mutation in the family, or
- any of the above with Ashkenazi Jewish ancestry.

## Inheritance

Up to 10% of all breast cancer and 15% of all ovarian cancer is associated with an inherited gene mutation, with BRCA1 and BRCA2 accounting for about 20-25% of all hereditary cases.<sup>1,3-5</sup>

BRCA mutations are inherited in an autosomal dominant manner. When a parent has a BRCA mutation, each offspring have a 50% risk of inheriting the mutation.<sup>1</sup>

## Prevalence

About 1 in 400 people in the general population has a BRCA1 or BRCA2 mutation. The prevalence of mutations is higher in people of Norwegian, Dutch, or Icelandic ethnicity.<sup>1,3</sup>

The prevalence of BRCA mutations varies among African Americans, Hispanics, Asian Americans, and non-Hispanic whites.<sup>3</sup>

### Ashkenazi Jewish ancestry

About 1 in 40 people of Ashkenazi Jewish ancestry has a BRCA1 or BRCA2 mutation. The majority of the risk in the Ashkenazi Jewish population is associated with three common founder mutations, two of which are in the BRCA1 gene and one in the BRCA2 gene.<sup>1,6,7</sup> These three mutations account for 99% of identified mutations in the Ashkenazi Jewish population.<sup>1</sup>

## Cancer risks

People with a BRCA mutation have an increased risk of various types of cancer.<sup>1</sup>

| Type of cancer     | Risk                      |
|--------------------|---------------------------|
| Breast cancer      | 38-87%                    |
| Ovarian cancer     | 16.5-63%                  |
| Male breast cancer | 1-9%                      |
| Prostate cancer    | up to 20%                 |
| Pancreatic cancer  | 1-7%                      |
| Melanoma           | Increased risk with BRCA2 |



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**Note** The risk for breast and ovarian cancer varies among family members and between families.

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### Screening and prevention

Screening and prevention options are available to specifically address the increased risk of these cancers in a person with a BRCA mutation.<sup>1</sup>

### Breast cancer risk and other genes

Other inherited cancer syndromes that can include breast cancer are Li-Fraumeni syndrome (TP53 gene), Cowden syndrome (PTEN), Hereditary Diffuse Gastric Cancer syndrome (CDH1), and Peutz Jeghers syndrome (STK11). Additionally, other genes that can increase the risk for breast cancer are ATM, CHEK2, NBN, NF1, and PALB2.<sup>1,6-9</sup>

## Test information

### Introduction

BRCA testing may include Ashkenazi Jewish founder mutation testing, full gene sequencing, deletion/duplication analysis, known familial mutation analysis, or multigene panel testing.

### Ashkenazi Jewish founder mutation testing

This test is appropriate for those who meet criteria and have Ashkenazi Jewish ancestry.<sup>6-8</sup>

Ashkenazi Jewish founder mutation testing includes the three mutations most commonly found in the Ashkenazi Jewish population:

- 187delAG and 5385insC in BRCA1, and
- 6174delT in BRCA2.<sup>1</sup>

Testing for these three most common mutations detects about 98% of mutations in those with Ashkenazi Jewish ancestry.<sup>1,6</sup>

### Other testing options

See the BRCA Analysis guideline for other testing options:

- full sequence testing
- deletion/duplication analysis, or
- known familial mutation.

## Guidelines and evidence

### Introduction

This section includes relevant guidelines and evidence pertaining to BRCA Ashkenazi Jewish founder mutation testing.

### National Comprehensive Cancer Network

The National Comprehensive Cancer Network (NCCN, 2019)<sup>7</sup> evidence and consensus-based guidelines include unaffected women with a family history of cancer, those with a known mutation in the family, those with a personal history of breast cancer and/or ovarian cancer, those with a personal history of pancreatic and/or prostate (metastatic or Gleason score at least 7) cancer, and men with breast cancer.

Based on these guidelines, and the recommendations of the National Society of Genetic Counselors (2013)<sup>8</sup> the founder mutation analysis is appropriate for any individual with Ashkenazi Jewish ancestry with a personal history of breast, epithelial ovarian, fallopian tube, primary peritoneal, prostate (Gleason score at least 7), or pancreatic cancer.

These recommendations are Category 2A, defined as "lower-level evidence with uniform NCCN consensus."

### U.S. Preventive Services Task Force

The U.S. Preventive Services Task Force (USPSTF, 2013) recommendations address women who do not have a personal history of breast and/or ovarian cancer, but rather have a family history of these cancer types.<sup>10, 11</sup>

The USPSTF guideline recommends that primary care providers identify women who have a family history of breast, ovarian, fallopian tube, or peritoneal cancer with one of several screening tools. These tools are designed to identify women who may be at an increased risk to carry a BRCA mutation. Women identified as high risk should then be referred for genetic counseling and, if indicated after counseling, BRCA testing.

Women identified as high risk by these screening tools typically have one or more of the following characteristics.<sup>10-11</sup>

- a first or second degree relative with
  - breast cancer before 50 years old
  - ovarian cancer
  - bilateral/multifocal breast cancer, or
  - both breast and ovarian cancers
- a first or second degree male relative with breast cancer
- two or more relatives (first, second, third degree) with

- breast or ovarian cancer or both
- breast, prostate, or pancreatic cancer, or
- presence of Ashkenazi Jewish ancestry with any of the above

### **Grade B recommendation**

The USPSTF considers this a Grade B recommendation: “The USPSTF found at least fair evidence that [the service] improves important health outcomes and concludes that benefits outweigh harms.”

## **Criteria**

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy),<sup>7,8,10-12</sup> AND
- Previous Genetic Testing:
  - No previous full sequence testing, and
  - No previous deletion/duplication analysis, and
  - No previous Ashkenazi Jewish founder mutation testing, AND
- Age 18 years or older<sup>13</sup>, AND
- Diagnostic Testing for Symptomatic Individuals:<sup>7</sup>
  - Ashkenazi Jewish descent, and
    - Epithelial ovarian, fallopian tube, or primary peritoneal cancer diagnosis at any age, or
    - Male or female breast cancer diagnosis at any age, or
    - Personal history of pancreatic cancer, or
    - Personal history of high-grade prostate cancer (Gleason score at least 7) at any age, or
    - Personal history of metastatic prostate cancer (radiographic evidence of or biopsy-proven disease), OR
- Predisposition Testing for Presymptomatic/Asymptomatic Individuals:
  - Ashkenazi Jewish descent, and
  - A first or second degree relative who is Ashkenazi Jewish and meets at least one of the following:<sup>7</sup>

- Epithelial ovarian, fallopian tube, or primary peritoneal cancer diagnosis at any age, or
  - Male or female breast cancer diagnosis at any age, or
  - Pancreatic cancer, or
  - High-grade prostate cancer (Gleason score at least 7), and
  - The affected relative is deceased, unable, or unwilling to be tested<sup>†</sup>, or
  - Close blood relative (1st, 2nd, or 3rd degree) with a known founder mutation in a BRCA1/2 gene, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

\*\*First-degree relatives (parents, siblings, children); second-degree relatives (aunts, uncles, grandparents, grandchildren, nieces, nephews and half-siblings); and third-degree relatives (great-grandparents, great-aunts, great-uncles, and first cousins) on the same side of the family.

<sup>†</sup>Testing of unaffected individuals should only be considered when an affected family member is unavailable for testing due to the significant limitations in interpreting a negative result.

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**Note** Full gene sequencing of BRCA1/2 may be indicated if no founder mutations are detected by 81212 and the individual meets the criteria above. See BRCA Analysis guideline for criteria.

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# Breast Cancer Index for Breast Cancer Prognosis

MOL.TS.248.A

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## Introduction

Breast Cancer Index for breast cancer prognosis is addressed by this guideline.

## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedure addressed by this guideline | Procedure code |
|---------------------------------------|----------------|
| Breast Cancer Index                   | 81518          |

## What is Breast Cancer Index for breast cancer prognosis

### Definition

Breast Cancer Index<sup>®</sup> (BCI) is a commercial multigene expression profiling assay designed to assess prognosis in early-stage breast cancer patients.<sup>1</sup>

### Breast cancer recurrence

A large percentage of breast cancer patients (ER+/LN-) treated with endocrine therapy alone are free of disease 10+ years after initial diagnosis, and could forgo chemotherapy and its toxic side effects. Furthermore, a meta-analysis (n=~35,000 patients) reported a rate of recurrence of ~2% per year for breast cancer patients (ER+/LN-) receiving only tamoxifen.<sup>2</sup> Consequently, accurate prediction of the risk of breast cancer recurrence is important for establishing the most optimal course of treatment with endocrine therapy, adjuvant chemotherapy, or both for women with early-stage breast cancer.

### Risk assessment

Conventional methods of risk assessment including using the following clinicopathologic factors

- tumor size
- involvement of regional lymph nodes
- histologic grade

- expression of hormone receptors (estrogen and progesterone), and
- human epidermal growth factor receptor 2 (HER2) amplification.

These may not be sufficiently accurate to identify those subgroups of patients who are at low risk of recurrence and who are unlikely to benefit from extended endocrine therapy or adjuvant chemotherapy.<sup>3</sup>

As a result, alternative biomarker prognostic tests have been developed to more accurately predict individual risk of cancer recurrence and to better inform clinicians making treatment decisions for patients with early-stage breast cancer, including

- determining appropriate chemotherapy regimens
- decreasing treatment-associated complications, and
- avoiding unnecessary treatment.<sup>4</sup>

### **Intended use**

According to the manufacturer, "The Breast Cancer Index (BCI) Risk of Recurrence & Extended Endocrine Benefit Test is intended for use in patients diagnosed with estrogen receptor-positive (ER+), lymph node-negative (LN-) or lymph node positive (LN+; with 1-3 positive nodes) early-stage, invasive breast cancer, who are distant recurrence-free. BCI provides:

- A quantitative assessment of the likelihood of both late (post-5 years) and overall (0-10 year) distant recurrence following an initial 5 years of endocrine therapy (LN- patients) or 5 years of endocrine therapy plus adjuvant chemotherapy (LN+ patients), and
- Prediction of likelihood of benefit from extended (>5 year) endocrine therapy. BCI results are adjunctive to the ordering physician's workup; treatment decisions require correlation with all other clinical findings."<sup>1</sup>

## **Test information**

### **Introduction**

The test is intended to provide risk information beyond standard predictive and prognostic factors and identify those patients unlikely to benefit from extended endocrine therapy or adjuvant chemotherapy.<sup>1</sup>

### **Breast Cancer Index**

The Breast Cancer Index assay is an algorithmic gene expression-based signature, which combines 2 independent biomarkers (HOXB13:IL17BR [H:I or H/I]) and the 5-gene molecular grade index (MGI) to evaluate estrogen-mediated signaling and tumor grade.<sup>2</sup>

As a risk stratification tool, BCI attempts to stratify patients with early-stage estrogen-receptor positive (ER+), lymph-node negative (LN-) patients into three different risk groups, as well offer a continuous evaluation of an individual patient's risk of distant recurrence.<sup>2</sup>

## Guidelines and evidence

### Introduction

This section includes relevant guidelines and evidence pertaining to Breast Cancer Index testing.

### The National Comprehensive Cancer Network

The National Comprehensive Cancer Network (NCCN) 2018 Clinical Practice Guidelines for Breast Cancer state that Breast Cancer index (BCI) is considered evidence and consensus category 2A for prognostic assessment in node-negative hormone receptor positive, HER2 negative invasive breast cancer.<sup>5</sup> Use of the test for predictive purposes has not been determined.

### St. Gallen International Expert Consensus

St. Gallen International Expert Consensus (updated 2017)

- “The Panel did not recommend the use of gene expression signatures for choosing whether to recommend extended adjuvant endocrine treatment, as no prospective data exist and the retrospective data were not considered sufficient to justify the routine use of genomic assays in this setting.”<sup>6</sup>

### American Society of Clinical Oncology

The American Society of Clinical Oncology (ASCO, 2016) published a clinical practice guideline regarding the use of biomarkers to guide clinical decision-making on adjuvant systemic therapy among women with early-stage invasive breast cancer. Based on a review of the peer-reviewed scientific evidence, the following recommendations were published:<sup>7</sup>

- “If a patient has ER/PgR-positive, HER2-negative (node-negative) breast cancer, the clinician may use the Breast Cancer Index to guide decisions on adjuvant systemic therapy. Type: evidence based. Evidence quality: intermediate. Strength of recommendation: moderate.”
- “If a patient has ER/PgR-positive, HER2-negative (node-positive) breast cancer, the clinician should not use the Breast Cancer Index to guide decisions on adjuvant systemic therapy. Type: informal consensus. Evidence quality: insufficient. Strength of recommendation: strong.”



- “If a patient has HER2-positive breast cancer or TN breast cancer, the clinician should not use the Breast Cancer Index to guide decisions on adjuvant systemic therapy. Type: informal consensus. Evidence quality: insufficient. Strength of recommendation: strong.”

### Peer Reviewed Literature

Several retrospective and prospective-retrospective studies, published by the manufacturer, have assessed the clinical validity of the BCI test for women with early stage breast cancer (ER+/LN-) to guide clinical decision making regarding adjuvant therapy (prognostic) or regarding treatment response (predictive).<sup>2,8-11</sup> Results of clinical validity are generally consistent across these studies, reporting that women classified by the BCI test into higher risk categories tend to have worse rates of distant recurrence, and women in lower risk categories have better rates of distant recurrence.

Most recently, Sestak and colleagues (2018) performed a within-patient comparison of 6 prognostic signatures, including BCI, in 774 women (591 node negative) with early ER+ HER2- breast cancer who received 5 years of endocrine therapy (Tamoxifen or anastrozole, but not combination therapy) and no chemotherapy using data from the TransATAC trial.<sup>12</sup> They looked at both overall (0-10 year) and distant (5-10 year) recurrence. The Hazard Ratio for BCI for in node-negative patients for overall recurrence was 2.46, and for distant recurrence was 2.30. The authors note that all signatures performed worse in node positive patients, but that BCI and EndoPredict were the best performers in this category.

## Criteria

### Introduction

Requests for Breast Cancer Index testing are reviewed using these criteria.

### Criteria

- Previous Testing:
  - No repeat Breast Cancer Index testing on the same sample when a result was successfully obtained, and
  - No previous gene expression assay (e.g. OncotypeDx Breast) performed on the same sample when a result was successfully obtained, AND
- Required Clinical Characteristics:
  - Primary invasive breast cancer meeting all of the following criteria:
  - Unilateral tumor
    - Tumor size >0.5cm (5mm) in greatest dimension (T1b-T3), and

- Hormone receptor positive (ER+ or PR+), and
- HER2 negative, and
- Patient has no regional lymph node metastasis (pN0) or only micrometastases (pN1mi, malignant cells in regional lymph node(s) not greater than 2.0mm), and
- Adjuvant endocrine systemic chemotherapy is a planned treatment option for the patient or results from this Breast Cancer Index test will be used in making adjuvant chemotherapy treatment decisions, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

### Other Considerations

#### Testing Multiple Samples:

- When more than one ipsilateral breast cancer primary is diagnosed, testing should be performed on the tumor with the most aggressive histologic characteristics. If an exception is requested, the following criteria will apply:
  - There should be reasonable evidence that the tumors are distinct (e.g., different quadrants, different histopathologic features, etc.), AND
  - There should be no evidence from either tumor that chemotherapy is indicated with or without knowledge of the Breast Cancer Index test result (e.g., histopathologic features or previous Breast Cancer Index result of one tumor suggest chemotherapy is indicated), AND
  - If both tumors are to be tested, both tumors must independently meet the required clinical characteristics

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# Brugada Syndrome Genetic Testing

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## Introduction

Brugada syndrome genetic testing is addressed by this guideline.

## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedures address by this guideline                  | Procedure codes |
|---|-----------------|
| Brugada Syndrome Known Familial Mutation Analysis     | 81403           |
| SCN5A Sequencing                                      | 81407           |
| SCN5A Deletion/Duplication Analysis                   | 81479           |
| Brugada Syndrome Sequencing Multigene Panel           | 81413           |
| Brugada Syndrome Deletion/Duplication Panel           | 81414           |
| Brugada Syndrome Genetic Testing (SCN5A and Variants) | S3861           |

## What is Brugada syndrome

### Definition

Brugada syndrome (BrS) is an inherited channelopathy characterized by right precordial ST elevation. This can result in cardiac conduction delays at different levels, syncope, or a lethal arrhythmia resulting in sudden cardiac death.

### Onset

Although the typical presentation of BrS is sudden death in a male in his 40s with a previous history of syncope, BrS has been seen in individuals between the ages of 2 days and 85 years,<sup>1</sup> as well as females.<sup>2</sup> Symptoms often occur at rest or during sleep.

### Diagnosis

The diagnosis of BrS is based on ECG results, clinical presentation and family history. Findings of either type 1, 2, or 3 ECG pattern with a personal history of fainting spells,

ventricular fibrillation, self-terminating polymorphic ventricular tachycardia, or electrophysiologic inducibility can help identify those at risk for BrS. A family history of syncope, coved-type ECGs, or sudden cardiac death, especially in an autosomal dominant inheritance pattern, can help aid in the diagnosis.<sup>3,4</sup>

## Cause

BrS has been associated with at least 16 different genes and >400 mutations,<sup>3,5-7</sup> and is estimated to be seen in about 1 in 2000 individuals. Approximately 65-75% of families with a clinical diagnosis of BrS do not test positive for a mutation in one of the known genes, suggesting that there are other genes that have not been identified.<sup>3,5</sup>

- SCN5A is responsible for the majority of BrS cases (15-30%).
- There are reports that CACNA1C and CACNB2B may account for up to 11% of cases of BrS.<sup>6,8</sup>
- Each of the other genes comprise less than 5% of mutations in each case.

BrS has variable expression and incomplete penetrance. Approximately 25% of gene positive individuals have an ECG diagnostic of BrS.<sup>3, 5</sup> Additionally, 80% of individuals with a disease-causing mutation only present with symptoms when challenged with a sodium channel blocker.<sup>2,9</sup>

## Prevalence

BrS is found worldwide with a prevalence of approximately 1:2000 in endemic areas.<sup>3</sup> It seems to have a higher incidence in Southeast Asia. In countries such as Japan, the Philippines, Laos, and Thailand, a condition called Sudden Unexplained Nocturnal Death syndrome (SUNDS) has been associated with mutations in the SCN5A, suggesting that this condition is actually Brugada Syndrome.<sup>10,11</sup> In these countries, SUNDS is the second most common cause of death of men under age 40 years.<sup>3</sup>

## Inheritance

BrS is inherited in an autosomal dominant inheritance pattern, with the exception of KCNE5-related Brugada syndrome, which is inherited in an X-linked manner.<sup>3</sup> This means that an individual has a 50% chance of passing on a mutation to their children. Additionally, parents and siblings of known carriers have a 50% chance of being carriers of the same mutation.

When a mutation in a child is not found in the parents, it is assumed that there is a de novo mutation in the child. De novo mutations are estimated to occur in approximately 1% of cases.<sup>3</sup> Siblings would still need to be tested to rule out germline mutations.

A DNA test for BrS should be offered to the person who has the most obvious disease, as that individual will more likely test positive than someone without disease. At this time, population wide carrier screening for BrS is not recommended.<sup>5</sup>

## Test information

### Introduction

Testing for Brugada syndrome may include full sequence analysis, deletion/duplication testing, known familial mutation analysis, or multigene panels.

### Full sequence analysis

Full sequence analysis of the SCN5A gene is available through a number of commercial laboratories.

### Deletion/duplication testing

Deletion/duplication testing for SCN5A is available and is typically done in reflex to a negative result from full sequence analysis.

### Known familial mutation analysis

Known familial mutation analysis can be considered for individuals with a known mutation in the family. Once a deleterious mutation is identified in a family member, at-risk relatives can be tested for only that specific mutation. Testing by single site analysis is greater than 99% accurate.<sup>3</sup>

### Multigene panels

Multigene panels can be considered but this test is typically not recommended.

## Guidelines and evidence

### Introduction

This section includes relevant guidelines and evidence pertaining to Brugada syndrome testing.

### Heart Rhythm Society, European Heart Rhythm Association, and Asia Pacific Heart Rhythm Society

A 2013 expert consensus statement from the Heart Rhythm Society (HRS), the European Heart Rhythm Association (EHRA), and the Asia Pacific Heart Rhythm Society is silent on the role of genetic testing in diagnosis and management.<sup>12</sup>

### Heart Rhythm Society and European Heart Rhythm Association

A 2011 expert consensus statement from the Heart Rhythm Society (HRS) and the European Heart Rhythm Association (EHRA) recommends:<sup>5</sup>

- “Comprehensive or BrS1 (SCN5A) targeted BrS genetic testing can be useful for any patient in whom a cardiologist has established a clinical index of suspicion for BrS based on examination of the patient’s clinical history, family history, and expressed electrocardiographic (resting 12-lead ECGs and/or provocative drug challenge testing) phenotype.” (Class IIa)
- “Genetic testing is not indicated in the setting of an isolated type 2 or type 3 Brugada ECG pattern.”
- “Mutation-specific genetic testing is recommended for family members and appropriate relatives following the identification of the BrS-causative mutation in an index case.”

### **Multigene panels**

The clinical utility of Brugada multigene panels has not been well established. Mutations in SCN5A are responsible for 15-30% of cases of Brugada Syndrome, making it the most common known genetic cause of BrS. There are other genes associated with BrS, but mutations in each gene account for <5% of cases of BrS, therefore incremental mutation yield on a multi-gene panel is expected to be very low.<sup>5</sup>

## **Criteria**

### **Introduction**

Requests for Brugada syndrome testing are reviewed using these criteria.

### **Brugada Syndrome Known Familial Mutation Analysis**

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - No previous genetic testing for Brugada Syndrome, AND
- Diagnostic and Predisposition Testing:
  - Brugada Syndrome familial mutation identified in biologic relative(s), OR
- Prenatal Testing:
  - Brugada syndrome mutation identified in one biologic parent or 1<sup>st</sup> degree relative, AND
- Rendering laboratory is a qualified provider of service per the Health plan policy.

**Brugada Syndrome Full Sequence Analysis of SCN5A**

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - No previous sequence analysis of SCN5A, AND
- Diagnostic Testing for Symptomatic Individuals:
  - Type 1, 2, or 3 ECG results, and
  - Documented ventricular fibrillation, or
  - Self-terminating polymorphic ventricular tachycardia, or
  - A family history of sudden cardiac death, or
  - Coved-type ECGs in family members, or
  - Electrophysiologic inducibility, or
  - Syncope, or
  - Nocturnal agonal respiration (breaths that persist after cessation of heartbeat), OR
- Predisposition Testing for Presymptomatic/Asymptomatic Individuals:
  - Biologic relative(s) (1<sup>st</sup>, 2<sup>nd</sup>, or 3<sup>rd</sup> degree) diagnosed with BrS clinically, and no familial mutation identified, or
  - Sudden death in biologic relative(1<sup>st</sup>, 2<sup>nd</sup>, or 3<sup>rd</sup> degree), and
  - Type 1 ECG changes, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

**Brugada Deletion/Duplication Analysis of SCN5A**

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:(a)
  - No mutation identified with Brugada Syndrome sequence analysis of SCN5A, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.



## Brugada Syndrome Multigene Panels

- Brugada syndrome multigene panels are considered investigational and/or experimental.
  - Investigational and experimental (I&E) molecular and genomic (MolGen) tests refer to assays involving chromosomes, DNA, RNA, or gene products that have insufficient data to determine the net health impact, which typically means there is insufficient data to support that a test accurately assesses the outcome of interest (analytical and clinical validity), significantly improves health outcomes (clinical utility), and/or performs better than an existing standard of care medical management option. Such tests are also not generally accepted as standard of care in the evaluation or management of a particular condition.
  - In the case of MolGen testing, FDA clearance is not a reliable standard given the number of laboratory developed tests that currently fall outside of FDA oversight and FDA clearance often does not assess clinical utility.

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### Introduction

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# CADASIL Testing

MOL.TS.144.A

v1.0.2020

## Introduction

CADASIL testing is addressed by this guideline.

## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedures addressed by this guideline  | Procedure codes |
|---|-----------------|
| NOTCH3 Known Familial Mutation Analysis | 81403           |
| NOTCH3 Targeted Sequencing              | 81406           |
| NOTCH3 Deletion/Duplication Analysis    | 81479           |

## What is CADASIL

### Definition

CADASIL (Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy) is an adult-onset form of cerebrovascular disease. There are no generally accepted clinical diagnostic criteria for CADASIL and symptoms vary among affected individuals.

### Signs and symptoms

Typical signs and symptoms include<sup>1,2,3</sup>

- Transient ischemic attacks and ischemic stroke, occurs at a mean age of 47 years (age range 20-70 years), in most cases without conventional vascular risk factors
- cognitive disturbance, primarily affecting executive function, may start as early as age 35 years
- psychiatric or behavioral abnormalities
- migraine with aura, occurs with a mean age of onset of 30 years (age range 6-48 years), and

Less common symptoms include:

- recurrent seizures with onset in middle age, usually secondary to stroke
- acute encephalopathy, with a mean age of onset of 42 years

Life expectancy for men with CADASIL is reduced by approximately five years and for women by 1 to 2 years.<sup>4</sup>

## Diagnosis

Brain Magnetic Resonance Imaging (MRI) findings include T2-signal-abnormalities in the white matter of the temporal pole and T2-signal-abnormalities in the external capsule and corpus callosum.<sup>1,2</sup>

CADASIL is suspected in an individual with the clinical signs and MRI findings. A positive family history for stroke or dementia is also indicative of disease in symptomatic individuals. However, a negative family history should not exclude the diagnosis, as de novo mutations may occur, and affected family members are frequently misdiagnosed.<sup>1,5</sup>

Sequencing of all NOTCH3 exons encoding EGF-like domains fails to identify a mutation in up to 4% of individuals with CADASIL. Therefore, skin biopsy with histopathologic evaluation for characteristic granular osmiophilic material (GOM) deposits is appropriate for patients with a high index of clinical suspicion for CADASIL and negative genetic testing.<sup>2,4</sup>

For a firm diagnosis of CADASIL, at least one of the following is required:

- Documentation of a typical NOTCH3 mutation by genetic analysis.<sup>1,2,5</sup>
- Documentation of characteristic GOM deposits within small blood vessels by skin biopsy.<sup>1,2,5</sup>

## Prevalence

CADASIL is a rare disease. The exact prevalence is unknown. CADASIL is probably still underdiagnosed. The minimum prevalence is estimated to be between 2-5 per 100,000 based on multiple small and national registries.<sup>1,4</sup> A founder effect has been reported for Finnish individuals and patients in the Marche region of Italy.<sup>1</sup>

CADASIL is the most prevalent inherited cause of cerebral small-vessel disease.<sup>6</sup>

## Cause

CADASIL is an autosomal dominant disease caused by mutations in the NOTCH3 gene. Each offspring of an individual with CADASIL has a 50% chance of inheriting the disease-causing mutation.

To date, NOTCH3 is the only gene in which mutations are known to cause CADASIL.<sup>1</sup> NOTCH3 has 33 exons. All CADASIL pathogenic variants occur in exons 2–24, which encode the 34 epidermal growth factor repeats (EGFR).<sup>1,7</sup> The majority of pathogenic variants occur in exons 2-6.<sup>2</sup> NOTCH3 encodes a transmembrane receptor that is

primarily expressed in vascular smooth-muscle cells, preferentially in small arteries.<sup>1</sup> "In CADASIL, the extracellular domain of the Notch3 receptor accumulates within blood vessels. Accumulation takes place at the cytoplasmic membrane of VSMCs and pericytes in close vicinity to the granular osmiophilic deposits (GOM) that characterize the disease. NOTCH3 recruits other proteins into the extracellular deposits, among them vitronectin and tissue inhibitor of metalloproteinase-3 (TIMP3), which may be relevant for disease pathogenesis."<sup>2</sup> There is a hypothesis that structural abnormalities in the vascular smooth-muscle protein NOTCH3 trigger arterial degeneration, vascular protein accumulation, and cerebrovascular failure.<sup>6</sup>

No clear genotype-phenotype correlations exist for individuals with CADASIL.<sup>5,8</sup> Some studies describe phenotype-genotype correlations. There can be significant intrafamilial variability with the age of onset, disease severity, and disease progression. The genotype cannot be used to predict the phenotype.<sup>1,6</sup>

### **Management and treatment**

A correct diagnosis of CADASIL is important because the clinical course of disease is different from individuals with other types of cerebral small-vessel disease and proven therapies for stroke have not been validated in individuals with CADASIL.<sup>5</sup> However, no specific disease-modifying treatments for CADASIL exist. Management and treatment of individuals is generally symptomatic and supportive.<sup>1,2,3,5</sup>

## **Test information**

### **Introduction**

Testing for CADASIL may include a skin biopsy, sequence analysis, deletion/duplication analysis, or known familial mutation analysis.

### **Skin biopsy**

"The pathologic hallmark of CADASIL is electron-dense granules in the media of arterioles, and increased NOTCH3 staining of the arterial wall, which can be evaluated in a skin biopsy."<sup>1</sup> Specificity of skin biopsy findings is high as the characteristic deposits have not been documented in any other disorder. Sensitivity has been reported to range from 45%-100%. Sensitivity and specificity can be maximized to >90% by immunostaining for NOTCH3 protein.<sup>5</sup> When interpreted by an experienced (neuro) pathologist, combined analysis by electron microscopy and immunohistochemistry usually allows for a conclusive CADASIL diagnosis.

### **Sequence analysis**

NOTCH3 mutation detection may reach >95% in individuals with strong clinical suspicion of CADASIL1. To date, all mutations in NOTCH3 causing CADASIL have been in exons 2-24, including intron-exon boundaries.<sup>1</sup> In the United States,

laboratories offering CADASIL testing appear to perform sequencing of, at minimum, exons 2-24 at the time of this review.

### **Deletion/duplication analysis**

Deletion/duplication analysis will find gene rearrangements that are too large to be detected by sequencing. Large deletions and duplications in the NOTCH3 gene have not been reported.<sup>2</sup>

"Molecular testing approaches can include sequence analysis of exons 2-24 and intron-exon boundaries of NOTCH3 followed by deletion/duplication analysis if no pathogenic variant is found."<sup>1</sup>

### **Known familial mutation analysis**

Once a mutation in an affected individual has been identified, targeted testing of at risk individuals in the family is possible.

## **Guidelines and evidence**

### **Introduction**

This section includes relevant guidelines and evidence pertaining to CADASIL testing. No evidence-based U.S. testing guidelines have been identified.

#### **Pescini et al (2012)**

Pescini et al (2012) published a scale to help guide clinicians in selecting patients for NOTCH3 genetic analysis due to a high probability of a CADASIL genetic diagnosis. This scale assigns weighted scores to common features of CADASIL. The authors state that their scale is "accurate with optimal sensitivity and specificity values (96.7% and 74.2%, respectively); however, our results need to be confirmed and further validated."<sup>9</sup>

#### **Choi et al (2010)**

A two-center cohort study found that blood pressure and hemoglobin A1c levels were associated with cerebral mini bleeds in CADASIL patients.<sup>5</sup> Therefore, controlling blood pressure and glucose levels may improve the clinical course of the disease. It is also reasonable to control for high cholesterol and high blood pressure given the high rate of ischemic stroke seen in CADASIL.<sup>5</sup>

#### **Tikka et al (2009)**

Evidence from one 2009 retrospective cohort study suggests that an adequate skin biopsy for analysis of granular osmophilic material is a cost effective way to determine a diagnosis of CADASIL in symptomatic individuals.<sup>10</sup>

The authors suggest that biopsy results can be used to guide the decision for who should have genetic testing, particularly in individuals with no known familial mutation or from ethnic populations with no evidence of founder mutations.<sup>10</sup>

### **Clinical utility**

Patients with CADASIL should avoid anticoagulants, angiography, and smoking to avoid disease-related complications, so clinical utility is represented.<sup>1,5</sup> Because of the risk for cerebral hemorrhage, use of antiplatelets rather than anticoagulants is considered for prevention of ischemic attacks. Evidence against the use of intravenous tissue plasminogen activator (IV tPA) has been suggested due to the possibility of hemorrhage; however, this is not conclusive.<sup>11</sup> Statins are used for treatment of hypercholesterolemia and antihypertensive drugs are used for hypertension and hypertension treatment may have an additional benefit.<sup>2</sup>

### **Criteria**

#### **Introduction**

Requests for CADASIL testing are reviewed using these criteria.

#### **Known familial mutation testing**

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - No previous genetic testing for NOTCH3 mutations, AND
- Predictive Testing:
  - Member has a first-degree relative (i.e. parent, sibling, child) with an identified NOTCH3 gene mutation, and
  - Member is at least 18 years of age, OR
- Diagnostic Testing for Symptomatic Individuals:
  - Member has a first-degree relative (i.e. parent, sibling, child) with an identified NOTCH3 gene mutation, and
  - High index of suspicion for CADASIL diagnosis based on clinical findings, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

## NOTCH3 targeted sequencing

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - No previous genetic sequencing for NOTCH3 mutations, AND
- Diagnostic Testing for Symptomatic Individuals:
  - High index of suspicion for CADASIL diagnosis based on clinical findings, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

## NOTCH3 deletion/duplication analysis

- Member meets the above criteria for NOTCH3 targeted sequencing, AND
- NOTCH3 targeted sequencing performed and detected no mutations, AND
- No previous NOTCH3 deletion/duplication analysis

## References

### Introduction

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# Canavan Disease Testing

MOL.TS.145.A  
v1.0.2020

## Introduction

Canavan disease testing is addressed by this guideline.

## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedures addressed by this guideline | Procedure codes |
|--|-----------------|
| ASPA Known Familial Mutation Analysis  | 81403           |
| ASPA Targeted Mutation Analysis        | 81200           |
| ASPA Sequencing                        | 81479           |
| ASPA Deletion/Duplication Analysis     | 81479           |

## What is Canavan disease

### Definition

Canavan disease is a genetic disorder leading to progressive damage to the brain's nerve cells.<sup>1,2</sup>

### Prevalence

Canavan disease is most often found in Ashkenazi Jewish populations.<sup>1,2</sup>

- Between 1 in 40 and 1 in 82 people of Ashkenazi Jewish descent are carriers for Canavan disease.<sup>2</sup> Because of this relatively high carrier rate, population based screening in the Ashkenazi Jewish population is available. (See **Ashkenazi Jewish Carrier Screening**).
- Between 1 in 6,400 and 1 in 13,500 Ashkenazi Jews have the disease.<sup>1</sup>

Canavan disease occurs in all ethnic groups, and the prevalence among the general population is significantly lower than that in the Ashkenazi Jewish population.<sup>2</sup>

### Symptoms

Signs and symptoms of Canavan disease usually begin in infancy and include:<sup>1</sup>

- developmental delays including motor skills, learning disabilities, or problems sleeping
- weak muscle tone (hypotonia)
- large head size (macrocephaly)
- abnormal posture
- leukodystrophy on neuroimaging, and
- seizures.

### **Cause**

Canavan disease is caused by changes, or mutations, to the ASPA gene.<sup>1</sup> ASPA helps make an enzyme called aspartoacylase.<sup>1</sup>

This enzyme is essential to maintain the health of myelin, the nerve cells' protective covering, by breaking down harmful compounds that would otherwise degrade myelin.<sup>1</sup> The most significant of these compounds that break down myelin is called N-acetylaspartic acid (NAA).

In the absence of aspartoacylase, the myelin protective covering of the nerve is eventually destroyed. Without this protective covering, nerve cells malfunction and die.<sup>1</sup>

### **Inheritance**

Canavan disease is an autosomal recessive disorder, meaning that an affected individual must inherit two ASPA gene mutations - one from each parent.<sup>1,2</sup>

Individuals with only one mutation are called carriers. Carriers do not show symptoms of Canavan disease, but have a 50% chance of passing on the mutation to their children who will also be carriers.

If two unaffected carriers have children, each of their pregnancies has a 1 in 4 (25%) chance of being affected with Canavan disease.

### **Diagnosis**

Canavan disease is suspected when a patient presents with classic signs and symptoms. Diagnosis is confirmed by biochemical or genetic testing or both.<sup>2</sup> Biochemical tests analyze either NAA levels or aspartoacylase enzyme activity in someone with suspected Canavan disease.

- Affected individuals cannot break down NAA, so it accumulates in the blood or urine.
- Affected individuals will have severely reduced or nonexistent aspartoacylase enzyme activity.

Molecular genetic testing can be used for confirmation of the diagnosis and to help family planning by identifying individuals at risk of being carriers.<sup>2</sup>

## Survival

Canavan disease does not usually allow survival beyond childhood.<sup>1</sup>

## Test information

### Introduction

Testing for Canavan disease may include targeted mutation analysis, sequence analysis, deletion/duplication analysis, or known familial mutation analysis.

### Targeted mutation analysis

Targeted mutation analysis is the most common genetic test for Canavan disease. The panel looks for up to four of the most common mutations in the ASPA gene linked to Canavan disease, including the Glu285Ala and Tyr231X mutations, which account for 98% of all Ashkenazi Jewish cases.<sup>2,3</sup> The panel also includes the p.Ala305Glu mutation, which accounts for between 30% and 60% of all non-Ashkenazi Jewish cases.<sup>2,3</sup>

### Sequence analysis

Sequence analysis looks for mutations across the entire coding region of the ASPA gene. In addition to the more common mutations found in the Ashkenazi Jewish population, sequencing is also able to find less common mutations found in non-Ashkenazi Jews.<sup>2,3</sup> Sequence analysis has a detection rate of about 99% in all populations.<sup>2</sup>

### Deletion/duplication analysis

Deletion/duplication analysis will find gene rearrangements that are too large to be detected by sequencing. Large deletions in the ASPA gene have been reported but are believed to be uncommon.<sup>2</sup> Therefore, deletion/duplication analysis is unlikely indicated in most cases.

### Known familial mutation analysis

Once mutations have been identified in a symptomatic individual, carrier testing can be performed on at-risk relatives using this same targeted mutation panel or perhaps known familial mutation analysis for the specific mutation identified in the affected individual.

If both members of a couple are carriers with identified mutations, prenatal diagnosis of an at-risk pregnancy is possible using this same targeted mutation panel or known familial mutation analysis for the specific mutations identified in the parents.

## Guidelines and evidence

### Introduction

This section includes relevant guidelines and evidence pertaining to Canavan disease testing.

### 2018 expert-authored review

A 2018 expert-authored review states the following regarding molecular genetic testing for diagnostic purposes:<sup>2</sup>

- The targeted mutation panel may be used to confirm a clinical diagnosis, biochemical diagnosis, or both.
- “Targeted analysis for the pathogenic variants p.Glu285Ala, p.Tyr231Ter, and p.Ala305Glu can be performed first in individuals of Ashkenazi Jewish ancestry.”
- “Targeted analysis for the pathogenic variant p.Ala305Glu can be performed first in individuals of non-Ashkenazi Jewish ancestry.”
- “Sequence analysis of ASPA detects small intragenic deletions/insertions and missense, nonsense, and splice site variants; typically, exon or whole-gene deletions/duplications are not detected. Perform sequence analysis first. If only one or no pathogenic variant is found perform gene-targeted deletion/duplication analysis to detect intragenic deletions or duplications.”

### American College of Medical Genetics

The American College of Medical Genetics (ACMG, 2008) supports offering carrier testing for Canavan disease to individuals of Ashkenazi Jewish descent for the two common mutations. It is anticipated that the detection rate will be ~97%. This test should be offered to individuals of reproductive age, preferentially prior to pregnancy, with genetic counseling performed by a geneticist or genetic counselor. ACMG supports the testing of individuals of Ashkenazi Jewish descent, even when their partner is non-Ashkenazi Jewish. In this situation, testing would start with the individual who is Ashkenazi and reflex back to the partner if necessary.<sup>4</sup>

### American College of Obstetrics and Gynecologists

The American College of Obstetrics and Gynecologists (ACOG, 2009) recommends that individuals who are considering a pregnancy or are pregnant should consider testing if at least one member of the couple is Ashkenazi Jewish or has a relative with

Canavan disease. If the woman is pregnant, testing may need to be conducted on both partners simultaneously in order to receive results in a timely fashion. If one or both partners are found to be carriers of Canavan disease, genetic counseling should be provided, and prenatal testing offered, if appropriate.<sup>5</sup>

## Criteria

### Introduction

Requests for Canavan Disease testing are reviewed using these criteria.

#### ASPA Known Familial Mutation Analysis

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - No previous genetic testing of ASPA, AND
- Carrier Screening for Asymptomatic Individuals:
  - Known family mutation in ASPA in 1<sup>st</sup>, 2<sup>nd</sup>, or 3<sup>rd</sup> degree biologic relative, OR
- Prenatal Testing for At-Risk Pregnancies:
  - ASPA mutations identified in both biologic parents

#### ASPA Targeted Mutation Analysis for Common Mutations

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - No previous ASPA genetic testing, including Ashkenazi Jewish screening panels containing targeted mutation analysis for Canavan disease, AND
- Diagnostic Testing or Carrier Screening:
  - Ashkenazi Jewish descent, regardless of disease status and N-acetylaspartic acid (NAA) levels, OR
- Prenatal Testing for At-Risk Pregnancies:
  - ASPA Ashkenazi mutations identified in both biologic parents.

## ASPA Sequencing

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - No previous ASPA gene sequencing, and
  - No known ASPA mutation in family, and
  - No mutations or one mutation detected by common mutation panel, AND
- Diagnostic Testing for Symptomatic Individuals:
  - Increased levels of N-acetylaspartic acid (NAA) in urine, and
  - An individual age three to five months of age with a triad of hypotonia, macrocephaly and head lag, or
  - Failure to attain independent sitting, walking or speech, OR
- Testing for Individuals with Family History or Partners of Carriers:
  - 1<sup>st</sup>, 2<sup>nd</sup>, or 3<sup>rd</sup> degree biologic relative with Canavan disease clinical diagnosis, family mutation unknown, and testing unavailable, or
  - Partner is monoallelic or biallelic for ASPA mutation, and
    - Have the potential and intention to reproduce

## References

### Introduction

These references are cited in this guideline.

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# Celiac Disease Testing

**MOL.TS.146.A**  
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## Introduction

Celiac disease testing is addressed by this guideline.

## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedures addressed by this guideline                           | Procedure codes |
|--|-----------------|
| HLA Class II Typing- low resolution, multiple alleles            | 81376           |
| HLA Class II Typing- low resolution, one allele or allele group  | 81377           |
| HLA Class II Typing- high resolution, multiple alleles           | 81382           |
| HLA Class II Typing- high resolution, one allele or allele group | 81383           |

## What is Celiac disease

### Definition

Celiac disease is an immune-mediated disorder that mainly affects the digestive tract.<sup>1-4</sup>

### Symptoms

Symptoms include diarrhea, constipation, vomiting, abdominal pain and bloating, growth problems, iron deficiency anemia, osteoporosis, and other complications of malabsorption.<sup>1-4</sup>

### Prevalence

Celiac disease affects infants, children, and adults and can present at any age. It affects about 1 in every 100 people in the U.S.<sup>2,3</sup>

## Cause

Celiac disease is caused by multiple factors, including environmental trigger in a genetically predisposed person, upon exposure to dietary gluten, which is a protein molecule found in wheat, barley and rye.<sup>1-4</sup>

## Diagnosis

An initial diagnosis of celiac disease is highly suspected based on serologic testing and is confirmed by finding characteristic changes on intestinal biopsy. Intestinal biopsy remains the gold standard for making a diagnosis of celiac disease.<sup>1-4</sup>

## Increased risk

Patients with certain medical conditions and relatives of people with celiac disease are known to have an increased risk of developing the condition.<sup>2,3</sup>

## Test information

### Introduction

Two HLA haplotypes are associated with celiac disease: HLA-DQ2 and HLA-DQ8, and are determined by molecular testing of the genes HLA-DQA1 and HLA-DQB1. These haplotypes are present in about 30-40% of the general population, but more than 99% of patients with celiac disease have one or more of these variants<sup>1</sup>. If a person suspected of having celiac disease is found not to have one of these markers, the diagnosis can be essentially excluded.<sup>2-4</sup>

## Guidelines and evidence

### Introduction

This section includes relevant guidelines and evidence pertaining to Celiac disease testing.

### Guidelines

Consensus-based guidelines from the American Gastroenterological Association (2006), the National Institutes of Health (2005), North American Society for Pediatric Gastroenterology, Hepatology and Nutrition (2005) and the 2013 American College of Gastroenterology Practice Guidelines state that HLA typing for celiac disease should be used as outlined in this table.<sup>2-5</sup>

| Test type  | Use   | Level of recommendation                           |
|--|---|---|
| Human leukocyte antigen DQ2/DQ8 testing            | Do not use routinely in the initial diagnosis of Celiac disease         | Strong recommendation, moderate level of evidence |
| Human leukocyte antigen DQ2/DQ8 genotyping testing | Use to effectively rule out the disease in selected clinical situations |   |

The European Society for Pediatric Gastroenterology, Hepatology, and Nutrition Guidelines for the Diagnosis of Coeliac Disease (2012) suggest screening in asymptomatic children and adolescents who are at risk for the disorder.<sup>6</sup> Included in this group are individuals with type I diabetes, Down Syndrome, autoimmune thyroid disease, Turner Syndrome, Williams Syndrome, selective IgA deficiency, autoimmune liver disease, and first degree relatives diagnosed with celiac disease.<sup>6</sup>

The World Gastroenterology Organisation Global Guidelines on Celiac disease (2016) state that for first degree relatives of individuals with celiac disease, no further work-up is necessary in those who test negative for HLA-DQ2/8.<sup>7</sup>

## Criteria

### Introduction

Requests for Celiac disease testing are reviewed using these criteria.

### Criteria

Testing may be considered in individuals who meet the following criterion:

- Celiac disease is in the differential diagnosis, but the individual has had ambiguous or indeterminate results from serology and biopsy.

## References

### Introduction

These references are cited in this guideline.

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# CellSearch Circulating Tumor Cell Count for Breast Cancer Prognosis

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## Introduction

CellSearch circulating tumor cell count is addressed by this guideline.

## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedures addressed by this guideline       | Procedure codes |
|--|-----------------|
| CellSearch Circulating Tumor Cell (CTC) Test | 86152           |
| CTC Physician Interpretation and Report      | 86153           |

## What are circulating tumor cells

### Definition

Circulating tumor cells (CTCs) are cells whose source is unknown, but may have broken away from tumor tissue and are circulating in the blood stream.<sup>1-3</sup> CTCs are rare in healthy individuals, but often present in people with metastatic cancer.<sup>1</sup>

### CTCs and breast cancer

The presence of CTCs in breast cancer patients may predict metastasis of an aggressive primary tumor.<sup>1,2</sup>

A 2004 study found that individuals undergoing treatment for metastatic breast cancer with greater than or equal to 5 CTCs/7.5 mL had shorter progression-free survival (PFS) and shorter overall survival (OS) than individuals with less than 5 CTCs/7.5 mL.<sup>2</sup>

The results of these and other studies suggest that measuring CTCs could be a useful prognostic tool for individuals with metastatic breast cancer.

CTCs may be measured before the start of therapy, and then after each therapy cycle (usually 4-5 weeks).<sup>3</sup>

## Test information

### Introduction

The CellSearch® Circulating Tumor Cells Test measures CTC levels in the blood of breast cancer patients to identify risk for distant metastasis.<sup>3</sup>

### CellSearch

The purpose of CellSearch is to distinguish normal cells from CTCs with fluorescent nucleic acid dye.<sup>3</sup>

Results are generally reported at number of CTCs per 7.5 ml of whole blood.<sup>2,4</sup>

It has been reported that CellSearch correctly measures the levels of CTCs in 99.7% of breast cancer patients.<sup>1</sup>

CellSearch was cleared by the FDA in 2004.<sup>4</sup>

## Guidelines and evidence

### Introduction

This section includes relevant guidelines and evidence pertaining to circulating tumor cells.

### National Comprehensive Cancer Network

The NCCN Guidelines for Breast Cancer (2019) state that the clinical use of CTC in metastatic breast cancer is “not yet included in the NCCN Guidelines for Breast Cancer for disease assessment and monitoring.” They also state that “In spite of its prognostic ability, CTC count has failed to show a predictive value.”<sup>5</sup>

### American Society of Clinical Oncology

The American Society of Clinical Oncology (ASCO, 2016) states the following regarding circulating tumor cells with regard to early stage invasive breast cancer:<sup>6</sup>

- "The clinician should not use circulating tumor cells to guide decisions on adjuvant systemic therapy. Type: evidence based. Evidence quality: intermediate. Strength of recommendation: strong.

The American Society of Clinical Oncology (ASCO, 2015) states the following regarding circulating tumor cells with regard to metastatic breast cancer:<sup>7</sup>

- "There is no evidence at this time that changing therapy based solely on circulating biomarker results improves health outcomes, quality of life, or cost effectiveness."
- "...comparison of progression-free survival and overall survival showed no difference in outcome when patients were switched to an alternate regimen on the

basis of CTC level. These data also illustrate the frequency observed result that biomarkers may be prognostic but not predictive of clinical benefit when used to guide or influence decisions on systemic therapy for breast cancer."

## Literature Review

The evidence is insufficient to support the use of CellSearch as a prognostic or predictive test. The clinical utility of CellSearch to predict or monitor treatment response has not been established.<sup>8-17</sup>

- Results of several observational studies suggest that the use of CellSearch in metastatic breast cancer patients has prognostic value to assess risk of disease progression. Study results showed that enumerated CTC levels by CellSearch were significantly associated with overall survival outcomes in metastatic breast cancer patients. However, results of an RCT reported that use of CellSearch had no impact on survival outcomes in metastatic breast cancer patients who either maintained first-line treatment or opted to change treatment based on CTC levels. A good quality RCT reported that use of CellSearch had no impact on survival outcomes in metastatic breast cancer patients who were undergoing first-line treatment. Thus, clinical utility of CellSearch to predict and monitor treatment response has not been established. There is a lack of evidence regarding how CellSearch compares with variant analysis of tumor biopsy tissue. It is unclear whether molecular subtypes of breast cancer affect the status of CTC under various treatments. There is also a lack of evidence evaluating the use of CellSearch in early stage or non-metastatic breast cancer, or in screening settings.

## Criteria

### Introduction

Requests for CellSearch circulating tumor cell count are reviewed using these criteria.

### Criteria

This test is considered investigational and/or experimental.

- Investigational and experimental (I&E) molecular and genomic (MolGen) tests refer to assays involving chromosomes, DNA, RNA, or gene products that have insufficient data to determine the net health impact, which typically means there is insufficient data to support that a test accurately assesses the outcome of interest (analytical and clinical validity), significantly improves health outcomes (clinical utility), and/or performs better than an existing standard of care medical management option. Such tests are also not generally accepted as standard of care in the evaluation or management of a particular condition.

- In the case of MolGen testing, FDA clearance is not a reliable standard given the number of laboratory developed tests that currently fall outside of FDA oversight and FDA clearance often does not assess clinical utility.

## References

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# Charcot-Marie-Tooth Neuropathy Testing

**MOL.TS.148.A**  
v1.0.2020

## Introduction

Testing for Charcot-Marie-Tooth disease is addressed by this guideline.

## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedures addressed by this guideline                          | Procedure codes |
|---|-----------------|
| Charcot-Marie-Tooth Neuropathy Known Familial Mutation Analysis | 81403           |
| DNM2 Sequencing   | 81479           |
| EGR2 Sequencing   | 81404           |
| FGD4 Sequencing   | 81479           |
| FIG4 Sequencing   | 81406           |
| GARS Sequencing   | 81406           |
| GDAP1 Sequencing  | 81405           |
| GJB1 Deletion/Duplication Analysis                              | 81479           |
| GJB1 Sequencing   | 81403           |
| HSPB1 Sequencing  | 81404           |
| HSPB8 Sequencing  | 81479           |
| LITAF Sequencing  | 81404           |
| LMNA Sequencing   | 81406           |
| MFN2 Sequencing   | 81406           |
| MPZ Sequencing  | 81405           |
| MTMR2 Sequencing  | 81479           |
| NDRG1 Sequencing  | 81479           |
| NEFL Sequencing   | 81405           |
| PMP22 Deletion/Duplication Analysis                             | 81324           |

| Procedures addressed by this guideline   | Procedure codes |
|--|-----------------|
| PMP22 Known Familial Mutation Analysis   | 81326           |
| PMP22 Sequencing   | 81325           |
| PRX Sequencing   | 81405           |
| RAB7A Sequencing   | 81405           |
| SBF2 Sequencing  | 81479           |
| SH3TC2 Sequencing  | 81406           |
| TRPV4 Sequencing   | 81479           |
| YARS Sequencing  | 81479           |
| Hereditary peripheral neuropathies (eg, Charcot-Marie-Tooth, spastic paraplegia), genomic sequence analysis panel, must include sequencing of at least 5 peripheral neuropathy-related genes (eg, BSCL2, GJB1, MFN2, MPZ, REEP1, SPAST, SPG11, SPTLC1) | 81448           |

## What is Charcot-Marie-Tooth Hereditary Neuropathy

### Definition

Charcot-Marie-Tooth Hereditary Neuropathy (CMT) is a group of inherited genetic conditions characterized by chronic motor and sensory polyneuropathy.<sup>1</sup> The key finding in CMT is symmetric, slowly progressive distal motor neuropathy of the arms and legs, usually beginning in the first to third decade and resulting in weakness and atrophy of the muscles in the feet and/or hands. This is expressed as distal muscle weakness and atrophy, weak ankle dorsiflexion, depressed tendon reflexes, and pes cavus foot deformity (i.e. high arched feet).<sup>1</sup>

### Diagnosis

The clinical diagnosis of CMT in a symptomatic person is based on characteristic findings of peripheral neuropathy on medical history and physical examination.<sup>1</sup> CMT needs to be distinguished from the following entities: systemic disorders with neuropathy, other types of hereditary neuropathy, distal myopathies, hereditary sensory neuropathies (HSN), and acquired disorders.<sup>1</sup>

Molecular genetic testing can be used to establish a specific diagnosis, which aids in understanding the prognosis and risk assessment for family members.<sup>1</sup>

## Prevalence

CMT is the most common inherited neurological disorder. The prevalence of all CMT types is 1 in 2,500.<sup>1</sup>

## Types and subtypes

As more genes causing CMT were identified and as the overlap of neuropathy phenotypes and modes of inheritance became apparent, the previous alphanumeric classification system proved unwieldy and inadequate. In 2018, Magy et al proposed a gene-based classification of inherited neuropathies, which includes a comprehensive list of CMT-associated genes and correlation with the alphanumeric classification.<sup>5</sup> An additional advantage of this classification system is that a patient's findings can be described in terms of mode of inheritance, neuropathy type, and gene.

More than 80 different genes are associated with CMT and establishing a specific genetic cause of CMT hereditary neuropathy can aid in discussions of prognosis.<sup>1</sup>

## Inheritance

CMT can be inherited in an autosomal dominant, autosomal recessive, or an X-linked manner.<sup>1</sup>

## Test information

### Introduction

Testing for CMT may include gene sequencing, deletion/duplication analysis, or panel testing.

### Genetic testing

There are various methods used to test for mutations in genes which can cause CMT neuropathy.

- Single gene analysis
- Deletion/duplication analysis, particularly for the 1.5-Mb duplication at 17p11.2 that includes PMP22
- Panel testing using next-generation sequencing (NGS)

### CMT panel testing

CMT multi-gene panels include a wide variety of genes associated with CMT neuropathy. Multi-gene panels may also include genes believed to be associated with CMT neuropathy but with a lower impact on risk than recognized syndromes. Results

for such genes are of less clear value because there often are not clear management recommendations for mutation-positive individuals.

Under certain circumstances, technologies used in multi-gene testing may fail to identify mutations that might be identifiable through single-gene testing. If high clinical suspicion remains for a particular syndrome after negative multi-gene test results, consultation with the testing lab and/or additional targeted genetic testing may be warranted.

Multi-gene tests vary in technical specifications (e.g., depth of coverage, extent of intron/exon boundary analysis, methodology of large deletion/duplication analysis).

Since genes can be easily added or removed from multi-gene tests over time by a given lab, medical records must document which genes were included in the specific multi-gene test used from each patient, and in which labs they were performed.

## Guidelines and evidence

### Introduction

This section includes relevant guidelines and evidence pertaining to CMT testing.

### American Academy of Neurology

Evidence-based guidelines from the American Academy of Neurology (2009; reaffirmed in 2013) recommend testing for CMT, but with a tiered approach:<sup>2</sup>

- “Genetic testing should be conducted for the accurate diagnosis and classification of hereditary neuropathies.”
  - This is considered a level A recommendation which is defined as “established as effective, ineffective or harmful (or established as useful/predictive or not useful/predictive) for the given condition in the specified population.”
- “Genetic testing may be considered in patients with cryptogenic polyneuropathy who exhibit a hereditary neuropathy phenotype. Initial genetic testing should be guided by the clinical phenotype, inheritance pattern, and electrodiagnostic features and should focus on the most common abnormalities which are CMT1A duplication/HNPP deletion, Cx32 (GJB1), and MFN2 mutation screening.”
  - This is considered a level C recommendation which is defined as “possibly effective, ineffective or harmful (or possibly useful/predictive or not useful/predictive) for the given condition in the specified population.”
- “There is insufficient evidence to determine the usefulness of routine genetic testing in patients with cryptogenic polyneuropathy who do not exhibit a hereditary neuropathy phenotype.”

- This is considered a level U recommendation which is defined as “data inadequate or conflicting; given current knowledge, treatment (test, predictor) is unproven.”

### Peer Reviewed Literature

DiVincenzo et al. [2014] described their experience testing more than 17,000 patients for CMT using a commercially available comprehensive panel of 14 genes.<sup>3</sup> Overall, they identified a mutation in 18.5% of patients. Notably they state that “Among patients with a positive genetic finding in a CMT-related gene, 94.9% were positive in one of four genes (PMP22, GJB1, MPZ, or MFN2). The results of our study in a population in over 17,000 individuals support the initial genetic testing of four genes (PMP22, GJB1, MPZ, and MFN2) followed by an evaluation of rarer genetic causes in the diagnostic evaluation of CMT.”<sup>3</sup>

Dohrne et al. [2017] examined over 600 patients with either a CMT phenotype, hereditary sensory neuropathy, familial amyloid neuropathy, or small fiber neuropathy using a NGS multigene panel.<sup>6</sup> At least one putative pathogenic mutation was identified in 121 cases (19.8%), with the most frequently affected genes

PMP22, GJB1, MPZ, SH3TC2, and MFN2. Likely or known pathogenic variants in HINT1, HSPB1, NEFL, PRX, IGHMBP2, NDRG1, TTR, EGR2, FIG4, GDAP1, LMNA, LRSAM1, POLG, TRPV4, AARS, BIC2, DHTKD1, FGD4, HK1, INF2, KIF5A, PDK3, REEP1, SBF1, SBF2, SCN9A, and SPTLC2 were detected with a declining frequency. One pathogenic variant in MPZ was identified after being previously missed by Sanger sequencing. The authors conclude that panel-based NGS “is a useful, time- and cost-effective approach to assist clinicians in identifying the correct diagnosis and enable causative treatment considerations”.<sup>6</sup>

Bacquet et al [2018] compared the diagnostic yield of targeted NGS with their previous step-wise Sanger sequencing strategy.<sup>7</sup> A cohort of 123 unrelated patients affected with diverse forms of inherited peripheral neuropathies including CMT (23% CMT1, 52% CMT2), distal hereditary motor neuropathy (9%), hereditary sensory and autonomic neuropathy (7%), and intermediate CMT (6.5%) were evaluated using an 81-gene NGS panel. Pathogenic variants were identified in 49 of 123 patients (~40%). In this cohort, the most frequently mutated genes

were: MFN2, SH3TC2, GDAP1, NEFL, GAN, KIF5A and AARS, respectively. “Panel-based NGS was more efficient in familial cases than in sporadic cases (diagnostic yield 49% vs 19%, respectively). NGS-based search for copy number variations, allowed the identification of three duplications in three patients and raised the diagnostic yield to 41%. This yield is two times higher than the one obtained previously by gene Sanger sequencing screening. The impact of panel-based NGS screening is particularly important for demyelinating CMT (CMT1) subtypes, for which the success rate reached 87% (36% only for axonal CMT2).”<sup>7</sup> While NGS panels were able to identify causal variants in a shorter and more cost-effective time, the authors caution that this approach, “leads to the identification of numerous variants of unknown significance, which interpretation requires interdisciplinary collaborations between molecular geneticists, clinicians and (neuro) pathologists”.<sup>7</sup>

## Expert-authored review

In an expert-authored review, the following step-wise genetic testing strategy is recommended:<sup>1</sup>

- Step 1: “Single-gene testing for PMP22 duplication/deletion is recommended as the first test in all probands with CMT. PMP22 duplication (a 1.5-Mb duplication at 17p11.2 that includes PMP22) accounts for as much as 50% of all CMT.”
- Step 2: “A multigene panel that includes the seven most commonly involved genes (i.e., GDAP1, GJB1, HINT1, MFN2, MPZ, PMP22, and SH3CT2) as well as some or all of the other CMT-associated genes is most likely to identify the genetic cause of the neuropathy at the most reasonable cost while limiting identification of variants of uncertain significance and pathogenic variants in genes that do not explain the underlying phenotype.”
- Step 3: “Comprehensive genomic testing - which does not require the clinician to determine which gene(s) are likely involved – may be considered if a genetic cause has not been identified in Step 1 and Step 2. Exome sequencing is most commonly used; genome sequencing is also possible. Exome array (when clinically available) may be considered if exome sequencing is nondiagnostic.”
- “Given the complexity of the genetics of CMT, health care providers should consider referring at-risk relatives to a neurogenetics center or genetic counselor specializing in neurogenetics.”
- “For asymptomatic minors at risk for adult-onset conditions for which early treatment would have no beneficial effect on disease morbidity and mortality, predictive genetic testing is considered inappropriate, primarily because it negates the autonomy of the child with no compelling benefit. Further, concern exists regarding the potential unhealthy adverse effects that such information may have on family dynamics, the risk of discrimination and stigmatization in the future, and the anxiety that such information may cause.”

## Comprehensive CMT panels

Comprehensive CMT panels test most known genes related to CMT simultaneously, but this is not usually necessary or cost-effective, and therefore not recommended as first line tests.<sup>1,4</sup>

## Criteria

### Introduction

Requests for CMT testing are reviewed using these criteria.

**Known Familial Mutation Analysis**

- Previous Genetic Testing:
  - No previous genetic testing for the familial mutation, and
  - Pathogenic CMT-related mutation in a 1st or 2nd degree biologic relative, AND
- Diagnostic Testing for Symptomatic Individuals:
  - Distal muscle weakness and atrophy, or
  - Weak ankle dorsiflexion (e.g. foot drop), or
  - Distal sensory loss, or
  - Depressed or absent tendon reflexes, or
  - Foot deformity (e.g. high arches, hammer toes, pes cavus), or
  - Electrodiagnostic studies consistent with a peripheral neuropathy, OR
- Predisposition Testing for Presymptomatic/Asymptomatic Individuals:
  - Age 18 years or older

**PMP22 Deletion/Duplication Analysis**

- Previous Genetic Testing:
  - No previous PMP22 deletion/duplication analysis, and
  - No known CMT-related mutation in the member's family, AND
- Diagnostic Testing for Symptomatic Individuals:
  - Distal muscle weakness and atrophy, or
  - Weak ankle dorsiflexion (e.g. foot drop), or
  - Distal sensory loss, or
  - Depressed or absent tendon reflexes, or
  - Foot deformity (e.g. high arches, hammer toes, pes cavus), AND
- The member does not have a known underlying cause for their neuropathy (e.g. diabetic neuropathy, vitamin B12 deficiency, chronic inflammatory demyelinating polyneuropathy, known mutation), AND
- Member's electrodiagnostic studies are consistent with a primary demyelinating neuropathy



## CMT Neuropathy Multigene Panel

When a multi-gene panel is being requested and will be billed with the appropriate CPT panel code, 81448, the panel will be considered medically necessary when the following criteria are met:

- Previous Genetic Testing:
  - No previous CMT neuropathy multi-gene panel testing, and
  - No known CMT-related mutation in the member's family, AND
- Diagnostic Testing for Symptomatic Individuals:
  - Distal muscle weakness and atrophy, or
  - Weak ankle dorsiflexion (e.g. foot drop), or
  - Distal sensory loss, or
  - Depressed or absent tendon reflexes, or
  - Foot deformity (e.g. high arches, hammer toes, pes cavus), AND
- The member does not have a known underlying cause for their neuropathy (e.g. diabetic neuropathy, vitamin B12 deficiency, chronic inflammatory demyelinating polyneuropathy, known mutation), AND
- The panel includes the genes with the highest diagnostic yield for the member's suspected CMT neuropathy subtype, AND
- Member's electrodiagnostic studies are consistent with an axonal neuropathy or combined axonal and demyelinating neuropathy (e.g., CMT1 is NOT the most likely diagnosis), OR
- Member's electrodiagnostic studies are consistent with a primary demyelinating neuropathy (e.g., CMT1 is the most likely diagnosis) and PMP22 deletion/duplication analysis was previously performed and was negative

### Billing and reimbursement considerations

- When separate procedure codes will be billed for individual CMT-related genes (e.g., Tier 1 MoPath codes 81200-81355 or Tier 2 MoPath codes 81400-81408), the entire panel will be approved if the above criteria are met. However, the laboratory will be redirected to the use of an appropriate panel CPT code, 81448, for billing purposes.
- The billed amount should not exceed the list price of the test.
- Broad CMT neuropathy panels may not be medically necessary when a narrower panel is available and more appropriate based on the clinical findings.

- Genetic testing is only necessary once per lifetime. Therefore, a single gene included in a panel or a multi-gene panel may not be reimbursed if testing has been performed previously. Exceptions may be considered if technical advances in testing demonstrate significant advantages that would support a medical need to retest.
- If a panel was previously performed and an updated, larger panel is being requested, only testing for the medically necessary, previously untested genes will be reimbursable. Therefore, only the most appropriate procedure codes for those additional genes will be considered for reimbursement.
- If the laboratory will not accept redirection to 81448 due to their panel not sequencing at least 5 genes, the medical necessity of each billed component procedure will be assessed independently.
  - In general, only a limited number of panel components that are most likely to explain the member's presentation will be reimbursable. The remaining individual components will not be reimbursable.
  - When the test is billed with multiple stacked codes, only sequencing of the following genes may be considered for reimbursement, based on electrodiagnostic findings and the family history:
    - Primary demyelinating neuropathy with negative PMP22 deletion/duplication analysis (CMT1 suspected): MPZ, PMP22, LITAF (SIMPLE) and EGR2.
    - Primary axonal neuropathy (CMT2 suspected): MFN2, MPZ and HSPB1 (HSP27). If there is no evidence of male-to-male transmission in the family, GJB1 (for CMTX) is also reimbursable.
    - Combined axonal and demyelinating neuropathy (intermediate CMT suspected): DNM2, YARS, MPZ, and GNB4.

## References

### Introduction

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# Chromosomal Microarray for Prenatal Diagnosis

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v1.0.2020

## Introduction

Chromosomal microarray analysis for prenatal diagnosis is addressed by this guideline.

## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedures addressed by this guideline              | Procedure codes |
|---|-----------------|
| Chromosomal Microarray [BAC or CGH], Constitutional | 81228           |
| Chromosomal Microarray [SNP], Constitutional        | 81229           |

## What are copy number variants in developmental disorders

### Introduction

Copy number variants (CNVs) are deletions and duplications of genetic material that are too small to be seen with routine chromosome analysis (karyotyping). CNVs account for a significant proportion of congenital anomalies and developmental disorders without a clear etiology based on clinical findings.<sup>1,2</sup> CNVs are detected using chromosomal microarray analysis (CMA). CMA is known by several names including comparative genomic hybridization (CGH) and single-nucleotide polymorphism arrays (SNP-array).<sup>1,2</sup>

### Prevalence

Intellectual disability (ID) and congenital birth defects affect approximately 3-4% of the general population.<sup>1,2</sup> Sixty to eighty percent of major structural birth defects are identified prenatally by ultrasound evaluation.<sup>3</sup>

## Cause

The etiology of congenital anomalies is complex. Some developmental problems may be caused by environmental factors, such as injury and infection. However, genetic causes also play a significant role.<sup>1,2</sup>

## First-line test

Routine chromosome analysis (karyotyping) by chorionic villus sampling (CVS) or amniocentesis has historically been the first-line test in the evaluation of a pregnancy identified with congenital birth defects.<sup>4</sup> In 2010, CMA was recommended as the first-line postnatal test for individuals with developmental disabilities or congenital anomalies.<sup>1</sup> In 2012, a large multi-center study showed that prenatal CMA detected more clinically significant chromosomal abnormalities and CNVs than karyotyping. The additional yield was 6% when ultrasound showed a fetal abnormality and 1.7% when the reason for testing was maternal age or abnormal maternal serum screen results.<sup>5</sup>

CMA on chorionic villi or amniocytes is indicated in any pregnancy in which diagnostic testing for chromosome abnormalities and CNVs is desired.<sup>6,7</sup> Identifying an underlying genetic cause in these patients may<sup>1</sup>

- provide diagnostic and prognostic information
- guide prenatal management and decision-making, and
- allow for testing of family members and accurate recurrence risk counseling.

## CNV detected in fetus

If a unique CNV is detected in a fetus, it is usually necessary to test both parents to determine whether the CNV is inherited or a new (de novo) genetic change. This information along with parental clinical findings can be used to weigh the possibilities of a benign vs. pathogenic variant. However, even with parental studies, the clinical outcome may remain unclear.<sup>8</sup> A de novo variant is more likely to represent a pathogenic abnormality.<sup>8</sup>

## Test information

### Introduction

Prenatal diagnosis may include chromosomal microarray (CMA) testing.

### Chromosomal microarray

Chromosomal microarray (CMA) testing generally works by fluorescently tagging DNA from a patient test sample with one color and combining it with a control sample tagged in a different color. The two samples are mixed and then added to the array chip, where they compete to hybridize with the DNA fragments on the chip. By comparing

the test sample versus the control, computer analysis can determine where genetic material has been deleted or duplicated in the individual.

### **Coverage and resolution**

There are a growing number of CMA testing platforms, including non-chip based applications, which differ in approach and resolution. Testing guidelines do not endorse one CMA over another. However, international consensus guidelines do suggest that CMAs should have coverage better than that offered by a standard karyotype (~5 Mb), and resolution of greater than or equal to 400 kb throughout the genome.<sup>4</sup>

### **Subtelomeric and disease-specific FISH tests not needed**

CMAs include the subtelomeric regions and all known chromosome microdeletion syndrome regions, such as those for 22q11.2 (DiGeorge) syndrome, Williams syndrome (7p11.2), and Smith-Magenis syndrome (17p11.2). Therefore, subtelomeric and disease-specific FISH tests are not needed in parallel with CMA, or as follow-up to normal CMA results.

### **Cell division in culture not required**

In contrast to typical chromosome analysis, CMA testing does not require dividing cells in culture. This makes testing possible in samples that may be difficult to culture, such as those from perinatal losses.<sup>5,6</sup>

### **Limitations of CMA**

While there are significant advantages of CMA over conventional karyotyping with regard to resolution and yield, there are disadvantages as well. Limitations of CMA include

- the inability to detect
  - balanced translocations or inversions
  - certain forms of polyploidy
  - low level mosaicism
  - some marker chromosomes, and
- the detection of CNVs of uncertain clinical significance
- the inability to differentiate free trisomies from unbalanced Robertsonian translocations, and
- the high cost of testing as compared to traditional karyotyping.<sup>2</sup>

## Guidelines and evidence

### Introduction

This section includes relevant guidelines and evidence pertaining to CMA for prenatal diagnosis.

### American College of Obstetricians and Gynecologists Committee on Genetics and the Society for Maternal-Fetal Medicine

The American College of Obstetricians and Gynecologists and the Society for Maternal-Fetal Medicine (2016) published a joint practice bulletin regarding the application of chromosomal microarray in the prenatal setting. This practice bulletin recommended CMA "as the primary test (replacing conventional karyotype) for patients undergoing prenatal diagnosis for the indication of a fetal structure abnormality detected by ultrasound examination...It is recommended that chromosomal microarray analysis be made available to any patient choosing to undergo invasive diagnostic testing."<sup>6</sup>

### Diagnostic yield of CMA

Diagnostic yield of CMA testing differs based on clinical presentation. The results of one recent multicenter trial of CMA in the prenatal setting were published in 2012.<sup>5</sup> This study reported that CMA identified a clinically relevant deletion or duplication in 6% of prenatal cases with a structural anomaly and normal karyotype. In addition, 1.7% of prenatal cases with an indication of advanced maternal age or positive screening results and normal karyotype had a clinically relevant deletion or duplication identified by CMA.<sup>5</sup>

In a large series of fetuses with ultrasound anomalies and normal conventional karyotype, CMA detected chromosome abnormalities in 5% of fetuses and up to 10% in those with 3 or more anatomic abnormalities.<sup>12</sup>

### Criteria

- Genetic Counseling:
  - Pre- and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - No previous chromosomal microarray testing in the same pregnancy, AND
- Diagnostic Prenatal Testing:<sup>‡</sup>
  - The member has sufficient risk of fetal CNV to justify invasive prenatal diagnosis. [It is important to note that invasive diagnostic procedures such as chorionic villus sampling and amniocentesis are associated with risks; the

provider and patient must have determined that the associated benefits outweigh the risks.]

‡Microarray may also be used in association with in utero fetal demise, stillbirth, or neonatal death. If microarray will be performed on fetal tissue after delivery, reference the Chromosomal Microarray Testing for Developmental Disorders guideline.

### Exclusions and other considerations

- If routine karyotype and CMA are ordered simultaneously, only the most appropriate test based on clinical history will be considered for coverage.
- If CMA has been performed, the following tests are often excessive and thus not considered medically necessary. Each test may require medical necessity review.
  - Routine karyotype: Full karyotype in addition to CMA is typically considered excessive. However, a limited 5 cell analysis may be approved in addition to CMA if criteria for CMA are met. This approval may be subject to claims review to ensure that the appropriate procedure code for a limited 5 cell analysis is billed (CPT 88261 x1, 88230 x1, 88291 x1).
  - FISH analysis
  - Telomere analysis
  - More than one type of microarray analysis (i.e. if 81228 performed, 81229 is not medically necessary)

### Billing and reimbursement considerations

- FISH or other procedure codes that do not accurately describe the test methodology performed (e.g. 88271) are not eligible for reimbursement of CMA.

## References

### Introduction

These references are cited in this guideline.

1. Manning M, Hudgins L; for the Professional Practice and Guidelines Committee. American College of Medical Genetics Practice Guidelines: Array-based technology and recommendations for utilization in medical genetics practice for detection of chromosomal abnormalities. *Genet Med*. 2010 Nov 12(11);742-5. Available at: <http://www.acmg.net/ACMG/UploadedPDFS/PDFDocuments/Array-Based-Technology-and-Recommendations-for-Utilization-in-Medical-Genetics-Practice-for-Detectio.aspx>
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  12. Hillman SC, Pretlove S, Coomarasamy A, et al. Additional information from array comparative genomic hybridization technology over conventional karyotyping in prenatal diagnosis: a systemic review and meta-analysis. *Ultrasound Obstet Gynecol* 2011 Jan;37(1):6-14.

# Chromosomal Microarray Testing For Developmental Disorders

**MOL.TS.150.A**  
v1.0.2020

## Introduction

Chromosomal microarray testing for developmental disorders is addressed by this guideline.

## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedures addressed by this guideline       | Procedure codes |
|--|-----------------|
| Chromosomal Microarray [BAC], Constitutional | 81228           |
| Chromosomal Microarray [SNP], Constitutional | 81229           |
| Chromosomal Microarray [CGH], Constitutional | S3870           |

## What are copy number variants in developmental disorders

### Introduction

Copy number variants (CNVs) are small deletions and duplications of genetic material and account for a significant proportion of developmental disorders without a clear etiology based on clinical findings. CNVs are detected using chromosomal microarray (CMA) testing. CMA is known by several names including array-comparative genomic hybridization (aCGH) and single-nucleotide polymorphism arrays (SNP-array).

### Prevalence

Intellectual disability (ID) and congenital birth defects affect approximately 3-4% of the general population.<sup>1</sup> Autism spectrum disorders (ASD), which now includes autistic disorder, pervasive developmental disorder not otherwise specified (PDD-NOS), and Asperger syndrome are also of increasing concern, with recent CDC incidence figures estimating 1 in 59 affected children.<sup>2</sup>

## Cause

The etiology of developmental disorders is complex. Some developmental problems may be caused by environmental factors, such as injury and infection. However, genetic causes also play a significant role.<sup>1,3</sup>

A causative explanation can be determined in about 40-60% of patients with ID<sup>3</sup> and in over 30% of patients with ASD.<sup>3</sup> Identifying an underlying genetic cause in these patients may:<sup>3,4</sup>

- provide diagnostic and prognostic information
- improve health screening and prevention for some conditions
- allow for testing of family members and accurate recurrence risk counseling, and
- empower the patient and family to acquire needed services and support.

## Diagnostic yield

Diagnostic yield differs based on clinical presentation:

- Approximately 10-19% of people with unexplained ID or developmental delay (DD) will have CNVs.<sup>5-8</sup>
- A similar diagnostic yield for ASD is estimated at 7-10%.<sup>3</sup>
- About 13% of spontaneous pregnancy losses had CNVs identified in one small prospective study.<sup>9</sup>
- Chromosomal microarray may also be useful in the workup of non-immune fetal hydrops.<sup>10</sup>

## Parental testing

If a CNV is detected in a child, it may be helpful to test both parents to determine whether the CNV is inherited or a new (de novo) genetic change. This information along with parental findings can be used to weigh the possibilities of a benign vs. pathogenic variant. However, even with parental studies, the clinical outcome may remain unclear.<sup>6</sup> A de novo variant is more likely to represent a pathologic abnormality.<sup>6,7</sup>

## Test information

### Introduction

Testing for developmental disorders may include chromosomal microarray testing.

## Chromosomal microarray

Chromosomal microarray (CMA) testing generally works by fluorescently tagging DNA from a patient test sample with one color and combining it with a control sample tagged in a different color. The two samples are mixed and then added to the array chip, where they compete to hybridize with the DNA fragments on the chip. By comparing the test sample versus the control, computer analysis can determine where genetic material has been deleted or duplicated in the individual.

### Coverage and resolution

There are a growing number of CMA testing platforms, including non-chip based applications, which differ in approach and resolution. Testing guidelines do not endorse one CMA over another. However, international consensus guidelines do suggest that CMAs should have coverage better than that offered by a standard karyotype (~5 Mb), and resolution of greater than or equal to 400 kb throughout the genome.<sup>4</sup>

### Subtelomeric and disease-specific FISH tests not needed

CMAs include the subtelomeric regions and all known chromosome microdeletion syndrome regions, such as those for 22q11.2 (DiGeorge) syndrome, Williams syndrome (7p11.2), and Smith-Magenis syndrome (17p11.2). Therefore, subtelomeric and disease-specific FISH tests are not needed in parallel with CMA, or as follow-up to normal CMA results.

### Cell division in culture not required

In contrast to typical chromosome analysis, CMA testing does not require dividing cells in culture. This makes testing possible in samples that may be difficult to culture, such as those from perinatal losses.<sup>5,6</sup>

### Limitations of CMA

While there are significant advantages of CMA over conventional karyotyping with regard to resolution and yield, there are disadvantages as well. Limitations of CMA include

- the inability to detect
  - balanced translocations or inversions
  - certain forms of polyploidy
  - low level mosaicism
  - some marker chromosomes, and
- the detection of CNVs of uncertain clinical significance
- the inability to differentiate free trisomies from unbalanced Robertsonian translocations, and

- the high cost of testing as compared to traditional karyotyping.<sup>2</sup>

## Guidelines and evidence

### Introduction

This section includes relevant guidelines and evidence pertaining to chromosomal microarray testing for developmental disorders.

### American College of Medical Genetics

The American College of Medical Genetics (ACMG, 2010) Professional Practice and Guidelines Committee recommends CMA as a first-tier test for the evaluation of “multiple anomalies not specific to a well-defined genetic syndrome, apparently non-syndromic developmental delay/intellectual disability, and autism spectrum disorders.”<sup>5</sup>

### International Standard Cytogenomic Array Consortium

The International Standard Cytogenomic Array Consortium (ISCA, 2010) recommends offering CMA as a first-tier genetic test, in place of karyotype, for patients with unexplained developmental delay/intellectual disability, autism spectrum disorders, or birth defects.<sup>6</sup>

### American College of Obstetricians and Gynecologists and Society for Maternal Fetal Medicine

The American College of Obstetricians and Gynecologists (ACOG, 2016) and Society for Maternal Fetal Medicine (SMFM, 2016) joint committee opinion on chromosomal microarray states that:<sup>11</sup>

- “In cases of intrauterine fetal demise or stillbirth when further cytogenetic analysis is desired, chromosomal microarray analysis on fetal tissue (i.e. amniotic fluid, placenta, or products of conception) is recommended because of the increased likelihood of obtaining results and improved detection of causative abnormalities.”
- “Additional information is needed regarding the clinical use and cost-effectiveness in cases of recurrent miscarriage and structurally normal pregnancy losses at less than 20 weeks of gestation.”
- “The routine use of whole-genome or whole-exome sequencing for prenatal diagnosis is not recommended outside of the context of clinical trials until sufficient peer-reviewed data and validation studies are published.”

### Society for Maternal Fetal Medicine

The Society for Maternal Fetal Medicine (SMFM, 2016) published a consult series that states:<sup>12</sup>

- “We recommend that CMA be offered when genetic analysis is performed in cases with fetal structural anomalies and/or stillbirth and replaces the need for fetal karyotype in these cases (GRADE 1A).”

## Criteria

### Introduction

Requests for chromosomal microarray testing for developmental disorders are reviewed using these criteria.

### Criteria

- Genetic Counseling:
  - Pre- and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:\*\*
  - No previous chromosomal microarray (CMA) testing, AND
- Diagnostic Testing for Symptomatic Individuals:
  - Testing performed on living child or adult, and
  - Diagnosis cannot be made on clinical evaluation alone, and
  - Common aneuploidy (trisomy 13, 18, 21, or sex chromosome) is not a suspected diagnosis, and
  - One of the following presentations:
    - Apparently nonsyndromic DD/ID, or
    - Autism spectrum disorder, or
    - Multiple congenital anomalies† not specific to a well-delineated genetic syndrome, OR
- Diagnostic Testing for Intrauterine Fetal Demise or Stillbirth:
  - Common aneuploidy (trisomy 13, 18, 21, or sex chromosome) is not a suspected diagnosis, and
  - Multiple congenital anomalies† not specific to a well-delineated genetic syndrome, or
  - Fetal demise or stillbirth occurred at 20 weeks of gestation or later

†Multiple congenital anomalies defined as 1) two or more major anomalies affecting different organ systems or 2) one major and two or more minor anomalies affecting

different organ systems. [Major structural abnormalities are generally serious enough as to require medical treatment on their own (such as surgery) and are not minor developmental variations that may or may not suggest an underlying disorder.]

\*\*Microarray is considered a first tier test in the evaluation of postnatal developmental disorders. Therefore, it often is not necessary to do chromosome analysis or FISH in conjunction with microarray. Microarray requests following such testing will require review.

### Exclusions and other considerations

- CMA is not considered medically necessary in cases of family history of chromosome rearrangement in phenotypically normal individuals
- CMA is not considered medically necessary in individuals experiencing infertility, structurally normal pregnancy losses that occur at less than 20 weeks, or recurrent pregnancy loss.<sup>10</sup>
- If routine karyotype and CMA are ordered simultaneously, only the most appropriate test based on clinical history will be considered for coverage.
- If CMA has been performed, the following tests are often excessive and are not considered medically necessary. Each test may require medical necessity review:
  - Routine karyotype: Full karyotype in addition to CMA is typically considered excessive. However, a limited 5 cell analysis may be approved in addition to CMA if criteria for CMA are met. This approval may be subject to claims review to ensure that the appropriate procedure code for a limited 5 cell analysis is billed (CPT 88261 x1).
  - FISH analysis
  - Telomere/subtelomere analysis
  - More than one type of microarray analysis (i.e. if 81228 performed, 81229 is not medically necessary)
- When a multigene deletion/duplication panel is being requested and billed using a microarray procedure code (typically 81228 or 81229), please refer to the *Genetic Testing by Multigene Panels* clinical use guideline; do not apply the criteria in this guideline.

### Billing and reimbursement considerations

FISH or other procedure codes that do not accurately describe the test methodology performed (e.g. 88271) are not eligible for reimbursement of CMA.

## References

### Introduction

These references are cited in this guideline.

1. Manning M, Hudgins L; for the Professional Practice and Guidelines Committee. American College of Medical Genetics Practice Guidelines: Use of array-based technology in the practice of medical genetics. *Genet Med*. 2007;9(9):650-3.
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# Chromosome Analysis for Reproductive Disorders, Prenatal Testing, and Developmental Disorders

MOL.CS.289.A

v1.0.2020

## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedures addressed by this guideline | Procedure codes                           |
|--|---|
| Chromosome Analysis, Amniocentesis     | 88235<br>88267<br>88269<br>88291<br>88280 |
| Chromosome Analysis, Blood             | 88230<br>88262<br>88280<br>88291          |
| Chromosome Analysis, CVS               | 88235<br>88267<br>88269<br>88291<br>88280 |
| Chromosome Analysis; count 5 cells     | 88261                                     |

## What is a chromosome abnormality

### Definition

A chromosome abnormality is any difference in the structure, arrangement, or amount of genetic material packaged into the chromosomes.<sup>1</sup>

Humans usually have 23 pairs of chromosomes. Each chromosome has a characteristic appearance that should be the same in each person.<sup>1</sup>

Chromosome abnormalities can lead to a variety of developmental and reproductive disorders. Common chromosome abnormalities include Down syndrome (trisomy 21), trisomy 18, trisomy 13, Turner syndrome, and Klinefelter syndrome.

About 1 in 200 newborns has some type of chromosome abnormality.<sup>2</sup> A higher percentage of pregnancies are affected but lost during pregnancy. According to the American College of Obstetricians and Gynecologists (ACOG), "Fetuses affected with Down syndrome often do not survive pregnancy; between the first trimester and full term, an estimated 43% of pregnancies end in miscarriage or stillbirth."<sup>3</sup>

Chromosome abnormalities are also seen in pregnancy losses. 2-5% of cases of recurrent pregnancy loss are due to chromosome abnormalities, primarily balanced reciprocal translocations.<sup>4</sup> Individuals with balanced translocations will typically not experience any outward symptoms of the chromosome abnormality. However, they may conceive pregnancies with an unbalanced rearrangement, resulting in an increased risk for miscarriage, stillbirth, and live-born children with developmental disorders. Offspring who inherit the balanced translocation are usually asymptomatic, but will have the same reproductive risks as their parent.

## Test information

### Introduction

Chromosome analysis can be done on blood or tissue. This testing can also be performed prenatally on fetal cells from amniotic fluid (amniocentesis) or placenta (CVS).

Chromosome analysis, also called karyotyping, requires stimulating cells to divide, arresting cell division at metaphase when the chromosomes can be seen microscopically, and staining to visualize the banding patterns. Routine chromosome analysis allows visualization of about 400-550 bands per karyotype.<sup>5</sup> High resolution chromosome analysis allows visualization of finer details and up to 1000 bands per karyotype.<sup>5</sup>

Chromosome analysis can be done on blood or tissue. This testing can also be performed prenatally on fetal cells from amniotic fluid (amniocentesis) or placenta (CVS).

Once the chromosomes are prepared, chromosome analysis will identify any differences from normal that can be seen under the microscope. This includes entire missing or extra chromosomes, deletions or duplications within a chromosome that are large enough to be seen by microscope, and rearrangements including translocations and inversions.

Chromosome analysis will not detect submicroscopic abnormalities, such as Y chromosome microdeletions that cause 16% of azoospermia and severe oligospermia.<sup>3</sup> Specific probes or array CGH is required.

Chromosome analysis also cannot detect any single gene disorders (such as cystic fibrosis, Tay-Sachs, etc.).

## Guidelines and evidence

### Introduction

The following section includes relevant guidelines and evidence pertaining to chromosome analysis for purposes of prenatal testing, reproductive purposes, and developmental disorders.

### Prenatal Testing

Prenatal diagnosis through amniocentesis and CVS is standard of care in obstetrics practice.

#### **American College of Obstetricians and Gynecologists**

Consensus guidelines from the American College of Obstetricians and Gynecologists (ACOG, 2016) recommend that:<sup>6</sup>

- “All pregnant women should be offered prenatal assessment for aneuploidy by screening or diagnostic testing regardless of maternal age or other risk factors.”
- “Prenatal genetic testing cannot identify all abnormalities or problems in a fetus, and any testing should be focused on the individual patient’s risk, reproductive goals, and preferences.”
- “Genetic testing should be discussed as early as possible in pregnancy, ideally at the first obstetrics visit, so that first trimester options are available.”

#### **Society for Assisted Reproductive Technology and American Society for Reproductive Medicine**

Practice Committee opinion from the Society for Assisted Reproductive Technology (SART) and American Society for Reproductive Medicine (ASRM, 2008) indicates that “Prenatal diagnostic testing to confirm the results of PGD is encouraged strongly because the methods used for PGD have technical limitations that include the possibility for a false negative result.”<sup>7</sup>

#### **Society of Obstetricians and Gynaecologists of Canada**

The Genetics Committee of the Society of Obstetricians and Gynaecologists of Canada (SOGC, 2006) indicate, “Couples considering IVF-ICSI for male-factor

infertility should receive information, and if necessary formal genetic counseling, about the increased risk of de novo chromosomal abnormalities (mainly sex chromosomal anomalies) associated with their condition. Prenatal diagnosis by chorionic villus sampling (CVS) or amniocentesis should be offered to these couples if they conceive. (Evidence level II-2A).”<sup>8</sup>

## Reproductive Disorders

### American Society for Reproductive Medicine

The ASRM (2015)<sup>9</sup> did not include chromosome analysis in their recommendations for the routine evaluation of female infertility.

In a later guideline for the evaluation of male infertility, the ASRM (2015)<sup>10</sup> reaffirmed their previous recommendation for chromosome analysis in men with nonobstructive azoospermia or severe oligospermia prior to ICSI.

### American Society for Reproductive Medicine and Society for Male Reproduction and Urology

In 2008, the American Society for Reproductive Medicine (ASRM) and Society for Male Reproduction and Urology issued the following recommendation for the evaluation of azoospermia: “[...] men with nonobstructive azoospermia or severe oligospermia should be karyotyped before their sperm are used for ICSI.”<sup>11</sup>

### American College of Obstetricians and Gynecologists

The American College of Obstetricians and Gynecologists (ACOG, 2014) guidelines on ovarian insufficiency recommend the following for diagnosis:<sup>12</sup>

- “Menstrual irregularity for at least 3 consecutive months”
- “Follicle-stimulating hormone and estradiol levels (two random tests at least 1 month apart)”
- “Prolactin and thyroid function test”
- If the diagnosis is confirmed, karyotype is among the tests recommended for further evaluation.

### National Institute for Health and Clinical Excellence

In 2004 (updated 2013), the National Institute for Health and Clinical Excellence (NICE) made the following recommendations regarding the evaluation of infertility:<sup>13</sup>

- “Where a specific genetic defect associated with male infertility is known or suspected, couples should be offered appropriate genetic counseling and testing.”
- “Where the indication for ICSI is a severe deficit of semen quality or nonobstructive azoospermia, the man’s karyotype should be established.”

### **American Society for Reproductive Medicine**

ASRM (2012) guidelines state that evaluation of recurrent pregnancy loss can proceed after two consecutive clinical pregnancy losses, and should include peripheral karyotype of the parents.<sup>4</sup>

The ASRM 2008 guideline for the evaluation of amenorrhea state:<sup>14</sup>

- “The history and physical examination should include a thorough assessment of the external and internal genitalia.”
- “When the physical examination is normal (the majority of cases), the initial investigations should exclude pregnancy and estimate FSH and prolactin concentrations.”
- “Ovarian failure is confirmed by documenting an FSH level persistently in the menopausal range. In women under 30 with ovarian failure, a karyotype should be obtained to rule out sex chromosome translocation, short arm deletion, or the presence of an occult Y chromosome, which is associated with an increased risk of gonadal tumors.”

### **American College of Obstetricians and Gynecologists**

ACOG (2002) guidelines on recurrent pregnancy loss state, “Parental cytogenetic analysis should be offered to all couples with recurrent pregnancy loss. In addition, all couples in which one partner has been found to have a balanced translocation or inversion should be offered prenatal genetic diagnosis because of the increased risk of a karyotypic abnormality in the conceptus.”<sup>15</sup>

## **Developmental Disorders**

### **American Academy of Pediatric**

The American Academy of Pediatrics (2014)<sup>16</sup> published recommendations for the evaluation of children with intellectual disability or developmental delay. They state the following:

- “CMA now should be considered a first tier diagnostic test in all children with GDD/ID for whom the causal diagnosis is not known.”
- “G-banded karyotyping historically has been the standard first-tier test for detection of genetic imbalance in patients with GDD/ID for more than 35 years. CMA is now the standard for diagnosis of patients with GDD/ID, as well as other conditions, such as autism spectrum disorders or multiple congenital anomalies.”

### **American Academy of Child and Adolescent Psychiatry**

The American Academy of Child and Adolescent Psychiatry (AACAP, 2014) states that as a clinical standard, clinicians should coordinate an appropriate multidisciplinary assessment of children with ASD. This includes the following:<sup>17</sup>

- “All children with ASD should have a medical assessment, which typically includes physical examination, a hearing screen, a Wood’s lamp examination for signs of tuberous sclerosis, and genetic testing, which may include G-banded karyotype, fragile X testing, or chromosomal microarray.”
- “Unusual features in the child (e.g., history of regression, dysmorphology, staring spells, family history) should prompt additional evaluations... Genetic or neurologic consultation, neuroimaging, EEG, and additional laboratory tests should be obtained when relevant, based on examination or history (e.g., testing for the MECP2 gene in cases of possible Rett’s disorder).”

### **American College of Medical Genetics and Genomics**

American College of Medical Genetics and Genomics (2013) included a list of first tier tests in the evaluation of an individual with autism spectrum disorder. These include:<sup>18</sup>

- “Three-generation family history with pedigree analysis
- Initial evaluation to identify known syndromes or associated conditions
  - Examination with special attention to dysmorphic features
  - If specific syndromic diagnosis is suspected, proceed with targeted testing
  - If appropriate clinical indicators present, perform metabolic and/or mitochondrial testing (alternatively, consider a referral to a metabolic specialist
- Chromosomal microarray: oligonucleotide array-comparative genomic hybridization or single-nucleotide polymorphism array
- DNA testing for Fragile X (to be performed routinely for male patients only.”

American College of Medical Genetics and Genomics (2005) published recommendations for cytogenetic evaluation for developmental delay. The final recommendations stated:<sup>19</sup>

- “For any child with unexplained MR/DD, even in the absence of dysmorphic facial features, other clinical features or positive family history, routine chromosome analysis (minimum 550-band resolution) is indicated.”
- “For children with clinical features of known chromosomal abnormality syndromes (e.g., Down syndrome), cytogenetic analysis should be performed. The identification of a translocation may affect the family’s recurrence risk.”
- “High-resolution chromosome analysis is not routinely indicated unless a specific chromosomal region is to be investigated or there is a family history of a particular abnormality. These studies should be limited in focus and used when FISH is not available.”

- “For children with clinical features suggestive of a particular microdeletion/microduplication syndrome, FISH or other molecular techniques should be performed prior to or concurrently with chromosome analysis.”
- “If chromosome analysis is normal at 550-band resolution, subtelomere FISH testing may be considered.”

## Criteria

### Introduction

Requests for chromosome analysis are reviewed using the following criteria.

- Genetic Counseling:
  - Pre- and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - No previous chromosomal microarray (CMA) testing on the individual or the fetus, and
  - No previous chromosome analysis performed on the individual or the fetus, AND
- Prenatal Testing:
  - The member has sufficient risk of fetal aneuploidy to justify invasive prenatal diagnosis. [It is important to note that invasive procedures such as chorionic villus sampling and amniocentesis are associated with risks; the provider and patient must have determined that the associated benefits outweigh the risks), OR
- Testing for Individuals with Reproductive Disorders:
  - Males with azoospermia or severe oligozoospermia, defined as <5 million sperm per mL, without obstruction or congenital absence of the vas deferens, or
  - Females with ovarian failure (cessation of menses with elevated FSH) prior to age 30, or
  - Evidence of gonadal dysgenesis on physical examination or ultrasound, or
  - Two or more spontaneous, unexplained pregnancy losses conceived by the member, or
  - Abnormal chromosome arrangement (e.g. translocation or inversion) in a first-, second-, or third-degree biologic relative, OR
- Diagnostic Testing for Symptomatic Individuals with Developmental Disorders:



- Testing is performed on living child or adult, and
- Diagnosis cannot be made on clinical evaluation alone, and
- Common aneuploidy (trisomy 13, 18, 21, or sex chromosome) is a suspected diagnosis

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**Note** This guideline only addresses chromosome analysis for prenatal testing, reproductive disorders, and developmental disorders. This guideline does not address chromosome analysis for cancer. Please see the guideline Chromosome Analysis for Blood, Bone Marrow, and Solid Tumor Cancers for this indication.

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### **Billing and Reimbursement Considerations**

Chromosomal microarray (CMA) is considered a first tier test in the evaluation of postnatal developmental disorders. Therefore, it often is not necessary to do chromosome analysis or FISH in conjunction with CMA. The following claims will be subject to review:

- Claims for chromosome analysis (CPT 88230), in conjunction with all other billed cytogenetics codes (CPT 88230-88291), will require medical necessity documentation and review if CMA (CPT 81228, 81229) has already been paid for the member on any date of service.
- Claims for CMA (CPT 81228, 81229) will require medical necessity documentation and review if chromosome analysis (88230) has already been paid for the member on any date of service.

If routine karyotype and CMA are ordered simultaneously, only the most appropriate test based on clinical history will be considered for reimbursement.

Full karyotype in addition to CMA is considered excessive. However, a limited 5 cell analysis may be approved in addition to CMA if criteria for CMA are met. This approval may be subject to claims review to ensure that the appropriate procedure code for a limited 5 cell analysis is billed (CPT 88261 x 1).

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# Chromosome Analysis for Blood, Bone Marrow, and Solid Tumor Cancers

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## Introduction

Chromosome analysis for blood, bone marrow, and solid tumors is addressed by this guideline.

## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedures addressed by this guideline    | Procedure codes         |
|---|-------------------------|
| Chromosome Analysis, Blood or Bone Marrow | 88237<br>88264<br>88291 |
| Chromosome Analysis, Solid Tumor          | 88239<br>88264<br>88291 |

## What are chromosome abnormalities in cancer

### Introduction

A chromosome abnormality is any difference in the structure, arrangement, or amount of genetic material packaged into the chromosomes. Chromosome abnormalities have been identified in many types of cancer, including leukemias, lymphomas, and solid tumors.<sup>1</sup>

### Chromosome abnormalities

Chromosome abnormalities can include

- deletions
- duplications
- balanced or unbalanced rearrangements, and

- gain or loss of whole or partial chromosomes.

Some chromosome abnormalities are characteristic of certain types of malignancy, and can be used to classify a type or subtype of cancer. For example, the “Philadelphia chromosome” is defined by a common translocation between chromosomes 9 and 22, and indicates chronic myelogenous leukemia in most cases.<sup>1</sup>

### **Disease monitoring and treatment response**

These abnormalities can play a key role in the development, diagnosis, and monitoring of cancer.<sup>1</sup>

The cytogenetics of a cancer can also change over time or in response to treatment. Therefore, chromosome analysis can be used to monitor disease progression and treatment response.<sup>1</sup>

## **Test information**

### **Introduction**

Chromosome analysis is routinely performed on bone marrow biopsy for the diagnosis and monitoring of leukemia, lymphoma, and other hematological disorders.

### **Chromosome analysis**

Chromosome analysis (karyotyping) requires stimulating cells to divide, arresting cell division at metaphase when the chromosomes can be seen microscopically, and staining to visualize the banding patterns.<sup>2</sup>

Chromosome analysis identifies any differences from normal that can be seen under the microscope. This includes all of the following:

- entire missing or extra chromosomes
- deletions or duplications within a chromosome that are large enough to be seen by microscope, and
- rearrangements including translocations and inversions.

### **Chromosome microarray**

Smaller copy number changes can be identified using chromosome microarray.<sup>3</sup>

## **Guidelines and evidence**

### **Introduction**

This section includes relevant guidelines and evidence pertaining to chromosome

analysis for blood, bone marrow, and solid tumors.

### **National Comprehensive Cancer Network**

The National Comprehensive Cancer Network (NCCN) considers chromosome analysis of a bone marrow biopsy to be routine standard of care in the evaluation of acute myeloid leukemia (AML), chronic myelogenous leukemia (CML), multiple myeloma (MM), myelodysplastic syndromes (MDS), and Burkitt's lymphoma (BL).<sup>4</sup>

### **American College of Medical Genetics**

The American College of Medical Genetics (ACMG, 2016) provides technical standards and guidelines for chromosome analysis in acquired chromosomal abnormalities of blood and bone marrow:<sup>5</sup>

- Bone marrow is the preferred specimen for cytogenetic analysis hematopoietic neoplasms.
- “Cytogenetic analyses of hematological neoplasms are performed to detect and characterize clonal chromosomal abnormalities that have important diagnostic, prognostic, and therapeutic implications.”
- “Furthermore, cytogenetic analysis can provide crucial information regarding specific genetically defined subtypes of these neoplasms that have targeted therapies.”
- “At time of relapse, cytogenetic analysis can be used to confirm recurrence of the original neoplasm, detect clonal disease evolution, or uncover a new unrelated neoplastic process.”
- Acute Myeloid Leukemia: “G-banded chromosome analysis should preferably be performed first. However, interphase FISH analysis for KMT2A (MALL) gene rearrangement is highly recommended on all diagnostic AML samples because these abnormalities are often cryptic and have a pronounced prognostic impact.”
- Acute Lymphocytic Leukemia: “B-lineage ALL is more frequent, accounting for 85% of pediatric ALL and 75% of adult ALL.1 - In pediatric/young adult B-lineage ALL, G-banded chromosome analysis should be performed simultaneously with interphase FISH.”

### **Criteria**

Chromosome analysis on a bone marrow biopsy is considered medically necessary when performed in the evaluation of leukemia, lymphoma, and other hematological disorders.

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### Introduction

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# ConfirmMDx for Prostate Cancer Risk Assessment

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## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedure addressed by this guideline | Procedure code |
|---------------------------------------|----------------|
| ConfirmMDx for Prostate Cancer        | 81551          |

## What is ConfirmMDx testing for prostate cancer

### Definition

The ConfirmMDx™ test (MDx Health) is a proprietary epigenetic assay that measures gene methylation associated with the presence of cancer. Results are intended to assist in determining which patients likely have a true negative biopsy, and which patients are at increased risk for occult cancer. Results may prevent unnecessary repeat biopsies in unaffected men, and triage higher risk patients for repeat biopsies and treatment, as needed.<sup>1</sup>

- Prostate cancer is the most common cancer among men, with over 150,000 new cases identified each year in the United States.<sup>2,3</sup> The median age at diagnosis is 66 years.<sup>4</sup> Older men are more likely to be affected than younger men, and African American men have higher rates compared to men of other ethnic backgrounds.<sup>4</sup>
- Screening programs for prostate cancer may allow for its early detection. Screening is typically performed by prostate-specific antigen (PSA) test and/or digital rectal examination (DRE).<sup>3</sup>
- Diagnosis is confirmed by prostate biopsy.<sup>5-7</sup> Biopsy is typically performed by collecting approximately 12 needle biopsy cores.<sup>7</sup>
- Initial biopsies only detect 65-77% of prostate cancers, and repeat biopsies are frequently performed.<sup>8,9</sup> The false negative rate of biopsy may be as high as 25%.<sup>10</sup>

### Test information

- ConfirmMDx™ measures the methylation levels (using quantitative methylation PCR) of 3 genes (GSTP1, APC and RASSF1) associated with prostate cancer. The



test is performed on formalin-fixed, paraffin-embedded prostate specimens from a 12-core biopsy.

- Results are reported with methylation positive/negative for each biopsy core, along with a map of the regions where methylation is distributed.<sup>1</sup>
- Negative predictive value of the test is approximately 90%, based on results of a large, blinded clinical evaluation study.<sup>11</sup>

## Guidelines and evidence

### National Comprehensive Cancer Network (NCCN)

- The National Comprehensive Cancer Network (NCCN, 2019) Clinical Practice Guidelines in Oncology for Prostate Cancer Early Detection state the following:<sup>7</sup>
  - “Those patients with negative prostate biopsies should be followed with DRE and PSA. Tests that improve specificity in the post-biopsy state—including percent PSA, 4Kscore, PHI, PCA3, and ConfirmMDx—should be considered in patients thought to be higher risk despite a negative prostate biopsy.”
  - “Biomarkers that improve the specificity of detection are not, as yet, recommended as firstline screening tests. However, there may be some patients who meet PSA standard for consideration of prostate biopsy, but for whom the patient and/or physician wish to further define the probability of high-grade cancer. A percent free PSA <10%, PHI >35, EPI score greater than 15.6, or 4K score (which provides an estimate of the probability of high-grade prostate cancer) are potentially informative in patients who have never undergone biopsy or after a negative biopsy; a PCA3 score >35 is potentially informative after a negative biopsy.”

### Literature Review

A number of peer-reviewed expert-authored studies that evaluate ConfirmMDx for detection of prostate cancer are available.<sup>8-17</sup> Most of these studies demonstrate the potential for the assay to help urologists accurately determine which patients likely have a true negative biopsy, and which patients are at increased risk for occult cancer.

### Criteria

Coverage for ConfirmMDx will be granted when the following criteria are met:

- No previous ConfirmMDx testing on the same sample when a result was successfully obtained, AND
- No previous 4Kscore testing performed after the most recent negative biopsy when a result was successfully obtained, AND

- Member is not under active surveillance for low stage prostate cancer, AND
- Negative prostate biopsy within the past 24 months, AND
- Member is considered at higher risk for prostate cancer by one or more of the following:
  - Family history of 1<sup>st</sup> degree relative with prostate cancer diagnosed younger than age 65 years,<sup>7,18,19,20</sup> and/or
  - Family history of two or more first-degree relatives with prostate cancer diagnosed at any age,<sup>19</sup> and/or
  - African American race,<sup>7,18,19,20</sup> and/or
  - Known mutation in a gene associated with increased risk of prostate cancer (e.g., BRCA1/2, HOXB13 (G84E mutation carriers), MLH1, MSH2, MSH6, PMS2, EPCAM),<sup>7,18</sup> and/or
  - PSA level of greater than 10 ng/ml,<sup>21</sup> and/or
  - PSA level increase of greater than 0.35 ng/ml/year if PSA level less than or equal to 10 ng/ml,<sup>7,22</sup> and/or
  - PSA doubling time of less than 3 years, when initial PSA level is greater than or equal to 4 ng/ml and other causes of rising PSA (i.e., infection, inflammation) have been ruled out for individuals whose PSA doubling occurred in less than 2 years<sup>23,24</sup>

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# Corus CAD for Obstructive Coronary Artery Disease

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## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedure addressed by this guideline | Procedure code |
|---------------------------------------|----------------|
| Corus CAD Gene Expression Test        | 81493          |

## What is the Corus CAD test for obstructive CAD

### Definition

Corus CAD is a blood-based test designed to exclude the presence of obstructive CAD in symptomatic patient.

- Heart disease is the leading cause of death for both men and women, accounting for 1 in 6 US deaths.<sup>1</sup> Coronary heart disease is the most common type of heart disease.<sup>2</sup>
- Patients with signs and symptoms of obstructive CAD, the result of a chronic inflammatory process that ultimately results in progressive luminal narrowing and acute coronary syndromes, may be evaluated with a variety of tests according to risk. Coronary angiography is the gold standard for diagnosing obstructive CAD, but it is invasive and associated with a low but finite risk of harm. Thus, coronary angiography is recommended solely for patients at high risk of CAD.<sup>3</sup>
- For patients initially assessed to be at low-to-intermediate risk, observation and noninvasive diagnostic methods, which may include imaging methods such as coronary computed tomography angiography (CCTA) or Myocardial Perfusion Imaging (MPI), may be recommended.
- Even noninvasive imaging methods, however, have potential risks of exposure to radiation and contrast material. Despite efforts to risk stratify patients with noninvasive testing, the subsequent yield of coronary angiography remains low. In one study of nearly 400,000 patients without known CAD undergoing elective coronary angiography, only approximately 38% were found to have obstructive CAD (if the definition of obstructive CAD was broadened to include stenosis of 50% or more in any coronary vessel, the prevalence increased to 41%).<sup>4</sup>

- If symptoms are atypical, then they should be concurrent with at least one risk factor such as high cholesterol, hypertension, family history, smoking, post-menopausal state, morbid obesity, and known non-cardiac vascular disease. <sup>4</sup>
  - It is suggested as a first-line diagnostic modality in the ambulatory care setting ahead of noninvasive imaging to rule out obstructive CAD as the cause of a patient's symptoms.
  - Corus CAD is intended for use in adult patients with stable, non-acute presentation of symptoms suggestive of obstructive CAD who:<sup>5</sup>
    - are not diabetic
    - have not been diagnosed with prior myocardial infarction (MI) nor have had a previous revascularization procedure
    - are not currently taking steroids, immunosuppressive agents or chemotherapeutic agents
    - have a known history of obstructive CAD

## Test information

- Corus CAD is a gene expression test that integrates the mRNA activity of 23 genes known to be involved in the development of and/or response to atherosclerosis into a single score, which can identify patients without obstructive CAD.<sup>6</sup>
  - Obstructive CAD is defined as:<sup>7</sup>
    - >50% stenosis in at least one coronary artery by Quantitative Coronary Angiography (QCA) core lab.
    - >50% QCA stenosis corresponds to 65 – 75% stenosis on clinical angiography.
- Some of these genes are sex-specific, accounting for key biological differences between men and women in the development of CAD.<sup>6</sup>
- A proprietary algorithm converts gene expression changes to a score that ranges from 1 to 40. The specific numeric value is translated into a percentage likelihood of the patient having obstructive CAD.<sup>7,8</sup>
  - Patients with scores less than or equal to 15 (“low score”) have a low likelihood (<8%) of having obstructive CAD.<sup>8</sup>
- The test potentially eliminates 46% of patients (those with scores less than or equal to 15) from further cardiac workup due to the low likelihood of their symptoms being caused by obstructive CAD.<sup>8</sup>
- Test performance in the intended use population (disease prevalence of about 15%):<sup>8</sup>

- Sensitivity = 89%
- Specificity = 52%
- Negative predictive value (NPV) = 96%

## Guidelines and evidence

- Corus CAD is not mentioned in any of the current applicable American College of Cardiology (ACC) or American Heart Association (AHA) guidelines, policy statements or scientific statements. <sup>9,10,11,12</sup>
- Clinical validity studies:
  - PREDICT<sup>7</sup>
    - Prospective, multi-center, blinded study in 39 U.S. sites.
    - 1569 non-diabetic patients undergoing cardiac catheterization.
    - The predictive accuracy of the Corus CAD score was good, with AUC =  $0.70 \pm 0.02$ .
    - Corus CAD significantly improved the ability to detect underlying obstructive CAD compared with clinical assessment (based on the Diamond-Forrester [D-F] clinical risk score).
    - Test significantly improved MPI accuracy in identifying underlying obstructive CAD.
  - COMPASS<sup>8</sup>
    - Prospective, multi-center study in 19 U.S. sites.
    - 431 non-diabetic symptomatic patients scheduled for MPI.
    - Primary end point: Receiver-operating characteristics (ROC) analysis to discriminate less than or equal to 50% stenosis by QCA.
    - Corus CAD significantly improved the ability to detect underlying obstructive CAD compared to MPI.
    - Corus CAD outperformed clinical factors as assessed by D-F criteria and Morise score.
    - Six-month follow-up on 97% of patients showed that 27 of 28 patients with major adverse cardiovascular events (MACE) or revascularization had scores >15.
- Clinical utility studies:
  - IMPACT-CARD<sup>13</sup>

- Prospective, single-center study at Vanderbilt University Medical Center.
- 83 prospective non-diabetic symptomatic patients presenting to the cardiologist's office with 83 matched historical controls.
- A change in the diagnostic testing pattern pre/post Corus CAD testing was noted in 48/83 patients (58% observed vs. 10% expected change,  $p < 0.001$ ).
  - Low Score (less than or equal to 15): 56% decreased intensity of testing; 44% had no change.
  - High Score ( $>15$ ): 52% increased intensity of testing; 39% had no change.
- 71% reduced testing rate in prospective group vs. historical cohort ( $p < 0.001$ ).
- Follow-up (chart review/phone call) in 180 d to ensure plan was followed & get MACE.
  - 0 patients of 161 (0.0%; 97% follow-up) had MACE.
- IMPACT-PCP<sup>14</sup>
  - Prospective, multi-center study of 4 practice sites.
  - 251 non-diabetic symptomatic patients presenting to the primary care physician's (PCP) office.
  - 51% of patients had a low score (less than or equal to 15).
  - A change in the diagnostic testing pattern pre/post Corus CAD testing was noted in 145/251 patients (58% observed vs. 10% expected change,  $p < 0.001$ ).
    - Low Score (less than or equal to 15): 60% decreased intensity of testing; 38% had no change.
    - High Score ( $>15$ ): 40% increased intensity of testing; 47% had no change.
  - Follow-up (chart review/phone call) in 30 days to ensure plan was followed & record MACE.
    - 1 patient of 247 (0.4%) had "MACE" (hemorrhagic CVA 5 days after testing, later determined not to meet criteria for MACE).
- REGISTRY-1<sup>15</sup>
  - Prospective, multi-center chart review of non-diabetic patients with typical and/or atypical symptoms suggestive of obstructive CAD at 7 sites.
  - 342 patients presenting to PCP office.



- Study designed for 670 patients with an interim look at 335.
- Study stopped early due to meeting primary endpoint.
- 49% of patients had a low score (less than or equal to 15).
- Patients with low Corus CAD score (less than or equal to 15) had 94% decreased odds of referral versus patients with high score (> 15) (p < 0.0001).
- For every 10 point decrease in score, had 14x decreased likelihood of referral to cardiology or advanced cardiac testing (p < 0.0001).
- Referral rate: 6% for low scores, 70% for high scores.
- Followed for minimum of 180 days (Avg. F/U = 267 days).
- 21 cardiac cath, 2 from patients with low scores; 19 from patients with high scores.
- MACE rate = 1.5% (5/342); 1 in low score group (percutaneous coronary intervention [PCI]), 3 in high score group (PCI x 2 and myocardial infarction [MI]) plus another not judged to be related to CV disease.
- Recently completed clinical trials
  - The PRESET Registry: A Registry to Evaluate Patterns of Care Associated With the Use of Corus<sup>®</sup> CAD in Real World Clinical Care Settings. ClinicalTrials.gov Identifier: NCT01677156.<sup>16</sup>
    - Primary outcome measures: “To describe referral patterns for cardiac care and testing within 1 month after gene expression testing.”<sup>16</sup>
  - Effect of Exercise Stress Testing on Peripheral Gene Expression Using CORUS<sup>™</sup> CAD Diagnostic Test. ClinicalTrials.gov Identifier: NCT01486030.<sup>17</sup>
    - Primary outcome measures: “Gene expression score difference between peak exercise and baseline.”<sup>17</sup>
  - PROspective Multicenter Imaging Study for Evaluation of Chest Pain - The PROMISE Trial. ClinicalTrials.gov Identifier: NCT01174550.<sup>18</sup>
    - Primary outcomes measures: “Time to primary endpoint as defined as a composite of death, myocardial infarction (MI), major complications from cardiovascular (CV) procedures or testing, and unstable angina hospitalization. The Kaplan-Meier events rates (cumulative percentage of participants with an event) were estimated for the anatomic and functional diagnostic test groups.”<sup>18</sup>
- While clinical utility studies have demonstrated that Corus CAD results can influence clinical decision making, there is insufficient data to demonstrate that

these decisions improve health outcomes as measured by the presence of major adverse cardiovascular events (MACE).

- The relatively small number of patients in the clinical utility trials (total n = 676) and the distribution of these patients (across less than a dozen practice sites) also raises questions about whether these results are generalizable to the entire US.

## Criteria

- This test is considered investigational and/or experimental.
  - Investigational and experimental (I&E) molecular and genomic (MolGen) tests refer to assays involving chromosomes, DNA, RNA, or gene products that have insufficient data to determine the net health impact, which typically means there is insufficient data to support that a test accurately assesses the outcome of interest (analytical and clinical validity), significantly improves health outcomes (clinical utility), and/or performs better than an existing standard of care medical management option. Such tests are also not generally accepted as standard of care in the evaluation or management of a particular condition.
  - In the case of MolGen testing, FDA clearance is not a reliable standard given the number of laboratory developed tests that currently fall outside of FDA oversight and FDA clearance often does not assess clinical utility.

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# Cxbladder

MOL.TS.236.A  
v1.0.2020

## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedure addressed by this guideline | Procedure code |
|---------------------------------------|----------------|
| Cxbladder Detect                      | 0012M          |
| Cxbladder Monitor                     | 0013M          |
| Cxbladder Triage                      | 81599          |
| Cxbladder Resolve                     | 81599          |

## What is Cxbladder

### Definition

Cxbladder is a family of non-invasive urinary biomarker tests manufactured by Pacific Edge Diagnostics. Cxbladder was developed as an alternative or adjunct to conventional tests for the initial diagnosis of bladder cancer or for later disease recurrence.<sup>1,2</sup>

- Bladder cancer is typically diagnosed using a combination of cytologic evaluation of urine, imaging tests, and cystoscopy. However, patients have reported that cystoscopy is uncomfortable and expensive, and as a result, investigators are exploring alternative methods to detect bladder cancer.
- The following tests are included in the Cxbladder family:<sup>2</sup>
  - Cxbladder Triage: used to rule out bladder cancer at an early stage.<sup>2</sup>
  - Cxbladder Detect: used to assess the probability of bladder cancer.<sup>2</sup>
  - Cxbladder Monitor: used to assess the probability of disease recurrence.<sup>2</sup>
  - Cxbladder Resolve: used to identify patients with high grade or late stage bladder cancer.<sup>2</sup>

## Test information

- According to the manufacturer, levels of messenger RNA (mRNA) of five biomarker genes, including MDK, HOXA13, CDC2, IGFBP5, CXCR2, are believed to be in higher concentrations in urine samples of patients with bladder cancer.
- The Cxbladder test involves the extraction, purification, and quantification of mRNA of the 5 biomarkers by reverse transcription (RT) quantification polymerase chain reaction (RT-qPCR).<sup>2</sup>
  - Cxbladder Triage
    - Combines bladder cancer risk factors as well as urinary biomarkers to rule out the presence of bladder cancer.<sup>2</sup>
  - Cxbladder Detect
    - Analyzes five urinary biomarkers to identify bladder cancer.<sup>2</sup>
  - Cxbladder Monitor
    - Combines clinical information and urinary biomarkers to assess the chance that bladder cancer has recurred.<sup>2</sup>
  - Cxbladder Resolve
    - Used to identify high grade or late stage bladder cancer in patients with haematuria.<sup>2</sup>

## Guidelines and evidence

### The National Comprehensive Cancer Network (NCCN)

- The National Comprehensive Cancer Network (2018) Clinical Practice Guidelines state the following regarding the use of available urinary biomarkers:<sup>3</sup>
  - “Consideration may be given to FDA-approved urinary biomarker testing by fluorescence in situ hybridization or nuclear matrix protein 22 in monitoring for recurrence.”
  - “For cTa high grade, cT1, and Tis, follow-up is recommended with a urinary cytology and cystoscopy at 3- to 6- month intervals for the first 2 years, and at increasing intervals as appropriate thereafter. Imaging of the upper tract should be considered every 1 to 2 years for high grade tumors (see Follow-up in the algorithm). Urine molecular tests for urothelial tumor markers are now available. Most of these tests have a better sensitivity for detecting bladder cancer than urinary cytology, but specificity is lower. However, it remains unclear whether these tests offer additional information that is useful for detection and management of non-muscle-invasive bladder tumors. Therefore, the panel considers this to be a category 2B recommendation.”

## The American Urological Association (AUA)

The American Urological Association (2016) states:<sup>4</sup>

- Urinary biomarkers are insufficiently accurate to replace cystoscopy for diagnosis/surveillance, though some appear to have predictive ability for assessing response to intravesical BCG and may help interpret indeterminate cytology.
- “At the time of first disease evaluation and treatment, none of the existent risk stratification tools or urinary biomarkers are sufficiently sensitive and specific to predict which patient will have an early tumor recurrence. Therefore, the most reliable way to know whether patients are at risk for early recurrence is by cystoscopic evaluation.”

## US Preventive Services Task Force (USPSTF)

In 2011, the U.S. Preventive Services Task Force updated its 2004 evidence review with regard to bladder cancer screening, and reported the following:<sup>5</sup>

- “no study evaluated the sensitivity or specificity of tests for hematuria, urinary cytology, or other urinary biomarkers for bladder cancer in asymptomatic persons without a history of bladder cancer. The positive predictive value of screening is less than 10% in asymptomatic persons, including higher-risk populations. No study evaluated harms associated with treatment of screen-detected bladder cancer compared with no treatment.”
- “screening tests that might be feasible for primary care include tests for hematuria, urinary cytology, and other urinary biomarkers. The U.S. Preventive Services Task Force (USPSTF) last reviewed the evidence on bladder cancer screening in 2004 but found insufficient evidence to guide a recommendation.”

## Peer Reviewed Literature

The accuracy of CxBladder tests has been evaluated in multiple peer reviewed studies.<sup>1,6-12</sup> Multiple limitations are noted, including indirect, low quality evidence; use of overlapping patient populations; non-blinded analysis; small sample sizes; short follow-up period, and/or bias in study design. For some tests in the suite, there is a lack of peer reviewed literature. There are no available studies that evaluated the effects on patient-relevant outcomes (survival, quality of life) of Cxbladder testing.

Sathianathen and colleagues carried out a systematic meta-analysis of published studies of urinary biomarker assays used to evaluate the clinical significance of primary hematuria.<sup>13</sup> The Cxbladder assay was included in the review. The authors concluded that:

- “The current diagnostic performance of biomarkers are inadequate to replace cystoscopy in the primary hematuria setting.”<sup>13</sup>

- “Given the current evidence, the use of these markers as an adjunct to cystoscopy for the evaluation of hematuria should be considered investigational.”<sup>13</sup>

Additional research is needed to assess how Cxbladder testing will be used in the disease management of patients with cancer. Questions persist regarding if Cxbladder has sufficient clinical utility to replace invasive cystoscopy or if Cxbladder has the potential to augment or clarify uncertain results obtained using conventional diagnostic methods.

### Ongoing Clinical Trials

The Cxbladder Monitoring Study: A Clinical, Non-Intervention Study of the Cxbladder Urine Test for the Detection of Recurrent Urothelial Carcinoma (UC).<sup>14</sup>

- NCT02700659
- Primary outcome measures
  - “Proportion of participants with bladder cancer who are correctly identified as having cancer (true positives) and no cancer (true negatives) by the Cxbladder test.”
    - “The Cxbladder test results will be compared to that of cystoscopy, which is the gold standard method for diagnosing urothelial cancer; the true positive and true negative rates will be measured, along with the false positive and false negative rates of the test. The results will be reported as sensitivity and specificity of the Cxbladder test for detecting urothelial cancer in patients with recurrent disease.”
  - “Probability that patients identified as having cancer and no cancer by the Cxbladder test truly have cancer (positive predictive value; PPV), and truly have no cancer (negative predictive value; NPV) respectively.”

### Criteria

- These tests are considered investigational and/or experimental.
  - Investigational and experimental (I&E) molecular and genomic (MolGen) tests refer to assays involving chromosomes, DNA, RNA, or gene products that have insufficient data to determine the net health impact, which typically means there is insufficient data to support that a test accurately assesses the outcome of interest (analytical and clinical validity), significantly improves health outcomes (clinical utility), and/or performs better than an existing standard of care medical management option. Such tests are also not generally accepted as standard of care in the evaluation or management of a particular condition.
  - In the case of MolGen testing, FDA clearance is not a reliable standard given the number of laboratory developed tests that currently fall outside of FDA oversight and FDA clearance often does not assess clinical utility.



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# CYP2C19 Variant Analysis for Clopidogrel Response

MOL.TS.155.A

v1.0.2020

## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedure addressed by this guideline | Procedure code |
|---------------------------------------|----------------|
| CYP2C19 Genotyping                    | 81225          |

## What is CYP2C19 testing for clopidogrel response

### Definition

Clopidogrel (Plavix®) is a prodrug that must be converted by CYP2C19 to an active form to inhibit clot formation. Variants in the CYP2C19 gene can result in reduced or enhanced enzyme function, which in turn affects clopidogrel activity.<sup>1,2</sup> The CYP2C19\*2 genetic variant alone accounts for about 12% of the variability in clopidogrel response.<sup>3</sup>

- CYP2C19 variant testing can be used to predict response to clopidogrel and modify the therapeutic strategy when necessary.<sup>1,2</sup> CYP2C19 variant testing determines if a person is a poor, intermediate, extensive, or ultrarapid metabolizer.
  - A person with two nonfunctional alleles (any combination of \*2-\*8) is classified as a poor metabolizer.<sup>1,2</sup> About 2-3% of Caucasians and blacks and up to 20% East Asians are poor metabolizers.<sup>4</sup>
  - People with one loss-of-function allele (\*1 and any combination of \*2-\*8) are intermediate metabolizers and represent 30-50% and 40-45% of these populations, respectively.<sup>4,5</sup>
  - The CYP2C19\*17 variant is associated with increased enzyme function or gain of function carriers. The CYP2C19\*17 allele has multiethnic frequencies of 3-21%, on average.<sup>6</sup>
- Several studies have demonstrated a reduced effectiveness of clopidogrel in people with reduced CYP2C19 metabolism. Poor metabolizers may be at increased risk of nonfatal stroke, MI, or death from any cause in patients with poor metabolism.<sup>3,7-10</sup> In contrast, an analysis of the CURE trial and ACTIVE trial, involving 5059 genotyped patients with acute coronary syndromes, did not find an effect of CYP2C19 genotype on outcome in homozygous, heterozygous or in those who were not carriers of the loss of function alleles.<sup>11</sup>

- CYP2C19 ultrarapid metabolizers (\*17 carriers) may be at increased risk for clopidogrel-related bleeding.<sup>6,12</sup> However, a recent study showed ultrarapid metabolizers had a greater benefit from clopidogrel therapy than non-carriers, without increased bleeding events.<sup>11</sup>

## Test information

- CYP2C19 testing identifies the most common gene variants and is performed on buccal or blood samples.
  - CYP2C19\*1 is the normal functioning allele.
  - The most common loss of function alleles are \*2 and \*3.
  - CYP2C19\*4, \*5, \*6, \*7, and \*8 alleles are much less common and are associated with absent or reduced CYP2C19 enzyme function.<sup>2</sup>
  - CYP2C19\*17 allele is associated with increased enzyme function or gain-of-function carriers.

## Guidelines and evidence

- U.S. Food and Drug Administration (FDA) approved product labeling for clopidogrel (Plavix®) was updated in July 2017 to revise a boxed warning of the diminished effectiveness in individuals with poor CYP2C19 metabolism. The revised boxed warning provides more general guidance about the impact of reduced platelet activity:<sup>1,2</sup>
  - “The effectiveness of Plavix® results from its antiplatelet activity, which is dependent on its conversion to an active metabolite by the cytochrome P450 (CYP) system, principally CYP2C19.”
  - “Plavix® at recommended doses forms less of the active metabolite and so has a reduced effect on platelet activity in patients who are homozygous for nonfunctional alleles of the CYP2C19 gene, (termed ‘CYP2C19 poor metabolizers’).”
  - “Tests are available to identify patients who are CYP2C19 poor metabolizers. Consider use of another platelet P2Y12 inhibitor in patients identified as CYP2C19 poor metabolizers.”
- In January 2015, the American Heart Association published a Scientific Statement on Basic Concepts and Potential Applications of Genetics and Genomics for Cardiovascular and Stroke Clinicians.<sup>13</sup> They noted:
  - “No cardiovascular pharmacogenetic application has yet been fully validated or widely adopted.”

- “In aggregate, the available data suggests that patients at highest risk for cardiovascular events (those who have undergone PCI [percutaneous coronary intervention] and are in the acute period after the procedure) may have worse outcomes on clopidogrel if they are reduced-function variant carriers.”
- “To date, no clinical trials assessing the utility of a CYP2C19 genotype test to guide and tailor therapy in a way that leads to improved patient outcomes have been published (although such clinical trials are underway)...”
- In December 2013, the American Heart Association published a Scientific Statement on Genetics and Genomics for the Prevention and Treatment of Cardiovascular Disease.<sup>14</sup> They surmised:
  - “...the magnitude of benefit of clopidogrel in a given patient population influences the risk associated with CYP2C19 loss-of-function variants. Specifically, if the magnitude of benefit is small, the impact of genotype on clopidogrel efficacy may also be small. Therefore, the risk of genotype appears to be greatest among patients for whom clopidogrel has the greatest efficacy (i.e., largest risk reduction), specifically those undergoing percutaneous coronary intervention with stenting. Meta-analyses suggest that this group may be at up to 3- to 4-fold increased risk for stent thrombosis among \*2 variant carriers.”
- In July 2013, the Clinical Pharmacogenetics Implementation Consortium published an update to their antiplatelet therapy recommendations for acute coronary syndrome (ACS) patients undergoing percutaneous coronary intervention (PCI) based on CYP2C19 status.<sup>6</sup> They concluded:
  - Genotype-directed therapy could identify those with ACS/PCI who benefit most from alternative antiplatelet therapy. Current data do not support the use of CYP2C19 genotype data to guide treatment in other scenarios.
  - Standard dosing of clopidogrel, as recommended in the product label, is warranted among ACS/PCI patients with a predicted CYP2C19 extensive metabolizer or ultrarapid metabolizer phenotype (i.e., \*1/\*1, \*1/\*17, and \*17/\*17).
  - If genotyping identifies a patient as a CYP2C19 PM (i.e., any combination of \*2 through \*8), literature supports the use of an alternative antiplatelet agent (e.g., prasugrel (Effient®) or ticagrelor (Brilinta®) when not contraindicated.
  - Data support switching to an alternative antiplatelet agent for CYP2C19 IMs (e.g., \*1/\*2, \*1/\*3, and \*2/\*17) when not contraindicated. However, given the wide inter-individual variability in residual platelet activity observed among clopidogrel-treated IMs, other factors that may place an IM at increased risk of a CV event (or adverse bleeding event) must be considered to most effectively individualize therapy.
  - It is currently premature to support an increased dosing strategy based on CYP2C19 genotype. Large clinical trials that evaluated higher-dose clopidogrel in ACS/PCI patients with high on-treatment platelet reactivity have concluded

that adjusting clopidogrel dose on the basis of platelet function monitoring alone does not reduce the incidence of death from CV causes, nonfatal myocardial infarction, or stent thrombosis.

- In August 2012, the American College of Cardiology Foundation (ACCF)/American Heart Association (AHA) Task Force on Practice Guidelines, in collaboration with the American College of Emergency Physicians, the Society for Cardiovascular Angiography and Interventions, and the Society of Thoracic Surgeons<sup>15</sup>, commented:
  - Genetic testing for CYP2C19 loss-of-function alleles may be considered on a case-by-case basis, especially for patients who experience recurrent ACS events despite ongoing therapy with clopidogrel.
- In May 2012, the American Heart Association published a Policy Statement on Genetics and Cardiovascular Disease.<sup>16</sup> They concluded:
  - "...it is now unambiguously clear that the use of standard doses of clopidogrel in patients with CYP2C19 loss-of-function variants is associated with an increased frequency of major adverse cardiovascular events and, in particular, of in-stent thrombosis among patients receiving drug-eluting stents."
- In July 2010, the American College of Cardiology Foundation (ACCF) and the American Heart Association (AHA) issued a Clopidogrel Clinical Alert for approaches to the FDA black box warning which include the following points:<sup>4,5</sup>
  - An emphasis on adherence to the existing ACCF/AHA guidelines for the use of antiplatelet therapy.
  - Clinicians should be aware that genetic variability in CYP enzymes alter clopidogrel metabolism and that diminished responsiveness to clopidogrel has been associated with adverse patient outcomes in registry experiences and clinical trials.
  - The predictive value of pharmacogenomic testing is very limited at this time, but studies are ongoing.
  - Evidence is insufficient to recommend routine genetic testing or platelet function testing but may be considered for people at moderate to high risk for poor outcomes. If a person is tested and found to be a poor metabolizer, other therapies should be considered:
    - For coronary patients - consider prasugrel (Effient®) (NOTE: Or ticagrelor (Brilinta®), now that it has been approved).
    - For TIA/stroke patients - consider aspirin or aspirin plus extended release dipyridamole. Prasugrel is contraindicated in TIA/stroke (NOTE: ticagrelor (Brilinta®) should not be used in patients with active pathological bleeding or a history of intracranial hemorrhage).

- For people who experience adverse reactions (i.e. adverse CV event or thrombosis, not bleeding) on clopidogrel several options exist:
  - Clopidogrel can be switched to prasugrel (NOTE: Or ticagrelor, now that it has been approved).
  - Clopidogrel dose can be increased (though little data exists).
  - Platelet function testing may be performed to determine if patients are clopidogrel non-responders.
  - For stroke patients, aspirin alone or combination of aspirin plus extended-release dipyridamole can be considered.
- Higher loading doses and maintenance doses of clopidogrel have been found to improve platelet inhibition and might be considered alternatives for high-risk patients who respond poorly to clopidogrel. New antiplatelet drugs such as prasugrel and if approved, ticagrelor (NOTE: ticagrelor has been approved), are additional alternatives. Other possibilities are adding cilostazol (Pletal®) to standard doses of aspirin and clopidogrel, though data with this combination is still accruing. Follow up platelet function testing might be considered to ensure adequate platelet inhibition.
- Ongoing clinical trial:
  - NCT Number NCT01742117<sup>17</sup>
    - The TAILOR-PCI (Tailored Antiplatelet Initiation to Lesson Outcomes Due to Decreased Clopidogrel Response After Percutaneous Coronary Intervention) trial is evaluating clinical outcomes of CYP2C19-based treatment decisions in patients with acute coronary syndrome (ACS) or stable coronary artery disease (CAD). The trial plans to enroll 5,300 patients and randomize participants to either a conventional treatment arm or a CYP2C19 genotype-based antiplatelet therapy selection approach. Participants who are heterozygous or homozygous for CYP2C19\*2 or \*3 will be treated with ticagrelor instead of clopidogrel. The primary endpoint will include cardiovascular mortality, non-fatal MI, non-fatal stroke, severe recurrent ischemia, and stent thrombosis. The estimated study completion date is March 2020.

## Criteria

- Previous Testing:
  - No previous genetic testing of CYP2C19, AND
- Personal History:
  - Currently on clopidogrel therapy, or

- Use of clopidogrel therapy is being proposed for a patient at moderate to high risk for a poor outcome, such as:
  - Experiencing symptoms consistent with ACS when percutaneous coronary intervention is an option, and/or
  - Considering a drug-eluting stent

## Exclusions

- Current data do not support the use of CYP2C19 genotype data to guide treatment in other scenarios, including altering the dosing recommendation for clopidogrel based on the CYP2C19 genotype.

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# CYP2C9, VKORC1, and CYP4F2 Testing for Warfarin Response

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v1.0.2020

## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedures addressed by this guideline                                | Procedure codes |
|---|-----------------|
| CYP2C9 Genotyping   | 81227           |
| VKORC1 Genotyping   | 81355           |
| CYP4F2 Genotyping   | 81479           |
| Warfarin responsiveness testing by genetic technique using any method | G9143           |

## What is Warfarin sensitivity testing?

### Definition

Warfarin (Coumadin®) is a commonly prescribed anticoagulant with a narrow therapeutic range and a 20-fold inter-individual variation in dose requirements.<sup>1</sup> Incorrect dosage, especially during the initial dosing phase, is associated with either severe bleeding or failure to prevent thromboembolism.<sup>2</sup>

- Approximately 21% of patients who receive anticoagulant therapy will experience a major or minor bleeding event.<sup>2</sup> Environmental and genetic factors combined influence 55% of warfarin dose variability and include: age, height, body mass index (BMI), gender, diet, genetic variations in CYP2C9 and VKORC1, use of concomitant medications and indication for warfarin.<sup>3,4</sup>
- The activity of two genes [cytochrome P450 2C9 (CYP2C9) and vitamin K epoxide reductase complex subunit-1 (VKORC1)] impact the rate of warfarin metabolism and account for up to 40% of the inter-individual dose requirements for warfarin.<sup>4</sup> The addition of a third gene, cytochrome P450 4F2 (CYP4F2) accounts for an additional 2% of warfarin dosing variability.<sup>5</sup>
- CYP2C9 is a p450 enzyme that influences warfarin pharmacokinetics by impacting the rate of metabolism. Poor or intermediate metabolizing 2C9 variants are seen in between 2% to 20% of the population depending on ethnicity.<sup>2</sup> Carriers of alleles \*2

and \*3 have decreased warfarin metabolism and may require lower warfarin doses.<sup>4,6</sup>

- Vitamin K activity is important to the blood's ability to clot. VKORC1 influences the pharmacodynamics and sensitivity of warfarin on the vitamin K cycle. Approximately 14% to 89% of the population display VKORC1 enzyme inhibition making them more sensitive to warfarin.<sup>2</sup> Carriers of VKORC1 AA genotype (high warfarin sensitivity) require a significantly lower warfarin dose compared to individuals with genotype GA or GG.<sup>4,6</sup>
- CYP4F2 is a p450 enzyme that counteracts the effects of VKORC1 by limiting the excessive accumulation of Vitamin K. Depending on ethnicity, carriers of the \*3 allele (AA or GA genotypes) have a moderate 8-11% increase in warfarin dosing requirements compared to individuals with genotype GG.<sup>5,6</sup>
- Testing these three genes predicts variability in warfarin dosage requirements. The presence of gene variants in CYP2C9, VKORC1, and CYP4F2 indicate that more careful dosing and monitoring is required to achieve therapeutic anticoagulation and to decrease risk of bleeding or clotting during warfarin dose titration.<sup>6</sup>

## Test information

- The CYP2C9 and VKORC1 alleles are thought to be the predominant cause of the variation of warfarin dosing.<sup>6,7</sup>
- There are approximately 37 alleles reported in the CYP complex, however many do not have a functional impact.
  - Two alleles, \*2 and \*3 (CYP2C9\*2 and CYP2C9\*3) are linked to a slower metabolism of warfarin, thereby needing an increase in warfarin dose. These alleles are found in approximately 12.2% and 7.9%, respectively, of the European Caucasian population.<sup>8,9</sup>
  - Other variants, \*4, \*5, and \*6 are seen in the Asian and African American populations, but typically around a <1% incidence.<sup>9</sup>
- Diagnosis of these alleles can occur through sequence analysis of the CYP2C9, VKORC1, and CYP4F2 genes. Analytical sensitivity of alleles is expected to be greater than 98%.<sup>5,8</sup>

## Guidelines and evidence

- There has been a mixed response to genotyping from professional associations, payors, and other organizations, largely because data supporting the utility of genetic testing to improve clinical endpoints is conflicting. For example, two recent meta-analyses came to opposite conclusions:

- A genotype-guided dosing strategy did not result in a greater percentage of time that the INR was within the therapeutic range, fewer patients with an INR greater than 4, or a reduction in major bleeding or thromboembolic events compared with clinical dosing algorithms.<sup>7</sup>
- Genotype-guided initial dosing is able to reduce serious bleeding events by approximately 50% (RR = 0.47; 95% CI, 0.23-0.96; P = 0.040) compared with clinically-guided dosing approaches.<sup>10</sup>
- The Clinical Pharmacogenetics Implementation Consortium (CPIC, 2017) guidelines state “This guideline recommends that pharmacogenetic warfarin dosing be accomplished through the use of one of the pharmacogenetic dosing algorithms...The two algorithms provide very similar dose recommendations...The warfarindosing.org website contains both algorithms, the Gage algorithm as the primary algorithm and the IWPC [International Warfarin Pharmacogenetics Consortium] algorithm as the secondary algorithm...” It also notes “In patients of African ancestry, CYP2C9\*5, \*6, \*8, \*11 are important for warfarin dosing. If these genotypes are not available, warfarin should be dosed clinically without consideration for genotype.”<sup>6</sup>
- In January 2015, the American Heart Association published a Scientific Statement on Basic Concepts and Potential Applications of Genetics and Genomics for Cardiovascular and Stroke Clinicians.<sup>11</sup> They noted:
  - “No cardiovascular pharmacogenetic application has yet been fully validated or widely adopted.”
  - “Building on these early findings, additional clinical studies of warfarin pharmacogenetics are underway.”
- The American College of Medical Genetics (ACMG, 2008) and the American College of Chest Physicians (ACCP, 2008) both suggest against routine genotyping to guide warfarin dosing until better evidence is available to support a policy decision, but the ACMG does say that testing might be useful to explain unexpected warfarin responses.<sup>8,12</sup>
- Product labeling for Coumadin (warfarin) has been updated based on FDA recommendation to include a table recommending initial dosing ranges for patients with different combinations of CYP2C9 and VKORC1 genotypes. Labeling also includes the range of expected therapeutic warfarin doses based on CYP2C9 and VKORC1 genotypes.<sup>4</sup>
- Publications based on the European Pharmacogenetics of Anticoagulant Therapy (EU-PACT) trial data have indicated that pharmacogenomics-driven dosing algorithms may need to be age- and ethnicity-specific. This revised approach to dosing algorithms will need additional research and validation.<sup>13,14</sup>
- A systematic meta-analysis review of 9 studies suggest that while “CYP2C9 testing could identify high-risk patients who might benefit from conservation induction regimens, lower maintenance doses, and more frequent clinical and laboratory

monitoring," there is insufficient evidence to support routine genotyping of patients due to a lack of established clinical utility and cost-effectiveness of testing.<sup>9</sup>

## Criteria

This test is considered investigational and/or experimental.

- Investigational and experimental (I&E) molecular and genomic (MolGen) tests refer to assays involving chromosomes, DNA, RNA, or gene products that have insufficient data to determine the net health impact, which typically means there is insufficient data to support that a test accurately assesses the outcome of interest (analytical and clinical validity), significantly improves health outcomes (clinical utility), and/or performs better than an existing standard of care medical management option. Such tests are also not generally accepted as standard of care in the evaluation or management of a particular condition.
- In the case of MolGen testing, FDA clearance is not a reliable standard given the number of laboratory developed tests that currently fall outside of FDA oversight and FDA clearance often does not assess clinical utility.

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# CYP2D6 Variant Analysis for Drug Response

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v1.0.2020

## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedures addressed by this guideline  | Procedure codes |
|---|-----------------|
| CYP2D6 Genotyping for Drug Response   | 81226           |
| CYP2D6 Common Variants and Copy Number, Mayo Clinic   | 0070U           |
| CYP2D6 Full Gene Sequencing, Mayo Clinic  | 0071U           |
| CYP2D6-2D7 Hybrid Gene Targeted Sequence Analysis, Mayo Clinic                                      | 0072U           |
| CYP2D7-2D6 Hybrid Gene Targeted Sequence Analysis, Mayo Clinic                                      | 0073U           |
| CYP2D6 trans-duplication/ multiplication nonduplicated gene targeted sequence analysis, Mayo Clinic | 0074U           |
| CYP2D6 5' gene duplication/ multiplication targeted sequence analysis, Mayo Clinic                  | 0075U           |
| CYP2D6 3' gene duplication/ multiplication targeted sequence analysis, Mayo Clinic                  | 0076U           |

## What is CYP2D6 testing

### Definition

The cytochrome P450 2D6 (CYP2D6) enzyme is involved in metabolizing many medications, including tamoxifen, tetrabenazine, deutetrabenazine and eliglustat.<sup>1,2,3,4</sup>

- Studies suggest that certain variations (polymorphisms) in the CYP2D6 gene result in reduced or absent enzyme function, which may lead to lower levels of active tamoxifen metabolites and reduced treatment efficacy.<sup>5-7</sup>

- CYP2D6 testing has, therefore, been proposed to guide adjuvant therapy decisions in some circumstances.
  - Tamoxifen users:
    - Pre and Postmenopausal women have a choice between tamoxifen and aromatase inhibitors.<sup>8</sup> Current data does not support the use of CYP2D6 testing to determine the effectiveness of tamoxifen therapy and testing is not recommended.
    - Testing is not recommended for patients considering tamoxifen in the preventative setting.<sup>8,9</sup>
  - Tetrabenazine (Xenazine) users:
    - Tetrabenazine is a “vesicular monoamine transporter 2 (VMAT) inhibitor indicated for the treatment of chorea associated with Huntington’s disease.”<sup>2</sup>
    - CYP2D6 testing is used to help guide tetrabenazine dosage in patients that are being considered for a tetrabenazine dose greater than 50mg.<sup>2</sup>
    - For extensive and intermediate metabolizers, “the maximum recommended daily dose is 100 mg and the maximum recommended single dose is 37.5 mg.”<sup>2</sup>
    - “In poor metabolizers, the initial dose and titration is similar to extensive metabolizers except that the recommended maximum single dose is 25 mg, and the recommended daily dose should not exceed a maximum of 50 mg.”<sup>2</sup>
  - Deutetrabenazine (Austedo) users:
    - Deutetrabenazine is a “reversible depletor of monoamines (such as dopamine, serotonin, norepinephrine, and histamine) from nerve terminals”. Metabolites of deutetrabenazine, are reversible vesicular monoamine transporter 2 (VMAT2) inhibitors.<sup>4</sup>
    - Deutetrabenazine is indicated for the treatment of chorea associated with Huntington’s disease.<sup>4</sup>
    - Maximum recommended daily dosage of deutetrabenazine is 48 mg. In patients who are poor CYP2D6 metabolizers, however, the total daily dosage should not exceed 36 mg (with a maximum single dose of 18 mg).<sup>4</sup>
  - Eliglustat (Cerdelga) users:
    - Eliglustat is a “glucosylceramide synthase inhibitor indicated for the long-term treatment of adult patients with Gaucher disease type 1 who are CYP2D6 extensive metabolizers (EMs), intermediate metabolizers (IMs), or poor metabolizers (PMs) as detected by an FDA-cleared test.”<sup>3</sup>



- CYP2D6 intermediate metabolizers and extensive metabolizers are recommended to take a dose of 84 mg twice daily. This dosage requirement is decreased to 84 mg once a day for CYP2D6 poor metabolizers.<sup>3</sup>

## Test information

- CYP2D6 testing is usually performed on a buccal swab or blood sample using either polymerase chain reaction (PCR) or next generation sequencing (NGS) to look for certain common variants.
- More than 100 variants of the CYP2D6 gene have been noted to date.<sup>10,11</sup> Variants most commonly included in available test panels are: \*2, \*3, \*4, \*5, \*6, \*7, \*8, \*9, \*10, \*11, \*12, \*14, \*15, \*17, \*41, \*1XN, \*2XN, \*4XN, \*10XN, \*17XN, \*41XN.
- Genotype results are generally assigned a metabolizer phenotype:

| Phenotype                     | Genotype(s) <sup>11</sup>   |
|-------------------------------|---|
| Poor Metabolizer (PM)         | Two CYP2D6 inactive variants  |
| Intermediate Metabolizer (IM) | One normal and one inactive variant<br>One inactive and one reduced-activity variant<br><br>Two reduced-activity variants |
| Extensive Metabolizer (EM)    | Two normal CYP2D6 alleles   |
| Ultrarapid Metabolizer (UM)   | More than two copies of the normal CYP2D6 allele  |

- The frequency of the CYP2D6 metabolizer phenotypes varies with ethnicity. About 5-10% of Caucasians, 0-19% of African Americans, and approximately 1% of Chinese are poor metabolizers.<sup>10</sup>

## Guidelines and evidence

- Tetrabenazine, deutetabenazine, and eliglustat:
  - CYP2D6 is listed as an FDA-approved biomarker for both tetrabenazine and eliglustat.<sup>12</sup>
  - Product labeling for tetrabenazine, deutetabenazine, and eliglustat address CYP2D6 testing.<sup>2,3,4</sup>
- Tamoxifen
  - The FDA product labeling for tamoxifen states, "The impact of CYP2D6 polymorphisms on the efficacy of tamoxifen is not well established."<sup>12</sup>

- Evidence-based guidelines from the National Comprehensive Cancer Network (NCCN, 2019) state: “At this time, based on current data the [NCCN Breast Cancer] panel recommends against CYP2D6 gene testing for women being considered for tamoxifen therapy.”<sup>8</sup> (category 2A: The recommendation is based on lower level evidence and there is uniform NCCN consensus)
- Practice guidelines from the American Society of Clinical Oncologists (ASCO, 2009) state: “Given the limited evidence, CYP2D6 testing is currently not recommended in the preventive setting.”<sup>9</sup>
- Two important large clinical trials have most directly addressed clinical utility of CYP2D6 testing for tamoxifen response.<sup>13,14</sup> Both found that CYP2D6 genotype did not predict long-term outcome among tamoxifen users.
  - Regan et al. performed CYP2D6 variant testing on tumor tissue from 4393 patients enrolled in the BIG 1-98 trial and evaluated the association with breast cancer recurrence. BIG 1-98 was an international, randomized double-blind trial that compared tamoxifen monotherapy, letrozole (an aromatase inhibitor) monotherapy, and sequential therapy (2 years of one and 3 years of another). Patients were mostly Caucasian and all had postmenopausal, hormone receptor-positive, operable breast cancer. Results found a non-statistically significant association between metabolizer phenotype and recurrence (poor metabolizer vs. extensive metabolizer HR = 0.58, 95% CI = 0.28 to 1.21). The authors concluded “The results of this study do not support using the presence or absence of hot flashes or the pharmacogenetic testing of CYP2D6 to determine whether to treat postmenopausal breast cancer patients with tamoxifen.”<sup>13</sup>
  - Similarly, Rae et al. found no association between CYP2D6 genotype and breast cancer recurrence in people treated with tamoxifen from the randomized double-blind Arimidex, Tamoxifen, Alone or in Combination (ATAC) trial (n=1203; poor metabolizer vs. extensive metabolizer HR = 1.25, 95% CI = 0.55 to 3.15). The authors conclude “The results do not support the hypothesis that CYP2D6 genotype predicts clinical benefit of adjuvant tamoxifen treatment among postmenopausal breast cancer patients.”<sup>14</sup>
- The Clinical Pharmacogenomics Implementation Consortium (CPI, 2018) has evaluated outcomes using CYP2D6 genotyping to guide tamoxifen treatment for breast cancer. Because of wide variability of breast cancer types where tamoxifen is administered (breast cancer prevention, ductal carcinoma in situ, metastatic breast cancer, etc.) the guideline focused only on the use of CYP2D6 genotyping in estrogen-receptor positive (ER+) breast cancer. Using this narrow focus, there was moderate evidence to support improvements in breast cancer recurrence or event-free survival for patients treated with tamoxifen who were poor metabolizers (PM). However, there was weak evidence to support improvements in breast cancer-specific-survival and overall survival in this same PM group. The evidence regarding potential improvements in outcomes for patients treated with tamoxifen who were intermediate metabolizers (IM), normal metabolizers (NM) [also called

extensive metabolizers (EM)] and ultra-rapid metabolizers (UM) was also judged to be weak.<sup>15</sup>

## Criteria

CYP2D6 testing will be granted when the following criteria are met:

### Testing for Tetrabenazine Response

- No previous CYP2D6 testing performed, AND
- Member has a diagnosis of Huntington's disease, AND
- Treatment with tetrabenazine is being considered in a dosage greater than 50mg per day, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

### Testing for Deutetrabenazine Response

- No previous CYP2D6 testing performed, AND
- Member has a diagnosis of Huntington's disease, AND
- Treatment with deutetrabenazine is being considered in a dosage greater than 36mg per day, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

### Testing for Eliglustat Response

- No previous CYP2D6 testing performed, AND
- Member has a diagnosis of Gaucher disease, AND
- Treatment with eliglustat is being considered, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

### Exclusions and other considerations

- CYP2D6 testing for tamoxifen response is considered investigational/experimental and, therefore, not eligible for reimbursement.
- Additional CYP2D6 tests, denoted by CPT codes 0071U–0076U, are typically not medically necessary. Requests for these tests will be reviewed on a case by case basis.
- CYP2D6 testing for all other indications is addressed by the Pharmacogenomic Testing for Drug Toxicity and Response guideline.

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CYP2D6

# Cystic Fibrosis Testing

**MOL.TS.158.A**  
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## Introduction

Cystic fibrosis testing is addressed by this guideline.

## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedures addressed by this guideline | Procedure codes |
|--|-----------------|
| CFTR Targeted Mutation Analysis        | 81220           |
| CFTR Known Familial Mutation Analysis  | 81221           |
| CFTR Sequencing                        | 81223           |
| CFTR Deletion/Duplication Analysis     | 81222           |
| CFTR Poly T Tract (5T) Genotyping      | 81224           |

## What is cystic fibrosis

### Definition

Classic cystic fibrosis (CF) is a genetic disorder that causes chronic lung disease, pancreatic insufficiency, and male infertility.<sup>1,2</sup> It is caused by mutations in the CFTR gene.<sup>1</sup>

### Prevalence

CF affects approximately 1 in 3200 live births of northern European background.<sup>1</sup> While CF is most common in this background, it can occur in any ethnic group.<sup>2</sup>

### Inheritance

CF is an autosomal recessive condition. Males and females are equally likely to be affected.<sup>1</sup> If both parents are carriers of CF, the risk for a pregnancy to be affected is 1 in 4 (25%).<sup>1</sup> If one partner is affected with CF and the other partner is a carrier, the risk for a pregnancy to be affected is 1 in 2 (50%). Preimplantation and prenatal diagnosis are available for couples known to be at-risk.

## Prognosis and Treatment

Patient registry data from 2017 indicate that the median predicted survival for people with classic CF is about 44 years.<sup>3</sup> Treatment advances continue to extend the life of patients with CF. Several therapies in development or currently available target specific CFTR gene mutations, such as the FDA-approved Kalydeco™ for people with the G551D and other approved mutations, Orkambi™ for people with two copies of F508del; and Symdeko® for people with two copies of F508del or a single copy of 26 other specific mutations.<sup>4</sup> The genotype must be confirmed by molecular genetic testing in order to direct CFTR mutation-specific therapies.

## Detection

Most signs of CF cannot be identified on prenatal ultrasound examination. However, pregnancies in which fetal echogenic bowel is identified on ultrasound are at an increased risk to be affected with CF.<sup>1</sup>

Prenatal diagnosis for CF can be performed on a sample from chorionic villus sampling (CVS) or amniocentesis:<sup>1</sup>

- If both parents are known carriers, a mutation panel that includes both parental mutations is typically the test of choice.
- If only one parent is a carrier, or if testing is indicated because of echogenic bowel, testing with a large mutation panel or sequencing and deletion/duplication analysis offers greater sensitivity.

Newborn screening (NBS) programs include screening for CF, though the screening protocol may vary by state.<sup>5</sup>

## CFTR-related disorders and CF screen-positive, inconclusive diagnosis

Several other conditions that share some clinical similarities to CF, are also caused by mutations in the CFTR gene, but do not meet the diagnostic criteria for CF. These are called “CFTR-related disorders” and include congenital bilateral absence of vas deferens (CBAVD/CAVD), acute recurrent or chronic pancreatitis, and some respiratory tract conditions such as bronchiectasis, sinusitis, and nasal polyps.<sup>6</sup>

CBAVD is frequently identified after semen analysis shows absent sperm (azoospermia). CBAVD is often caused by one severe CFTR mutation and one mild mutation (including the 5T allele). At least one CFTR mutation can be found in up to 80% of men with CAVD.<sup>1</sup> Because of this association, CFTR analysis is routinely performed for men with azoospermia.

CF screen-positive, inconclusive diagnosis (CF-SPID), also referred to as *CFTR*-related metabolic syndrome (CRMS) "is used to describe an infant with an elevated trypsinogen on newborn screening, sweat chloride values  $\leq 60$  mEq/L, and up to two CFTR variants, at least one of which is not clearly categorized as a pathogenic variant and therefore not meeting diagnostic criteria for CF. These infants are typically asymptomatic, and knowledge of the natural history of CRMS continues to evolve."<sup>1,2</sup>

## Test information

### Introduction

Testing for cystic fibrosis tests may include CFTR mutation panels, CFTR sequencing, CFTR deletion/duplication analysis, intron 8 poly-T analysis, or CFTR known-familial mutation analysis.

### CFTR mutation panels

The American College of Medical Genetics has defined a panel of 23 common, pan-ethnic mutations that occur at a frequency of at least 0.1% in patients with cystic fibrosis.<sup>7</sup> While this panel was created for carrier screening purposes, the CF diagnostic guidelines also endorse its use in that setting for most patients.<sup>2</sup> Laboratories performing mutation panel testing routinely include all of these mutations. Many laboratories expand their panels with more mutations intended to increase the detection rate, particularly in non-Caucasian populations. Expanded mutation panels generally test for 70 or more CFTR mutations. The detection rates of expanded panels vary by laboratory and depend on the mutations included and the patient's ethnicity.<sup>1</sup>

### CFTR sequencing

CFTR sequencing detects more than 97% of mutations.<sup>1</sup> Sequencing is generally performed in reflex to normal mutation panel results, and reserved for specific situations in which a mutation panel is insufficient.

### CFTR deletion/duplication analysis

CFTR deletion/duplication analysis identifies mutations that sequencing would not find. This test is generally performed in reflex to normal sequencing results.<sup>1</sup>

### CFTR known familial mutation analysis

Once the mutations in affected or carrier family members have been identified, other relatives and at-risk pregnancies can be tested for those mutations.<sup>1</sup> Mutation panels are often used in this situation, as long as they include the family mutation(s). If a family mutation is rare or unique, testing targeted for that mutation may be needed.

### Intron 8 poly-T analysis

Intron 8 poly-T analysis identifies the number of thymidine bases in intron 8 of the CFTR gene. The three common variants are 5T, 7T, and 9T.<sup>1</sup> The 5T variant is considered a mild mutation with reduced penetrance, while 7T and 9T are considered normal variants.<sup>1</sup>

Testing is typically done in reflex to the identification of an R117H mutation by CFTR mutation panel testing.<sup>1,7,8</sup> The 5T variant also modifies the effect of the R117H mutation if the two mutations are located on the same chromosome.<sup>1</sup> R117H is a mild CFTR mutation included in the standard panel recommended by the American College



of Medical Genetics.<sup>7,8</sup> If R117H is identified by CF testing, reflex testing for the 5T variant is indicated to provide information relevant to genetic counseling.<sup>1,7,8</sup>

### **5T variant analysis**

5T variant analysis may also be included in CFTR testing panels when the testing is done specifically to evaluate a man with CAVD.<sup>1,8</sup> The 5T variant is more commonly found in men with CAVD in the absence of other symptoms of CF. In one large study, 25% of men with CAVD who had CFTR mutations identified had at least one copy of the 5T variant identified.<sup>9</sup>

## **Guidelines and evidence**

### **Introduction**

This section includes relevant guidelines and evidence pertaining to cystic fibrosis testing.

### **American College of Obstetrics and Gynecology**

Evidence-based guidelines from the American College of Obstetrics and Gynecology (ACOG) (2017) recommend that CF carrier screening using a mutation panel be offered to all couples who are pregnant or planning a pregnancy or those with a family history of CF.<sup>10</sup>

ACOG adds, "Cystic fibrosis is more common among the non-Hispanic white population compared with other racial and ethnic populations; however, because of the increasing difficulty in assigning a single ethnicity to individuals, in 2005, the American College of Obstetricians and Gynecologists recommended offering cystic fibrosis carrier screening to all patients."<sup>10</sup>

These ACOG guidelines state:<sup>10</sup>

- "Cystic fibrosis carrier screening should be offered to all women who are considering pregnancy or are currently pregnant.
- If the patient is a cystic fibrosis carrier, then her partner should be tested. During pregnancy, concurrent screening of the patient and her partner is suggested if there are time constraints for decisions regarding prenatal diagnostic evaluation.
- Current guidelines, revised by the American College of Medical Genetics and Genomics in 2004, recommend use of a panel that contains, at a minimum, the 23 most common mutations. A number of expanded mutation panels are now commercially available and can be considered to enhance the sensitivity for carrier detection, especially in non-Caucasian ethnic groups.
- Complete analysis of the CFTR gene by DNA sequencing is not appropriate for routine carrier screening. This type of testing generally is reserved for patients with cystic fibrosis, patients with negative carrier screening result but a family history of

cystic fibrosis (especially if family test results are not available), males with congenital bilateral absence of the vas deferens, or newborns with a positive newborn screening result when mutation testing (using the standard 23-mutation panel) has a negative result.

- For couples in which both partners are unaffected but one or both has a family history of cystic fibrosis, genetic counseling and medical record review should be performed to determine if CFTR mutation analysis in the affected family member is available.
- If a woman's reproductive partner has cystic fibrosis or apparently isolated congenital bilateral absence of the vas deferens, the couple should be provided follow-up genetic counseling by an obstetrician–gynecologist or other health care provider with expertise in genetics for mutation analysis and consultation.
- If both partners are found to be carriers of a genetic condition, genetic counseling should be offered."

### **American Society for Reproductive Medicine in partnership with the Society for Male Reproduction and Urology**

Consensus-based guidelines from the American Society for Reproductive Medicine in partnership with the Society for Male Reproduction and Urology (2018) recommend cystic fibrosis testing for men with CAVD and their partners, stating:<sup>11</sup>

- "...failure to identify a CFTR abnormality in a man with CBAVD does not exclude a mutation entirely, because 10%–40% are undetectable using common clinically available methods. During comprehensive screening with CFTR gene sequencing (as opposed to the commonly used delta F508, 30-mutation, or 100-mutation panels), a small fraction of CBAVD men will have no identifiable mutations." "Before any treatments using sperm from a man with CBAVD or congenital unilateral absence of the vas deferens (CUAVD), testing should be offered to his female partner to exclude the possibility (~4%) that she too may be a carrier."
- These guidelines do not specify a preferred testing methodology.

### **Cystic Fibrosis Foundation**

Consensus-based guidelines from the Cystic Fibrosis Foundation (2017) outline the ways in which a CF diagnosis can be established (see below). Characteristic features of CF include chronic sinopulmonary disease (such as persistent infection with characteristic CF pathogens, chronic productive cough, bronchiectasis, airway obstruction, nasal polyps, and digital clubbing), gastrointestinal/nutritional abnormalities (including meconium ileus, pancreatic insufficiency, chronic pancreatitis, liver disease, and failure to thrive), salt loss syndromes, and obstructive azoospermia in males (due to CAVD).<sup>2</sup>

**When at least one characteristic feature is present, a diagnosis of CF can be established by:**

- Two abnormal sweat chloride values; or
- Identification of two CF-causing CFTR gene mutations; or
- Characteristic transepithelial nasal potential difference (NPD)

**In the absence of symptoms, a CF diagnosis can be established in:**

- A newborn with two CF-causing CFTR gene mutations identified via newborn screening

"Individuals who are screen-positive and meet sweat chloride criteria for CF diagnosis should undergo CFTR genetic testing if the CFTR genotype was not available through the screening process or is incomplete." "Even in the presence of a positive sweat test, the identification of 2 CF-causing mutations should be confirmed in a clinical genetics laboratory capable of performing in-depth genetic analysis when required to further define CF risk (eg, the length of polyT tracts with the c.350G>A [legacy:R117H] CFTR mutation). Confirmation of genetic testing results with an FDA-approved companion diagnostic test also has additional value in therapy selection and access."<sup>2</sup>

These guidelines further state that, "Individuals presenting with a positive newborn screen, symptoms of CF, or a positive family history, and sweat chloride values in the intermediate range (30- 59 mmol/L) on 2 separate occasions may have CF. They should be considered for extended CFTR gene analysis and/ or CFTR functional analysis."<sup>2</sup>

### **Society of Obstetricians and Gynaecologists of Canada**

No US evidence-based guidelines have been identified that specifically address CF prenatal diagnosis for echogenic bowel. However, it is standard practice and evidence-based guidelines from the Society of Obstetricians and Gynaecologists of Canada (SOGC, 2005) state:<sup>12</sup>

- "Grade 2 and 3 echogenic bowel is associated with both chromosomal and nonchromosomal abnormalities. Expert review is recommended to initiate the following:...laboratory investigations that should be offered, including fetal karyotype, maternal serum screening, DNA testing for cystic fibrosis (if appropriate), and testing for congenital infection (II-2 A)." [Evidence level II-2: "Evidence from well-designed cohort (prospective or retrospective) or case-control studies, preferably from more than one centre or research group." Recommendation classification A: "There is good evidence to support the recommendation for use of a diagnostic test, treatment, or intervention."]

## Criteria Introduction

Requests for cystic fibrosis testing are reviewed using these criteria.

### CFTR Standard Panel Testing

- Genetic Counseling:
  - Pre- and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - No previous genetic testing for CFTR mutation(s), AND
- Diagnostic Testing for Symptomatic Individuals:
  - Individuals with an intermediate range/equivocal sweat chloride test (30-59mmol/L), or
  - Individuals with a negative sweat chloride test when symptoms of CF are present, or
  - Infants with meconium ileus or other symptoms indicative of CF and are too young to produce adequate volumes of sweat for sweat chloride test, or
  - Infants with an elevated IRT value on newborn screening, or
  - Males with oligospermia/azoospermia/congenital absence of vas deferens (CAVD)<sup>8</sup>, OR
- Mutation Identification to Guide Pharmacologic Therapy Selection
  - Individuals who meet diagnostic criteria for CF and are eligible for FDA-approved CFTR mutation-specific therapies, OR
- Carrier Screening:
  - Be of reproductive age and have potential and intention to reproduce, OR
- Prenatal Testing:
  - Either biological parent has a diagnosis of CF, or
  - Family history of CF in a first degree relative, or
  - Both parents are carriers of CF mutations included in the panel, or
  - Echogenic bowel has been identified on ultrasound in a fetus, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

## CFTR Known Familial Mutation Analysis

- Genetic Counseling:
  - Pre- and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Testing:
  - No previous genetic testing for the known CFTR family mutation(s), AND
- Diagnostic Testing for Symptomatic Individuals:
  - Individuals who have a suspected diagnosis of cystic fibrosis and the familial mutations to be tested were identified in a 1st degree biologic relative with CF, OR
- Mutation Identification to Guide Pharmacologic Therapy Selection
  - Individuals who meet diagnostic criteria for CF and are eligible for FDA-approved CFTR mutation-specific therapies, OR
- Carrier Screening:
  - Be of reproductive age and have potential and intention to reproduce, and
  - Familial CFTR mutation(s) in known biologic relative, OR
- Prenatal Testing:
  - Either biological parent is a known carrier of a CFTR mutation, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

## CFTR Sequencing

- Genetic Counseling:
  - Pre- and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - Previous CFTR standard panel was negative (no mutation found) or only one mutation was found, AND
- Diagnostic Testing for Symptomatic Individuals:
  - Individuals with a negative or equivocal sweat chloride test, and unexplained COPD or bronchiectasis with unexplained chronic or recurrent sinusitis and abnormal pulmonary function tests (PFTs), or
  - Infants with meconium ileus or other symptoms indicative of CF and are too young to produce adequate volumes of sweat for sweat chloride test, or

- Infants with an elevated IRT value on newborn screening and 0 or 1 mutations identified on standard panel testing, OR
- Mutation Identification to Guide Pharmacologic Therapy Selection
  - Individuals who meet diagnostic criteria for CF and are eligible for CFTR FDA-approved genotype-based therapies, or
  - No CFTR mutations that have FDA-approved genotype-based therapies identified by standard panel testing, OR
- Carrier Screening:
  - Be of reproductive age and have potential and intention to reproduce, and
  - An individual with a family history of CF with an unknown mutation, or
  - An individual whose reproductive partner is a known CF carrier, has a diagnosis of CF, or has a diagnosis of CAVD, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

#### **CFTR Deletion/Duplication Analysis**

- Genetic Counseling:
  - Pre- and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - No previous CFTR deletion/duplication testing, and
  - Previous CFTR gene sequencing was negative (no mutation found) or only one mutation was found, and
  - No known familial mutation, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

#### **CFTR Intron 8 Poly T Analysis**

- Genetic Counseling:
  - Pre- and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - No previous CFTR intron 8 poly T testing, AND
- Diagnostic Testing:

- CFTR mutation analysis performed and R117H mutation detected, or
- Diagnosis of male infertility (congenital absence of vas deferens [CAVD], obstructive azoospermia), or
- Diagnosis of non-classic CF, OR
- Carrier Testing:
  - CFTR mutation analysis performed and R117H mutation detected, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

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**Note** This guideline does not apply to CFTR testing for individuals with pancreatitis. CFTR testing for this indication is addressed by the guideline *Genetic Testing for Hereditary Pancreatitis*.

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# Genetic Testing for Dilated Cardiomyopathy

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## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedures addressed by this guideline                                    | Procedure codes |
|---|-----------------|
| Known Familial Mutation Analysis  | 81403           |
| LMNA Mutation Analysis  | 81406           |
| MYBPC3 Mutation Analysis  | 81407           |
| MYH6 Mutation Analysis  | 81407           |
| MYH7 Mutation Analysis  | 81407           |
| SCN5A Mutation Analysis   | 81407           |
| TNNT2 Mutation Analysis   | 81406           |
| TTN Mutation Analysis   | 81479           |
| Hereditary Cardiomyopathy Panel (at least 5 cardiomyopathy-related genes) | 81439           |

## What is Dilated Cardiomyopathy

### Definition

Dilated cardiomyopathy is a heart condition characterized by an enlarged left ventricle and systolic dysfunction in the absence of coronary artery disease or other structural heart disease.

### Incidence or prevalence

The best estimates of prevalence range from 1/250 to 1/1700.<sup>1</sup> However, large scale studies have failed to determine accurate incidence or prevalence data given that DCM is likely underdiagnosed.

## Symptoms

Average age of onset of DCM is in the 40s, but onset can begin as early as childhood. Enlargement of the left ventricle causes a weakened contraction of the heart muscle which in turn may lead to arrhythmias, including ventricular tachycardia or ventricular fibrillation, congestive heart failure, or thromboembolic disease.

## Cause

Between 20 and 50% of idiopathic dilated cardiomyopathy (IDCM) cases are thought to have a genetic etiology.<sup>1,2</sup> Approximately 35% of familial dilated cardiomyopathy cases are thought to have a genetic etiology.<sup>1,2</sup>

Syndromic causes include muscular dystrophies such as Duchenne and Becker muscular dystrophy, limb girdle muscular dystrophy, myotonic dystrophy, facioscapulohumeral muscular dystrophy, Friedreich's ataxia, and Emery-Dreifuss muscular dystrophy. Other syndromic causes include atypical Werner syndrome and Dunnigan-type familial partial lipodystrophy.

Non-genetic causes include infection, toxin exposure, metabolic disease, autoimmune disease, tachyarrhythmia, sarcoidosis, and coronary artery disease.<sup>3</sup>

## Inheritance

Familial DCM can be inherited in an autosomal dominant, autosomal recessive, or X-linked pattern, depending on the underlying syndrome or causative gene. While mitochondrial causes exist, they are exceedingly rare and often syndromic. Penetrance is reduced and age-dependent.<sup>1,4</sup> Variable expressivity has also been noted.<sup>1</sup> Several studies have identified 40 genes that are consistently linked to DCM.<sup>1,3,5,6</sup>

A strong genotype-phenotype correlation exists for LMNA mutations resulting in high risk for sudden death and significant conduction system disease. As such, recommendations have been made for those harboring such a mutation to be restricted from competitive sports.<sup>5</sup>

## Diagnosis

Diagnosis of DCM can be established through echocardiogram or MRI to visualize left ventricular enlargement. Systolic dysfunction (ejection fraction below 50%) should be measured through 2D echocardiogram. While an ECG/EKG may be used as a screening tool to evaluate for hypertrophy, conduction abnormalities, and arrhythmias, it is not sufficient for a diagnosis of dilated cardiomyopathy.

A diagnosis of IDCM is given when syndromic genetic causes and non-genetic causes are ruled out.

Familial IDCM is diagnosed when two or more patients who are first or second degree relatives have individually met criteria for dilated cardiomyopathy. The presence of peripartum or pregnancy associated cardiomyopathy can be counted toward a

diagnosis of familial dilated cardiomyopathy when present in a relative of an affected individual.

Pre-symptomatic diagnosis of DCM has been shown to prevent symptoms and increase life expectancy. Therefore, screening with ECG and echocardiogram starting in childhood is recommended for first degree relatives of DCM patients without a clear etiology.<sup>3</sup>

Evidence suggests testing symptomatic minors or testing minors for a known familial mutation can change their management and prevent sudden cardiac death.<sup>5</sup>

## Treatment

Early stages of DCM are often asymptomatic, but the natural history can be altered through treatment with reverse remodeling medications, pacemakers, or cardiac defibrillator device implantations. Severe or late stage disease otherwise refractory to these treatments is treated with heart transplant.<sup>4</sup>

## Survival

Survival depends on the etiology of DCM and whether the individual is symptomatic. In patients with heart failure, the survival is 20-30% eight years post-diagnosis.<sup>7</sup>

## Test information

### Introduction

Testing for dilated cardiomyopathy may include known familial mutation analysis, single gene sequence analysis, deletion/duplication analysis, or multi-gene panels testing.

### Sequence analysis

Until recently, most sequencing tests used the Sanger sequencing methodology that was originally developed in the 1970s. Sanger sequencing is labor intensive and did not lend itself to high-throughput applications.

Next generation sequencing (NGS), which is also sometimes called massively parallel sequencing, was developed in 2005 to allow larger scale and more efficient gene sequencing. NGS relies on sequencing many copies of small pieces of DNA simultaneously and using bioinformatics to assemble the sequence. NGS may not perform as well as Sanger sequencing in some applications.

NGS tests vary in technical specifications (e.g., depth of coverage, extent of intron/exon boundary analysis, methodology of large deletion/duplication analysis).

Sequence analysis detects single nucleotide substitutions and small (several nucleotide) deletions and insertions. Regions analyzed typically include the coding sequence and intron/exon boundaries. Promoter regions and intronic sequences may

also be sequenced if disease-causing mutations are known to occur in these regions of a gene.

The efficiency of NGS has led to an increasing number of large, multi-gene testing panels. NGS panels that test several genes at once are particularly well-suited to conditions caused by more than one gene or where there is considerable clinical overlap between conditions.

Results may be obtained that cannot be adequately interpreted based on the current knowledge base. When a sequence variation is identified that has not been previously characterized or shown to cause the disorder in question, it is called a variant of uncertain significance (VUS). VUSs are relatively common findings when sequencing large amounts of DNA with NGS.

Under certain circumstances, technologies used in multi-gene testing may fail to identify mutations that might be identifiable through single-gene testing. If high clinical suspicion exists for a particular syndrome testing for that syndrome should be performed instead of a broad multi-gene panel.

Since genes can be easily added or removed from multi-gene tests over time by a given lab, medical records must document which genes were included in the specific multi-gene test used and in which labs they were performed.

Additionally, tests should be chosen to

- maximize the likelihood of identifying mutations in the genes of interest
- contribute to alterations in patient management
- minimize the chance of finding variants of uncertain clinical significance

### **DCM Sequence Analysis**

The most common genetic causes of DCM include TTN, TNNT2, MYH7, MYH6, SCN5A, MYBPC3, and LMNA.<sup>5,8</sup>

Larger panels may include genes that are considered rare causes of DCM. These include the following: ABCC9, ACTC1, ACTN2, ANKRD1, BAG3, CRYAB, CSRP3, DES, DMD, DSG2, EMD, EYA4, ILK, LAMP2, LDB3/ZASP, MYPN, NEBL, NEXN, PDLIM3, PLN, PSEN1, PSEN2, RBM20, SGCD, TAZ, TCAP, TMPO, TNNC1, TNNI3, TPM1, TTR, TXNRD2, VCL.<sup>5</sup>

Test yield has not been demonstrably higher when large scale testing is used versus disease specific panels.<sup>9</sup>

No evidence exists to suggest testing of asymptomatic individuals when there is not a known familial mutation. This testing has not been shown to be effective due to the high volume of variants found with large cardiac panels. Instead, unaffected individuals with a suspicious family history should follow clinical monitoring guidelines.<sup>5</sup>

## Deletion/duplication analysis

Analysis for deletions and duplications can be performed using a variety of technical platforms including exon array, MLPA, and NGS data analysis.

These assays detect gains and losses too large to be identified through sequencing technology, often single or multiple exons or whole genes.

## Known familial mutation analysis

Analysis for known familial mutations is typically performed by Sanger sequencing, but if available, a targeted mutation panel that includes the familial mutation may be performed.

Known familial mutations analysis is performed when a causative mutation has been identified in a close relative of the individual requesting testing.

## Guidelines and evidence

### Introduction

The following section includes relevant guidelines and evidence pertaining to DCM testing.

### Heart Failure Society

The Heart Failure Society (2018) states:<sup>10</sup>

- “Guideline 4: Genetic testing is recommended for patients with cardiomyopathy (Level of evidence A)”
  - “4a: Genetic testing is recommended for the most clearly affected family member.”
  - “4b: Cascade genetic testing of at-risk family members if recommended for pathogenic and likely pathogenic variants.”
- “Genetic testing is recommended to determine if a pathogenic variant can be identified to facilitate patient management and family screening.”
- “Testing should ideally be initiated on the person in a family with the most definitive diagnosis and most severe manifestations. This approach would maximize the likelihood of obtaining diagnostic results and detecting whether multiple pathogenic variants may be present and contributing to variable disease expression or severity.”
- “Molecular genetic testing for multiple genes with the use of a multigene panel is now the standard of practice for cardio-vascular genetic medicine. Furthermore, multigene panel genetic testing is recommended over a serial single-gene testing

approach owing to the genetically heterogeneous nature of cardiomyopathy. Genetic testing and cascade screening have been shown to be cost-effective.”

- “In DCM, there is evidence for prognostication value of genetic testing and management implications for specific genetic findings, such as consideration of ICD placement for primary prevention in carriers of LMNA pathogenic variants.”

### **American College of Medical Genetics and Genomics**

The American College of Medical Genetics and Genomics (ACMG, 2018) published a practice resource on genetic testing for cardiomyopathies. This practice resource is an abbreviated version of the Heart Failure Society Guidelines above, on which ACMG collaborated. They state the following:<sup>11,12</sup>

- “Recommendation 1. Genetic testing is recommended for patients with cardiomyopathy.”
- “(a) Genetic testing is recommended for the most clearly affected family member.”
- “(b) Cascade genetic testing of at-risk family members is recommended for pathogenic and likely pathogenic variants.”
- “(c) In addition to routine newborn screening tests, specialized evaluation of infants with cardiomyopathy is recommended, and genetic testing should be considered.”

### **American College of Cardiology**

The American College of Cardiology does not have specific testing guidelines. However, the following recommendations have been published in the Journal for the American College of Cardiology (2016):<sup>5</sup>

- Sequence analysis for disease specific gene panel is appropriate in an affected proband, regardless of family history.
- At risk relatives should be tested for pathogenic or likely pathogenic alterations identified in the proband.
- Testing of at risk relatives is not recommended if no mutation is identified in the proband. Instead, clinical monitoring is recommended.

### **Heart Rhythm Society and European Society of Cardiology**

The Heart Rhythm Society and European Society of Cardiology (2011) states:<sup>13</sup>

- “Comprehensive or targeted (LMNA and SCN5A) DCM genetic testing is recommended for patients with DCM and significant cardiac conduction disease (i.e., first-, second-, or third-degree heart block) and/or a family history of premature unexpected sudden death.”

- “Mutation-specific genetic testing is recommended for family members and appropriate relatives following the identification of a DCM-causative mutation in the index case.”
- “Genetic testing can be useful for patients with familial DCM to confirm the diagnosis, to recognize those who are at highest risk of arrhythmia and syndromic features, to facilitate cascade screening within the family, and to help with family planning.”
- Genetic testing is appropriate on post-mortem samples when there is sudden cardiac death.

## Criteria

### Introduction

Requests for DCM testing are reviewed using the following criteria.

#### Known Familial Mutation analysis

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - No previous full sequence testing or deletion/duplication analysis, and
  - Known disease-causing mutation in a DCM gene identified in 1st or 2nd degree relative(s), AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy

#### Multi-Gene Panel Testing

- Genetic counseling:
  - Pre- and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing
  - No previous full sequencing of requested genes, and
  - No known mutation identified by previous analysis, AND
- Diagnostic Testing for Symptomatic Individuals
  - Personal History

- Confirmed diagnosis of dilated cardiomyopathy by appropriate imaging and/or electrophysiology modality (e.g. echocardiogram, electrocardiogram, MRI, angiogram), and
- No evidence of a specific syndrome in patient or family, and
- Non-genetic causes such as infection, toxin exposure, and metabolic/autoimmune disease have been ruled out, OR
- Personal & Family History Combination
  - A diagnosis of IDCM with one or more first or second degree relatives with a diagnosis of IDCM or peripartum cardiomyopathy, or
  - A diagnosis of IDCM with a suspicious family history including a first or second degree relative with sudden adult death or young cardiac or thromboembolic event, or
  - Mildly affected individual (defined as having dilated left ventricle but normal ejection fraction) with a first or second degree relative with a known diagnosis of IDCM who is deceased or otherwise unavailable for testing, AND
- Documentation from ordering provider indicating clear and specific impact result will have on medical care for the individual (e.g. change in surveillance or treatment plan), AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

### Deletion/Duplication Analysis

- Genetic Counseling:
  - Pre- and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - Member does not have a known mutation in a DCM gene, and
  - No previous deletion/duplication analysis for DCM genes, and
  - Meets criteria for full sequence analysis of DCM, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy

### Billing and Reimbursement Considerations

When multiple CPT codes are billed for components of a panel and there is a more appropriate CPT code representing the panel, eviCore will redirect to the panel code(s).



If the laboratory will not accept redirection to a panel code, the medical necessity of each billed component procedure will be assessed independently.

- In general, only a limited number of panel components that are most likely to explain the member's presentation will be reimbursable. The remaining panel components will not be reimbursable.
- When the test is billed with multiple stacked codes, only the following genes may be considered for reimbursement:
  - TTN
  - TNNT2
  - MYH7
  - MYH6
  - SCN5A
  - MYBPC3
  - LMNA

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# Decipher Prostate Cancer Classifier

MOL.TS.294.A

v1.0.2020

## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedures addressed by this guideline | Procedure codes |
|--|-----------------|
| Decipher Prostate Cancer Classifier    | 81479           |

## What are gene expression profiling tests for prostate cancer

### Definition

Prostate cancer (PC) is the most common cancer and a leading cause of cancer-related deaths worldwide. It is considered a heterogeneous disease with highly variable prognosis.<sup>1</sup>

- High-risk prostate cancer (PC) patients treated with radical prostatectomy (RP) undergo risk assessment to assess future disease prognosis and determine optimal treatment strategies. Post-RP pathology findings, such as disease stage, baseline Gleason score, time of biochemical recurrence (BCR) after RP, and PSA doubling-time, are considered strong predictors of disease-associated metastasis and mortality. Following RP, up to 50% of patients have pathology or clinical features that are considered at high risk of recurrence and these patients usually undergo post-RP treatments, including adjuvant or salvage therapy or radiation therapy, which can have serious risks and complications. According to clinical practice guideline recommendations, high risk patients should undergo 6 to 8 weeks of radiation therapy (RT) following RP. However, approximately 90% of high-risk patients do not develop metastases or die of prostate cancer, and instead may be appropriate candidates for alternative treatment approaches, including active surveillance (AS). As such, many patients may be subjected to unnecessary follow-up procedures and their associated complications, highlighting the need for improved methods of prognostic risk assessment.<sup>2,3</sup>
- Several genomic biomarkers have been commercially developed to augment the prognostic ability of currently available routine clinical and pathological tests and identify those patients most and least likely to benefit from a specific treatment strategy. Prognostic genomic tests, including gene expression profiling tests, may help to avoid overtreatment by reclassifying those men originally identified as high risk, but who are unlikely to develop metastatic disease. Genomic biomarkers may

also play a role in assisting clinicians to tailor personalized and more appropriate treatments for subgroups of PC patients, and improve overall health outcomes.<sup>2,3</sup>

## Test information

- Gene expression profiles (GEPs) evaluate the expression of several genes using one sample. Gene expression is determined through RNA analysis, using either reverse transcriptase (RT) polymerase chain reaction (PCR) or DNA microarrays.<sup>4</sup>
- Decipher<sup>®</sup> Prostate Cancer Classifier (GenomeDX Biosciences, Inc.)<sup>5</sup>
  - According to the manufacturer, the Decipher test is a tissue-based tumor genomic test that predicts the probability of metastasis within 5 years of RP, and provides an independent assessment of tumor aggressiveness, information that is distinct from that provided by the Gleason score or PSA.
  - Decipher analyzes a small tissue sample removed during surgery that is routinely archived or stored by the pathology lab. This test is intended for PC patients with stage T2 disease with positive margins, stage T3 disease, or rising serum PSA after RP. The test evaluates the expression of 1.4M RNA (44,000 genes) using RNA extracted from formalin-fixed paraffin-embedded (FFPE) tumor specimens of the index lesion, defined as the highest tumor stage or Gleason score.
  - The Decipher test result is expressed as a continuous risk score; a genomic classifier (GC) that ranges from 0 (lowest) to 1 (highest). Each score is associated with the probability of 5-year metastasis.

## Guidelines and evidence

### National Comprehensive Cancer Network

- The National Comprehensive Cancer Network (NCCN) 2018 Clinical Practice Guidelines on Prostate Cancer state the following regarding molecular assays:<sup>6</sup>
  - “Men with low or favorable intermediate risk disease may consider the use of the following tumor-based molecular assays: Decipher, Oncotype DX Prostate, Prolaris, Promark. Retrospective studies have shown that molecular assays performed on prostate biopsy or radical prostatectomy specimens provide prognostic information independent of NCCN risk groups.”
  - According to NCCN, the Molecular Diagnostic Services Program (MoIDX) recommendations stated the following:<sup>6</sup>
    - Decipher: “Cover post-RP for 1) pT2 with positive margins; 2) any pT3 disease; 3) rising PSA (above nadir)”

- Prolaris: “Cover post-biopsy for NCCN very-low, low-risk, and favorable intermediate-risk prostate cancer in patients with at least 10 years life expectancy who have not received treatment for prostate cancer and are candidates for active surveillance or definitive therapy.”
  - Oncotype DX Prostate: “Cover post-biopsy for NCCN very-low, low-risk, and favorable intermediate-risk prostate cancer in patients with at least 10 years life expectancy who have not received treatment for prostate cancer and are candidates for active surveillance or definitive therapy.”
  - ProMark: “Cover post-biopsy for NCCN very-low and low-risk prostate cancer in patients with at least 10 years life expectancy who have not received treatment for prostate cancer and are candidates for active surveillance or definitive therapy.”
- “These molecular biomarker tests have been developed with extensive industry support, guidance, and involvement, and have been marketed under the less rigorous FDA regulatory pathways for biomarkers. Although full assessment of their clinical utility requires prospective randomized clinical trials, which are unlikely to be done, the panel believes that men with low or favorable intermediate disease may consider the use of Decipher, Oncotype DX Prostate, Prolaris, or ProMark during initial risk stratification. In addition, Decipher may be considered during workup for radical prostatectomy PSA persistence or recurrence (category 2B).”

### **American Association of Clinical Urologists**

The American Association of Clinical Urologists has issued a position statement on genomic testing in prostate cancer that states the following:<sup>7</sup>

- “The AACU supports the use of tissue-based molecular testing as a component of risk stratification in prostate cancer treatment decision making.”

### **American Urological Association, ASTRO, and the Society of Urologic Oncology**

The AUA/ASTRO/SUO guideline for clinically localized prostate cancer states the following:<sup>8</sup>

- “Among most low-risk localized prostate cancer patients, tissue based genomic biomarkers have not shown a clear role in the selection of candidates for active surveillance.”

### **Decipher**

Decipher Literature Review<sup>9-34</sup>

- There is currently limited evidence in the peer-reviewed literature to support the widespread use of the Decipher test to accurately provide prognostic risk stratification among patients with prostate cancer who have undergone RP in

routine clinical practice. The relatively large evidence base, published primarily by the test manufacturer, consists of retrospective case-control and retrospective cohort studies evaluating the strength of the association between the Decipher score and incidence of disease recurrence (e.g., biochemical recurrence, metastasis) or PC-associated mortality. Hazard and odds ratios from univariate and multivariate logistic regression analyses show significant associations between the test and clinical endpoint. Also, study results indicate that Decipher consistently discriminates between men at 5-year risk of metastatic disease progression after RP and men without disease progression with reasonable AUC and c-index estimates. Several studies reported reclassification rates using the Decipher test, indicating that patient risk could be stratified differently based on Decipher results. These types of reclassification calculations are useful since the clinical usefulness of a prognostic test has been reported to be reliant on its ability to categorize patients into different and more accurate prognostic groups, providing accurate predictions about their future disease state, and ultimately guiding optimal treatment regimens. However, these various estimates may be subject to bias and confounders given the several limitations that weaken the quality of the individual studies, including publication bias; patient overlap; insufficient follow-up periods and small number of metastatic event cases; bias associated with retrospective analyses; lack of observer or investigator blinding; missing or flawed registry data; Decipher sampling issues; and considerable heterogeneity between cases and controls for various demographic, disease risk factors, and treatment regimens.

- It is not clear how results of the Decipher test will impact patient disease management and treatment strategies, and if any changes will translate into improved morbidity and mortality for high-risk PC patients. Results of new peer-reviewed studies of clinical utility will potentially provide higher quality evidence to better inform clinicians regarding patient selection criteria and appropriate use of the Decipher test among high-risk PC patients who are weighing the risk and benefits of various treatment options.
- Results of a meta-analysis of 5 studies showed that Decipher moderately correlates with clinicopathologic measures and does appear to add benefit more than standard clinicopathologic measures to accurately assess prognosis and predict metastases in men who have undergone RP.

## Clinical Trials

Observational prospective cohort study: A Validation Study on the Impact of Decipher® Testing on Treatment Recommendations in African-American and Non-African American Men With Prostate Cancer (VANDAAM Study)<sup>35</sup>

- “The primary purpose of this study is to determine whether a tumor test recently developed by GenomeDx Biosciences known as Decipher® can predict aggressive prostate cancer with the same accuracy in African-American men (AAM) as in non-African-American men (NAAM).”
- NCT02723734

- Recruiting

Observational patient registry study: Decipher Genomics Resource Information Database (GRID)<sup>36</sup>

- “To prospectively evaluate the utility of genomic expression data as a tool to better characterize the tumors of individual patients, and to understand how genomic information from individual patients undergoing routine clinical testing can be used in population-level analysis to improve treatment and outcomes.”
- NCT02609269
- Recruiting

Genomics in Michigan Impacting Observation or Radiation (G-MINOR)<sup>37</sup>

- “To determine the impact of Decipher test results on adjuvant treatment decisions of high-risk post-RP patients with undetectable post-op prostate specific antigen (PSA) compared to clinical factors alone.”
- NCT02783950
- Active, not yet recruiting

## Criteria

- This test is considered investigational and/or experimental.
  - Investigational and experimental (I&E) molecular and genomic (MolGen) tests refer to assays involving chromosomes, DNA, RNA, or gene products that have insufficient data to determine the net health impact, which typically means there is insufficient data to support that a test accurately assesses the outcome of interest (analytical and clinical validity), significantly improves health outcomes (clinical utility), and/or performs better than an existing standard of care medical management option. Such tests are also not generally accepted as standard of care in the evaluation or management of a particular condition.
  - In the case of MolGen testing, FDA clearance is not a reliable standard given the number of laboratory developed tests that currently fall outside of FDA oversight and FDA clearance often does not assess clinical utility.

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# DecisionDx Uveal Melanoma

MOL.TS.254.A  
v1.0.2020

## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedures addressed by this guideline | Procedure codes |
|--|-----------------|
| DecisionDx Uveal Melanoma              | 0081U           |
| DecisionDx-PRAME                       | 81401           |
| DecisionDx-UMSeq                       | 81479           |

## What is DecisionDx Uveal Melanoma

### Definition

Melanoma is the most deadly form of skin cancer.<sup>1</sup> The median age at diagnosis is 59 years.<sup>2</sup> According to NCCN, “The incidence of melanoma continues to increase dramatically, at an overall rate of 33% for men and 23% for women from 2002 to 2006.”<sup>2</sup>

- Risk factors for melanoma include the following: “skin type, previous history of melanoma, multiple clinically atypical moles or dysplastic nevi, and a positive family history of melanoma.”<sup>2</sup> Some individuals have a genetic mutation which increases their risk of melanoma.
- Screening for melanoma typically involves a visual skin examination. A skin biopsy of the lesion and pathological examination can diagnose melanoma.
- “Uveal Melanoma, commonly known as ocular or choroidal melanoma, is a rare cancer of the eye.”<sup>3</sup> Although this type of melanoma is treatable in most cases, approximately 50% of individuals with uveal melanoma will develop metastasis.<sup>3</sup>
- DecisionDx Uveal Melanoma (DecisionDx-UM) is a test designed to assess an individual’s risk of metastasis.

### Test information

- DecisionDx-UM measures gene expression of 15 genes present in an ocular melanoma tumor. This test is designed to assess the risk of metastasis within 5 years.

- DecisionDx-UM test results are reported as follows:
  - Class 1A – very low risk (2%) of metastasis within 5 years<sup>4</sup>
  - Class 1B – moderate risk (21%) of metastasis within 5 years<sup>4</sup>
  - Class 2 – high risk (72%) of metastasis within 5 years<sup>4</sup>
- DecisionDx-PRAME is a test that can be added on to the DecisionDx-UM assay. According to Castle Biosciences, “PRAME (preferentially expressed antigen in melanoma) is a cancer testis antigen gene that is not expressed at appreciable levels in normal adult tissues but its expression can become aberrantly increased in some types of cancer, including sarcoma, hematological malignancies, breast cancer, and melanoma.”<sup>5</sup>
- The manufacturer also offers the DecisionDX-UMSeq test, which is a 7-gene panel that identifies the following: mutations at hotspots in GNAQ, GNA11, CYSLTR2, PLCB4, and SF3B1; mutations in exons 1-2 of EIF1AX; and all coding exon mutations in the BAP1 gene.<sup>6</sup> This test uses next generation sequencing (NGS) to identify somatic mutations in patients with UM and can be ordered in addition to DecisionDX-UM using the same tissue specimen.
- The DecisionDx-UMSeq reports on clinically relevant mutations identified in any of the 7 gene targets. For each mutation found, the report describes any of the following:<sup>6</sup>
  - Genomic location of the mutation
  - Type of mutation
  - Functional change that occurs because of the mutation
  - Frequency that the mutation was detected in the sample; and
  - Potential consequences of that mutation on gene function and relevant literature references

## Guidelines and evidence

### National Comprehensive Cancer Network (NCCN)

- The National Comprehensive Cancer Network (NCCN, 2018) states the following regarding gene expression tests for uveal melanoma:<sup>7</sup>
  - “Biopsy of the primary tumor does not impact outcome, but may provide prognostic information that can help inform frequency of follow-up and may be needed for eligibility for clinical trials. Specimen should be sent for histology, chromosome analysis, and/or gene expression profiling. The risk/benefits of biopsy for prognostic analysis should be carefully considered and discussed.”

## Literature Review

Based on the review of the available peer-reviewed published literature, the DecisionDx-UM 15-gene assay has sufficient evidence for use as a prognostic test in patients diagnosed with primary, localized uveal melanoma to assist clinicians with predicting disease severity and improving disease management strategies.<sup>8-18</sup>

### DecisionDX PRAME and DecisionDX-UMSeq

There is currently insufficient evidence from 2 analytical validity studies regarding use of DecisionDX PRAME.<sup>19,20</sup> No clinical validity or clinical utility studies were identified. There is also no evidence evaluating use of DecisionDX-UMSeq. As a result, no conclusions can be drawn regarding the value and usefulness of these two additional tests.

## Criteria

- DecisionDx-UM testing is considered medically necessary when the following criteria are met:
  - No previous DecisionDx-UM testing performed after current diagnosis when a result was successfully obtained, AND
  - Member has primary, localized uveal melanoma, AND
  - No evidence of metastatic disease, AND
  - Rendering laboratory is a qualified provider of service per the Health Plan policy.

### DecisionDx-PRAME

- This test is considered investigational and/or experimental.
  - Investigational and experimental (I&E) molecular and genomic (MolGen) tests refer to assays involving chromosomes, DNA, RNA, or gene products that have insufficient data to determine the net health impact, which typically means there is insufficient data to support that a test accurately assesses the outcome of interest (analytical and clinical validity), significantly improves health outcomes (clinical utility), and/or performs better than an existing standard of care medical management option. Such tests are also not generally accepted as standard of care in the evaluation or management of a particular condition.
  - In the case of MolGen testing, FDA clearance is not a reliable standard given the number of laboratory developed tests that currently fall outside of FDA oversight and FDA clearance often does not assess clinical utility.

### DecisionDx-UMSeq

This test is considered investigational and/or experimental.

- Investigational and experimental (I&E) molecular and genomic (MolGen) tests refer to assays involving chromosomes, DNA, RNA, or gene products that have insufficient data to determine the net health impact, which typically means there is insufficient data to support that a test accurately assesses the outcome of interest (analytical and clinical validity), significantly improves health outcomes (clinical utility), and/or performs better than an existing standard of care medical management option. Such tests are also not generally accepted as standard of care in the evaluation or management of a particular condition.
- In the case of MolGen testing, FDA clearance is not a reliable standard given the number of laboratory developed tests that currently fall outside of FDA oversight and FDA clearance often does not assess clinical utility.

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# Dentatorubral-Pallidolusian Atrophy Testing

MOL.TS.159.A

v1.0.2020

## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedure addressed by this guideline | Procedure code |
|---------------------------------------|----------------|
| ATN1 Expansion Analysis               | 81177          |

## What is Dentatorubral-Pallidolusian Atrophy

### Definition

Dentatorubral-pallidolusian atrophy (DRPLA) is a progressive neurologic disorder.

- Age of onset ranges from one year of age to 72 years of age; the mean age of onset is 31.5 years of age.<sup>1</sup> The mean age of death is 49 years.<sup>2</sup> It demonstrates no sex bias, affecting males and females equally.<sup>2</sup>
  - In adults (over ~age 20), DRPLA presents as ataxia, choreoathetosis, and dementia or character changes.<sup>1</sup>
  - In people under ~age 20, DRPLA typically manifests with progressive intellectual deterioration, behavior changes, ataxia, myoclonus, and seizures.<sup>1</sup>
  - Neuropathology demonstrates degeneration of the dentatorubral and pallidolusian systems.<sup>3</sup> In addition, white matter lesions have been described.<sup>1</sup>
- DRPLA is also known as Naito-Oyanagi Disease; Haw River Syndrome;<sup>1</sup> Myoclonic Epilepsy with Choreoathetosis; Ataxia, Chorea, Seizures, and Dementia; and Dentatorubropallidolusian atrophy.<sup>4</sup>
- Although initially thought to be a disorder of the Japanese population, DRPLA has been diagnosed in people from a variety of other ethnic backgrounds.<sup>5</sup> DRPLA is most commonly recognized in populations of Japanese ancestry with an incidence of 2-7 per million.<sup>2</sup>
- The diagnosis of DRPLA is based on presenting findings and family history of DRPLA or by the results of molecular genetic testing demonstrating an expansion of the CAG trinucleotide/polyglutamine tract in ATN1.<sup>1</sup>
  - Normal alleles typically have a repeat length of 6 to 35.



- Individuals with DRPLA have a full penetrance allele with repeat length greater than or equal to 48 repeats, usually 48-93.<sup>1</sup>
- Alleles of 35–47 repeat length ("mutable normal alleles") are incompletely penetrant and have been associated with a milder DRPLA clinical phenotype in a small number of cases.<sup>2</sup> Mutable normal alleles are unstable and may increase in size when transmitted to offspring.<sup>1</sup>
- The age of onset and clinical presentation is inversely correlated with the size of the expansion. On average, people with large expansions have earlier onset than those with a smaller number of repeats.<sup>1,3</sup>
  - Although the size of the trinucleotide repeat is inversely correlated with the age of onset, the number of repeats cannot be used for specific prediction of symptoms or age of onset in an asymptomatic person. Repeat length is estimated to account for 50-68% of the variability in age of onset, the other contributing factors are not known.<sup>6</sup>
- DRPLA is inherited in an autosomal dominant manner. Males and females are equally likely to be affected. A person with DRPLA has a 50% chance of passing an ATN1 expansion mutation to each of his/her children.
  - Most individuals with DRPLA have inherited the mutation from a parent. The parent may not have had signs of DRPLA because the number of repeats he or she had were below the "threshold" for manifesting symptoms ("mutable normal" or "intermediate" alleles) or the number of repeats was within the disease-causing range, but small in number thus the parent with the abnormal allele has not yet developed symptoms.
  - Unaffected persons with mutable normal or intermediate alleles may pass this allele to offspring and the allele may undergo intergenerational expansion to a disease-causing range. The amount of that expansion depends upon the size of the repeat and gender of the transmitting parent. When the expansion is inherited from the father, increase in size of the expansion tends to be larger than when the disease-causing allele is inherited from the mother.<sup>1</sup> As a result, individuals who inherit the mutation from their father tend to have onset of disease 26-29 years earlier than their affected parent; when inheritance is from the mother, the onset of disease is about 14-15 years earlier.<sup>1</sup>

## Test information

- DRPLA molecular genetic testing identifies the number of CAG trinucleotide/polyglutamine repeats in ATN1. A repeat length of greater than or equal to 48 confirms the diagnosis of disease. Testing is >99% accurate. Once the diagnosis is confirmed in an affected relative, pre-symptomatic/predictive testing, prenatal diagnosis, and preimplantation genetic diagnosis are available to at-risk family members.

## Guidelines and evidence

- No U.S. guidelines exist for genetic testing for DRPLA.
- A 2018 expert-authored review states:<sup>2</sup>
  - “No established clinical diagnostic criteria have been established for DRPLA, with the genetic diagnosis typically made during the investigation of symptomatic individuals.”
  - “Diagnostic genetic testing should be considered in any individual with an autosomal dominant pattern of family history involving cognitive impairment, dementia, or movement disorder.”
  - “Consensus guidance on testing within adult-onset ataxia for DRPLA focuses on clinical findings, Asian ancestry, and family history as being important factors to consider.”
  - “Genetic testing is typically via polymerase chain reaction amplification across the ATN1 CAG repeat region followed by gel or capillary electrophoresis, which identifies 100% of pathogenic expansions of >48 CAG repeats. Although next-generation sequencing technologies are promising they have not been widely used or validated for the ATN1 repeat expansion and diagnosis of DRPLA, and repetitive genomic elements remain problematic to assay via short-read next generation sequencing technologies.”
- A 2016 expert-authored review states:<sup>1</sup>
  - Dentatorubral-pallidolusian atrophy (DRPLA) should be suspected in individuals with the following:
    - “Clinical features (by age):
      - Age <20 years: Ataxia, myoclonus, seizures, progressive intellectual deterioration
      - Age >20 years: Ataxia, choreoathetosis, dementia, psychiatric disturbance
    - Brain MRI findings: Cerebellar and brain stem atrophy
    - Family history: Consistent with autosomal dominant inheritance and Asiatic (mainly Japanese) familial origin. Note: (1) Absence of a family history of DRPLA does not preclude the diagnosis. (2) DRPLA is extremely rare outside of Asiatic populations.”
  - “The diagnosis of DRPLA is established in a proband with suggestive clinical findings and a family history of DRPLA or by the identification of a heterozygous pathogenic CAG trinucleotide expansion in ATN1 by molecular genetic testing. The CAG repeat length in individuals with DRPLA ranges from 48 to 93.”

- “Most individuals diagnosed with DRPLA have an affected parent. It is appropriate to evaluate both parents of an affected individual with molecular genetic testing even if they are asymptomatic.”
- “It is appropriate to consider testing symptomatic individuals regardless of age in a family with an established diagnosis of DRPLA.”
- “Testing of asymptomatic at-risk adults for DRPLA in the presence of nonspecific or equivocal symptoms is predictive testing, not diagnostic testing. When testing at-risk individuals for DRPLA, it is helpful to test for the CAG expansion in an affected family member to confirm the molecular diagnosis in the family.”
- “Testing of asymptomatic, healthy at-risk adults for DRPLA can be performed, taking into consideration their autonomy of choice and right to privacy.”
- “Potential consequences of such testing [predictive testing] (including but not limited to socioeconomic changes and the need for long-term follow up and evaluation arrangements for individuals with a positive test result) as well as the capabilities and limitations of predictive testing should be discussed in the context of formal genetic counseling prior to testing.”
- “Predictive testing of minors for adult-onset disorders for which no treatment exists is not considered appropriate. Such testing negates the autonomy of the child with no compelling benefit. Further, concern exists regarding the potential unhealthy adverse effects that such information may have on family dynamics, the risk of discrimination and stigmatization in the future, and the anxiety that such information may cause.”
- “If the disease-causing mutation has been identified in the family, prenatal diagnosis for pregnancies at increased risk is possible by analysis of DNA extracted from fetal cells obtained by amniocentesis (usually performed at ~15-18 weeks’ gestation) or chorionic villus sampling (usually performed at ~10-12 weeks’ gestation).”
- “Once the ATN1 (DRPLA) CAG trinucleotide repeat expansion has been identified in an affected family member, prenatal testing for a pregnancy at increased risk and preimplantation genetic diagnosis for DRPLA are possible.”

## Criteria

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Testing:
  - No previous ATN1 expanded repeat testing for DRPLA, AND
- Diagnostic Testing for Symptomatic Individuals:

- less than 20 years of age and 2 or more of the following:
  - Ataxia
  - Myoclonus
  - Seizures
  - Progressive intellectual deterioration/behavior changes
  - Brain MRI demonstrating cerebellar and brain stem atrophy
  - Affected 1<sup>st</sup> degree biologic relative or Japanese/Haw River descent, OR
- 20 years of age or older and 2 or more of the following:
  - Ataxia
  - Choreoathetosis
  - Dementia/psychiatric disturbance
  - Brain MRI demonstrating cerebellar and brain stem atrophy
  - Affected 1<sup>st</sup> degree biologic relative or Japanese/Haw River descent, OR
- Predisposition Testing for Presymptomatic/Asymptomatic Individuals:
  - ATN1 CAG trinucleotide expansion detected in 1<sup>st</sup> degree biologic relative, or
  - Suspected DRPLA in a deceased 1<sup>st</sup>, 2<sup>nd</sup> or 3<sup>rd</sup> degree biologic relative who was not genetically diagnosed

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# DermTech Pigmented Lesion Assay

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## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedures addressed by this guideline | Procedure code |
|--|----------------|
| DermTech Pigmented Lesion Assay        | 0089U          |

## What is melanoma

### Definition

According to the American Academy of Dermatology (AAD), the incidence of primary cutaneous melanoma has been increasing substantially for several decades. The incidence of melanoma has been reported to be increasing at a rate of 3% to 7% annually among fair-skinned Caucasian populations, which is faster than other major cancers.<sup>1</sup>

Melanoma accounts for the majority of skin cancer related deaths, but treatment is nearly always curative with early detection of disease. Minimal depth (thin) melanomas have a cure rate of nearly 100%, while tumors with a Breslow depth of greater than 4mm have a 10-year survival rate of less than 50%.<sup>1</sup>

Standard of care for the assessment of clinically suspicious pigmented skin lesions is surgical biopsy and subsequent histopathology. However, histopathology is believed to have inherent limitations. Some lesions that are likely to be true melanomas based on clinical behavior do not meet the complete set of histologic criteria to establish a melanoma diagnosis.<sup>1</sup> There is also considerable interrater variability with visual image and pattern recognition of skin lesions.<sup>2</sup> In an effort to improve patient survival, a number of novel noninvasive techniques have been developed to classify pigmented skin lesions at an earlier stage.<sup>3</sup>

## Test information

### Introduction

The Pigmented Lesion Assay (PLA) is a non-invasive method for the biopsy of clinically atypical pigmented lesions or moles using an adhesive patch to obtain mRNA from the surface of the suspicious lesion.

According to the manufacturer, the PLA assesses gene expression consistent with melanoma and is intended as a decision making aid for the clinician to determine whether or not to biopsy a pigmented skin lesion, clinically suspicious for melanoma.<sup>4</sup> The test is intended for use on pigmented lesions suspicious for melanoma that meet at least one of the A (asymmetry) B (border) C (color) D (diameter) E (evolving) criteria for which the clinician would like additional information prior to surgical biopsy. Uses of the PLA include the following: lesions being followed for change; lesions in cosmetically sensitive areas of the body; lesions on patients with possible risks for complications during surgical biopsy; or lesions among patients who refuse biopsy.<sup>4</sup>

The PLA is a non-invasive method for the biopsy of clinically atypical pigmented lesions or moles using an adhesive patch to obtain mRNA from the surface of the suspicious lesion. The method of adhesive tape stripping has been used for to obtain RNA from the stratum corneum for gene expression of other disorders, such as allergic and irritant skin reactions and psoriasis.<sup>5</sup> The PLA detects the expression of 2 specific genes, PRAME and LINC00518, both of which are believed to play key roles in oncogenesis and both of which have been shown to be elevated in melanoma. If one or more of the genes is detected by the PLA, the gene expressive is considered positive. The positive lesions generally undergo surgical biopsy to definitively establish a melanoma diagnosis.<sup>4</sup> The test manufacturer notes that this assay cannot be used on mucous membranes, palms of the hands, and soles of the feet.<sup>2</sup>

## Guidelines and evidence

### Introduction

The following section includes relevant guidelines and evidence pertaining to DermTech PLA.

#### American Academy of Dermatology (AAD)

The American Academy of Dermatology (AAD) acknowledges that the clinical and prognostic significance of the use of biomarkers and mutational analysis is still unclear and there are gaps regarding their clinical usefulness that have yet to be addressed.<sup>3</sup> The 2019 guideline states: "Routine molecular testing, including GEP, for prognostication is discouraged until better use criteria are defined. The application of molecular information for clinical management (eg, sentinel lymph node eligibility, follow-up, and/or therapeutic choice) is not recommended outside of a clinical study or trial"<sup>3</sup>

### Literature review

The evidence is currently insufficient to support the use of the PLA to accurately differentiate melanoma lesions from nonmelanoma lesions.<sup>1,2,5-9</sup> Study limitations include the small study populations, lack of generalizability of study results to more diverse melanoma subtypes, lack of blinding of primary readers, as well as early reports of insufficient RNA obtained from study samples. Independent prospective

clinical utility studies are currently lacking and it is unclear if the use of the PLA versus conventional diagnostic tools leads to changes in health care decision making and improvement in patient survival.

### **Additional studies**

Additional well-designed studies in larger patient populations with diverse melanoma subtypes are needed to add to the evidence base and corroborate the early study findings.

## **Criteria**

### **Introduction**

Requests for DermTech PLA are reviewed using the following criteria.

This test is considered investigational and/or experimental.

- Investigational and experimental (I&E) molecular and genomic (MolGen) tests refer to assays involving chromosomes, DNA, RNA, or gene products that have insufficient data to determine the net health impact, which typically means there is insufficient data to support that a test accurately assesses the outcome of interest (analytical and clinical validity), significantly improves health outcomes (clinical utility), and/or performs better than an existing standard of care medical management option. Such tests are also not generally accepted as standard of care in the evaluation or management of a particular condition.
- In the case of MolGen testing, FDA clearance is not a reliable standard given the number of laboratory developed tests that currently fall outside of FDA oversight and FDA clearance often does not assess clinical utility.

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### **Introduction**

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# DPYD Variant Analysis for 5-FU Toxicity

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## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedure addressed by this guideline | Procedure code |
|---------------------------------------|----------------|
| DPYD Genotyping                       | 81232          |

## What is dihydropyrimidine dehydrogenase (DPYD) testing for 5-FU toxicity

### Definition

5-fluorouracil (5-FU) and its prodrug capecitabine (Xeloda®) are common, broad-spectrum chemotherapeutic agents.<sup>1,2</sup>

- Dihydropyrimidine dehydrogenase (DPD) is the enzyme involved in the first step of the breakdown of 5-fluorouracil (5-FU), to 5-fluoro-5, 6-dihydro-fluorouracil (FUH2).<sup>3-5</sup>
- More than 80% of a dose of 5-fluorouracil is metabolized by DPD to FUH2. This metabolite has much lower toxicity than 5-FU.<sup>4</sup>
- A small percentage ( $\leq 10\%$ ) of 5-FU patients develop grade III-IV toxicity (neutropenia, nausea, vomiting, severe diarrhea, stomatitis, mucositis, and neuropathy),<sup>6,7</sup> which can be life-threatening.
- One primary cause for toxicity is DPD deficiency.<sup>4,6,7</sup> An estimated 0.1-3% of the population has DPD deficiency, caused by variants in the dihydropyrimidine dehydrogenase (DPYD) gene.<sup>5,8</sup> In particular, about 1% of the population has the DPYD IVS14 +1G>A variant (also called DPYD\*2A) that is found to be associated with a seven-fold increased risk for grade III/IV 5-FU toxicity.<sup>9-11</sup>
- Individuals found to have a DPYD genetic variant require lowered drug doses or alternative therapies.<sup>7,9</sup>
- Testing may also be used to investigate a possible cause of toxicity if a person experiences adverse effects while on a 5-FU based therapy.<sup>6</sup>

## Test information

- Testing for the DPYD variants DPYD\*2A (rs3918290), DPYD\*13 (rs55886062), and rs67376798 A (on the positive chromosomal strand) should be considered prior to initiating treatment with 5-fluorouracil and capecitabine for most patients.
- Testing is widely available and highly accurate for the DPYD\*2A (rs3918290) variant and for other variants with no activity or significantly reduced activity.

## Guidelines and evidence

- The **FDA** has acknowledged DPD deficiency as a risk factor for 5-FU related toxicity on multiple drug inserts. However, testing is not explicitly recommended or required prior to treatment initiation.
  - The 2016 updated prescribing information for Xeloda<sup>®</sup> lists DPD deficiency as a contraindication.<sup>12</sup>
    - "...patients with certain homozygous or certain compound heterozygous mutations in the DPD gene that result in complete or near complete absence of DPD activity are at increased risk for acute early-onset of toxicity and severe, life-threatening, or fatal adverse reactions caused by XELODA..."
    - "Patients with partial DPD activity may also have increased risk of severe, life-threatening, or fatal adverse reactions caused by XELODA."
    - "No XELODA dose has been proven safe for patients with complete absence of DPD activity. There is insufficient data to recommend a specific dose in patients with partial DPD activity as measured by any specific test."
  - Similar warnings exist about use in patients with DPD enzyme deficiency in the Contraindications section of the prescribing information for Carac<sup>®</sup> Cream and Efudex<sup>®</sup> topical solutions and cream.<sup>13, 14</sup>
  - DPYD variant testing is listed by the FDA as a valid biomarker in the context of approved drug labeling.<sup>15</sup>
- In October 2013, the Clinical Pharmacogenetics Implementation Consortium (CPIC) published a guideline on *Dihydropyrimidine Dehydrogenase Genotype and Fluoropyrimidine Dosing*.<sup>16</sup> They recommended:
  - "...alternative drug for patients who are homozygous for DPYD non-functional variants—\*2A (rs3918290), \*13 (rs55886062), and rs67376798 A (on the positive chromosomal strand)—as these patients are typically DPD deficient."
  - "Patients who are homozygous for DPYD\*2A, \*13, or rs67376798 may demonstrate complete DPD deficiency and the use of 5-fluorouracil or capecitabine is not recommended in these patients."
  - "However, available evidence does not clearly indicate a degree of dose reduction needed to prevent fluoropyrimidine related toxicities..."

- “Consider a 50% reduction in starting dose for heterozygous patients (intermediate activity).”
- “...followed by an increase in dose in patients experiencing no or clinically tolerable toxicity to maintain efficacy, a decrease in dose in patients who do not tolerate the starting dose to minimize toxicities...”
- Though not specified in other professional guidelines, there is general consensus that given the large number of patients treated each year with 5-FU, and the human and economical cost of severe toxic side effects, pre-therapeutic detection of DPD deficiency should be considered.<sup>7,17,18</sup>

## Criteria

DPD testing for genetic variants DPYD\*2A (rs3918290), DPYD\*13 (rs55886062), and rs67376798 A (on the positive chromosomal strand) is indicated in individuals considering or currently on therapy with any 5-FU containing drug including, but not limited to:

- 5-fluorouracil (Fluorouracil<sup>®</sup> , Aducril<sup>®</sup> )
- Capecitabine (Xeloda<sup>®</sup> )
- Fluorouracil topical formulations (Carac<sup>®</sup> , Efudex<sup>®</sup> , Fluoroplex<sup>®</sup> )

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# Duchenne and Becker Muscular Dystrophy Testing

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## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedures addressed by this guideline | Procedure codes |
|--|-----------------|
| DMD Known Familial Mutation Analysis   | 81403           |
| DMD Deletion/Duplication Analysis      | 81161           |
| DMD Sequencing                         | 81408           |

## What are Duchenne and Becker Muscular Dystrophy

### Definition

Duchenne muscular dystrophy (DMD) is an X-linked inherited neuromuscular disorder.<sup>1</sup>  
<sup>2</sup> The prevalence of DMD has been reported as 15.9 cases per 100,000 live male births in the USA and 19.5 cases per 100,000 live male births in the UK.<sup>2</sup> It is caused by pathogenic variants in the DMD gene. It is typically diagnosed by age 5.

- The main clinical findings of DMD include:<sup>1</sup>
  - rapidly progressive skeletal muscle weakness and wasting that is more proximal than distal
  - a delay in motor milestones (such as walking at 18 months)
  - calf pseudohypertrophy
  - wheelchair dependency by 13 years
  - dilated cardiomyopathy
  - reduced life expectancy
  - greatly elevated serum creatine kinase (CK) concentration
- Genetic testing confirms a clinical diagnosis in affected males. Muscle biopsy may be used for diagnosis when molecular testing does not find a mutation.<sup>2</sup>
- Although this is an X-linked disorder, some carrier females may exhibit symptoms, sometimes later in life, including muscle weakness and cardiomyopathy.<sup>1</sup>

- Physiotherapy and treatment with glucocorticoids remain the mainstays of DMD treatment and should continue after loss of ambulation. The benefits of long-term glucocorticoid therapy have been shown to include loss of ambulation at a later age, preserved upper limb and respiratory function, and avoidance of scoliosis surgery. The FDA has also granted full approval for deflazacort, making this the first glucocorticoid with a labeled indication specifically for DMD.<sup>2</sup>
- “In September, 2016, the US Food and Drug Administration (FDA) approved use of eteplirsen, which targets the approximately 13% of boys with a mutation in the dystrophin gene that is amenable to exon 51 skipping, via an accelerated approval pathway. Ataluren and eteplirsen are the first of a series of mutation-specific therapies to gain regulatory approval.”<sup>2</sup> However, the manufacturer is required to conduct a trial to determine whether eteplirsen improves motor function of individuals with DMD with an amenable dystrophin gene pathogenic variant. Ataluren is not approved for treating DMD in the US. Other therapies are under investigation.<sup>1</sup>
- Becker muscular dystrophy (BMD) is a similar disorder, caused by mutations in the same gene, which has a later age of onset and is less common than DMD. It is typically diagnosed by age 10, and people with BMD are often still able to walk into their 20s. The typical features include:<sup>1</sup>
  - progressive skeletal muscle weakness, proximal more than distal
  - wheelchair dependence after age 16 years if at all
  - flexion contractures of the elbows
  - preservation of neck flexor muscle strength (differentiates BMD from DMD)
  - dilated cardiomyopathy
  - greatly elevated serum CK concentration

## Test information

- DMD deletion/duplication testing is the best first test, which detects genetic changes in about 65-80% of males with DMD and up to 95% of males with BMD.<sup>1</sup> DMD deletion/duplication testing can also be used to identify a mutation in a known or suspected carrier female if an affected male is not available for molecular analysis.<sup>1</sup>
- DMD sequence analysis will identify about 20-35% of DMD genetic changes.<sup>1</sup> DMD sequencing analysis can also be used to identify a mutation in a known or suspected carrier female, if an affected male is not available for molecular analysis.<sup>1</sup>
- Once the familial mutation is identified, at-risk family members can have reliable and accurate testing for just that mutation.<sup>1</sup>

- “If genetic testing does not confirm a clinical diagnosis of DMD, then a muscle biopsy sample should be tested for the presence of dystrophin protein by immunohistochemistry of tissue cryosections or by western blot of a muscle protein extract. Skeletal muscle biopsy continues to be used only rarely in the diagnosis of dystrophinopathies.”<sup>1</sup>

## Guidelines and evidence

- The Centers for Disease Control and Prevention (CDC) selected the Care Considerations Working Group (2018) to create guidelines for diagnosis and management of DMD:<sup>2</sup>
  - “If deletion/duplication testing is negative, then dystrophin gene sequencing should be done to look for the remaining types of mutations that are attributed to DMD [e.g., point mutations or small deletions/insertions]”<sup>2</sup>
- American Academy of Pediatrics (2005, reaffirmed 2008) guidelines on cardiac care address screening for DMD/BMD carriers.<sup>3</sup>
  - “Carriers of DMD or BMD should be made aware of the risk of developing cardiomyopathy and educated about the signs and symptoms of heart failure.”<sup>3</sup>
  - “Carriers of DMD or BMD should be referred for evaluation by a cardiac specialist with experience in the treatment of heart failure and/or neuromuscular disorders. Patients should undergo initial complete cardiac evaluation in late adolescence or early adulthood or at the onset of cardiac signs and symptoms, if these signs or symptoms appear earlier.”<sup>3</sup>
  - “Carriers should be screened with a complete cardiac evaluation at a minimum of every 5 years starting at 25 to 30 years of age.”<sup>3</sup>
  - “Treatment of cardiac disease is similar to that outlined for boys with DMD or BMD.”<sup>3</sup>

## Criteria

### DMD Known Familial Mutation Analysis

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - No previous genetic testing of DMD by a method that would detect the familial variant, AND



- Diagnostic Testing for Symptomatic Individuals:
  - DMD mutation identified in 1<sup>st</sup>, 2<sup>nd</sup>, or 3<sup>rd</sup> degree biologic relative(s), OR
- Carrier Screening and Predictive Testing for Presymptomatic/Asymptomatic At-Risk Individuals:
  - DMD mutation identified in 1<sup>st</sup>, 2<sup>nd</sup>, or 3<sup>rd</sup> degree biologic relative(s), OR
- Prenatal Testing for At-Risk Pregnancies:
  - DMD mutation identified in mother or sibling

### **DMD Deletion/Duplication Analysis**

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Testing:
  - No previous deletion/duplication analysis of DMD, and
  - If sequence analysis of DMD was performed, no mutations detected, AND
- Diagnostic Testing for Symptomatic Individuals:
  - Progressive symmetric muscle weakness (proximal greater than distal)—e.g., leg, pelvic and shoulder girdle muscles, and calf hypertrophy, and positive Gower maneuver, or
  - Elevated serum CK concentration, and
  - Progressive symmetric muscle weakness (proximal greater than distal)-e.g., leg, pelvic and shoulder girdle muscles, or
  - Calf hypertrophy, or
  - Positive Gower maneuver, or
  - Male gender, or
  - Onset of symptoms by early adulthood (usually by adolescence), or
  - Delayed motor milestones, or
  - Gait problems; waddling gait or
  - Learning difficulties, or
  - Quadriceps weakness; activity-induced cramping, or
  - Family history consistent with X-linked inheritance, OR

- Carrier Screening and Predictive Testing for Presymptomatic/Asymptomatic at Risk Individuals:
  - DMD or BMD diagnosed in 1<sup>st</sup> or 2<sup>nd</sup> degree family member and no known mutation at time of testing, AND
  - Family history consistent with X-linked inheritance

### DMD Sequencing

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous testing:
  - No mutations detected by deletion/duplication analysis in DMD, and
  - No previous full sequencing analysis of DMD

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# Early Onset Familial Alzheimer Disease (EOFAD) Genetic Testing

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## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedures addressed by this guideline | Procedure codes |
|--|-----------------|
| PSEN1 Sequencing                       | 81405           |
| PSEN1 Deletion/Duplication             | 81479           |
| PSEN1 Known Familial Mutation          | 81403           |
| APP Sequencing                         | 81406           |
| APP Deletion/Duplication               | 81479           |
| APP Known Familial Mutation            | 81403           |
| PSEN2 Sequencing                       | 81406           |
| PSEN2 Known Familial Mutation          | 81403           |
| EOFAD Multigene panel                  | 81479           |

## What is early onset familial Alzheimer disease

### Definition

Alzheimer disease (AD) is characterized by adult onset, progressive dementia with cerebral cortical atrophy, beta amyloid plaque formation, and intraneuronal neurofibrillary tangles.<sup>1</sup> Common findings include memory loss, confusion, speech issues, hallucinations, and personality and behavioral changes such as poor judgment, agitation, and withdrawal.<sup>2,3</sup> Symptoms of Alzheimer disease usually start after 60-65 years old.<sup>2</sup>

- Of all people with Alzheimer disease, about 25% have at least two affected relatives (called “familial Alzheimer disease”). Most familial Alzheimer disease is late-onset, but in about 5% of cases symptoms start at an unusually young age (called “early onset familial Alzheimer disease” or EOFAD).<sup>2</sup>
- EOFAD is suspected when:<sup>1</sup>
  - More than one family member has Alzheimer disease.

- Symptoms consistently start before age 60 to 65 and often before 55.

## Genetics

- Table 1 below summarizes three subtypes of EOFAD. While not clinically distinguishable, the underlying genetic cause differs. Among families with EOFAD, 40-80% will have a detectable mutation in the APP, PSEN1, or PSEN2 gene.<sup>1</sup>Therefore, some families with EOFAD will not have an identifiable mutation by current testing. There may be other disease causing genes that have not been identified to date.
- EOFAD is inherited in an autosomal dominant fashion.<sup>1</sup>
- A person who is found to have a mutation in one of the genes known to cause EOFAD has a 50% chance to pass the mutation to his/her children.
- Most people with EOFAD have an affected parent. In cases where there appears to be no parent affected, most people have a second degree relative with the condition. De novo (new) mutations are possible. However, they have not been reported in EOFAD.<sup>1,2</sup>
- The presence of a mutation in the PSEN1 gene has complete penetrance by the age of 65, meaning that when a mutation is present symptoms present by this age. Mutations in PSEN2 have a penetrance of approximately 95%.The penetrance of APP is unknown.<sup>1</sup>

Table 1

| EOFAD type D | Gene  | Proportion of EOFAD cases | Average age of onset            | Likelihood of symptoms with a mutation |
|--------------|-------|---------------------------|---------------------------------|--|
| Type 1       | APP   | 10% to 15%                | 40's to 50's (occasionally 60s) | Unknown                                |
| Type 3       | PSEN1 | 20% to 70%                | 40s to early 50's               | ~100% by 65                            |
| Type 4       | PSEN2 | ~ 5%                      | 40 to 75                        | ~95% by 80s                            |

## Test information

- EOFAD is clinically diagnosed based on family history and age of onset. Genetic testing can confirm a clinical diagnosis in symptomatic individuals. Positive results also allow reliable presymptomatic predictive testing for at-risk family members.<sup>1</sup>
- Sequence analysis is available for each gene individually or as panel. Given the significant overlap in clinical manifestations and age of onset in AD, single-gene testing is typically not recommended.<sup>1,2</sup> A multigene panel that includes PSEN 1/2

and APP is most likely to identify the genetic cause of the condition while limiting identification of variants of uncertain significance.<sup>2</sup>

- Once the disease-causing mutation is identified, predictive testing of adult first-degree relatives (primarily siblings and adult offspring) may be considered. The detection rate for a known familial mutation is greater than 99%. Because of the implications of predictive testing, “Those seeking testing should be counseled about possible problems that they may encounter with regard to health, life, and disability insurance coverage, employment and educational discrimination, and changes in social and family interaction.”<sup>1</sup>

## Guidelines and evidence

- The Amyloid Imaging Task Force, Society of Nuclear Medicine and Molecular Imaging, and the Alzheimer’s Association (2013) reference genetic testing in their recommendations:<sup>4</sup>
  - “The use of amyloid PET in lieu of genotyping for suspected autosomal dominant mutation carriers is considered inappropriate. The optimal clinical evaluation in these cases is careful collection of a family history, followed (if appropriate) by genetic counseling prior to and after genetic testing for known mutations. Future use of amyloid PET in autosomal dominant mutation carriers could include determination of whether the amyloid deposition phase of their illness has begun. In the setting of a complete clinical evaluation, including serial neuropsychological testing, this information may be useful in identifying one disease-related milestone that, along with the genetic information, aids decision making.”
- A 2012 expert-authored review states that:<sup>1</sup>
  - “EOFAD is diagnosed in families with multiple affected individuals with mean age of onset before 65 years and/or with a documented disease-causing mutation in one of the genes known to be associated with EOFAD.”
  - “Establishing the diagnosis in a proband requires molecular genetic testing to identify a disease-causing mutation in one of the three genes known to be associated with EOFAD.”
  - “Predictive testing for at-risk asymptomatic adult family members requires prior identification of the disease-causing mutation in the family.”
- American College of Medical Genetics and The National Society of Genetic Counselors (2011):<sup>5</sup>
  - “Testing for genes associated with early-onset autosomal dominant AD should be offered in the following situations:”
    - “A symptomatic individual with EOAD in the setting of a family history of dementia or in the setting of an unknown family history (e.g., adoption).”

- “Autosomal dominant family history of dementia with one or more cases of EOAD.”
- “A relative with a mutation consistent with EOAD (currently PSEN 1/2 or APP).”
- The European Federation of Neurological Societies (2010)<sup>6</sup> Alzheimer's diagnosis and management guidelines address genetic testing: “Screening for known pathogenic mutations can be undertaken in patients with appropriate phenotype or a family history of an autosomal dominant dementia.” (No evidence level assigned.) They add, “Testing of patients with familial dementia and of unaffected at-risk-relatives should be accompanied by neurogenetic counseling and undertaken only after full consent and by specialist centers. Pre-symptomatic testing may be performed in at risk member of family-carrying mutation. It is recommended that the Huntington’s disease protocol is followed for pre-symptomatic testing.”

## Criteria

### **PSEN1, PSEN2, or APP Known familial mutation testing**

- Clinical Consultation:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - No previous genetic testing of PSEN1, PSEN2, or APP, and
  - PSEN1, PSEN2, or APP mutation identified in a 1<sup>st</sup> or 2<sup>nd</sup> degree biological relative, AND
- Diagnostic Testing for Symptomatic Individuals:
  - Dementia diagnosed ≤65 years of age, OR
- Predictive Testing
  - Age 18 years or older, and
  - No previous genetic testing of PSEN1, PSEN2, or APP, and
  - PSEN1, PSEN2, or APP mutation identified in 1<sup>st</sup> or 2<sup>nd</sup> degree biological relative

### **PSEN1 full sequence and deletion/duplication analysis**

- Clinical Consultation:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND

- Previous Testing:
  - No previous PSEN1 sequencing or deletion/duplication analysis, and
  - No known PSEN1, PSEN2, or APP mutation in the family, AND
- Diagnostic Testing for Symptomatic Individuals:
  - Dementia diagnosed  $\leq 65$  years of age, and
  - Family history of dementia in 1st or 2nd degree relative, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

### **APP sequence and deletion/duplication analysis**

- Criteria for PSEN1 analysis are met, AND
- No previous genetic testing for APP, AND
- No mutations detected in PSEN1 analysis

### **PSEN2 full sequence analysis**

- Criteria for PSEN1 analysis are met, AND
- No previous genetic testing for PSEN2, AND
- No mutations detected in PSEN1 or APP analysis

### **Multigene Panel (PSEN1, APP, and PSEN2 ONLY)**

#### Paragraph

- Clinical Consultation:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Testing:
  - No previous testing for EOFAD, and
  - No known PSEN1, PSEN2, or APP mutation in the family, AND
- Diagnostic Testing for Symptomatic Individuals:
  - Dementia diagnosed less than or equal to 65 years of age, and
  - Family history of dementia in 1<sup>st</sup> of 2<sup>nd</sup> degree relative, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

EOFAD

## Billing and Reimbursement Considerations

### Paragraph

- When multiple CPT codes are billed for components of a panel and there is a more appropriate CPT code representing the panel, eviCore will redirect to the panel code(s).
- If the laboratory will not accept redirection to a panel code, the medical necessity of each billed component procedure will be assessed independently.
  - In general, only a limited number of panel components that are most likely to explain the member's presentation will be reimbursable. The remaining panel components will not be reimbursable.
  - When the test is billed with multiple stacked codes, only the following genes may be considered for reimbursement in a tiered fashion:
    - PSEN1
    - APP
    - PSEN2

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# EGFR Testing for Non-Small Cell Lung Cancer TKI Response

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## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedure addressed by this guideline | Procedure code |
|---------------------------------------|----------------|
| EGFR Targeted Mutation Analysis       | 81235          |

## What is EGFR testing in non-small cell lung cancer

### Definition

Non-small cell lung cancer (NSCLC) is the most common type of lung cancer, and is associated with exposure to cigarette smoking.<sup>1</sup>

- About 80-85% of NSCLC tumors express the epidermal growth factor receptor (EGFR).<sup>1</sup> EGFR is a cell surface receptor that promotes cell growth via activation of its intracellular tyrosine kinase domain when epidermal growth factor binds to its extracellular region. Constitutive activation of the tyrosine kinase domain (via mutation) or overexpression of EGFR results in increased proliferation and survival of cells, leading to the growth of tumors.<sup>1</sup>
- The drugs erlotinib (Tarceva<sup>®</sup>), afatinib (Gilotrif<sup>®</sup>), and gefitinib (Iressa<sup>®</sup>) are used in the treatment of people with advanced NSCLC.<sup>1</sup> These drugs are tyrosine kinase inhibitors (TKIs). They directly inhibit the EGFR pathway by binding to the mutated epidermal growth factor receptor and blocking downstream signaling resulting in reduced tumor growth.<sup>1,2</sup>
  - People can develop resistance to erlotinib, afatinib and gefitinib therapy through the development of an EGFR T790M mutation. A third-generation TKI, osimertinib (Tagrisso<sup>®</sup>), can be used to treat people who have developed this specific mutation.<sup>3</sup>
- The presence of a mutation in the tyrosine kinase domain of EGFR is associated with positive response to TKIs. About 10-15% of Caucasian and up to 40% of Asian NSCLC patients have these mutations in EGFR. Mutations occur more often in patients with adenocarcinoma, women, and patients who never smoked.<sup>1,2,4</sup>
- Testing an NSCLC patient for EGFR mutations can be helpful to select patients who are more likely to respond to TKI therapy.<sup>1</sup>

- Patients with activating mutations in exons 18, 19, 20 or 21 of the EGFR gene, which encodes the tyrosine kinase domain of EGFR, are considered good candidates for treatment with erlotinib, afatinib, or gefitinib. Patients found to be wild type are unlikely to respond to erlotinib, afatinib, or gefitinib and there is insufficient evidence at this time to support their use in individuals with other exon mutations. Other treatment options should be considered for these patients.<sup>1,5-7</sup>
- Patients who develop an EGFR T790M mutation become resistant to treatment with the first- and second-generation TKIs erlotinib, afatinib and gefitinib. This mutation frequently develops after a median of 9 to 13 months of initial TKI therapy and may respond to treatment with the third-generation TKI osimertinib.<sup>3,8</sup>
- EGFR is upstream from another gene, KRAS, in the signaling pathway. Overlapping EGFR mutations and KRAS mutations occur in <1% of patients with lung cancer: patients with NSCLC may have an EGFR mutation or a KRAS mutation, but co-occurrence of both mutations is very rare.<sup>1</sup>

## Test information

- Targeted analysis of the EGFR gene can be performed by two different methods:
  - **Defined mutation panels** check specifically for the most common activating mutations in exons 18 to 21 of the EGFR gene. The two most common types of activating EGFR mutations are in-frame deletions in exon 19, and the point mutation, L858R. These mutations account for up to 85% of all EGFR mutations.<sup>1</sup>
  - **Sequencing of specific exons (18-21)** will find any mutation in the region encoding the tyrosine kinase domain, including deletions in exon 19. The T790M mutation is located on exon 20 and the L858R mutation is located on exon 21.<sup>1,8</sup>
- Testing by either method is sensitive and accurate,<sup>1</sup> and both methods are commonly used by commercial laboratories doing testing.
- Roche's cobas EGFR Mutation Test v2 is designed to identify exon 19 deletions (E19del) or exon 21 (L858R) substitution mutations in the EGFR gene in patients with non-small cell lung cancer (NSCLC) eligible for treatment with erlotinib. It is also designed to identify the T790M mutation, exon 19 deletions (E19del), or exon 21 (L858R) substitution mutations in patients eligible for treatment with osimertinib. This testing is performed on circulating tumor DNA (ctDNA) from a plasma sample and is commonly known as a liquid biopsy test.<sup>9,10</sup>
- Roche's cobas EGFR Mutation Test v2 had previously been approved for this same indication using formalin-fixed paraffin-embedded (FFPE) tissue specimens on November 13, 2015.<sup>11</sup>

## Guidelines and evidence

- The National Comprehensive Cancer Network (NCCN, 2019) guidelines state:<sup>1</sup>
  - “Numerous gene alterations have been identified that impact therapy selection. Testing of lung cancer specimens for these alterations is important for identification of potentially efficacious targeted therapies, as well as avoidance of therapies unlikely to provide clinical benefit.”
  - The EGFR gene is included in the list of gene targets recommended for testing by NCCN.
- The National Comprehensive Cancer Network (NCCN, 2019) states the following in regards to liquid biopsy testing for EGFR mutations:<sup>1</sup>
  - “Cell-free/circulating tumor DNA testing should not be used in lieu of a tissue diagnosis.”
  - “The use of cell-free/circulating tumor DNA testing can be considered in specific clinical circumstances, most notably:
    - If a patient is medically unfit for invasive tissue sampling
    - In the initial diagnostic setting, if following pathologic confirmation of a NSCLC diagnosis there is insufficient material for molecular analysis, cell-free/circulating tumor DNA should be used only if follow-up tissue-based analysis is planned for all patients in which an oncogenic driver is not identified
    - In patients with a sensitizing EGFR mutation at progression, tissue biopsy.”
- The American Society of Clinical Oncology (ASCO, 2018) Endorsement of the College of American Pathologists/International Association for the Study of Lung Cancer/Association for Molecular Pathology Guideline states that:<sup>2</sup>
  - “The ASCO Expert Panel determined that the recommendations from the CAP/IASLC/AMP molecular testing guideline are clear, thorough, and based upon the most relevant scientific evidence. ASCO endorsed the guideline with minor modifications. Recommendations: This update clarifies that any sample with adequate cellularity and preservation may be tested and that analytical methods must be able to detect mutation in a sample with as little as 20% cancer cells. It strongly recommends against evaluating epidermal growth factor receptor (EGFR) expression by immunohistochemistry for selection of patients for EGFR-targeted therapy.”
- The U.S. Food and Drug Administration approved Roche’s cobas EGFR Mutation Test v2 through their Premarket Approval (PMA) pathway in June 2016:<sup>9</sup>
  - “The U.S. Food and Drug Administration approved cobas EGFR Mutation Test v2 (Roche Molecular Systems, Inc.) using plasma specimens as a companion diagnostic test for the detection of exon 19 deletions or exon 21 (L858R) substitution mutations in the epidermal growth factor receptor (EGFR) gene to

- identify patients with metastatic non-small cell lung cancer (NSCLC) eligible for treatment with Tarceva (erlotinib).”
- “Patients who are negative by this test should undergo routine biopsy and testing for EGFR mutations with the FFPE tissue sample type.”
  - The U.S. Food and Drug Administration approved a label extension of Roche’s cobas EGFR Mutation Test v2 as a companion diagnostic test for osimertinib (Tagrisso®):<sup>10</sup>
    - According to the company, the test can now “be used as a companion diagnostic test (CDx) with Tagrisso for the first line of patients diagnosed with metastatic NSCLC whose tumors have epidermal growth factor receptor (EGFR) exon 19 deletions or exon 21 L858R mutations.” The test was previously FDA-approved as a companion diagnostic test for osimertinib for second-line treatment and beyond in NSCLC patients with EGFR T790M mutations.
  - EGFR is listed as an FDA-approved biomarker for erlotinib, afatinib, gefitinib, and osimertinib.<sup>12</sup>
  - Product labeling for erlotinib, afatinib, and gefitinib address EGFR testing. All three products are listed as “first line treatments for patients with metastatic non-small cell lung cancer whose tumors have epidermal growth factor receptor (EGFR) exon 19 deletions or exon 21 (L858R) substitution mutations as detected by an FDA-approved test.”<sup>5-7</sup>
  - Product labeling for osimertinib notes the product is “a kinase inhibitor indicated for the treatment of patients with metastatic epidermal growth factor receptor (EGFR) T790M mutation-positive non-small cell lung cancer (NSCLC), as detected by an FDA-approved test, whose disease has progressed on or after EGFR TKI therapy.”<sup>8</sup>
  - Osimertinib may also be used as first-line therapy for patients who have mutations in the EGFR gene that sensitize the tumor to earlier-generation tyrosine-kinase inhibitors, or for patients who have the T790M mutation that creates resistance to earlier-generation tyrosine-kinase inhibitors.<sup>13</sup> NCCN guidelines indicate that osimertinib is the preferred TKI drug to use when a sensitizing EGFR mutation is detected before first-line systemic therapy is initiated.<sup>1</sup>

## Criteria

EGFR targeted mutation testing is indicated in individuals with metastatic non-small cell lung cancer prior to initiation of treatment with erlotinib, afatinib, gefitinib, or osimertinib therapy.

For patients whose disease progresses either on or after TKI therapy, repeat EGFR testing to identify the emergence of a T790M mutation may be considered to determine whether further treatment with osimertinib would be indicated.

## Other considerations

Liquid biopsy testing for EGFR targeted mutations will be considered medically necessary for individuals meeting the above criteria and when billed as an individual tumor marker. All other liquid biopsy testing, including panels of genes, is addressed by the guideline **Liquid Biopsy Testing - Solid Tumors**.

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# Ehlers-Danlos Syndrome Testing

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## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedures addressed by this guideline | Procedure codes |
|--|-----------------|
| Known Familial Mutation Analysis       | 81403           |
| ADAMTS2 Sequencing                     | 81479           |
| ADAMTS2 Deletion/Duplication Analysis  | 81479           |
| B3GALT6 Sequencing                     | 81479           |
| B3GALT6 Deletion/Duplication Analysis  | 81479           |
| B4GALT7 Sequencing                     | 81479           |
| B4GALT7 Deletion/Duplication Analysis  | 81479           |
| C1R Sequencing                         | 81479           |
| C1R Deletion/Duplication Analysis      | 81479           |
| C1S Sequencing                         | 81479           |
| C1S Deletion/Duplication Analysis      | 81479           |
| CHST14 Sequencing                      | 81479           |
| CHST14 Deletion/Duplication Analysis   | 81479           |
| COL1A1 Sequencing                      | 81408           |
| COL1A1 Deletion/Duplication Analysis   | 81479           |
| COL1A2 Sequencing                      | 81408           |
| COL1A2 Deletion/Duplication Analysis   | 81479           |
| COL12A1 Sequencing                     | 81479           |
| COL12A1 Deletion/Duplication Analysis  | 81479           |
| COL3A1 Sequencing                      | 81479           |
| COL3A1 Deletion/Duplication Analysis   | 81479           |
| COL5A1 Sequencing                      | 81479           |
| COL5A1 Deletion/Duplication Analysis   | 81479           |
| COL5A2 Sequencing                      | 81479           |



| Procedures addressed by this guideline | Procedure codes |
|--|-----------------|
| COL5A2 Deletion/Duplication Analysis   | 81479           |
| DSE Sequencing                         | 81479           |
| DSE Deletion/Duplication Analysis      | 81479           |
| FKBP14 Sequencing                      | 81479           |
| FKBP14 Deletion/Duplication Analysis   | 81479           |
| PLOD1 Sequencing                       | 81479           |
| PLOD1 Deletion/Duplication Analysis    | 81479           |
| PRDM5 Sequencing                       | 81479           |
| PRDM5 Deletion/Duplication Analysis    | 81479           |
| SLC39A13 Sequencing                    | 81479           |
| SLC39A13 Deletion/Duplication Analysis | 81479           |
| TNXB Sequencing                        | 81479           |
| TNXB Deletion/Duplication Analysis     | 81479           |
| ZNF469 Sequencing                      | 81479           |
| ZNF469 Deletion/Duplication Analysis   | 81479           |

## What is Ehlers-Danlos Syndrome

### Definition

Ehlers-Danlos syndrome (EDS) is a heterogeneous group of connective tissue disorders. Although all types of EDS affect the joints and skin, additional features vary by type.<sup>1</sup>

- An unusually large range of joint movement (hypermobility) occurs with most forms of EDS, and is especially prominent in the hypermobile type.<sup>1</sup>
  - Generalized joint hypermobility is typically assessed using a 9-point scale called the Beighton criteria. Adults 50 or younger with a Beighton score of  $\geq 5$ , adults older than 50 with a Beighton score  $\geq 4$ , and pre-pubertal children and adolescents with a Beighton score  $\geq 6$ , are considered to have generalized joint hypermobility.<sup>2-4</sup> In people with a Beighton score 1 point below the age-specific cut-off, a positive 5-point questionnaire result (2 or more positive answers) can be taken as evidence of generalized joint hypermobility.<sup>4</sup>
  - Generalized joint hypermobility is relatively common, occurring in 2-57% of different populations.<sup>2</sup>

- Joint hypermobility can be a feature of other connective tissue disorders (e.g. Marfan syndrome, skeletal dysplasias, and other disorders), myopathic disorders, and other chromosomal and molecular disorders. Joint hypermobility may also occur as an isolated, nonsyndromic finding.<sup>3</sup>
- Joint hypermobility may be asymptomatic, or associated with musculoskeletal complications such as chronic pain and disturbed proprioception. Individuals with symptomatic joint hypermobility who do not have hypermobile EDS or another identifiable cause are considered to have “hypermobility spectrum disorders (HSDs).”<sup>3</sup>
- The combined prevalence of all types of EDS appears to be at least 1 in 5,000 individuals worldwide, with the most common being the hypermobile type.<sup>1</sup>
- Six types of EDS were originally delineated in 1997.<sup>5</sup> In 2017, clinical criteria were updated and revised to include thirteen EDS types:<sup>4</sup>
  - Classical EDS
  - Classical-like EDS
  - Cardiac-valvular EDS
  - Vascular EDS
  - Hypermobile EDS
  - Arthrochalasia EDS
  - Dermatosparaxis EDS
  - Kyphoscoliotic EDS
  - Brittle cornea syndrome
  - Spondylodysplastic EDS
  - Musculocontractural type
  - Myopathic EDS
  - Periodontal EDS

## Genetics of Ehlers-Danlos Syndrome

Genetics of EDS (summarized in the table below):<sup>4</sup>

- Some EDS types follow an autosomal dominant pattern, meaning only one mutation is required to cause disease. In these cases, children, siblings, and parents of an affected person each have a 50% chance of having the same disease-causing mutation.
- Other types are autosomal recessive. Two mutations are required to cause recessive types, and usually only siblings are at risk for also being affected. There is rarely parent-to-child transmission.

| EDS Type                | Inheritance                     | Genetic basis   | Protein                                   |
|-------------------------|---------------------------------|---|---|
| Classical EDS           | Autosomal dominant              | Major: COL5A1,<br>COL5A2<br>Rare: COL1A1<br>c.934C>T  | Type V collagen<br>Type I collagen        |
| Classical-like EDS      | Autosomal recessive             | TNXB  | Tenascin XB                               |
| Cardiac valvular EDS    | Autosomal recessive             | COL1A2 (biallelic mutations that lead to COL1A2 NMD & absence of pro $\alpha$ 2(I) collagen chains) | Type I collagen                           |
| Vascular EDS            | Autosomal dominant              | Major: COL3A1<br>Rare: COL1A1<br>c.934C>T,<br>c.1720C>T,<br>c.3227C>T                               | Type III collagen<br>Type I collagen      |
| Hypermobility EDS       | Autosomal dominant              | Unknown   | Unknown                                   |
| Arthrochalasia EDS      | Autosomal dominant              | COL1A1<br>COL1A2  | Type I collagen                           |
| Dermatosparaxis EDS     | Autosomal recessive             | ADAMTS2   | ADAMTS-2                                  |
| Kyphoscoliotic EDS      | Autosomal recessive             | PLOD1<br>FKBP14   | LH1<br>FKBP22                             |
| Brittle cornea syndrome | Autosomal recessive             | ZNF469<br>PRDM5   | ZNF469<br>PRDM5                           |
| Spondylodysplastic EDS  | Autosomal recessive             | B4GALT7<br>B3GALT6<br>SLC9A13   | $\beta$ 4GalT7<br>$\beta$ 3GalT6<br>ZIP13 |
| Musculocontractural EDS | Autosomal recessive             | CHST14<br>DSE   | D4ST1<br>DSE                              |
| Myopathic EDS           | Autosomal recessive or dominant | COL12A1   | Type XII collagen                         |
| Periodontal type        | Autosomal dominant              | C1R<br>C1S  | C1r<br>C1s                                |

## Test information

- Clinical genetic testing is available for most types of EDS (see table above), and is used to confirm the final diagnosis when it is clinically suspected.<sup>4</sup>
  - Hypermobile EDS (hEDS) continues to require a clinical diagnosis, since the genetic etiology of this type is not yet known.<sup>4,8</sup>
- **Single gene analysis** — EDS genetic testing may be performed with Sanger sequencing or next generation sequencing (NGS). Deletion/duplication analysis may be considered. Mutation detection rates vary by type:
  - >90% of individuals with classical EDS have a mutation in COL5A1 or COL5A2.<sup>4,6</sup>
  - >95% of individuals with vascular EDS have a mutation in COL3A1.<sup>7</sup>
  - Mutation detection rates for the rarer EDS types are mostly unknown.
- **Multi-gene panel testing** — With the availability of NGS technology, EDS genetic testing is increasingly performed as a panel test that includes multiple EDS genes. In addition, these panels often include other hereditary connective tissue disorders with overlapping phenotypes. Panel testing is addressed in the guideline: Hereditary Connective Tissue Disorder Testing .

## Guidelines and evidence

- An expert-authored review (updated in 2018)<sup>8</sup> states the following regarding hEDS: “If a patient’s personal or family history is suggestive of one of the other types of EDS or another hereditary disorder of connective tissue or arterial fragility syndrome, analysis of an associated gene or multi-gene connective tissue disease panel may be appropriate. Failure to identify a pathogenic variant with such multiple gene testing reduces the likelihood of an arterial fragility syndrome, but does not completely rule it out, especially in the setting of a positive personal or family history of arterial fragility. Negative testing for an arterial fragility syndrome also does not confirm a diagnosis of EDS, hypermobility type. Therefore, such testing is not recommended in the absence of specific suggestive signs, symptoms, or family history.”
- According to the International Consortium on the Ehlers-Danlos Syndromes (2017):<sup>4</sup>
  - “In view of the vast genetic heterogeneity and phenotypic variability of the EDS subtypes, and the clinical overlap between many of these subtypes, but also with other hereditary connective tissue disorders, the definite diagnosis relies for all subtypes, except hEDS, on molecular confirmation with identification of (a) causative variant(s) in the respective gene.”
  - “Molecular diagnostic strategies should rely on NGS technologies, which offer the potential for parallel sequencing of multiple genes. Targeted resequencing of

a panel of genes...is a time- and cost-effective approach for the molecular diagnosis of the genetically heterogeneous EDS. When no mutation (or in case of an autosomal recessive condition only one mutation) is identified, this approach should be complemented with a copy number variant (CNV) detection strategy to identify large deletions or duplications, for example Multiplex Ligation-dependent Probe Amplification (MLPA), qPCR, or targeted array analysis.”

- “The diagnosis of hEDS remains clinical as there is yet no reliable or appreciable genetic etiology to test for in the vast majority of patients.”

## 2017 International Criteria for Classical EDS

Minimal criteria suggestive for Classical EDS (cEDS):<sup>4</sup>

- Major criterion 1, PLUS either:
  - Major criterion 2, and/or
  - At least three minor criteria.

| Major criteria for cEDS  | Minor criteria for cEDS  |
|--|--|
| <ol style="list-style-type: none"> <li>1. Skin hyperextensibility and atrophic scarring</li> <li>2. Generalized joint hypermobility</li> </ol> | <ol style="list-style-type: none"> <li>1. Easy bruising</li> <li>2. Soft, doughy skin</li> <li>3. Skin fragility (or traumatic splitting)</li> <li>4. Molluscoid pseudotumors</li> <li>5. Subcutaneous spheroids</li> <li>6. Hernia (or history thereof)</li> <li>7. Epicanthal folds</li> <li>8. Complications of joint hypermobility (e.g., sprains, luxation/subluxation, pain, flexible flatfoot)</li> <li>9. Family history of a first-degree relative who meets clinical criteria</li> </ol> |

## 2017 International Criteria for Classical-like EDS

Minimal criteria suggestive for Classical-like EDS (clEDS):<sup>4</sup>

- All three major criteria, AND
- A family history compatible with autosomal recessive transmission.

| Major criteria for cEDS   | Minor criteria for cEDS   |
|---|---|
| <ol style="list-style-type: none"> <li>1. Skin hyperextensibility, with velvety skin texture and absence of atrophic scarring</li> <li>2. Generalized joint hypermobility with or without recurrent dislocations (most commonly shoulder and ankle)</li> <li>3. Easy bruisable skin/spontaneous ecchymoses</li> </ol> | <ol style="list-style-type: none"> <li>1. Foot deformities: broad/plump forefoot, brachydactyly with excessive skin; pes planus; hallux valgus; piezogenic papules</li> <li>2. Edema in the legs in absence of cardiac failure</li> <li>3. Mild proximal and distal muscle weakness</li> <li>4. Axonal polyneuropathy</li> <li>5. Atrophy of muscles in hands and feet</li> <li>6. Acrogeric hands, mallet finger(s), clinodactyly, brachydactyly</li> <li>7. Vaginal/uterus/rectal prolapse</li> </ol> |

### 2017 International Criteria for Cardiac-Valvular EDS

Minimal criteria suggestive for Cardiac-Valvular EDS (cvEDS)

- Major criterion 1, AND
- A family history compatible with autosomal recessive inheritance, PLUS either:
  - One other major criterion, and/or
  - At least two minor criteria.

| Major criteria for cvEDS   | Minor criteria for cvEDS  |
|--|---|
| <ol style="list-style-type: none"> <li>1. Severe progressive cardiac-valvular problems (aortic valve, mitral valve)</li> <li>2. Skin involvement: skin hyperextensibility, atrophic scars, thin skin, easy bruising</li> <li>3. Joint hypermobility (generalized or restricted to small joints)</li> </ol> | <ol style="list-style-type: none"> <li>1. Inguinal hernia</li> <li>2. Pectus deformity (especially pectus excavatum)</li> <li>3. Joint dislocations</li> <li>4. Foot deformities: pes planus, pes planovalgus, hallux valgus</li> </ol> |

### 2017 International Criteria for Vascular EDS

Minimal criteria suggestive for Vascular EDS (vEDS):

- A family history of the disorder, and/or

- Arterial rupture or dissection in individuals less than 40 years of age, and/or
- Unexplained sigmoid colon rupture, and/or
- Spontaneous pneumothorax in the presence of other features consistent with vEDS, and/or
- A combination of the other minor clinical features listed below.

| Major criteria for vEDS  | Minor criteria for vEDS  |
|--|--|
| 1. Family history of vEDS with documented causative variant in COL3A1  | 1. Bruising unrelated to identified trauma and/or in unusual sites such as cheeks and back |
| 2. Arterial rupture at a young age   | 2. Thin, translucent skin with increased venous visibility                                 |
| 3. Spontaneous sigmoid colon perforation in the absence of known diverticular disease or other bowel pathology             | 3. Characteristic facial appearance  |
| 4. Uterine rupture during the third trimester in the absence of previous C-section and/or severe peripartum perineum tears | 4. Spontaneous pneumothorax  |
| 5. Carotid-cavernous sinus fistula (CCSF) formation in the absence of trauma   | 5. Acrogeria   |
|  | 6. Talipes equinovarus   |
|  | 7. Congenital hip dislocation  |
|  | 8. Hypermobility of small joints   |
|  | 9. Tendon and muscle rupture   |
|  | 10. Keratoconus  |
|  | 11. Gingival recession and gingival fragility  |
|  | 12. Early onset varicose veins (under 30 and nulliparous if female)                        |

### 2017 International Criteria for Hypermobile EDS

Diagnosis of Hypermobile EDS (hEDS) requires the simultaneous presence of criteria 1 AND 2 AND 3:

- Criteria 1: Generalized joint hypermobility
- Criterion 2: Two or more among the features (A-C) listed in the table below must be present (for example: A and B; A and C; B and C; A and B and C).
- Criterion 3: All of the following prerequisites must be met:
  - Absence of unusual skin fragility, and
  - Exclusion of other heritable and acquired connective tissue disorders, including autoimmune rheumatologic conditions, and

- Exclusion of alternative diagnoses that may also include joint hypermobility by means of hypotonia and/or connective tissue laxity.

| Feature A  | Feature B   | Feature C  |
|--|---|--|
| <p>A total of 5 must be present:</p> <ol style="list-style-type: none"> <li>1. Unusually soft or velvety skin</li> <li>2. Mild skin hyperextensibility</li> <li>3. Unexplained striae</li> <li>4. Bilateral piezogenic papules of the heel</li> <li>5. Recurrent or multiple abdominal hernia(s)</li> <li>6. Atrophic scarring involving at least two sites</li> <li>7. Pelvic floor, rectal, and/or uterine prolapses in children, men or nulliparous women without a history of morbid obesity or other known predisposing medical condition</li> <li>8. Dental crowding and high or narrow palate</li> <li>9. Arachnodactyly</li> <li>10. Arm span-to-height <math>\geq</math> 1.05</li> <li>11. Mitral valve prolapse (MVP)</li> <li>12. Aortic root dilatation with Z-score <math>&gt;</math> +2</li> </ol> | <p>Positive family history, with one or more first degree relatives independently meeting the current diagnostic criteria for hEDS.</p> | <p>Must have at least one</p> <ol style="list-style-type: none"> <li>1. Musculoskeletal pain in two or more limbs, recurring daily for at least 3 months.</li> <li>2. Chronic, widespread pain for <math>\geq</math> 3 months</li> <li>3. Recurrent joint dislocations or frank joint instability, in the absence of trauma:               <ol style="list-style-type: none"> <li>a. Three or more atraumatic dislocations in the same joint or two or more atraumatic dislocations in two different joints occurring at different times, or</li> <li>b. Medical confirmation of joint instability at two or more sites not related to trauma</li> </ol> </li> </ol> |

### 2017 International Criteria for Arthrochalasia EDS

Minimal criteria suggestive for Arthrochalasia EDS (aEDS):

- Major criterion 1, PLUS either:



- Major criterion 3, and/or
- Major criterion 2 and at least two other minor criteria.

| Major criteria for aEDS  | Minor criteria for aEDS                       |
|--|---|
| 1. Congenital bilateral hip dislocation  | 1. Muscle hypotonia                           |
| 2. Severe generalized joint hypermobility, with multiple dislocations/subluxations | 2. Kyphoscoliosis                             |
| 3. Skin hyperextensibility   | 3. Radiologically mild osteopenia             |
|  | 4. Tissue fragility, including atrophic scars |
|  | 5. Easy bruisable skin                        |

### 2017 International Criteria for Dermatosparaxis EDS

Minimal criteria suggestive for Dermatosparaxis EDS (dEDS):

- Major criterion 1, AND
- Major criterion 2, PLUS either:
  - One other major criterion, and/or
  - Three minor criteria.

| Major criteria for dEDS  | Minor criteria for dEDS  |
|--|--|
| <ol style="list-style-type: none"> <li>1. Extreme skin fragility with congenital or postnatal skin tears</li> <li>2. Characteristic craniofacial features, which are evident at birth or early infancy, or evolve later in childhood</li> <li>3. Redundant, almost lax skin, with excessive skin folds at the wrist and ankles</li> <li>4. Increased palmar wrinkling</li> <li>5. Severe bruisability with a risk of subcutaneous hematomas and hemorrhage</li> <li>6. Umbilical hernia</li> <li>7. Postnatal growth retardation</li> <li>8. Short limbs, hands and feet</li> <li>9. Perinatal complications due to connective tissue fragility</li> </ol> | <ol style="list-style-type: none"> <li>1. Soft and doughy skin texture</li> <li>2. Skin hyperextensibility</li> <li>3. Atrophic scars</li> <li>4. Generalized joint hypermobility</li> <li>5. Complications of visceral fragility (e.g., bladder rupture, diaphragmatic rupture, rectal prolapse)</li> <li>6. Delayed motor development</li> <li>7. Osteopenia</li> <li>8. Hirsutism</li> <li>9. Tooth abnormalities</li> <li>10. Refractive errors (myopia, astigmatism)</li> <li>11. Strabismus</li> </ol> |

### 2017 International Criteria for Kyphoscoliotic EDS

Minimal criteria suggestive for Kyphoscoliotic EDS (kEDS):

- Major criterion 1, AND
- Major criterion 2, PLUS either:
  - Major criterion 3, and/or
  - Three minor criteria (either general or gene-specific criteria).

| Major criteria for kEDS  | Minor criteria for kEDS  | Gene-specific minor criteria for kEDS  |
|--|--|--|
| <ol style="list-style-type: none"> <li>1. Congenital muscle hypotonia</li> <li>2. Congenital or early onset kyphoscoliosis (progressive or non-progressive)</li> <li>3. Generalized joint hypermobility with dislocations/subluxations (shoulders, hips, and knees in particular)</li> </ol> | <ol style="list-style-type: none"> <li>1. Skin hyperextensibility</li> <li>2. Easy bruisable skin</li> <li>3. Rupture/aneurysm of a medium-sized artery</li> <li>4. Osteopenia/osteoporosis</li> <li>5. Blue sclerae</li> <li>6. Hernia (umbilical or inguinal)</li> <li>7. Pectus deformity</li> <li>8. Marfanoid habitus</li> <li>9. Talipes equinovarus</li> <li>10. Refractive errors (myopia, hypermetropia)</li> </ol> | <p>PLOD1</p> <ol style="list-style-type: none"> <li>1. Skin fragility (easy bruising, friable skin, poor wound healing), widened atrophic scarring</li> <li>2. Scleral and ocular fragility/rupture</li> <li>3. Microcornea</li> <li>4. Facial dysmorphology</li> </ol> <p>FKBP14</p> <ol style="list-style-type: none"> <li>1. Congenital hearing impairment (any type)</li> <li>2. Follicular hyperkeratosis</li> <li>3. Muscle atrophy</li> <li>4. Bladder diverticula</li> </ol> |

### 2017 International Criteria for Brittle Cornea Syndrome

Minimal criteria suggestive for Brittle Cornea Syndrome (BCS):

- Major criterion 1, PLUS either:
  - At least one other major criterion, and/or
  - Three minor criteria.

| Major criteria for BCS   | Minor criteria for BCS  |
|--|---|
| <ol style="list-style-type: none"> <li>1. Thin cornea, with or without rupture (central corneal thickness often &lt;400 µm)</li> <li>2. Early onset progressive keratoconus</li> <li>3. Early onset progressive keratoglobus</li> <li>4. Blue sclerae</li> </ol> | <ol style="list-style-type: none"> <li>1. Enucleation or corneal scarring as a result of previous rupture</li> <li>2. Progressive loss of corneal stromal depth, especially in central cornea</li> <li>3. High myopia, with normal or moderately increased axial length</li> <li>4. Retinal detachment</li> <li>5. Deafness (often mixed, progressive, higher frequencies often more severely affected)</li> <li>6. Hypercompliant tympanic membranes</li> <li>7. Developmental dysplasia of the hip</li> <li>8. Hypotonia in infancy, usually mild if present</li> <li>9. Scoliosis</li> <li>10. Arachnodactyly</li> <li>11. Hypermobility of distal joints</li> <li>12. Pes planus, hallux valgus</li> <li>13. Mild contractures of fingers (especially fifth)</li> <li>14. Soft, velvety skin, translucent skin</li> </ol> |

### 2017 International Criteria for Spondylodysplastic EDS

Minimal criteria suggestive for Spondylodysplastic EDS (spEDS):

- Major criterion 1, AND
- Major criterion 2, PLUS
- Characteristic radiographic findings and at least 3 other minor criteria (general or type-specific).

| Major criteria for spEDS   | Minor criteria for spEDS   | Gene-specific minor criteria for spEDS   |
|--|--|--|
| <ol style="list-style-type: none"> <li>1. Short stature (progressive in childhood)</li> <li>2. Muscle hypotonia (ranging from severe congenital, to mild later-onset)</li> <li>3. Bowing of limbs</li> </ol> | <ol style="list-style-type: none"> <li>1. Skin hyperextensibility, soft, doughy skin, thin translucent skin</li> <li>2. Pes planus</li> <li>3. Delayed motor development</li> <li>4. Osteopenia</li> <li>5. Delayed cognitive development</li> </ol> | <p><b>B4GALT7</b></p> <ol style="list-style-type: none"> <li>1. Radioulnar synostosis</li> <li>2. Bilateral elbow contractures or limited elbow movement</li> <li>3. Generalized joint hypermobility</li> <li>4. Single transverse palmar curve</li> <li>5. Characteristic craniofacial features</li> <li>6. Characteristic radiographic findings</li> <li>7. Severe hypermetropia</li> <li>8. Clouded cornea</li> </ol> |
|  |  | <p><b>SLC39A13</b></p> <ol style="list-style-type: none"> <li>1. Protuberant eyes with bluish sclerae</li> <li>2. Hands with finely wrinkled palms</li> <li>3. Atrophy of the thenar muscles, tapering fingers</li> <li>4. Hypermobility of distal joints</li> <li>5. Characteristic radiologic findings</li> </ol>  |

| Major criteria for spEDS | Minor criteria for spEDS | Gene-specific minor criteria for spEDS   |
|--------------------------|--------------------------|--|
|                          |                          | <p>B3GALT6</p> <ol style="list-style-type: none"> <li>1. Kyphoscoliosis (congenital or early onset, progressive)</li> <li>2. Joint hypermobility, generalized or restricted to distal joints, with joint dislocations</li> <li>3. Joint contractures (congenital or progressive) (especially hands)</li> <li>4. Peculiar fingers (slender, tapered, arachnodactyly, spatulate, with broad distal phalanges)</li> <li>5. Talipes equinovarus</li> <li>6. Characteristic craniofacial features</li> <li>7. Tooth discoloration, dysplastic teeth</li> <li>8. Characteristic radiographic findings</li> <li>9. Osteoporosis with multiple spontaneous fractures Ascending aortic aneurysm</li> <li>10. Lung hypoplasia, restrictive lung disease</li> </ol> |

### 2017 International Criteria for Musculocontractural EDS

Minimal criteria suggestive for Musculocontractural EDS (mcEDS):

- At birth or in early childhood:
  - Major criterion 1, AND
  - Major criterion 2

- In adolescence and in adulthood:
  - Major criterion 1, AND
  - Major criterion 3.

| Major criteria for mcEDS  | Minor criteria for mcEDS  |
|---|---|
| <ol style="list-style-type: none"> <li>1. Congenital multiple contractures, characteristically adduction-flexion contractures, and/or talipes equinovarus (clubfoot)</li> <li>2. Characteristic craniofacial features, which are evident at birth or in early infancy</li> <li>3. Characteristic cutaneous features including skin hyperextensibility, easy bruisability, skin fragility with atrophic scars, increased palmar wrinkling</li> </ol> | <ol style="list-style-type: none"> <li>1. Recurrent/chronic dislocations</li> <li>2. Pectus deformities (flat, excavated)</li> <li>3. Spinal deformities (scoliosis, kyphoscoliosis)</li> <li>4. Peculiar fingers (tapering, slender, cylindrical)</li> <li>5. Progressive talipes deformities (valgus, planus, cavum)</li> <li>6. Large subcutaneous hematomas</li> <li>7. Chronic constipation</li> <li>8. Colonic diverticula</li> <li>9. Pneumothorax/pneumohemothorax</li> <li>10. Nephrolithiasis/cystolithiasis</li> <li>11. Hydronephrosis</li> <li>12. Cryptorchidism in males</li> <li>13. Strabismus</li> <li>14. Refractive errors (myopia, astigmatism)</li> <li>15. Glaucoma/elevated intraocular pressure</li> </ol> |

### 2017 International Criteria for Myopathic EDS

Minimal criteria suggestive for Myopathic EDS (mEDS):

- Major criterion 1, PLUS either:
  - One other major criterion and/or
  - Three minor criteria

| Major criteria for mEDS   | Minor criteria for mEDS  |
|---|--|
| <ol style="list-style-type: none"> <li>1. Congenital muscle hypotonia, and/or muscle atrophy, that improves with age</li> <li>2. Proximal joint contractures (knee, hip, and elbow)</li> <li>3. Hypermobility of distal joints</li> </ol> | <ol style="list-style-type: none"> <li>1. Soft, doughy skin</li> <li>2. Atrophic scarring</li> <li>3. Motor developmental delay</li> <li>4. Myopathy on muscle biopsy</li> </ol> |

### 2017 International Criteria for Periodontal EDS

Minimal criteria suggestive for Periodontal EDS (pEDS):

- Major criterion 1, OR major criterion 2, PLUS
  - At least two other major criteria and one minor criterion.

| Major criteria for pEDS  | Minor criteria for pEDS   |
|--|---|
| <ol style="list-style-type: none"> <li>1. Severe and intractable periodontitis of early onset (childhood or adolescence)</li> <li>2. Lack of attached gingiva</li> <li>3. Pretibial plaques</li> <li>4. Family history of a first-degree relative who meets clinical criteria</li> </ol> | <ol style="list-style-type: none"> <li>1. Easy bruising</li> <li>2. Joint hypermobility, mostly distal joints</li> <li>3. Skin hyperextensibility and fragility, abnormal scarring (wide or atrophic)</li> <li>4. Increased rate of infections</li> <li>5. Hernias</li> <li>6. Marfanoid facial features</li> <li>7. Acrogeria</li> <li>8. Prominent vasculature</li> </ol> |

### Criteria

#### EDS Known Familial Mutation Analysis

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy) , AND
- Previous Genetic Testing:
  - No previous testing of the requested gene, AND
- Diagnostic Testing for an Autosomal Dominant EDS:



- Known mutation identified in 1st degree biological relative. (Note: 2nd or 3rd degree relatives may be considered when 1st degree relatives are unavailable or unwilling to be tested), OR
- Carrier Screening for an Autosomal Recessive EDS:
  - Known mutation(s) identified in 1<sup>st</sup>, 2<sup>nd</sup>, or 3<sup>rd</sup> degree biologic relative(s), OR
- Prenatal Testing for At-Risk Pregnancies:
  - Family history of an autosomal dominant type of EDS with a known mutation identified in a previous child or either parent, or
  - Both parents carry a known mutation for an autosomal recessive type of EDS, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

### EDS Gene Analysis

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - No previous sequencing of the requested gene, AND
- The member does not have a known underlying cause for their symptoms (e.g. known genetic condition), AND
- The member does not have a family history of a known EDS gene mutation that would explain their clinical symptoms, AND
- The member meets the above 2017 minimal criteria suggestive for an EDS type associated with the requested gene test:
  - For COL5A1 and/or COL5A2 analysis: criteria for classical EDS met, or
  - For TNXB analysis: criteria for classical-like EDS met, or
  - For COL1A1\* analysis: criteria met for one of the following EDS types:
    - Classical EDS, or
    - Vascular EDS, or
    - Arthrochalasia EDS, or
    - Member displays one or more of the following:<sup>4</sup>
      - Arterial rupture at a young age, or

- Spontaneous sigmoid colon perforation in the absence of known diverticular disease or other bowel pathology, or
  - Uterine rupture during the third trimester in the absence of previous C-section and/or severe peripartum perineum tears, or
  - Carotid-cavernous sinus fistula (CCSF) formation in the absence of trauma, or
  - Member has one minor criterion for vEDS and a family history of arterial rupture, colonic rupture, uterine rupture, or carotid-cavernous sinus fistula (CCSF), OR
- For COL1A2\* analysis: criteria met for one of the following EDS types:
    - Cardiac valvular EDS, or
    - Arthrochalasia EDS, or
  - For COL3A1\* analysis: criteria for vascular EDS met, or
    - Member displays one or more of the following:<sup>4</sup>
      - Arterial rupture at a young age, or
      - Spontaneous sigmoid colon perforation in the absence of known diverticular disease or other bowel pathology, or
      - Uterine rupture during the third trimester in the absence of previous C-section and/or severe peripartum perineum tears, or
      - Carotid-cavernous sinus fistula (CCSF) formation in the absence of trauma, or
      - Member has one minor criterion for vEDS and a family history of arterial rupture, colonic rupture, uterine rupture, or carotid-cavernous sinus fistula (CCSF), OR
  - For ADAMTS2 analysis: criteria for dermatosparaxis EDS met, or
  - For PLOD1 and/or FKBP14 analysis: criteria for kyphoscoliotic EDS met, or
  - For ZNF469 and/or PRDM5 analysis: criteria for brittle cornea syndrome met, or
  - For B3GALT6, B4GALT7, and/or SLC39A13 analysis: criteria for spondylodysplastic EDS met, or
  - For CHST14 and/or DSE analysis: criteria for musculocontractural EDS met, or
  - For COL12A1 analysis: criteria for myopathic EDS met, or
  - For C1R and/or C1S analysis: criteria for periodontal EDS met, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

\* For non-EDS indications, refer to any available disorder-specific guidelines or general guidelines, **Hereditary Connective Tissue Disorder Testing** or *Genetic Testing for Non-Cancer Conditions*, as appropriate. COL1A1 and COL1A2 are also associated with osteogenesis imperfecta, Caffey disease, and skeletal dysplasias. COL3A1 is also associated with familial thoracic aortic aneurysm and dissection (TAAD).

Panel testing is addressed in the guideline: *Hereditary Connective Tissue Disorder Testing*.

### Exceptions and other considerations

The following are specifically non-reimbursable indications for EDS gene sequencing and deletion/duplication analysis:

- Member's personal and/or family history are suggestive of hypermobile EDS or the related clinical entity, "joint hypermobility syndrome"
- Isolated nonsyndromic joint hypermobility, including both asymptomatic and symptomatic forms (e.g., "hypermobility spectrum disorders")

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# EndoPredict for Breast Cancer Prognosis

MOL.TS.234.A

v1.0.2020

## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedure addressed by this guideline | Procedure codes |
|---------------------------------------|-----------------|
| EndoPredict Breast Cancer Assay       | 81599<br>S3854  |

## What is EndoPredict for breast cancer prognosis

### Definition

EndoPredict<sup>®</sup> is a commercial multigene expression profiling assay designed to assess prognosis in early-stage breast cancer patients.<sup>1</sup>

- The assay combined with results of the tumor size and nodal status is intended to predict the likelihood of women with early stage, node-negative, hormone receptor positive, and HER2 negative breast cancer of developing metastasis within 10 years of initial diagnosis.<sup>1</sup>
- This test identifies 12 genes related to tumor proliferation and hormone receptor activity, but does not assess ER or HER2 status.<sup>1</sup>
- Test results of the 12-gene risk score are designed to guide decisions regarding adjuvant systemic chemotherapy in women with early-stage invasive breast cancer with known hormone receptor and human epidermal growth factor receptor 2 (HER2) status following surgical management of breast cancer.<sup>1</sup>

### Test information

- The EndoPredict assay analyzes the gene expression level of 8 breast-cancer related genes and 4 reference genes (12 genes in total) within a breast tumor to determine an EndoPredict score (EP), ranging from 0 to 15. Each score corresponds to a specific likelihood of breast cancer recurrence within 10 years after the initial diagnosis. Based on the calculated score, the patient is categorized as follows:
  - Low risk: 0 to <5

- High risk: 5 to 15 for distant recurrence under endocrine therapy.<sup>1</sup>
- When combining the score with clinical risk factors, such as tumor size and node status, a combined molecular and clinical risk score, EPclin, is established. The EPclin score assigns patients into low- and high-risk groups. Patients placed in the high-risk group may be recommended to have chemotherapy, but those in the low-risk group may be able to forego chemotherapy and be spared its associated complications.<sup>1</sup>

## Guidelines and evidence

### National Comprehensive Cancer Network

- The National Comprehensive Cancer Network (NCCN) 2019 Clinical Practice Guidelines for Breast Cancer consider the 12-gene EndoPredict assay suitable for prognostic purposes (with evidence category 2A):<sup>2</sup>
  - “For patients with T1 and T2 hormone receptor-positive, HER2-negative, and lymph node-negative tumors, a 12-gene low- risk score, regardless of T size, places the tumor into the same prognostic category as T1a–T1b, N0, M0. In ABCSG 6/8, patients in the low risk group has risk of distant recurrence of 4% at 10 years and in the TransATAC study, patients with 1-3 positive nodes in the low-risk group had a 5.6% risk of distant recurrence at 10 years.”
  - These guidelines consider the therapeutic predictive value of this assay as “not determined”.

### National Institute for Health and Care Excellence

The National Institute for Health and Care Excellence (NICE) 2018 stated the following:<sup>3</sup>

- “EndoPredict (EPclin score), Oncotype DX Breast Recurrence Score and Prosigna are recommended as options for guiding adjuvant chemotherapy decisions for people with oestrogen receptor (RE)-positive, human epidermal growth factor receptor 2 (HER2)-negative and lymph node (LN)-negative (including micrometastatic disease; see section 5.4) early breast cancer, only if:”
  - “they have intermediate risk of distant recurrence using a validated tool such as PREDICT or the Nottingham Prognostic index”
  - “information provided by the test would help them choose, with their clinician, whether or not to have adjuvant chemotherapy taking into account their preference”.

## St. Gallen International Expert Consensus

The St. Gallen International Expert Consensus (updated 2017) states:<sup>4</sup>

- “The panel agreed that there was no role in clinical low risk cases [such as pT1a/b, grade 1 (G1), ER high, N0] and similar settings where chemotherapy would not be indicated under any circumstances.”
- “The Panel agreed that a number of gene expression signatures served as prognostic markers in the setting of adjuvant endocrine therapy in node-negative breast cancers, including the 21 gene recurrence score, the 70 gene signature, the PAM50 ROR score V R, the EpClin score V R, and the Breast Cancer Index V R. The Panel endorsed all of these assays for guiding the decision on adjuvant chemotherapy in node-negative tumors as they all identify node-negative cases at low risk, with an excellent prognosis that would not warrant chemotherapy.”
- “The Panel agreed that gene expression signatures offered information that can refine the prognosis for node-positive breast cancers. However, the Panel did not uniformly endorse the use of gene expression signatures for making treatment decisions regarding adjuvant chemotherapy in node positive cases.”
- “The Panel did not recommend the use of gene expression signatures for choosing whether to recommend extended adjuvant endocrine treatment, as no prospective data exist and the retrospective data were not considered sufficient to justify the routine use of genomic assays in this setting.”
- “In patients who are not candidates for adjuvant chemotherapy owing to comorbid health conditions or tumor stage/risk, or in patients who ‘obviously’ need adjuvant chemotherapy, typically including stage III breast cancer, there is no routine need for genomic tests.”
- “In general, the zone ‘in between’ is where genomic assays may be most valuable. These would often be patients with tumors between 1 and 3 cm, with zero to two or three positive lymph nodes, and intermediate proliferative fraction. Multigene assay should not be the only factor considered in making a decision to proceed or to avoid chemotherapy.”

## American Society of Clinical Oncology

The American Society of Clinical Oncology (ASCO, 2016) published a clinical practice guideline regarding the use of biomarkers to guide clinical decision-making on adjuvant systemic therapy among women with early-stage invasive breast cancer.<sup>5</sup> Based on a review of the peer-reviewed scientific evidence, the following recommendations were published:

- “If a patient has ER/PgR-positive, HER2-negative (node-negative) breast cancer, the clinician may use the 12-gene risk score (EndoPredict; Sividon Diagnostics, Koln, Germany) to guide decisions on adjuvant systemic chemotherapy. Type: evidence based. Evidence quality: intermediate. Strength of recommendation: moderate.”

- “If a patient has ER/PgR-positive, HER2-negative (node-positive) breast cancer, the clinician should not use the 12-gene risk score (EndoPredict) to guide decisions on adjuvant systemic chemotherapy. Type: evidence based. Evidence quality: insufficient. Strength of recommendation: moderate.”
- “If a patient has HER2-positive breast cancer or TN breast cancer, the clinician should not use the 12-gene risk score (EndoPredict) to guide decisions on adjuvant systemic therapy. Type: informal consensus. Evidence quality: insufficient. Strength of recommendation: strong.”

## European Society of Medical Oncology

The European Society of Medical Oncology (ESMO) (2015) states:<sup>6</sup>

- “Gene expression profiles, such as MammaPrint (Agendia, Amsterdam, the Netherlands), Oncotype DX Recurrence Score (Genomic Health, Redwood City, CA), Prosigna (Nanostring Technologies, Seattle, WA) and EndoPredict (Myriad Genetics), may be used to gain additional prognostic and/or predictive information to complement pathology assessment and to predict the benefit of adjuvant chemotherapy. The three latter tests are designed for patients with ER-positive early breast cancer only.”
- “In cases of uncertainty regarding indications for adjuvant chemotherapy (after consideration of other tests), gene expression assays, such as MammaPrint, Oncotype DX, Prosigna and Endopredict, may be used, where available.”
- “In cases when decisions might be challenging, such as luminal B HER2-negative and node-negative breast cancer, commercially available molecular signatures for ER-positive breast cancer, such as Oncotype DX, EndoPredict, Prosigna, and for all types of breast cancer (pN0–1), such as MammaPrint and Genomic Grade Index, may be used in conjunction with all clinicopathological factors, to help in treatment decision making.”

## Literature Review

Current review of the literature

- Two clinical validation studies were identified that used archived specimens from previous prospective RCTs (retrospective-prospective study).<sup>7,8</sup> Of the studies identified, these two prospective-retrospective studies are considered moderate quality evidence (Simon Level I evidence; category B; prospective using archived samples).<sup>9</sup>
  - Filipits et al. (2011) evaluated two groups of patients derived from two independent RCTs (ABC SG-6 and ABC SG-8) to assess the validity of both the EP and EPclin.<sup>3</sup> ABC SG-6 was a phase 3 RCT comparing tamoxifen alone for 5 years with tamoxifen in combination with aminoglutethimide for the first 2 years of treatment in postmenopausal women. In ABC SG-8, postmenopausal breast cancer patients were randomly assigned to receive tamoxifen for either 5 or 2



years followed by anastrozole for 3 years. Filipits et al. (2011) included women who had participated in the ABCSG-6 trial (n=378; tamoxifen-only arm; mean follow-up, 97.4 months) or the ABCSG-8 trial (n=1324; mean follow-up, 72.3 months). All tumor specimens were collected at the time of surgery before adjuvant therapy. Assessors of samples, qRT-PCR analyses and score calculations were blinded to clinical and outcome data. The primary outcome measure was distant disease recurrence. Study authors reported that qRT-PCR was successfully analyzed in ~96% and ~99% of the two patient groups.

- EPclin had significantly greater prognostic power compared with clinical pathology factors alone (c indices: 0.76 vs 0.75; P=0.024 [ABCSG-6; 0.726 vs 0.70; P=0.003 [ABCSG-8]).
- At 10 years, the distant recurrence rates were as follows:
  - Low EP and High EP (ABCSG-6): 8% (95% CI, 3-13%) and 22% (95% CI, 15-29%) (P<0.001)
  - Low EP and High EP (ABCSG-8): 6% (95% CI, 2-9%) and 15% (95% CI, 11-20%) (P<0.001)
  - Low EPclin and High EP (ABCSG-6): 4% (95% CI, 1-8%) and 28% (95% CI, 20-36%) (P<0.001)
  - Low EPclin and High EP (ABCSG-8): 4% (95% CI, 2-5%) and 22% (95% CI, 15-29%) (P<0.001)
- Buus et al. (2016) conducted a prospective-retrospective study to estimate the risk of distant recurrence in women with early-stage breast cancer (ER+/HER2-) considering adjuvant therapy.<sup>8</sup> Patients were evaluated in the prospective RCT (ATAC) evaluating the safety and efficacy of anastrozole compared with tamoxifen in postmenopausal women. Women with either node-positive or node-negative disease were included (n=928). The majority of the population had node-negative disease (n=680; 73%). (Study results that focused on node-negative disease will be discussed in this section.) Among node-positive patients, 59 had disease recurrence (8.6%). EP and EPclin were predictive of recurrence at 10 years of follow-up in both low- and high-risk groups. At 10 years, the distant recurrence rates based on Kaplan-Meier plots, stratified by pre-specified cut-off points, were as follows:
  - Low EP and High EP: 3% (95% CI, 1.5-6%) and 14.6% (95% CI, 11.3-18.8%)
  - EP hazard ratio (HR; 95% CI): 5.15 (2.44-10.85) (P<0.001)
  - Low EPclin and High EP: 5.9% (95% CI, 4-8.6%) and 20% (95% CI, 14.6-27%)
  - EPclin HR (95% CI): 3.90 (2.33-6.52%) (P<0.001)

- A prospective-retrospective study was conducted to evaluate the ability of the EndoPredict assay to identify those patients who would achieve the most benefit from continuing hormonal therapy after 5 years.<sup>10</sup> The study used archived samples from a population of ER-positive/HER2-negative post-menopausal women (node positive and node negative) from the ABCSG-6 (n=378; tamoxifen-only arm) and ABCSG-8 trials (n=1324) described in the earlier Dubsy study.<sup>11</sup> Patients were retrospectively classified to low- and high-risk EP categories based on the incidence of late recurrence. Based on Kaplan–Meier analysis of distant metastasis, assignment to the EP low-risk group was associated with a significantly reduced risk of recurrence between 0 to 5 years (HR 2.80; 95% CI, 1.81-4.34, P<0.001) and greater than 5 years (HR 3.28; 95% CI 1.47-7.24, P=0.002). Values for the EP high-risk group were not reported by study authors. When EndoPredict and clinical parameters were combined, the prediction of late recurrence was improved as evidenced by the improved c-index; the EPclin score had the highest c-index (0.786) in predicting late recurrence.
- In a prospective-retrospective study, Martin et al. (2014) evaluated the EP score in node-positive breast cancer patients (ER+/HER2-) who were treated with adjuvant chemotherapy followed by hormone therapy.<sup>12</sup> The study also evaluated whether EP scores could predict the efficacy of incorporating weekly paclitaxel into anthracycline-based regimens. Patients enrolled in the RCT (n=555; GEICAM 9906) were evaluated for distant metastasis-free survival (MFS). Rates of MFS at 10 years of follow-up were 93% for the EP low-risk group and 70% for the EP high-risk group, with an absolute risk reduction of 23% (HR 4.8; 95% CI 2.5-9.6; P<0.0001). Adding weekly paclitaxel treatment did not have an effect on the risk of relapse. The EPclin score c-index estimate of 0.70 was the highest compared with other risk factors.
- No direct evidence regarding clinical utility of EndoPredict to improve clinical decision making (e.g., predicting recurrence and/or selecting treatment approaches based on test results) and improve patient health outcomes in women with early-stage breast cancer considering adjuvant chemotherapy was identified. Weak indirect evidence from one small study (n=167) that evaluated treatment decisions assessed retrospectively suggests a potential for the test's clinical utility.<sup>13</sup> When pre- and post-test decisions were compared, a change of therapy was observed in nearly 38%. In addition, 16 patients (~12%) changed to a treatment strategy of additional chemotherapy; 33 patients (~25%) of patients changed to endocrine treatment alone. In addition to limitations of the retrospective study design, it is unclear how these projected and altered treatment recommendations would translate into improved morbidity and mortality outcomes in this patient population.
- There is adequate evidence in the peer-reviewed literature from two retrospective-prospective studies of moderate quality to support testing with EndoPredict in women with early stage (ER+/HER2-) node-negative breast cancer who are considering adjuvant chemotherapy. Moderate quality evidence indicates that use of the EndoPredict test may improve predictions regarding an individual's long-term prognosis up to 10 years and determine if they can safely avoid adjuvant chemotherapy.

- Evidence in the peer-reviewed literature regarding the use of EndoPredict in women with early stage (ER+/HER2-) node-positive breast cancer who are considering adjuvant chemotherapy remains limited.<sup>7,8,12,14</sup>
- **Ongoing clinical trials:**
  - NCT Number: NCT01805271<sup>15</sup>
    - Title: Safety Study of Adding Everolimus to Adjuvant Hormone Therapy in Women With High Risk of Relapse, ER+ and HER2- Primary Breast Cancer, Free of Disease After Receiving at Least One Year of Adjuvant Hormone Therapy

## Criteria

- Previous Testing:
  - No repeat EndoPredict testing on the same sample when a result was successfully obtained, and
  - No previous gene expression assay (e.g. OncotypeDx Breast) performed on the same sample when a result was successfully obtained, AND
- Required Clinical Characteristics:
  - Primary invasive breast cancer meeting all of the following criteria:
  - Unilateral tumor
    - Tumor size >0.5cm (5mm) in greatest dimension (T1b-T3)
    - Hormone receptor positive (ER+ or PR+), and
    - HER2 negative, and
  - Patient has no regional lymph node metastasis (pN0) or only micrometastases (pN1mi, malignant cells in regional lymph node(s) not greater than 2.0mm), and
  - Adjuvant endocrine systemic chemotherapy is a planned treatment option for the patient or results from this EndoPredict test will be used in making adjuvant chemotherapy treatment decisions, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

## Other considerations

- Testing Multiple Samples:
  - When more than one ipsilateral breast cancer primary is diagnosed, testing should be performed on the tumor with the most aggressive histologic characteristics. If an exception is requested, the following criteria will apply:

- There should be reasonable evidence that the tumors are distinct (e.g., different quadrants, different histopathologic features, etc.), AND
- There should be no evidence from either tumor that chemotherapy is indicated with or without knowledge of the EndoPredict test result (e.g., histopathologic features or previous EndoPredict result of one tumor suggest chemotherapy is indicated), AND
- If both tumors are to be tested, both tumors must independently meet the required clinical characteristics

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# Expanded Carrier Screening Panels

**MOL.TS.165.A**  
v1.0.2020

## Introduction

Expanded carrier screening panels are addressed by this guideline.

## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedures addressed by this guideline  | Procedure codes |
|---|-----------------|
| ASPA Targeted Mutation Analysis         | 81200           |
| BCKDHB Targeted Mutation Analysis       | 81205           |
| BLM Targeted Mutation Analysis          | 81209           |
| CFTR Targeted Mutation Analysis         | 81220           |
| CFTR Deletion/Duplication Analysis      | 81222           |
| CFTR Sequencing                         | 81223           |
| DMD Deletion/Duplication Analysis       | 81161           |
| FANCC Targeted Mutation Analysis        | 81242           |
| FMR1 Expansion Analysis                 | 81243           |
| FMR1 Methylation Analysis               | 81244           |
| G6PC Targeted Mutation Analysis         | 81250           |
| GBA Targeted Mutation Analysis          | 81251           |
| GJB2 Sequencing                         | 81252           |
| GJB6 Targeted Mutation Analysis         | 81254           |
| HBA1/HBA2 Targeted Mutation Analysis    | 81257           |
| HBA1/HBA2 Sequencing                    | 81259           |
| HBA1/HBA2 Deletion/Duplication Analysis | 81269           |
| HBB Targeted Mutation Analysis          | 81361           |
| HBB Deletion/Duplication Analysis       | 81363           |
| HBB Sequencing                          | 81364           |
| Hemoglobin Electrophoresis              | 83020           |
| HEXA Targeted Mutation Analysis         | 81255           |

| Procedures addressed by this guideline   | Procedure codes |
|--|-----------------|
| IKBKAP Targeted Mutation Analysis  | 81260           |
| MCOLN1 Targeted Mutation Analysis  | 81290           |
| SERPINA1 Targeted Mutation Analysis  | 81332           |
| SMN1 Gene Analysis; Dosage/Deletion Analysis (eg, carrier testing), includes SMN2 Analysis, if performed   | 81329           |
| SMPD1 Targeted Mutation Analysis   | 81330           |
| Ashkenazi Jewish Genetic Disorders Sequencing  | 81412           |
| Genetic testing for severe inherited conditions (eg, cystic fibrosis, Ashkenazi Jewish-associated disorders, genomic sequence analysis panel, must include sequencing of at least 15 genes (eg, ACADM, ARSA, ASPA, ATP7B, BCKDHA, BCKDHB, BLM, CFTR, DHCR7, FANCC, G6PC, GAA, GALT, GBA, GBE1, HBB, HEXA, IKBKAP, MCOLN1, PAH) | 81443           |
| Miscellaneous Carrier Screening Tests  | 81400           |
| Miscellaneous Carrier Screening Tests  | 81401           |
| Miscellaneous Carrier Screening Tests  | 81402           |
| Miscellaneous Carrier Screening Tests  | 81403           |
| Miscellaneous Carrier Screening Tests  | 81404           |
| Miscellaneous Carrier Screening Tests  | 81405           |
| Miscellaneous Carrier Screening Tests  | 81406           |
| Miscellaneous Carrier Screening Tests  | 81407           |
| Miscellaneous Carrier Screening Tests  | 81408           |
| Miscellaneous Carrier Screening Tests  | 81479           |

## What are expanded carrier screening panels

### Definition

Expanded carrier screening panels, also known as multiplex carrier screening panels, are designed to identify carrier status or predict risk for multiple genetic diseases in a single test. It is typically offered to individuals planning a pregnancy or currently

pregnant.

## Prevalence

The genetic diseases that are tested for range in severity from lethal in infancy to so mild an affected individual may never develop symptoms. Some conditions are quite common, especially in certain ethnic groups, while others are rare.

It is generally believed that all people carry several recessive gene mutations. An estimated 1 in 580 births has an autosomal recessive condition and 1 in 2000 have an X-linked condition.<sup>1</sup>

## Inheritance

A carrier has a single recessive gene mutation that does not cause symptoms for the person with the mutation.

Most commonly, both parents have to be carriers of the same genetic condition to have an affected child, which is autosomal recessive inheritance. In this case, each pregnancy has a 25% risk to be affected when both parents are carriers of mutations in the same gene.

Expanded carrier screening panels may include mutations for some X-linked conditions as well. In this case, a mother can be an unaffected carrier but is at risk to have a son with the genetic disease if she passes on that mutation. The father does not need to be a carrier to have an affected child in this situation.

## Common uses

Expanded carrier screening is most commonly done for reproductive planning, to identify couples at risk for having a child with a recessive inherited disorder. In most cases, couples who have a child with a recessive inherited disorder have no family history of that disorder or any other risk factors. Carrier screening for a specific disorder may be indicated when there is a positive family history, when a reproductive partner is a carrier of or affected with a recessive disorder, or when there is a known increased risk based on ethnicity or other factors.

## Test information

### Introduction

Expanded carrier screening panels determine carrier status for numerous genetic conditions simultaneously for the purposes of reproductive planning.

### Expanded carrier screening panels

Several expanded carrier screening panels are available. Each test has a unique set of diseases included in novel and proprietary genetic testing platforms. The number of



mutations tested varies considerably by condition, ranging from a single mutation for rare conditions to over 100 mutations for cystic fibrosis. Complete testing information, including a list of all conditions screened, can be found at a laboratory's website.

## Guidelines and evidence

### Introduction

This section includes relevant guidelines and evidence pertaining to expanded carrier screening.

### American College of Obstetrics and Gynecology

The American College of Obstetrics and Gynecology (ACOG, 2017)<sup>2</sup> published a committee opinion that stated the following regarding Expanded Carrier Screening:

- “Ethnic-specific, panethnic, and expanded carrier screening are acceptable strategies for prepregnancy and prenatal carrier screening. Each obstetrician–gynecologist or other health care provider or practice should establish a standard approach that is consistently offered to and discussed with each patient, ideally before pregnancy. After counseling, a patient may decline any or all carrier screening.”

“Given the multitude of conditions that can be included in expanded carrier screening panels, the disorders selected for inclusion should meet several of the following consensus-determined criteria: have a carrier frequency of 1 in 100 or greater, have a well-defined phenotype, have a detrimental effect on quality of life, cause cognitive or physical impairment, require surgical or medical intervention, or have an onset early in life. Additionally, screened conditions should be able to be diagnosed prenatally and may afford opportunities for antenatal intervention to improve perinatal outcomes, changes to delivery management to optimize newborn and infant outcomes, and education of the parents about special care needs after birth.”

- “Carrier screening panels should not include conditions primarily associated with a disease of adult onset.”

### American College of Medical Genetics and Genomics

The American College of Medical Genetics and Genomics (ACMG, 2013) published a position statement on prenatal and preconception carrier screening. This statement did not provide evidence-based guidance for specific tests or conditions. Rather, it provides general considerations for disease inclusion, clinical relevance, laboratory performance, reporting, and genetic counseling.<sup>3</sup>

## Concerns with large panels

Although large panels may be less expensive than doing each carrier screening test individually, most of the included tests are not indicated for each person being tested.

Issues with expanded carrier screening include:

- Many included tests have not been recommended for population-based carrier screening and should therefore only be performed when there is a specific known increased risk, such as a family history of the condition.
- Some conditions included in expanded carrier screens are exceedingly rare except in certain ethnicities.
- Mutation analysis may not be the preferred initial screening test for some conditions. For example, a CBC with RBC indices is the initial screening test for beta-thalassemia followed by hemoglobin analysis for individuals with microcytic anemia.<sup>3</sup> Measuring hexosaminidase A activity may be preferable to mutation analysis for Tay-Sachs carrier screening, especially in non-Jewish populations.<sup>4</sup>
- Some expanded carrier screens include testing for conditions that are relatively mild, treatable, or have onset in adulthood.
- Depending on ethnicity, current expanded carrier screening panels are expected to identify up to 40% of people tested as carriers of a recessive gene mutation. Therefore, if this screening is routinely offered, many patients will require counseling for a positive result, and partner testing must be offered. The most complete partner testing is often by full gene sequencing. Availability of partner testing, cost, turnaround time, and the possibility of identifying a variant of unknown significance by sequencing make this a complex clinical scenario to manage in the routine reproductive setting.

## Criteria

### Introduction

Requests for expanded carrier screening panels are reviewed using these criteria.

### Individually billed gene tests

Individual gene tests included in expanded carrier screening panels that will be separately billed should be evaluated based on the medical necessity criteria for each gene test.

Any gene tests that are separately billed and do not meet medical necessity criteria are not a reimbursable service. It will be at the laboratory, provider, and patient's discretion to determine if a multi-gene panel remains the preferred testing option, recognizing that only a portion of the panel may be reimbursed by insurance.

## Single panel code billed

Panel will be billed with a single procedure code, 81443, to represent all genes being sequenced.

- No single gene components of the panel have been performed and reimbursed previously, or billed separately on the same date of service, AND
- Medical necessity must be established for full gene **sequencing** of at least two conditions included in the panel. This does not include:
  - targeted mutation testing (i.e. cystic fibrosis carrier testing performed by a panel of mutations, or known familial mutation testing), or
  - molecular methodologies other than sequencing (i.e. fragile X testing; deletion/duplication analysis of any gene by MLPA or similar platform), or
  - non-molecular methodologies (i.e. hemoglobin electrophoresis for hemoglobinopathies)

## Billing and Reimbursement Considerations

The following conditions should not be billed as part of 81443 and should not count toward the requirement of two conditions meeting medical necessity requirements:

- Spinal muscular atrophy carrier testing should be billed separately using 81329
- Fragile X testing should be billed separately using 81243

Carrier testing performed due to the sole indication of Ashkenazi Jewish ancestry will be redirected to 81412.

## Coverage guidance

This table describes coverage guidance around the most commonly performed carrier screening tests. It also includes the test types addressed by population-based carrier screening guidelines. When the test is not addressed in this table, refer to the general guideline: Genetic Testing for Carrier Status. For these additional tests to be medically necessary, there will generally need to be a specific known increased risk for that condition such as a known family history or a reproductive partner who is known to be a carrier of or affected with the condition.

### **Coverage Guidance for Genes Included in Expanded Carrier Screening Multi-Gene Panels**

| Condition groups      | Condition       | Gene | CPT Code | Required Claim Code | Coverage   |
|-----------------------|-----------------|------|----------|---------------------|------------|
| Pan-Ethnic Conditions | Cystic fibrosis | CFTR | 81220    | NONE                | MOL.TS.158 |
|                       |                 |      | 81222    | NONE                | MOL.TS.158 |

| Condition groups | Condition                                 | Gene          | CPT Code | Required Claim Code | Coverage   |
|------------------|---|---------------|----------|---------------------|------------|
|                  |   |               | 81223    | NONE                | MOL.TS.158 |
|                  | Spinal muscular atrophy                   | SMN1/<br>SMN2 | 81329    | SMN1SMN2            | MOL.TS.225 |
|                  | Fragile X syndrome                        | FMR1          | 81243    | NONE                | MOL.TS.172 |
|                  |   |               | 81244    | NONE                | MOL.TS.172 |
|                  | Ashkenazi Jewish genetic disorders **     |               |          |                     | MOL.TS.129 |
|                  | Bloom syndrome                            | BLM           | 81209    | NONE                | MOL.TS.132 |
|                  | Canavan disease                           | ASPA          | 81200    | NONE                | MOL.TS.145 |
|                  | Dihydrolipoamide dehydrogenase deficiency | DLD           | 81479    | DLD                 | MOL.CU.110 |
|                  | Familial dysautonomia                     | IKBKAP        | 81260    | NONE                | MOL.CU.110 |
|                  | Familial hyperinsulinism                  | ABCC8         | 81401    | ABCC8               | MOL.CU.110 |
|                  | Fanconi anemia, type C                    | FANCC         | 81242    | NONE                | MOL.CU.110 |
|                  | Gaucher disease, type 1                   | GBA           | 81251    | NONE                | MOL.TS.173 |
|                  | Glycogen storage disease, type 1A         | G6PC          | 81250    | NONE                | MOL.CU.110 |
|                  | Joubert syndrome, type 2                  | TMEM216       | 81479    | TMEM216             | MOL.CU.110 |

Expanded Carrier Screening Panels

| Condition groups           | Condition                       | Gene      | CPT Code | Required Claim Code | Coverage             |
|----------------------------|---------------------------------|-----------|----------|---------------------|----------------------|
|                            | Maple syrup disease, type 1B    | BCKDHB    | 81205    | NONE                | MOL.CU.110           |
|                            | Mucopolipidosi s, type IV       | MCOLN1    | 81290    | NONE                | MOL.CU.110           |
|                            | Nemaline myopathy, type 2       | NEB       | 81400    | NEB                 | MOL.CU.110           |
|                            | Niemann-Pick disease, type A    | SMPD1     | 81330    | NONE                | MOL.TS.207           |
|                            | Tay-Sachs disease               | HEXA      | 81255    | NONE                | MOL.TS.226           |
|                            | Usher syndrome, type 1F         | PCDH15    | 81400    | PCDH15              | MOL.CU.110           |
|                            | Usher syndrome, type 3          | CLRN1     | 81400    | CLRN1               | MOL.CU.110           |
| Hemoglobinopathy screening | Hemoglobinopathies              | NONE      | 83020    | NONE                | Cover without review |
|                            | Sickle cell anemia, Thalassemia | HBB       | 81361    | HBB                 | MOL.TS.308           |
|                            |                                 |           | 81363    | HBB                 | MOL.TS.308           |
|                            |                                 |           | 81364    | HBB                 | MOL.TS.308           |
|                            | Alpha thalassemia               | HBA1/HBA2 | 81257    | NONE                | MOL.TS.308           |
|                            |                                 |           | 81269    | HBA1HBA2            | MOL.TS.308           |
|                            |                                 |           | 81259    | HBA1HBA2            | MOL.TS.308           |

**Note** \*\*The single Ashkenazi Jewish Carrier Screening guideline should be sufficient to assess the appropriateness of all tests in this category in most circumstances. The available individual gene test policies are provided should additional information be useful.

## References

### Introduction

These references are cited in this guideline.

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5. ACOG Committee Opinion. Number 318, October 2005. Screening for Tay-Sachs disease. *Obstet Gynecol.* 2005 Oct;106(4):893-4.

# Genetic Testing for Facioscapulohumeral Muscular Dystrophy

MOL.TS.290.A

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## Introduction

Facioscapulohumeral Muscular Dystrophy testing is addressed by this guideline.

## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedure addressed by this guideline         | Procedure code |
|---|----------------|
| D4Z4 region (FSHMD1A) deletion analysis       | 81404          |
| D4Z4 region (FSHMD1A) methylation analysis    | 81479          |
| FSHMD1 characterization of 4qA/4qB haplotypes | 81404          |
| SMCHD1 sequencing                             | 81479          |
| SMCHD1 deletion/duplication analysis          | 81479          |

## What is Facioscapulohumeral Muscular Dystrophy

### Definition

Facioscapulohumeral muscular dystrophy (FSHD) is both a genetic & epigenetic condition characterized by progressive muscle weakness involving facial, scapular, and humeral muscle groups early, and pelvic and peroneal muscle groups later.<sup>1,2</sup> There are two types of FSHD (FSHD1 and FSHD2) that are clinically identical, but distinguished by their different genetic causes.

### Incidence and Prevalence

Prevalence is estimated between 4-10 per 100,000. Approximately 95% of FSHD cases are FSHD1; the remaining cases are FSHD2.<sup>3</sup>

## Symptoms

Signs and symptoms can begin anytime between childhood and adulthood, but typical manifestations occur during the teenage years to early 20s in 90% of affected individuals.<sup>1,3</sup> There is a severe infantile form of FSHD in which muscle weakness is present from birth.<sup>3</sup>

Symptoms of FSHD include:

- Progressive facial muscle weakness (seen by difficulty with whistling) and shoulder girdle muscle weakness and atrophy
- Upper arm weakness and atrophy (“Popeye arms”), often asymmetric
- Pelvic muscle weakness and atrophy develop later
- Gait weakness, foot drop, calf hypertrophy
- Scapular winging
- Exercise intolerance
- Pain
- Extra-muscular manifestations include hearing loss (common) and vision deterioration (rare)

Severity ranges from almost asymptomatic weakness to severe restrictions of activities of daily living with some individuals requiring a wheel chair by 40 years of age.

## Cause

FSHD is caused by inappropriate expression of the DUX4 gene in muscle cells. The DUX4 gene is located within a microsatellite region called D4Z4, and relaxation of the chromatin in this region is believed to cause the aberrant expression.<sup>3</sup>

In FSHD1, the chromatin relaxation is caused by a deletion or contraction of a repeated stretch of DNA (called the D4Z4 repeat). Symptoms arise when this deletion occurs in the context of a permissive nearby haplotype (called 4A). Inheritance with another haplotype results in non-penetrance of the deletion, and FSHD1 is not likely.

In FSHD2, the chromatin relaxation is caused by the loss of methylation at D4Z4. This is commonly caused by a mutation in the SMCHD1 gene.<sup>2</sup>

## Inheritance

The pattern of inheritance differs between FSHD1 and FSHD2.

FSHD1 is inherited in an autosomal dominant pattern, with symptoms only occurring when the D4Z4 deletion occurs in the presence of the permissive haplotype. Without the presence of a specific chromosome 4A haplotype, a D4Z4 region deletion will not lead to the FSHD1 disorder.



FSHD2 inheritance is digenic, with symptoms only occurring when a mutation in SMCHD1 occurs with the permissive 4A haplotype. The inheritance is not simply autosomal dominant, as SMCHD1 sorts independently from the permissive 4A haplotype locus: they are not always inherited together or from the same parent, as is the case with FSHD1.

Between 10 and 30% of individuals diagnosed with FSHD have no family history. In these putative non-familial cases the genetic change occurred either de novo or the parents may be mosaic for the causative genetic change.

## Diagnosis

Diagnosis of FSHD is suggested by clinical phenotype and inheritance pattern, and confirmed by molecular testing. Because of the complex inheritance, careful correlation between clinical presentation and molecular result is essential.

- Diagnostic features should include a facial, scapular, humeral, and/or peroneal distribution of weakness and atrophy. Presence of a clinical phenotype more consistent with FSHD than other myopathies is an important diagnostic consideration. Note, myotonic dystrophy type 1 and 2 are very similar to FSHD and may only be distinguished by molecular testing.
- Biochemical abnormalities are nonspecific but point in the direction of muscle damage. Creatine kinase (CK) is normal to elevated, but it is not typically greater than 1500 IU/L.
- EMG shows mild myopathic changes.
- Muscle biopsy is usually reserved for cases in which molecular testing is inconclusive. If a muscle biopsy is performed, results typically show nonspecific, chronic myopathic changes and dystrophy. Occasionally there can be inflammatory changes present significant enough to suggest an inflammatory myopathy.

The University of Rochester's National Registry of Myotonic Dystrophy and Facioscapulohumeral Muscular Dystrophy defines definite FSHD diagnosis as:<sup>4</sup>

- Weakness of facial muscles, and
- Either of the following
  - Scapular weakness, or
  - Foot dorsiflexor weakness, AND
- Absence of eye involvement (ptosis or extraocular muscle weakness), and
- Absence of an alternative diagnosis on muscle biopsy, and
- EMG results that do not demonstrate myotonia or neurogenic changes

Probable FSHD diagnosis is defined as either:<sup>4</sup>

- Weakness of facial muscles, or

- Either of the following
  - Scapular weakness, or
  - Foot dorsiflexor weakness, and
- Absence of eye involvement (ptosis or extraocular muscle weakness), and
- Absence of an alternative diagnosis on muscle biopsy, and
- EMG results that do not demonstrate myotonia or neurogenic change

OR

- Weakness of facial muscles, and
- Either of the following
  - Scapular weakness, or
  - Foot dorsiflexor weakness, and

### **Treatment**

There are no disease-modifying treatments currently available for FSHD. Management is symptom driven and primarily consists of support needed to address loss of strength. Hearing loss and rarer sequelae such as vision impairment or decreased lung function should be assessed and addressed as needed.

Standard of care and management guidelines for confirmed FSDH diagnosis include:<sup>5</sup>

- Evaluation by physical therapy to address functional limitations
- Help determining standard follow-up schedules to monitor for complications (such as pulmonary function testing and ophthalmologic screenings), and the need for assistive devices
- Assessments for hearing and vision loss and other orthopedic interventions
- Pain management to avoid compounding existing mechanical limitations.

### **Survival**

FSHD is not typically life shortening, but does lead to increased morbidity.

### **Test information**

#### **Introduction**

Testing for FSHD may include Southern blot analysis and gene sequencing.

## **FSHD1 testing: Deletion assessment and Haplotyping**

Molecular testing for FSHD starts with assessment for the more common FSHD1. This testing consists of Southern blot analysis of the D4Z4 locus (reported as a number of D4Z4 repeats) and determination of the associated haplotype.

- The normal range is defined as 11-100 repeat units.
- The FSHD-associated repeat range is defined as 1-10; however, to be pathogenic, the contraction needs to occur in the context of the permissive 4A haplotype.
- Borderline repeat lengths of 10 or 11 require clinical phenotype to interpret, as they may or may not be associated with FSHD in a given individual, even in the presence of the 4A haplotype.

This analysis will detect causative variants in 95% of clinically affected individuals.<sup>3</sup>

## **FSHD2 testing: Methylation analysis and SMCHD1 sequencing**

Molecular testing for FSHD2 consists of determining the methylation status of the D4Z4 region.

- Southern blot analysis of the D4Z4 region: methylation levels below 25% are consistent with an FSHD2 diagnosis. Again, to be pathogenic, the contraction needs to occur in the context of the permissive 4A haplotype.
- If methylation analysis is abnormal, SMCHD1 gene sequencing may be performed to determine the causative mutation.
- SMCHD1 deletion/duplication analysis will find gene rearrangements that are too large to be detected by sequencing. Large deletions in SMCHD1 are infrequently reported; therefore, deletion/duplication analysis is done as second tier testing in FSHD2.

This analysis will detect causative variants in less than 5% of clinically affected individuals.<sup>3</sup>

## **Guidelines and evidence**

### **Introduction**

The following section includes relevant guidelines and evidence pertaining to FSHD testing.

### **American Academy of Neurology**

The American Academy of Neurology Evidenced-based Guideline for Clinicians (2015) considers the following to be Level B practice recommendations:<sup>5</sup>

- “Clinicians should obtain genetic confirmation of FSHD1 in patients with atypical presentations and no first-degree relatives with genetic confirmation of the disease.”
- “Large D4Z4 deletion sizes (contracted D4Z4 allele of 10-20kb) should alert the clinician that the patient is more likely to develop more significant disability and at an earlier age. Patients with large deletions are also more likely to develop symptomatic extramuscular manifestations.”

## European Neuromuscular Center

According to the 171st European Neuromuscular Center International Workshop: Standards of Care and Management of FSHD (2010): if a physician suspects FSHD clinically, genetic testing is the preferred diagnostic test.<sup>6,7</sup>

## Criteria

### Introduction

Requests for FSHD testing are reviewed using the following criteria.

### Known Familial Mutation Analysis

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - No previous genetic testing for the known familial mutation, AND
- Diagnostic Testing for Symptomatic Individuals:
  - D4Z4 deletion and permissive 4A haplotype in a 1<sup>st</sup>, 2<sup>nd</sup>, or 3<sup>rd</sup> degree biologic relative with a clinical diagnosis of FSHD, or
  - Abnormal D4Z4 methylation or disease-causing SMCHD1 mutation and permissive 4A haplotype in a 1<sup>st</sup>, 2<sup>nd</sup>, or 3<sup>rd</sup> degree biologic relative with a clinical diagnosis of FSHD, OR
- Presymptomatic Testing for Asymptomatic Individuals:
  - Member is 18 years of age or older, AND
  - One of the following has been identified in a 1<sup>st</sup>, 2<sup>nd</sup>, or 3<sup>rd</sup> degree biologic relative:
    - D4Z4 deletion and permissive 4A haplotype in a 1<sup>st</sup>, 2<sup>nd</sup>, or 3<sup>rd</sup> degree biologic relative with a clinical diagnosis of FSHD, or

- Abnormal D4Z4 methylation or disease-causing SMCHD1 mutation and permissive 4A haplotype in a 1<sup>st</sup>, 2<sup>nd</sup>, or 3<sup>rd</sup> degree biologic relative with a clinical diagnosis of FSHD, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

### **D4Z4 Deletion and Haplotype Analysis**

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - No redundant previous FSHD related testing, AND
- Diagnostic Testing for Symptomatic Individuals:
  - The member has a probable clinical diagnosis of FSHD based on the following:
    - Weakness of facial muscles, or
    - Either weakness of scapular stabilizers or foot dorsiflexors, and
    - Member has the following:
      - No involvement of the ocular muscles (including extraocular weakness or ptosis), and
      - Muscle biopsy, if available, is not consistent with another diagnosis, and
      - EMG, if available, does not show myotonia or neurogenic changes, and
      - Creatine kinase, if performed, is less than 1500 IU/L, AND
  - The member does not have a known underlying cause for their symptoms, AND
  - Rendering laboratory is a qualified provider of service per the Health Plan policy.

### **D4Z4 Methylation Analysis**

- Previous Genetic Testing:
  - No redundant previous FSHD related testing, AND
- Diagnostic Testing for Symptomatic Individuals:
  - The member meets the above criteria for D4Z4 deletion and haplotype analysis, and
  - The member has previously had negative D4Z4 deletion testing, and

- The member has a permissive 4A haplotype

### SMCHD1 Analysis

- Previous Genetic Testing:
  - No redundant previous FSHD related testing, AND
- Diagnostic Testing for Symptomatic Individuals:
  - The member meets the above criteria for D4Z4 methylation analysis, and
  - The member has low D4Z4 methylation analysis results (less than 25%).

### References

#### Introduction

This guideline cites the following references.

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# Factor II/Prothrombin Testing for Thrombophilia

MOL.TS.166.A

v1.0.2020

## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedure addressed by this guideline | Procedure code |
|---------------------------------------|----------------|
| F2 Targeted Mutation Analysis         | 81240          |

## What is prothrombin thrombophilia

### Definition

Prothrombin thrombophilia is a genetic disorder that increases one's risk for developing abnormal blood clots (venous thromboembolism or VTE).<sup>1</sup>

- Prothrombin thrombophilia is caused by a genetic change, or mutation, in the F2 gene called G20210A (20210G>A or c.\*97G>A).<sup>1-3</sup>
  - The F2 gene produces a protein that helps to initiate the formation of blood clots.<sup>1</sup>
  - The prothrombin mutation is a gain of function mutation that shifts the F2 gene into overdrive, increasing one's risk of VTE.<sup>1</sup>
  - The prothrombin mutation is one of several mutations linked to an increase risk for blood clotting.<sup>2,3</sup>
- The formation of abnormal blood clots can lead to conditions like deep vein thrombosis (DVT) and pulmonary embolism.<sup>1,2</sup>
- There has been conflicting evidence about the association of inherited thrombophilias and other pregnancy complications, such as severe preeclampsia, intrauterine growth restriction, and placental abruption.<sup>4,5</sup>
- About 1-3% of the European population have at least one prothrombin mutation.<sup>1,2,4</sup>
  - Inheriting one prothrombin mutation (heterozygous) increases one's risk for developing VTE approximately 2-fold to 4-fold compared to non-carriers.<sup>1,6</sup>
  - First-degree relatives of an individual who is heterozygous for the G20210A mutation are at 50% risk of carrying the same mutation.<sup>7</sup>



- Inheriting two prothrombin mutations (homozygous) is rare. The prevalence among the general population is 0.001-0.012% and 0.2-4% among individuals with VTE. The annual risk of VTE in homozygous is not clear but has been reported to be approximately 1.1%/year.<sup>8</sup>
- Inheriting a prothrombin mutation with other genetic risk factors such as Factor V Leiden also significantly increases the risk for developing VTE.<sup>1,8</sup>
- Definitive diagnosis of prothrombin thrombophilia relies on both clinical and genetic testing.<sup>2,3</sup>

## Test information

- Factor II/prothrombin analysis looks for the G20210A mutation, and determines how many copies of that mutation are present.<sup>2,3</sup> Understanding the number of prothrombin mutations in a suspected case is essential for proper diagnosis, management, and screening. The detection rate for prothrombin mutation analysis is virtually 100%.<sup>2,5</sup>
- Individuals with the prothrombin mutation often have mildly elevated prothrombin levels. These levels can be measured directly in suspected cases of prothrombin thrombophilia.<sup>2</sup> However, levels vary among individuals and even overlap significantly with the normal range.<sup>2</sup> Prothrombin levels are therefore not reliable for the diagnosis of prothrombin thrombophilia, and mutation analysis remains the best choice for definitive diagnosis.<sup>2</sup>

## Guidelines and evidence

- Thrombophilia in pregnancy guidelines from the American College of Obstetricians and Gynecologists (ACOG 2018) state:<sup>4</sup>
  - “Screening for inherited thrombophilias is useful only when results will affect management decisions, and it is not useful in situations in which treatment is indicated for other risk factors.”
  - Targeted assessment for inherited thrombophilia may also be considered in the following clinical scenarios: A personal history of VTE, with or without a recurrent risk factor, and no prior thrombophilia testing and a first-degree relative (e.g., parent or sibling) with a history of high-risk inherited thrombophilia. In this setting, targeted testing for the known thrombophilia can be considered if testing will influence management.
  - “Among women with personal histories of VTE, recommended screening tests for inherited thrombophilias should include factor V Leiden mutation; prothrombin G20210A mutation; and antithrombin, protein S, and protein C deficiencies.”

- “Screening for inherited thrombophilias is not recommended for women with a history of fetal loss or adverse pregnancy outcomes including abruption, preeclampsia, or fetal growth restriction because there is insufficient clinical evidence that antepartum prophylaxis with unfractionated heparin or low-molecular-weight heparin prevents recurrence in these patients.”
- Consensus guidelines from the College of American Pathologists (CAP, 2002) related to diagnostic issues in thrombophilia have been issued. These guidelines were obtained by evaluating the literature since 1996 and were accepted if 70% consensus were reached. The guidelines are summarized below:<sup>3</sup>
  - Prothrombin G20210A testing should be performed in the following individuals:
    - A first VTE before age 50 years
    - A first unprovoked VTE at any age
    - A history of recurrent VTE
    - Venous thrombosis at unusual sites such as the cerebral, mesenteric, portal, or hepatic veins
    - VTE during pregnancy or the puerperium
    - VTE associated with the use of oral contraceptives or hormone replacement therapy (HRT)
    - A first VTE at any age in an individual with a first-degree family member with a VTE before age 50 years
    - Women with unexplained fetal loss after the first trimester
  - Prothrombin G20210A testing may be considered in the following individuals/circumstances, but is more controversial:
    - Selected women with unexplained early-onset severe preeclampsia, placental abruption, or significant intrauterine growth retardation
    - A first VTE related to tamoxifen or other selective estrogen receptor modulators (SERM)
    - Female smokers under age 50 years with a myocardial infarction
    - Individuals older than age 50 years with a first provoked VTE in the absence of malignancy or an intravascular device
    - Asymptomatic adult family members of people with one or two known prothrombin G20210A alleles, especially those with a strong family history of VTE at a young age
    - Asymptomatic female family members of people with known prothrombin thrombophilia who are pregnant or considering oral contraception or pregnancy

- Prothrombin G20210A testing is not recommended for the following:
  - General population screening
  - Routine initial testing during pregnancy
  - Routine initial testing prior to the use of oral contraceptives, HRT, or SERMs
  - Prenatal or newborn testing
  - Routine testing in asymptomatic children
  - Routine initial testing in adults with arterial thrombosis
- A consensus statement from the American College of Medical Genetics (ACMG, 2001) on factor V Leiden mutation analysis also provided guidance about prothrombin testing. These older guidelines generally agree with the CAP guidelines of 2002.<sup>5</sup>
- A technical standard published by ACMG in 2018 states the following:<sup>8</sup>
  - "Testing for factor V Leiden and factor II c\*97G>A is recommended in the following circumstances:
    - A first unprovoked VTE, especially <50 years old
    - VTE at unusual sites (such as hepatic portal, mesenteric, and cerebral veins)
    - Recurrent VTE
    - Personal history of VTE with (a) two or more family members with a history of VTE or (b) one first-degree relative with VTE at a young age
    - Patients with low activated protein C (APC) resistance activity"<sup>8</sup>

An Agency for Health Care Research and Quality supported systematic review (AHRQ, 2009) found that, while mutation analysis is effective at identifying prothrombin mutations, "the incremental value of testing individuals with VTE for these mutations is uncertain. The literature does not conclusively show that testing individuals with VTE or their family members for FVL or prothrombin G20210A confers other harms or benefits. If testing is done in conjunction with education, it may increase knowledge about risk factors for VTE."<sup>9</sup>

- Evaluation of Genomic Applications in Practice and Prevention Working Group (EGAPP, 2011) found sufficient evidence to recommend against Prothrombin mutation analysis in the following scenarios:<sup>6</sup>
  - a) Adult with idiopathic VTE,
  - b) Asymptomatic adult family members of patient with VTE and a Prothrombin gene mutation for the purpose of considering primary prophylactic anticoagulation.

## Criteria

### Genetic Counseling

- Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND

### Previous Genetic Testing:

- No previous genetic testing for Factor II mutation, AND

Individual has at least one of the following clinical or family history factors suggesting a higher likelihood of having inherited thrombophilia:

- Provoked venous thromboembolism (VTE) at a young age (<50 years), or
- History of recurrent VTE, or
- VTE in an unusual site, such as those involving the hepatic, portal, mesenteric, or cerebral veins, or
- VTE associated with pregnancy or oral contraceptive use, or
- VTE associated with hormone replacement therapy, selective estrogen receptor modulators (SERMs), or tamoxifen, or
- Personal and close family history of VTE, or
- Unprovoked VTE at any age, or
- Family history of venous thrombosis at a young age (<50 years), or
- Women experiencing recurrent pregnancy loss (2 or more failed clinical pregnancies), or
- Women with a history of other unexplained poor pregnancy outcomes, including severe preeclampsia, placental abruption, fetal growth retardation, and stillbirth, or
- Family history of prothrombin gene mutation, particularly when results may impact oral contraceptive use or pregnancy management, or
- Myocardial infarction before age 50 in a female who smokes, AND

Rendering laboratory is a qualified provider of service per the Health plan policy.

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# Factor V Leiden Testing for Thrombophilia

MOL.TS.167.A

v1.0.2020

## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedure addressed by this guideline | Procedure code |
|---------------------------------------|----------------|
| F5 Leiden Genotyping                  | 81241          |

## What is Factor V Leiden thrombophilia

### Definition

About 1 in 1000 people in the U.S. experiences a first venous thromboembolism (VTE) each year, and about one-third of symptomatic patients will develop pulmonary embolism (PE).<sup>1</sup> VTE is a multifactorial condition, usually arising from a combination of genetic, acquired and circumstantial events and risk factors.

- A variant in the factor V gene (F5), called factor V Leiden (FVL), is the most common genetic risk factor for thrombophilia (hypercoagulability) among Caucasians.
  - F5 gene provides instructions for making a protein called coagulation factor V which plays a critical role in forming blood clots.<sup>2</sup>
  - A molecule called activated protein C (APC) keeps the size of clots in check by turning off F5 when clots have formed completely.<sup>2</sup>
  - The FVL variant prevents APC from inactivating F5, increasing the chance of developing abnormal blood clots.<sup>2</sup>
  - The FVL variant is one of several changes in the F5 gene that are reportedly linked to an increase risk of blood clotting.<sup>3</sup>
- The risk for FVL-related thrombosis depends on whether one or two FVL variants are present and additional risk factors, such as prothrombin gene variants.
  - A single FVL variant increases the risk for initial VTE up to 3-8 fold. Two FVL variants increases the risk more dramatically at 18-80 fold.<sup>3,4</sup> While the risk of subsequent VTE is significantly increased in anyone with a history of VTE, the risk for recurrent VTE attributable to a FVL variant after a first event is much

- more modest with a pooled odds ratio of 1.56 for single variant and 2.65 for two variants.<sup>4</sup>
- The increased risk for pregnancy-related VTE is estimated at 8 fold with a single FVL variant and 17-34 fold with two variants.<sup>3</sup>
  - The risk for oral contraceptive-related VTE is estimated at 16 fold with a single FVL variant and over 100 fold with two variants.<sup>3</sup>
  - FVL mutations have also appeared to have a small but significant association with some poor pregnancy outcomes in retrospective studies. However, more recent prospective data does not support an increased incidence of pregnancy loss among those with an FVL variant.<sup>5,6</sup> There has been conflicting evidence about the association of these variants with other pregnancy complications, such as severe preeclampsia, intrauterine growth restriction, and placental abruption.<sup>3,5,6</sup>
  - Inheriting an FVL variant with other genetic risk factors also significantly increases the risk for developing VTE. For example, inheriting both a single FVL variant and a single prothrombin variant appears to increase the risk for VTE 20 fold.<sup>3</sup>
- The frequency of FVL varies by ethnicity with about 5% of Caucasians, 2% of Hispanics, and 1% of African Americans in the US having one FVL variant.<sup>4</sup> About 1 in 1500 Caucasian people have two variants.<sup>4</sup>

## Test information

- Factor V Leiden genotyping looks specifically for the Leiden variant (1691G>A; R506Q) in the F5 gene. The detection rate for genotyping is virtually 100%.<sup>3</sup> Genotyping can determine how many Leiden variants a person has and therefore can provide information about relative risk of clotting. Understanding the number of Leiden variants in a suspected case is essential for proper diagnosis and management.
- In addition to factor V Leiden genotyping, the modified APC resistance assay is available to detect factor V Leiden thrombophilia. This assay makes use of the fact that the Leiden variant creates a protein that resists inactivation by activated protein C (APC). The APC resistance assay is effective, but does not determine how many copies of the Leiden variant are present. Therefore, if positive, factor V Leiden genotyping is recommended to confirm the findings and quantify the number of variants present.<sup>3</sup>
- Proposed uses for a positive test result include:
  - Treatment decisions for preventing recurrent VTE in an affected person
  - Primary prevention of VTE in at-risk relatives

- Decisions about use of oral contraceptives, hormone replacement therapy, or other estrogen-containing therapies
- Management decisions for preventing VTE or other possibly associated complications in pregnancy

## Guidelines and evidence

- Consensus statements from the American College of Medical Genetics (ACMG, 2001)<sup>7</sup> and the College of American Pathologists (CAP, 2002)<sup>8</sup> recommended factor V Leiden (FVL) variant testing in the populations most likely to have a mutation. These included:
  - VTE at a young age (<50 years)
  - Recurrent VTE
  - Unusual VTE site, such as those involving the hepatic, portal, mesenteric, or cerebral veins
  - VTE associated with pregnancy or oral contraceptive use
  - VTE associated with hormone replacement therapy, selective estrogen receptor modulators (SERMs), or tamoxifen
  - Personal and close family history of VTE
  - Unprovoked VTE at any age
  - Family history of VTE at a young age (<50 years)
- An Agency for Health Care Research and Quality (AHRQ, 2009) supported systematic review found that, while variant analysis is effective at identifying FVL variants, “the incremental value of testing individuals with VTE for these mutations is uncertain. The literature does not conclusively show that testing individuals with VTE or their family members for FVL or prothrombin G20210A confers other harms or benefits. If testing is done in conjunction with education, it may increase knowledge about risk factors for VTE.”<sup>9</sup>
- The Evaluation of Genomic Applications in Practice and Prevention (EGAPP, 2011), an initiative of the CDC Office of Public Health Genomics, evaluated the clinical utility evidence for two limited scenarios:<sup>4</sup>
  - a) anticoagulation duration to prevent recurrence in people with idiopathic VTE and
  - b) primary VTE prevention in their at-risk relatives. They specifically exclude individuals with other risk factors for VTE, such as estrogen-containing therapy use. EGAPP makes the following recommendations:<sup>4</sup>
    - [EGAPP] found adequate evidence to recommend against routine testing for Factor V Leiden (FVL) and/or prothrombin 20210G>A (PT) in the following circumstances: (1) adults with idiopathic venous thromboembolism (VTE). In



- such cases, longer term secondary prophylaxis to avoid recurrence offers similar benefits to patients with and without one or more of these mutations.
- (2) Asymptomatic adult family members of patients with VTE and an FVL or PT mutation, for the purpose of considering primary prophylactic anticoagulation. Potential benefits are unlikely to exceed potential harms.
- Because anticoagulation is associated with significant risks and these mutations are associated with relatively low absolute VTE risk, the potential harms of overtreatment in these scenarios appears to outweigh the benefits of testing. However, test results may be used for other treatment decisions, such as anticoagulation in high-risk situations (e.g., surgery, pregnancy, long-distance travel), avoidance of estrogen-containing therapies, or the use of low-risk preventive measures (e.g., compression hose, activity counseling, smoking cessation). The authors noted that the evidence was insufficient to determine if testing might have utility in some situations, such as for influencing patient behavior or identifying those with homozygous mutations or combined thrombophilias. Therefore, these findings have limited application to the broader decision about who should be tested.
- Several other organizations have issued guidelines that help inform a decision about clinical utility by defining the change, or lack of change, in management of patients with known FVL thrombophilia in specific clinical circumstances.
    - VTE management:
      - The American College of Chest Physicians (ACCP, 2012) recommends the same management for unprovoked VTE or VTE associated with a transient (reversible) risk factor (such as estrogen-containing therapies) irrespective of FVL results.<sup>10</sup>
      - These guidelines add that other factors (such as hereditary thrombophilia) predict risk of recurrence, but not strongly or consistently enough to influence recommendations on duration of therapy once the primary and secondary factors noted previously have been considered.<sup>9</sup>
      - The EGAPP (2011) study states “There is no evidence that knowledge of FVL/PT mutation status in patients with VTE affects anticoagulation treatment to avoid recurrence.” “Additionally, there is convincing evidence that anticoagulation beyond 3 months reduces recurrence of VTE, regardless of mutation status and there is no evidence that knowledge of FVL/PT mutation status among asymptomatic family members of patients with VTE leads to anticoagulation aimed at avoiding initial episodes of VTE.”<sup>4</sup>
    - Pregnancy management:
      - The American College of Chest Physicians (ACCP, 2012) recommends the same management for VTE in a current pregnancy or for those with a prior VTE history during or outside of pregnancy irrespective of FVL results. However, if a higher risk thrombophilia is present, such as two Leiden

variants or a combination of a Leiden and prothrombin variant, ACCP recommends some form of treatment and not simply surveillance.<sup>11</sup>

- Thrombophilia in pregnancy guidelines from the American College of Obstetricians and Gynecologists (ACOG, 2018) state:<sup>6</sup>
  - Screening for inherited thrombophilias is useful only when results will affect management decisions, and it is not useful in situations in which treatment is indicated for other risk factors. Targeted assessment for inherited thrombophilia may also be considered in the following clinical scenarios: A personal history of VTE, with or without a recurrent risk factor, and no prior thrombophilia testing and a first-degree relative (eg, parent or sibling) with a history of high-risk inherited thrombophilia. In this setting, targeted testing for the known thrombophilia can be considered if testing will influence management.
  - Among women with personal histories of VTE, recommended screening tests for inherited thrombophilias should include factor V Leiden mutation; prothrombin G20210A mutation; and antithrombin, protein S, and protein C deficiencies.
  - They add “Testing for inherited thrombophilias in women who have experienced recurrent fetal loss or placental abruption is not recommended because it is unclear if anticoagulation therapy reduces recurrence. Although there may be an association in these cases, there is insufficient clinical evidence that antepartum prophylaxis with unfractionated heparin or low molecular weight heparin (LMWH) prevents recurrence in these patients”
  - Screening for inherited thrombophilias is not recommended for women with a history of fetal loss or adverse pregnancy outcomes including abruption, preeclampsia, or fetal growth restriction because there is insufficient clinical evidence that antepartum prophylaxis with unfractionated heparin or low-molecular-weight heparin prevents recurrence in these patients.
- Estrogen-containing therapy decisions:
  - American College of Obstetricians and Gynecologists (ACOG, 2019) contraceptive use guidelines states: “The estrogenic component of combined hormonal contraceptives increases hepatic production of serum globulins involved in coagulation (including factor VII, factor X, and fibrinogen) and increases the risk of venous thromboembolism (VTE) in users. Although all combined hormonal contraceptives cause an increased risk of VTE, this risk remains half as high as the elevated risk observed in pregnancy. Women with certain conditions associated with VTE should be counseled for non-hormonal or progestin-only contraceptives. For women with a prior VTE, the risk of a recurrent VTE depends on whether the initial thrombosis was

- associated with a risk factor that is permanent (e.g., factor Leiden) or reversible (e.g., surgery).”<sup>12</sup>
- American Association of Clinical Endocrinologists (AACE, 2017) menopause guidelines: Women with a history of venous thromboembolic disease should be carefully advised about this risk with MHT is being considered. Recent evidence suggests that women at high risk for VTE should either avoid systemic HRT or choose a transdermal rather than oral delivery route.<sup>13</sup>
  - Family history of a Leiden variant:
    - The above referenced EGAPP (2011) statement specifically addresses this test use for VTE prophylaxis and found “There is no evidence that knowledge of FVL/PT mutation status among asymptomatic family members of patients with VTE leads to anticoagulation aimed at avoiding initial episodes of VTE.”<sup>4</sup>
    - American College of Obstetricians and Gynecologists (ACOG, 2018) states that testing should only be done when the results will change management. However, they add that screening “may be considered” for those with “A first-degree relative (eg, parent or sibling) with a history of high-risk thrombophilia.”<sup>6</sup>
    - Generally, estrogen-containing drugs must be approached with caution in anyone with a significant family history of VTE or known FVL and/or PT mutations, but no US evidence-based guidelines were identified that addressed testing in this scenario. Guidelines from the British Society for Haematology (BSH, 2010) most directly address FVL and PT testing in at-risk relatives for the purposes of deciding about estrogen-containing therapies. They recommend considering “alternative contraceptive or transdermal HRT [hormone replacement therapy]” when a first-degree relative: “has not been tested or is negative... Testing for heritable thrombophilia will provide an uncertain estimate of risk and is not recommended (1C).” or “has been tested and the result is positive... Offer alternative contraception, counsel that negative result would not exclude increased risk. However, testing may assist in counseling of selected women particularly if a high risk thrombophilia has been identified in the symptomatic relative (C).”<sup>14</sup>
  - The evidence supporting an association between FVL variants and thrombosis is adequate (clinical validity). However, there are no clinical situations in which FVL testing is either mandatory or specifically recommended in guidelines due to generally insufficient clinical utility data. Factor V Leiden genotyping may have some utility in limited circumstances where there is a recognized increased risk to have at least one mutation based on established risk factors, where the results will be used to direct management beyond the current VTE, and particularly when individuals are found to have a combination of more than one factor V Leiden mutation or additional genetic thrombophilias (despite the absence of reliable

indicators). If testing is performed, there should be a specific plan for how the results will impact management.

## Criteria

- Genetic Counseling
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - No previous genetic testing for Factor V Leiden mutation, AND
- Individual has at least one of the following risk factors suggesting a higher likelihood of having one or more factor V Leiden variants:
  - Unprovoked/idiopathic venous thromboembolism at any age, or
  - History of recurrent venous thromboembolism, or
  - Venous thrombosis at an unusual site (e.g., cerebral, mesenteric, hepatic, and portal veins), or
  - Venous thromboembolism during pregnancy or the puerperium, or
  - Venous thromboembolism associated with the use of estrogen-containing therapies (e.g., oral contraceptives or hormone replacement therapy), or
  - A personal history of any venous thromboembolism combined with a first-degree family member with venous thromboembolism before the age of 50 years, or
  - Known factor V Leiden variant(s) identified in at least one 1st degree relative (parent, sibling, child). (Note: 2nd or 3rd degree relatives may be considered when 1st degree relatives are unavailable or unwilling to be tested), AND
- Test results will be used for guiding management decisions beyond simply therapy of a current first venous thrombosis event or related future prophylaxis decisions, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

The following factor V Leiden genotyping test applications are specifically considered investigational and/or experimental:

- Testing without clear evidence of an increased likelihood of having at least one factor V Leiden variant. This includes but is not limited to:
  - Testing performed as part of expanded cardiovascular disease screening
  - Testing based on the presence of conditions with unclear evidence including stroke, myocardial infarction, pregnancy loss, and pregnancy complications

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# Familial Adenomatous Polyposis Testing

**MOL.TS.168.A**  
v1.0.2020

## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedures addressed by this guideline | Procedure codes |
|--|-----------------|
| APC Known Familial Mutation Analysis   | 81202           |
| APC Sequencing                         | 81201           |
| APC Deletion/Duplication Analysis      | 81203           |

## What is Familial Adenomatous Polyposis (FAP)

### Definition

FAP is an inherited colorectal cancer syndrome that accounts for up to 1 in 200 colorectal cancers.<sup>1</sup>

- FAP is clinically diagnosed when a person has 100 or more colorectal adenomatous polyps or fewer than 100 polyps and a family member with FAP. Polyposis typically begins before age 40. Virtually all people with classic FAP will develop colorectal cancer without intervention. Other clinical manifestations include:<sup>1</sup>
  - Modestly increased risk for other malignancies including cancers of the thyroid, small bowel, stomach, liver (hepatoblastoma, typically seen in children under 5), pancreas, brain (medulloblastoma), and bile duct.
  - Additional gastrointestinal manifestations including duodenal adenomas and gastric polyps.
  - Non-gastrointestinal manifestations including osteomas (often of the mandible or skull), dental abnormalities (supernumerary teeth, odontomas), desmoid tumors, soft tissue tumors (epidermoid cysts, fibromas), adrenal masses (adenomas), and congenital hypertrophy of retinal epithelium (CHRPE).<sup>1</sup> Isolated CHRPE may be found in the general population, but multiple or bilateral CHRPE in an at-risk family member may be suspicious for FAP.
  - FAP with osteomas or soft tissue tumors suggests the Gardner syndrome variant. FAP with medulloblastoma suggests the Turcot syndrome variant.

- Attenuated FAP (AFAP) is a milder form characterized by the presence of 10-99 polyps. Colon cancer generally presents at a later age than classic FAP. Individuals with 100 or more polyps occurring at later ages (35 to 40 years or older) may be found to have AFAP. A personal history of colorectal cancer before age 60 (without polyposis) and a family history of multiple adenomatous polyps may also be seen with AFAP. Currently, there is no consensus regarding precise diagnostic criteria for AFAP.<sup>1,2</sup>
- Almost all cases of FAP and some cases of AFAP are due to mutations in the adenomatous polyposis coli (APC) gene, a tumor suppressor gene. Most people inherit an APC mutation from an affected parent, but up to 1 in 4 people with FAP have a new mutation with no known affected family members. Parents of someone with FAP may also be unaffected due to germline mosaicism (a mix of normal and mutated copies of the APC gene are confined to the parent's eggs or sperm).<sup>1</sup>
- Management and prevention strategies for those affected with or at-risk for FAP/AFAP include annual flexible sigmoidoscopy or colonoscopy screening beginning at 10-15 years for FAP and every 2-3 years beginning in the late teens for AFAP. Prophylactic colectomy is generally recommended when sufficient polyps emerge such that polyposis cannot be managed endoscopically.<sup>3</sup>

## Test information

- APC sequence analysis is used to identify disease-causing mutations in those clinically diagnosed with FAP/AFAP.<sup>3-6</sup> Testing may be considered for close relatives of someone with FAP when an affected relative is unavailable for testing.<sup>5</sup>
  - Sequence analysis detects a mutation in up to 90% of individuals clinically diagnosed with FAP.<sup>1</sup> The mutation detection rate is lower for those with AFAP than classic FAP.<sup>2</sup>
- APC deletion/duplication testing is typically performed in reflex to negative analysis. Deletion/duplication testing detects an additional 8-12% of mutations in those with clinical suspicion of FAP.<sup>1</sup>
- Molecular genetic testing of MUTYH should be considered next if no APC mutation is found.<sup>1</sup>
- “Another strategy is to perform concurrent genetic testing of two or more genes known to be associated with colon cancer predisposition.”<sup>1</sup>
  - “Concurrent molecular genetic testing for both APC and MUTYH may be considered. These two genes may also be represented together on a multi-gene panel.”
  - “Multi-gene panels can be used for the simultaneous analysis of some or all of the genes known to be associated with intestinal polyposis conditions. These panels vary by methods used and genes included.”



- Once a disease-causing mutation has been identified, at-risk family members can be tested for that known familial mutation. This may be called single site mutation analysis. Those proven not to have inherited a known family mutation through genetic testing can avoid the additional screening required for those at-risk for FAP.<sup>1</sup>
- A common variant in the APC gene, called I1307K, may mildly increase the risk for colorectal cancer, but **does not cause FAP**. Testing for this variant is not widely accepted.

## Guidelines and evidence

- Consensus guidelines from the American Gastroenterological Association (AGA, 2001) recommend:<sup>4,5</sup>
  - APC gene testing in individuals age 10 or older to confirm the diagnosis of FAP or AFAP, or to provide presymptomatic screening in individuals age 10 or older with a first-degree relative with FAP or AFAP.
  - First testing an affected family member to establish if a detectable mutation is present in the family.
- Evidence- and consensus-based guidelines from the National Comprehensive Cancer Network (NCCN, 2018) state:<sup>3</sup>
  - “APC genetic testing is recommended in a proband to confirm a diagnosis of FAP and allow for mutation specific testing in family members. Additionally knowing the location of the mutation in the APC gene can be helpful for predicting severity of polyposis, rectal involvement and desmoid tumors.”
  - When the family mutation is known, APC gene testing is recommended for at-risk family members (defined as first-degree relatives or more distant relatives if closer relatives are unavailable or unwilling to be tested).
  - When the family mutation is not known, APC gene testing may be considered for first-degree relatives when an affected family member is not available or not willing to test first.
  - These recommendations are Category 2A, defined as “lower-level evidence with uniform NCCN consensus.”
  - Individuals with the APC I1307K mutation should have colonoscopy screening as determined by family history. For individuals not affected by colorectal cancer who have a first-degree relative with colorectal cancer, colonoscopy screening should occur every 5 years, beginning at age 40 years (or 10 years prior to the age at diagnosis for the affected relative). For individuals not affected by colorectal cancer who do not have a first-degree relative with colorectal cancer, colonoscopy screening should occur every 5 years, beginning at age 40 years.
- Evidence-based guidelines from the American College of Gastroenterology (ACG, 2009) recommend:<sup>6</sup>

- “patients with classic FAP (>100 adenomas) should be advised to pursue genetic counseling and genetic testing, if they have siblings or children who could potentially benefit from this testing.”[Grade 2B: “weak recommendation, moderate-quality evidence”].
- The American College of Gastroenterology (ACG, 2015) clinical guidelines state that “Individuals who have a personal history of >10 cumulative colorectal adenomas, a family history of one of the adenomatous polyposis syndromes, or a history of adenomas and FAP-type extracolonic manifestations (duodenal/ampullary adenomas, desmoid tumors, papillary thyroid cancer, congenital hypertrophy of the retinal pigment epithelium, epidermal cysts, osteomas) should undergo assessment for the adenomatous polyposis syndrome.”<sup>7</sup>

## Criteria

### APC Known Familial Mutation Analysis

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - No previous genetic APC mutation testing, AND
- Diagnostic or Predisposition Testing:
  - Family History:
    - Known family mutation in APC identified in 1<sup>st</sup> degree relative(s). (Note: 2<sup>nd</sup> or 3<sup>rd</sup> degree relatives may be considered when 1<sup>st</sup> degree relatives are unavailable or unwilling to be tested), AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

### APC Sequencing

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - No previous APC mutation testing, and
  - No known familial mutation, AND
- Diagnostic Testing for Symptomatic Individuals:

- Personal history:<sup>5,7</sup>
  - More than 10 cumulative adenomas (known or suspected diagnosis of FAP – 100 or more adenomas or AFAP – 10 to 100 adenomas), or
  - A desmoid tumor, hepatoblastoma, cribriform-morular variant of papillary thyroid cancer, or multifocal/bilateral CHRPE, OR
- Predisposition Testing for Presymptomatic/Asymptomatic Individuals:
  - Family history:
    - First degree relative of an individual with a diagnosis of FAP or AFAP. (Note: Whenever possible, an affected family member should be tested first), AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy

### **APC Duplication/Deletion Analysis**

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - No previous large rearrangement testing, and
  - Previous APC sequencing performed and no mutations found, and
  - No known familial mutation, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy

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# Familial Hypercholesterolemia Genetic Testing

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## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedures addressed by this guideline | Procedure codes |
|--|-----------------|
| LDLR Known Familial Mutation           | 81403           |
| APOB Known Familial Mutation           | 81403           |
| PCSK9 Known Familial Mutation          | 81403           |
| LDLR Sequencing                        | 81406           |
| LDLR Deletion/Duplication              | 81405           |
| APOB Targeted Mutation Analysis        | 81401           |
| APOB Sequencing                        | 81479           |
| PCSK9 Sequencing                       | 81406           |

## What is familial hypercholesterolemia

### Definition

Familial hypercholesterolemia (FH) is a genetic disorder characterized by very high levels of low-density lipoprotein (LDL) cholesterol.

- Familial hypercholesterolemia (FH) is a genetic disorder characterized by very high levels of low-density lipoprotein (LDL) cholesterol: usually >190 mg/dL in untreated adults. This leads to an increased risk for coronary heart disease (CHD), including heart attacks, at an early age.<sup>1,2,3</sup>
  - Men with untreated FH have a 50% risk for a coronary event by age 50.<sup>3,4</sup>
  - Women with untreated FH have a 30% risk for a coronary event by age 60.<sup>3,4</sup>
- People with untreated FH have about a 20 fold increase for coronary heart disease.<sup>3</sup>
- Early and aggressive LDL-lowering with high doses of potent statins or statin combination therapy significantly lowers CHD morbidity and mortality for people

with FH.<sup>5,6</sup> Statins are contraindicated during pregnancy due to concerns for teratogenicity and should be discontinued prior to conception.<sup>3</sup> Because there is considerable overlap between the LDL levels of those with FH and common multifactorial hypercholesterolemia, FH often goes undiagnosed until middle age, when much of the preventive value of cholesterol-lowering therapy is lost.<sup>7</sup>

- For FH patients who are not adequately controlled with statin therapy, or with intolerance to statins, PCSK9 inhibitors (e.g evolocumab, alirocumab) may be an effective alternative treatment.<sup>8</sup>
- Less than 10% of people with FH are adequately treated.<sup>9</sup>
- Various criteria for identifying FH clinically have been developed and are described below:<sup>4</sup>

### Diagnosis: MEDPED criteria

MEDPED criteria<sup>4</sup>

#### Total Cholesterol (LDL), mg/dL

| Patient's age | Patient has 1 <sup>st</sup> degree relative with FH | Patient has 2 <sup>nd</sup> degree relative with FH | Patient has 3 <sup>rd</sup> degree relative with FH | General population |
|---------------|---|---|---|--------------------|
| <18           | 220 (155)   | 230 (165)   | 240 (170)   | 270 (200)          |
| 20            | 240 (170)   | 250 (180)   | 260 (185)   | 290 (220)          |
| 30            | 270 (190)   | 280 (200)   | 290 (210)   | 340 (240)          |
| 40+           | 290 (205)   | 300 (215)   | 310 (225)   | 360 (260)          |

### Diagnosis: Dutch criteria

Definitive FH: 8 points or more; Probable FH: 6-7 points; Possible FH: 3-5 points<sup>4</sup>

| Points   | Description  |
|----------|--|
| 1 point  | First-degree relative with premature cardiovascular disease or LDL >95th percentile, or personal history of premature peripheral or cerebrovascular disease or LDL 155-189 mg/dL** |
| 2 points | First-degree relative with tendinous xanthoma or corneal arcus, or first-degree relative age <18 with LDL >95th percentile, or personal history of coronary artery disease         |
| 3 points | LDL 190-249 mg/dL**  |

| Points   | Description                            |
|----------|--|
| 4 points | Corneal arcus in patient age <45 years |
| 5 points | LDL 250-329 mg/dL**                    |
| 6 points | Tendon xanthoma                        |
| 8 points | LDL ≥330 mg/dL**                       |

**Note** \*\* Please note that these are LDL level cut offs for untreated individuals.

### Diagnosis: Simon Broome criteria

#### Definitive FH

- Total cholesterol (LDL): 290 (190) mg/dL in adults or 260 (155) mg/dL in pediatric patients and:
- DNA mutation

#### Probable FH

- Total cholesterol (LDL): 290 (190) mg/dL in adults or 260 (155) mg/dL in pediatric patients and:
- Tendon xanthoma in patient or in first-or second-degree relative

#### Possible FH

- Total cholesterol (LDL): 290 (190) mg/dL in adults or 260 (155) mg/dL in pediatric patients and:
- Family history of myocardial infarction (MI) at age <50 in second-degree relative or at age <60 in first-degree relative or family history of total cholesterol >290 mg/dL in first- or second-degree relative

### Prevalence

About 1 in 200-250 people worldwide have FH.<sup>3</sup> The risk is much higher in some South African Afrikaner, Amish, Lebanese, and Finnish populations.<sup>3</sup>

Approximately 1 in 300 to 500 people have heterozygous FH, which means they have one copy of the gene mutation.

Approximately one in 1 million people have homozygous FH, which means they have 2 copies of the gene mutation. This is much more severe than heterozygous FH.<sup>4</sup> People with this type of FH typically have severe coronary heart disease by their mid-20s; the rate of death or the need for surgical treatment of heart problems by the teenage years is high.<sup>3</sup>

## Cause

Most cases of FH are caused by mutations in one of three genes: LDLR, APOB, PCSK9.<sup>3</sup> However, mutations in these genes only account for approximately 60%-80% of FH.<sup>3</sup>

There are likely other genes that are not known at the present time that make up the remaining 20%-40% of cases of FH. Therefore, a negative genetic test does not rule out a diagnosis of FH.

## Inheritance

FH is an autosomal dominant condition, meaning that only one gene mutation is needed to cause the condition.

A person with heterozygous FH has a 50% chance to pass the mutation to each child.

Although not included in this guideline, it is important to note that there is an autosomal recessive form of hypercholesterolemia which is caused by mutations in the LDLRAP1 gene. There is also a milder autosomal dominant form, Familial Combined Hyperlipidemia, which is usually caused by mutations in the LPL gene.<sup>3</sup>

## Test information

- A clinical diagnosis of FH is suspected based on some combination of personal and family history of very high cholesterol, premature CHD, and cholesterol deposits, such as tendon xanthomas and corneal arcus.<sup>2</sup> At least three organizations have attempted to define clinical diagnostic criteria for FH, but all criteria have recognized limitations.<sup>10</sup> The three different criteria are described above.
- Genetic testing for FH can confirm a diagnosis of FH, particularly in borderline clinical cases.<sup>9,11,12</sup>
- Laboratories may offer evaluation of the LDLR, APOB, or PCSK9 genes individually, as panels, or with reflex options.
  - **LDLR:** Over 1000 mutations have been characterized so sequence analysis is required. Major gene deletions and rearrangements account for an estimated 9% of LDLR mutations and require specialized deletion testing to detect them.<sup>13</sup>
  - **APOB:** FH-causing APOB mutations are primarily found in a limited region of the gene, with the R3500Q mutation being most common.<sup>13</sup> Laboratory testing may be done by targeted mutation analysis for a limited number of APOB mutations or sequencing of the gene region where these mutations are generally found.<sup>3</sup> According to GeneReviews, as of 2016 there has been only one reported case of a deletion in APOB causing FH.<sup>3</sup>
  - **PCSK9:** “Gain of function mutations in PCSK9 cause fewer than 5% of cases in most studies.”<sup>14</sup> According to GeneReviews, as of 2016 there have been no deletions or duplications reported in PCSK9 that cause FH.<sup>3</sup>



- Once a mutation is found in an affected person, single-site testing should be offered to at-risk family members to allow for appropriately early intervention.<sup>15,16</sup>

## Genetic testing for FH

Proportion of FH attributed to each gene.

### *Molecular Genetic Testing for FH*

| Gene    | Proportion of FH Attributed to Mutations in Gene <sup>3</sup> | Test Method <sup>3</sup>   |
|---------|---|--|
| LDLR    | 60%-80%   | Sequence Analysis<br>Deletion/Duplication                        |
| APOB    | 1%-5%   | Targeted Analysis<br>Sequencing Analysis<br>Deletion/Duplication |
| PCSK9   | 0%-3%   | Targeted Analysis<br>Sequencing Analysis<br>Deletion/Duplication |
| Unknown | 20%-40%   | NA   |

## Guidelines and evidence

### Guidelines and evidence - genetic testing

- A Journal of the American College of Cardiology Scientific Expert Panel (2018) statement on clinical genetic testing for FH states:<sup>17</sup>
  - “Because FH is common yet underdiagnosed, it is expected that genetic testing will facilitate the diagnosis of FH, the initiation and intensity of recommended lipid-lowering therapy (LLT), and the identification of affected relatives, thus reducing the burden of cardiovascular disease in families with FH.”
- Evidence-based guidelines by the National Institute for Clinical Excellence of UK (NICE, 2008 (reaffirmed 2016)) support genetic testing for FH as follows:<sup>16</sup>
  - “A diagnosis of FH should be made using the Simon Broome criteria, which include a combination of family history, clinical signs (specifically tendon xanthomata), cholesterol concentration and DNA testing (see appendix E of the [NICE guideline](#)).”

- “Healthcare professionals should offer people with a clinical diagnosis of FH a DNA test to increase the certainty of their diagnosis and to aid diagnosis among their relatives.”
- “Healthcare professionals should inform all people who have an identified mutation diagnostic of FH that they have an unequivocal diagnosis of FH even if their LDL-C concentration does not meet the diagnostic criteria (see appendix E).”
- “In a family where a DNA mutation is identified, not all family members may have inherited the mutation. When DNA testing has excluded FH in a member of a family, healthcare professionals should manage the person's coronary heart disease risk as in the general population.”
- “In families in which a mutation has been identified, the mutation and not LDL-C concentration should be used to identify affected relatives. This should include at least the first- and second- and, when possible, third-degree biological relatives.”
- “In children at risk of FH because of one affected parent, the following diagnostic tests should be carried out by the age of 10 years or at the earliest opportunity thereafter. ”
  - “A DNA test if the family mutation is known.”
  - “LDL-C concentration measurement if the family mutation is not known. When excluding a diagnosis of FH a further LDL-C measurement should be repeated after puberty because LDL-C concentrations change during puberty.”
- The European Atherosclerosis Society Consensus Panel (2015) states the following:<sup>18</sup>
  - “Given the proven atherogenicity of LDL-C in experimental models and in humans with FH, with evidence that exposure to even moderate hypercholesterolaemia increases the long-term risk of a new CHD event, and given the lifelong benefit of genetically determined low LDL-C concentrations, there is an urgent need to identify and treat FH early to maximize therapeutic benefit.... Detection of a pathogenic mutation, usually in the LDLR gene, is the gold standard for diagnosis of FH.”
- Consensus-based guidelines from The Cardiac Society of Australia and New Zealand (CSANZ, 2013) state: “Although the clinical picture of FH will be clear-cut in many instances, the diagnostic criteria suggest that genetic testing can provide certainty of diagnosis in some cases where confounding factors such as borderline cholesterol levels, inconclusive family histories or tendon injuries have resulted in a diagnostic dilemma.”<sup>11</sup>
- The National Lipid Association expert panel on Familial Hypercholesterolemia (2011)<sup>14</sup> made the following recommendations regarding genetic testing:

- “Genetic screening for FH is generally not needed for diagnosis or clinical management but may be useful when the diagnosis is uncertain.”
- “Identification of a causal mutation may provide additional motivation for some patients to implement appropriate treatment.”
- “Importantly, a negative genetic test does not exclude FH, since approximately 20% of clinically definite FH patients will not be found to have a mutation despite an exhaustive search using current methods.”

### **Guidelines and evidence - drug treatment**

- The US Food and Drug Administration approved the following PCSK9 inhibitors as treatment for FH. However, there have been no guidelines recommending that genetic testing should be performed for the sole purpose of treatment decisions (i.e. PCSK9 inhibitors) in the absence of a clinical suspicion of FH:
  - “Praluent (alirocumab) injection in adult patients with heterozygous familial hypercholesterolemia or patients with clinical atherosclerotic cardiovascular disease such as heart attacks or strokes, who require additional lowering of LDL cholesterol.”<sup>19</sup>
  - “Repatha (evolocumab) injections for use in addition to diet and maximally-tolerated statin therapy in adult patients with heterozygous hypercholesterolemia, homozygous hypercholesterolemia, or clinical atherosclerotic cardiovascular disease, such as heart attacks or strokes, who require additional lowering of LDL cholesterol.”<sup>20</sup>

### **Criteria**

#### **LDLR, APOB, PCSK9 Known Familial Mutation Testing**

- Clinical Consultation:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - No previous genetic testing of LDLR, APOB, or PCSK9, and
  - LDLR, APOB, or PCSK9 mutation identified in 1<sup>st</sup>, 2<sup>nd</sup> or 3<sup>rd</sup> degree biological relative, AND
- Diagnostic Testing:
  - LDL cholesterol of >120 mg/dL in the absence of treatment

### **LDLR Full Sequence and Deletion/Duplication Analysis**

- Clinical Consultation:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Testing:
  - No previous LDLR sequencing or deletion/duplication testing, and
  - No known LDLR, APOB, or PCSK9 mutation in the family, AND
- Diagnostic Testing:
  - Member meets the MEDPED criteria or either the Dutch criteria or the Simon Broome criteria for possible or probable FH, and
  - Genetic testing is necessary because there is uncertainty in the clinical diagnosis

### **APOB Targeted Mutation Analysis or Full Sequence Analysis**

- Criteria for LDLR sequencing and deletion/duplication analysis is met, AND
- No previous full sequence analysis of APOB, AND
- No mutations detected in full sequencing or deletion/duplication testing of LDLR or PCSK9 sequencing, if previously performed

### **PCSK9 Full Sequence Analysis**

- Criteria for LDLR sequencing and deletion/duplication analysis is met, AND
- No previous genetic testing for PCSK9, AND
- No mutations detected in full sequencing or deletion/duplication analysis of LDLR or APOB sequencing, if previously performed

### **LDLR, APOB, PCSK9 Multigene Panels**

FH multi-gene panels, limited to testing for LDLR, APOB, and PCSK9, will be reimbursed when the following criteria are met:

- Clinical Consultation:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Testing:

- No previous LDLR, APOB, or PCSK9 sequencing or deletion/duplication testing, and
- No known LDLR, APOB, or PCSK9 mutation in the family, AND
- Diagnostic Testing:
  - Member meets the MEDPED criteria or either the Dutch criteria or the Simon Broome criteria for possible or probable FH, and
  - Genetic testing is necessary because there is uncertainty in the clinical diagnosis

### Exclusions

Genetic testing for the sole purpose of treatment decisions (i.e. PCSK9 inhibitors) in the absence of a clinical suspicion supported by either the MEDPED, Dutch, or Simon Broome criteria is considered investigational and/or experimental.

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# Familial Malignant Melanoma Testing

MOL.TS.170.A

v1.0.2020

## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedures addressed by this guideline  | Procedure codes |
|---|-----------------|
| CDKN2A Known Familial Mutation Analysis | 81403           |
| CDK4 Known Familial Mutation Analysis   | 81403           |
| CDKN2A Sequencing                       | 81404           |
| CDKN2A Deletion/Duplication Analysis    | 81479           |
| CDK4 Sequencing                         | 81479           |

## What is familial malignant melanoma

### Definition

Familial malignant melanoma (FMM) is a strongly inherited form of melanoma.

- The lifetime risk for a cutaneous melanoma for someone born in the U.S is 1 in 34 women and 1 in 53 men.<sup>1</sup> The incidence continues to rise dramatically.<sup>1</sup>
- Most melanoma is sporadic. It usually is the result of a combination of genetic susceptibility (probably from several relatively low risk gene variants such as those involved with pigment) and environmental risk factors such as sun exposure.<sup>1-4</sup>
- About 4-8% of people with melanoma have a family history of at least one first-degree relative (parent, child, sibling) with melanoma.<sup>3,5</sup> Less than 1% to 2% have multiple affected relatives, which suggests a stronger genetic susceptibility.<sup>2,5</sup>
- FMM is most likely in a family when there are three or more close relatives diagnosed with melanoma.<sup>2</sup> Other factors that may also suggest FMM include:<sup>2,4,5</sup>
  - Melanoma diagnosed younger than usual (average diagnosis age 30s versus 50s in people without FMM)
  - More than one melanoma primary in the same individual
  - Melanoma and pancreatic cancer in the same family

- Multiple, atypical moles, called dysplastic nevi that are often larger than 5mm in diameter with irregular borders. Melanoma with multiple nevi has also been called familial atypical mole-malignant melanoma syndrome. However, the presence or absence of such moles is no longer viewed as a reliable predictor of FMM in a family.
- Several genes have been linked to a higher risk of melanoma in families. CDKN2A gene mutations account for most of the currently identifiable FMM mutations, followed by CDK4 mutations.<sup>6</sup>
- FMM is an autosomal dominant condition, meaning that only one gene mutation is needed to increase susceptibility to melanoma. A person with FMM has a 50% chance to pass the mutation to each child.
- People who inherit an FMM mutation do not always develop melanoma. Data for CDKN2A mutations suggest that in the United States the melanoma risk is 50% by age 50 and 76% by age 80.<sup>4</sup> The likelihood may vary with geographic location and sun exposure.<sup>5</sup>
- Carriers of the CDKN2A p16-Leiden mutation have been found to have between 17% to 25% risk for pancreatic cancer. Estimates from studies using population based identification of subjects have shown a 7.4 relative-risk (95% CI 2.3 to 18.7) for pancreatic cancers in families with other CDKN2A/p16 mutations.<sup>7</sup>
- Familial melanoma is also associated with some other inherited cancer syndromes, like Li Fraumeni syndrome, inherited retinoblastoma, and xeroderma pigmentosum.<sup>2</sup> Additionally, germline mutations in the BAP1 gene have been identified in families with cutaneous and ocular melanoma.<sup>8</sup>

## Test information

- CDKN2A Sequencing: Identifies the majority of FMM-causing mutations, and is usually the first step in testing. The likelihood that genetic testing will identify an FMM mutation varies with the personal and family history. The chance of finding a CDKN2A mutation is:
  - 20-40% of people with melanoma from a family with at least 3 affected first-degree relatives.<sup>2,6</sup>
  - Less than 5% of those with only 2 affected first-degree relatives<sup>2</sup>
  - 15% in someone with multiple melanoma primaries and no known family history<sup>2</sup>
  - 25-40% in people diagnosed with familial atypical mole-malignant melanoma syndrome - a subset of FMM characterized by >50 atypical nevi with characteristic microscopy features<sup>9</sup>
  - 74% of families with FMM and pancreatic cancer<sup>6</sup>
- CDKN2A Deletion/Duplication Analysis: Tests for large deletions that cannot be identified by sequencing.



- CDK4 Sequencing: Sequencing, sometimes of only exon 2, is also available, but mutations are uncommon, accounting for only 2-3% of FMM cases.<sup>6</sup>
- CDKN2A Known Familial Mutation Analysis: When the family mutation is known, testing for only the family mutation can be performed in at-risk relatives. Test accuracy approaches 100%.<sup>2</sup>
- CDK4 Known Familial Mutation Analysis: When the family mutation is known, testing for only the family mutation can be performed in at-risk relatives. Test accuracy approaches 100%.<sup>2</sup>

## Guidelines and evidence

- No evidence-based U.S. guidelines were identified.
- FMM genetic testing outside of the research setting is not currently recommended for several reasons, including:
  - Currently available testing does not detect a mutation in a significant number of people who appear to have FMM. Therefore, a negative result cannot rule out FMM and should not change the prevention and screening plan for at-risk people.<sup>2</sup>
  - Individuals with FMM mutations need essentially the same prevention and screening as anyone at high risk for melanoma (family history, pigmentation, multiple moles, history of blistering sunburn).<sup>2</sup> Therefore, identifying an FMM-causing mutation is also not expected to change screening or treatment.<sup>5</sup>
  - When a family FMM mutation has been found, other relatives who test negative for that mutation at best only return to the background risk for melanoma (which may be as high as 1 in 25) and still need regular skin screening.<sup>2</sup>
  - A significant percentage of people with recognized FMM mutations do not develop melanoma, which is especially true when sun exposure is limited by geography or prevention.<sup>4</sup>
- The Melanoma Genetics Consortium (GenoMEL), an international research collaborative group, published a consensus statement in 1999 stating, “DNA testing for mutations in known melanoma susceptibility genes should only rarely be performed outside of defined research programs. With this general proviso, two distinct clinical situations need further consideration: families in which a CDKN2A mutation has been identified in a proband as part of a research study and families for which no prior testing of affected individuals has been conducted.”<sup>2</sup>
  - “Individuals who choose to undergo genetic testing [in a research setting] should have a second independent diagnostic (as distinct from research) DNA test performed in an accredited genetic testing laboratory.”<sup>2</sup>
  - For at-risk relatives with a known familial mutation, test sensitivity is virtually 100%. However, the likelihood of developing melanoma in mutation-positive

individuals is largely unknown and there is “lack of proved efficacy of prevention and surveillance strategies based on DNA testing, even for mutation carriers.” They do acknowledge potential benefits could include enhanced motivation to adhere to prevention and screening guidelines, earlier melanoma diagnosis if the biopsy threshold is lower, and lower anxiety for those who learn they are negative for a known family mutation.<sup>2</sup>

- The National Comprehensive Cancer Network (NCCN) Melanoma Guideline (updated 2019) includes family history as a melanoma risk factor and alters management based on this risk. However, these guidelines do not address genetic testing for FMM.<sup>1</sup>

## Criteria

- This test is considered investigational and/or experimental.
  - Investigational and experimental (I&E) molecular and genomic (MolGen) tests refer to assays involving chromosomes, DNA, RNA, or gene products that have insufficient data to determine the net health impact, which typically means there is insufficient data to support that a test accurately assesses the outcome of interest (analytical and clinical validity), significantly improves health outcomes (clinical utility), and/or performs better than an existing standard of care medical management option. Such tests are also not generally accepted as standard of care in the evaluation or management of a particular condition.
  - In the case of MolGen testing, FDA clearance is not a reliable standard given the number of laboratory developed tests that currently fall outside of FDA oversight and FDA clearance often does not assess clinical utility.

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# FibroTest/FibroSURE

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## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedures addressed by this guideline | Procedure codes |
|--|-----------------|
| HCV Fibrosure                          | 81596           |
| ASH Fibrosure                          | 0002M           |
| NASH Fibrosure                         | 0003M           |

## What is FibroTest/FibroSURE

### Definition

Liver fibrosis is a condition that can lead to cirrhosis, liver failure, and portal hypertension; it is defined by the accumulation of excess proteins such as collagen, which leads to the buildup of scar tissue.<sup>1</sup>

- There are many disease pathways that can lead to fibrosis, such as hepatitis B and C viruses (HBV and HCV, respectively), heavy alcohol use, and metabolic disease. Such diseases cause the liver cells, hepatocytes, to function improperly, which leads to the excess buildup of protein.
- Evaluating the extent of liver fibrosis is an important factor for clinicians making treatment decisions for patients with hepatitis B and C. Liver biopsy is currently considered to be the gold standard for evaluating liver fibrosis; however, obtaining a liver biopsy involves invasive surgery. As a result, several non-invasive alternatives have been developed, including FibroTest.
- FibroTest uses indirect markers to estimate the extent of fibrosis.<sup>1</sup> FibroTest (licensed in the United States as FibroSURE) was developed to be an alternative to liver biopsy in the assessment of liver fibrosis. The remainder of this guideline will refer to the test as FibroSURE.
- FibroSURE is a combination of five biochemical assays: alpha2-macroglobulin, haptoglobin, apolipoprotein A1, gamma glutamyl transpeptidase (GGT) and total bilirubin. An additional component – alanine aminotransferase (ALT) – is infrequently used to test for necroinflammatory lesions. This addition is known as ActiTest. The results of these assays are taken into account along with a patient's

age, gender, height and weight for the final FibroSURE score and/or ActiTest stage.<sup>2</sup>

- FibroSURE is intended for patients with chronic viral hepatitis B or C, alcoholic liver disease, and metabolic steatohepatitis (for those who are overweight, have diabetes, or hyperlipidemia). Under the name FibroMax, there are five different combinations of tests, which includes FibroSURE, ActiTest, SteatoTest, NashTest and AshTest.<sup>2</sup>

## Test information

- FibroSURE™ is a serum biomarker test that is designed to assess liver fibrosis in patients with chronic viral hepatitis B or C, alcoholic liver disease, and metabolic steatohepatitis (for those who are overweight, have diabetes, or hyperlipidemia).
- This test uses serum or plasma from a blood sample, preferably from a patient who has fasted or had a light meal prior to blood draw.
- The specific assays performed are as follows:
  - Alpha-2-macroglobulin
  - Haptoglobin
  - Apolipoprotein A1
  - Gamma-glutamyl transpeptidase (GGT)
  - Total bilirubin
  - ALT (additional component known as ActiTest)
- The FibroSURE score is a range from 0-1, which is proportional to the severity of fibrosis. FibroSURE scores have been assigned a corresponding METAVIR stage, as well as a Knodell and Ishak stage. Per the manufacturer, results should also come with a visual component that assigns three classes of severity: green=absent/minimal, orange=moderate, and red=significant.

## Guidelines and evidence

### American Association for the Study of Liver Disease

The American Association for the Study of Liver Disease published a practice guideline (2018) stating:<sup>3</sup>

- “Liver stiffness measurements (elastography) are more accurate than serum fibrosis panels (e.g. aspartate aminotransferase [AST] to platelet ratio index or FIB-4) in predicting significant or advanced fibrosis.(123,124) Noninvasive methods

overestimate fibrosis if high levels of necroinflammation, as reflected by elevated ALT, are present.”

- “Liver biopsy offers the only means of assessing both fibrosis and inflammation.”
- Of alternate/non-invasive methods, elastography is preferred.

## World Health Organization

The WHO has published documents on several liver-related diseases.

- Guidelines for the care and treatment of persons diagnosed with chronic hepatitis C virus infection (2018):<sup>4</sup>
  - “In resource-limited settings, WHO recommends that the assessment of liver fibrosis should be performed using non-invasive tests (e.g. aspartate/platelet ratio index (APRI) score or FIB-4 test, see existing recommendations, p. xvii). This can determine if there is cirrhosis before initiation of treatment.”
- Guidelines for the care and treatment of persons diagnosed with chronic hepatitis B virus infection (2015):<sup>5</sup>
  - “Aspartate aminotransferase (AST)-to-platelet ratio index (APRI) is recommended as the preferred non-invasive test (NIT) to assess for the presence of cirrhosis (APRI score >2 in adults) in resource-limited settings. Transient elastography (e.g., FibroScan) or FibroTest may be the preferred NITs in settings where they are available and cost is not a major constraint. (Conditional recommendation, low quality of evidence) ”

## World Gastroenterology Organisation

The World Gastroenterology Organisation has published documents on several liver-related diseases.

- Hepatitis C (2017)<sup>6</sup>
  - The extent of hepatic fibrosis should be checked using noninvasive measures:
    - “Studies have demonstrated that FibroScan is a sensitive alternative to liver biopsy. The amount of fibrosis can be quantified very easily and reliably in more than 95% of the patients [45]. A correct interpretation of transient elastography must have an interquartile range/median values of < 30% and serum ALT < 5 × upper limit of normal. There should be no ongoing excessive alcohol intake, and the patient’s BMI should be taken into account. If the BMI is over 30 kg/m<sup>2</sup>, using extralarge (XL) probes may be considered.”
    - “In resource limited regions, and in places where FibroScan is not readily available, scores such as the fibrosis 4 index (FIB4), AST to platelet ratio index (APRI), and acoustic radiation force impulse (ARFI) can be used. An

APRI score  $\geq 2$  can be used to predict the presence of cirrhosis. At its cut-off point, the ARFI score has a sensitivity of 48% but a specificity of 94% for predicting cirrhosis. It can also be used to predict the presence of significant fibrosis (stages 2–4). Using a cut-off value of 1.5, the sensitivity is 37% and the specificity is 95% for significant fibrosis [46,47].”

- Hepatitis B (2015)<sup>7</sup>
  - “Measurement of liver fibrosis by serological testing, FibroScan (transient elastography), or liver biopsy.”
  - Determination of the severity of liver disease:
    - “Laboratory tests for inflammation (ALT), hepatic function (bilirubin, albumin, coagulation factors and viral load (HBV DNA), if available”
    - “Hepatic ultrasound examination”
    - “Non-invasive methods to assess fibrosis (serum panels, transient elastography)”
  - Liver biopsy “can help exclude other coexistent causes of liver disease and clarify the diagnosis when ALT and HBV DNA levels are discordant.”
- Esophageal Varices (2014)<sup>8</sup>
  - In recommendations on “Esophageal varices”, the WGO states that the “predictive accuracy is still unsatisfactory” for noninvasive markers such as FibroSURE.

### European Association for the Study of the Liver

The European Association for the Study of the Liver (EASL) (2015) published a guideline entitled “Non-invasive test for evaluation of liver disease severity and prognosis”. In this document the EASL discusses the pros and cons of serum biomarkers of liver disease, stating that “further validation is warranted”.<sup>9</sup>

### British HIV Association

In a 2013 document the Association states:<sup>10</sup>

- “The Writing Group suggests hepatic transient elastography (TE) (FibroScan™ or Acoustic Radiation Force Impulse [ARFI]) as the non-invasive investigation of choice (2B) but if unavailable, or when reliable TE readings are not obtained, a blood panel test (aspartate transaminase to platelet ratio index [APRI], FIB-4, enhanced liver fibrosis [ELF], Fibrometer™, Forns Index, FibroTest™) as an alternative (2C).”

## Peer Reviewed Literature

- Several systematic reviews evaluating evidence of FibroSURE have pooled results of multiple studies; one review found AUROCs in the range of 0.75 to 0.84 for fibrosis; from 0.81-0.92 for cirrhosis in patients with HCV; 0.72-0.90 for detecting fibrosis; and from 0.75-0.92 for detecting cirrhosis in patients with HBV.<sup>11</sup> Another systematic review reported an HSROC of 0.84 for fibrosis, and 0.87 for cirrhosis in patients with HBV.<sup>12</sup> A pooled meta-analysis estimated a sensitivity of 71.2% and a specificity of 81.4% in patients with HBV.<sup>12</sup> In patients with ALD, the sensitivity for detecting fibrosis was 85% and specificity was 66%; for detecting cirrhosis, sensitivity rose to 91% and specificity to 87%.<sup>13</sup> Different analysis methods resulted in different AUROCs for diagnosing NAFLD; a weighting method produced an AUROC of 0.85, while a random effects model yielded an AUROC of 0.72.<sup>14</sup> In a prospective study of nearly 300 patients with alcohol-related liver disease, while FibroTest showed accuracy in predicting advanced fibrosis, it was outperformed by Enhanced Liver Fibrosis (ELF) test and elastography.<sup>15</sup>
- Despite many different types of studies and analysis methods, the diagnostic accuracy of FibroSURE generally seems to fall within a consistent, albeit moderate range. FibroSURE also generally seems to perform better in diagnosing cirrhosis than fibrosis.
- FibroSURE is intended as an alternative to liver biopsy, currently considered the gold standard for staging liver fibrosis and cirrhosis. As a blood-based test, FibroSURE is an appealing alternative to the invasive nature of a biopsy. However, the evidence as a whole is insufficient and does not yet fully support using FibroSURE as a stand-alone test.<sup>11-20</sup> Furthermore, several guideline organizations have published evidence-based recommendations regarding the treatment of liver disease and do not definitively recommend FibroSURE as a first-line choice; instead, they recommend the test as an alternative after other preferred tests, or in settings where resources are not constrained.

## Criteria

- This test is considered investigational and/or experimental.
  - Investigational and experimental (I&E) molecular and genomic (MolGen) tests refer to assays involving chromosomes, DNA, RNA, or gene products that have insufficient data to determine the net health impact, which typically means there is insufficient data to support that a test accurately assesses the outcome of interest (analytical and clinical validity), significantly improves health outcomes (clinical utility), and/or performs better than an existing standard of care medical management option. Such tests are also not generally accepted as standard of care in the evaluation or management of a particular condition.
  - In the case of MolGen testing, FDA clearance is not a reliable standard given the number of laboratory developed tests that currently fall outside of FDA oversight and FDA clearance often does not assess clinical utility.



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# Flow Cytometry

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## Introduction

Flow cytometry testing is addressed by this guideline.

## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedures addressed by this guideline  | Procedure codes |
|---|-----------------|
| Flow cytometry, cell cycle or DNA analysis  | 88182           |
| Flow cytometry, cell surface, cytoplasmic, or nuclear marker, technical component only; first marker  | 88184           |
| Flow cytometry, cell surface, cytoplasmic, or nuclear marker, technical component only; each additional marker (List separately in addition to code for first marker) | 88185           |
| Flow cytometry, interpretation; 2 to 8 markers  | 88187           |
| Flow cytometry, interpretation; 9 to 15 markers   | 88188           |
| Flow cytometry, interpretation; 16 or more markers  | 88189           |

## What is flow cytometry

### Definition

Flow cytometry is a method that uses lasers to detect cell characteristics, including their cell surface or cytoplasmic antigens, size, and granularity, by employing fluorescently-labeled antibodies. Specimens are most commonly fluids such as blood or bone marrow, but it is also possible to test ground-up solid samples.

## Flow cytometry procedure coding

The following combination(s) of CPT codes may be used unless more specific CPT codes exist (e.g., 86355-86367, 86828-86835). Any deviation from these CPT coding standards is subject to review and denial if not properly coded.

- 88184 is used to describe the technical component of the first marker applied (maximum one unit).
- 88185 is used for each additional marker applied and billed with the applicable number of units. Therefore, 88185 should not be billed without 88184.
- Because these two codes describe only the technical component, there are three other interpretation codes that may be applied based on the number of markers assessed (each billed with a maximum of one unit):
  - 88187 for evaluating 2 to 8 markers
  - 88188 for evaluating 9 to 15 markers
  - 88189 for evaluating 16 or more markers

## Common uses

A variety of disorders are associated with distinct biomarker patterns, which can be used to diagnose, subtype, or monitor these disorders.<sup>1</sup> The following are common uses of flow cytometry in medicine:

### Hematopoietic neoplasm evaluation and monitoring

Hematopoietic neoplasm evaluation and monitoring is the most common use, which includes leukemia and lymphoma phenotyping and minimal residual disease (MRD) detection. MRD is when individuals with acute leukemia appear to be in remission having levels of disease below morphologic detection on bone marrow samples but detectable through flow cytometry. Initial panels are generally smaller and made to account for all major cell populations while more extensive flow cytometry evaluation should be reserved for those cases with a higher likelihood of a new diagnosis of leukemia/lymphoma based on initial panel/evaluation.<sup>2,3</sup> In MRD, a limited panel based upon the patient's original disease immunophenotype may be employed.

### HIV infection monitoring

Flow cytometry is used for HIV infection monitoring to accurately and reliably evaluate the number of CD4 positive T lymphocytes.<sup>4</sup>

## Immunodeficiency

Flow cytometry is used for immunodeficiencies, which may be associated with absent or impaired cell proteins (primary disease), leukocyte dysfunction, and markers of immune status in lymphocytes (secondary disease).<sup>1</sup>

## Paroxysmal nocturnal hemoglobinuria

Paroxysmal nocturnal hemoglobinuria (PNH), a rare stem cell disorder, is diagnosed through the detection of deficient antigens on red blood cells, monocytes, and/or granulocytes by flow cytometry.<sup>1</sup>

## Criteria

### Introduction

Requests for flow cytometry testing are reviewed using these criteria. This guideline addresses common clinical applications of flow cytometry-based tests that are billed using CPT codes 88184-88189. It is not intended to encompass flow cytometry-based tests billed using more specific CPT codes (e.g., 86355-86367, 86828-86835).

### Hematopoietic Neoplasm Evaluation and Monitoring

#### ***Medical necessity requirements:***

Because the flow cytometry markers used to evaluate a sample are necessarily different based on clinical indication, information from other evaluations (e.g., morphology), sample type, and the laboratory setting, this guideline addresses general principles of marker panel selection.<sup>2</sup> Of note, many labs are now using  $\geq 8$  color flow cytometry panels; increased color panels provide more accuracy in identifying different cell populations, better sensitivity in detecting low levels of minimal residual disease (the finding of which can affect patient outcomes), and the ability to better analyze even very small or paucicellular specimens.<sup>3,5</sup>

- In the initial evaluation of suspected hematopoietic neoplasm:
  - Common non-neoplastic causes of the clinical presentation (e.g., infection or asplenia with leukocytosis, etc.) should be reasonably ruled out before flow cytometry is employed.
  - A limited but sufficient number of markers should be used in the initial evaluation that allows identification of all major categories of neoplasia (B, T, myeloid, or plasma cell lineages) under consideration based on the clinical indication.
  - Testing with additional markers is indicated to further characterize disease when the initial evaluation is suggestive.

- For staging or evaluating residual disease in patients with a known diagnosis of hematopoietic neoplasm, a limited panel of markers characteristic of that neoplasm should be used.

***Billing and reimbursement considerations:***

Most presentations, even non-specific indications that require evaluation of several lineages (e.g., anemia, thrombocytopenia, etc.), should rarely require more than 23 flow cytometry markers.<sup>2</sup> In those cases in which a new leukemia diagnosis needs confirmation and further characterization, up to 27 flow cytometry markers are usually employed.<sup>6</sup> Monitoring of a known hematopoietic neoplasia diagnosis requires fewer flow cytometry markers, usually less than 18.<sup>7</sup> Therefore:

- In addition to the one marker represented by CPT 88184, reimbursement will routinely be limited to 22 units of CPT 88185 for non-new leukemic cases, 26 units of CPT 88185 for new leukemia diagnoses, and 17 units of CPT 88185 for disease monitoring.
- ICD code information may be compared with units billed to identify cases with possible excess units that will require post-service medical necessity review. Expected unit number is based on the required cell lineage evaluation by medical indication outlined in reported flow cytometry guidelines.<sup>2,6,7</sup>
- When a laboratory routinely bills more than an average of 20 markers, claims from that laboratory will be subject to post-service medical necessity review.

**HIV Monitoring*****Medical necessity requirements:***

- Flow cytometry is an important method for determining the percentage of lymphocytes that express antigens used to identify CD4+ T cells, and to directly measure absolute T cell counts in the case of single-platform technology (SPT).<sup>4</sup>
- Four antibodies are routinely required (CD45, CD3, CD4, CD8), which may be applied in three- or four-color antibody panels.
- For pediatric patients, additional antibodies may be required to determine CD19+ B-cell values, which is an indicator of immune status in this population.

***Billing and reimbursement considerations:***

- The most commonly required flow cytometry studies for HIV are represented by marker-specific CPT codes (e.g., 86355-86367). The non-specific flow cytometry codes should not be used when a more specific code exists.
- Therefore, the non-specific CPT codes addressed in this policy should not routinely be required for HIV monitoring. Post-service medical necessity review may be employed when such codes are used for HIV monitoring as indicated by the following ICD codes:

- ICD10 Codes:
  - [ICD10 Codes Indicating HIV Positive Status](#)

### Non-Reimbursed Clinical Indications

#### ***Medical necessity requirements:***

Flow cytometry procedures will not be reimbursed for the evaluation of the following indications:

- Detection of sexually transmitted organisms, such as human papillomavirus
- Hypertension or cardiovascular disease risk

#### ***Billing and reimbursement considerations:***

Flow cytometry will not be reimbursed when billed with any of the following ICD codes:

- ICD10 Codes:
  - [ICD10 Codes Indicating Testing for STIs](#)
  - [ICD10 Codes Indicating Testing for Hypertension or Cardiovascular Disease Screening](#)

### Other Clinical Indications

#### ***Medical Necessity Requirements:***

Flow cytometry has a variety of applications that cannot all be adequately addressed by this guideline. All flow cytometry studies must be performed for well-validated and medically necessary indications.

#### ***Billing and reimbursement considerations:***

When flow cytometry is billed with ICD codes that do not suggest one of the other clinical indications addressed in this policy, post-service medical necessity review may be employed. See the Reimbursement Policy that addresses *Post-Service Medical Necessity Determination* for more information.

### ICD10 Codes

ICD10 codes in this section may be used to support or refute medical necessity as described in the above criteria.

#### ***ICD10 Codes Indicating HIV Positive Status***

| ICD10 Code or Range | Description                                |
|---------------------|--|
| B20                 | Human immunodeficiency virus [HIV] disease |

| ICD10 Code or Range | Description  |
|---------------------|--|
| B97.35              | Human immunodeficiency virus, type 2 [HIV-2]   |
| O98.7X              | Human immunodeficiency virus [HIV] disease complicating pregnancy, childbirth and the puerperium |
| R75                 | Inconclusive laboratory evidence of human immunodeficiency virus [HIV]                           |
| Z21                 | Asymptomatic human immunodeficiency virus [HIV] infection status                                 |

### ICD10 Codes Indicating Testing for STIs

| ICD10 Code or Range | Description   |
|---------------------|---|
| A50.X               | Congenital syphilis   |
| A51.X               | Early syphilis  |
| A52.X               | Late syphilis   |
| A53.X               | Other and unspecified syphilis  |
| A54.X               | Gonococcal infection  |
| A55                 | Chlamydial lymphogranuloma (venereum)                                       |
| A56.X               | Other sexually transmitted chlamydial diseases                              |
| A57                 | Chancroid   |
| A58                 | Granuloma inguinale   |
| A59.X               | Trichomoniasis  |
| A60.X               | Anogenital herpesviral [herpes simplex] infections                          |
| A63.X               | Other predominantly sexually transmitted diseases, not elsewhere classified |
| A64                 | Unspecified sexually transmitted disease                                    |
| A74.89              | Other chlamydial diseases   |
| A74.9               | Chlamydial infection, unspecified (includes childbirth and postpartum)      |
| B37.3               | Candidiasis of vulva and vagina   |
| B37.4X              | Candidiasis of other urogenital sites                                       |
| B97.7               | Papillomavirus as the cause of diseases classified elsewhere                |



| ICD10 Code or Range | Description   |
|---------------------|---|
| L29.3               | Anogenital pruritus, unspecified  |
| M02.30              | Reiter's disease, unspecified site  |
| N34.X               | Urethritis and urethral syndrome  |
| N35.111             | Postinfective urethral stricture, not elsewhere classified, male, meatal  |
| N37                 | Urethral disorders in diseases classified elsewhere                       |
| N39.0               | Urinary tract infection, site not specified                               |
| N39.9               | Disorder of urinary system, unspecified                                   |
| N70.X               | Salpingitis and oophoritis  |
| N71.X               | Inflammatory disease of uterus, except cervix                             |
| N72                 | Inflammatory disease of cervix uteri                                      |
| N73.X               | Other female pelvic inflammatory diseases                                 |
| N74                 | Female pelvic inflammatory disorders in diseases classified elsewhere     |
| N75.X               | Diseases of Bartholin's gland   |
| N76.X               | Other inflammation of vagina and vulva                                    |
| N77.X               | Vulvovaginal ulceration and inflammation in diseases classified elsewhere |
| N94.1               | Dyspareunia   |
| O09.X               | Supervision of high risk pregnancy  |
| O23.X               | Infections of genitourinary tract in pregnancy                            |
| O86.1X              | Other infection of genital tract following delivery                       |
| O86.2X              | Urinary tract infection following delivery                                |
| R87.5               | Abnormal microbiological findings in specimens from female genital organs |
| R87.6X              | Abnormal cytological findings in specimens from female genital organs     |
| R87.8X              | Other abnormal findings in specimens from female genital organs           |

| ICD10 Code or Range | Description  |
|---------------------|--|
| Z00.00              | Encounter for general adult medical examination without abnormal findings                            |
| Z00.8               | Encounter for other general examination  |
| Z01.4X              | Encounter for gynecological examination  |
| Z11.3               | Encounter for screening for infections with a predominantly sexual mode of transmission              |
| Z11.51              | Encounter for screening for human papillomavirus (HPV)   |
| Z11.59              | Encounter for screening for other viral diseases   |
| Z11.8               | Encounter for screening for other infectious and parasitic diseases                                  |
| Z11.9               | Encounter for screening for infectious and parasitic diseases, unspecified                           |
| Z12.4               | Encounter for screening for malignant neoplasm of cervix   |
| Z20.2               | Contact with and (suspected) exposure to infections with a predominantly sexual mode of transmission |
| Z20.6               | Contact with and (suspected) exposure to human immunodeficiency virus [HIV]                          |
| Z20.818             | Contact with and (suspected) exposure to other bacterial communicable diseases                       |
| Z20.828             | Contact with and (suspected) exposure to other viral communicable diseases                           |
| Z20.89              | Contact with and (suspected) exposure to other communicable diseases                                 |
| Z20.9               | Contact with and (suspected) exposure to unspecified communicable disease                            |
| Z30.X               | Encounter for contraceptive management   |
| Z31.X               | Encounter for procreative management   |
| Z32.X               | Encounter for pregnancy test and childbirth and childcare instruction                                |
| Z33.X               | Pregnant state   |
| Z34.X               | Encounter for supervision of normal pregnancy  |

| ICD10 Code or Range | Description  |
|---------------------|--|
| Z36                 | Encounter for antenatal screening of mother                      |
| Z39.X               | Encounter for maternal postpartum care and examination           |
| Z64.0               | Problems related to unwanted pregnancy                           |
| Z64.1               | Problems related to multiparity                                  |
| Z71.7               | Human immunodeficiency virus [HIV] counseling                    |
| Z72.5X              | High risk sexual behavior  |
| Z77.9               | Other contact with and (suspected) exposures hazardous to health |
| Z97.5               | Presence of (intrauterine) contraceptive device                  |

***ICD10 Codes Indicating Testing for Hypertension or Cardiovascular Disease Screening***

| ICD10 Code or Range | Description   |
|---------------------|---|
| I10                 | Essential (primary) hypertension  |
| I11.X               | Hypertensive heart disease  |
| I12.X               | Hypertensive chronic kidney disease   |
| I13.X               | Hypertensive heart and chronic kidney disease   |
| I15.X               | Secondary hypertension  |
| I20.X               | Angina pectoris   |
| I21.X               | ST elevation (STEMI) and non-ST elevation (NSTEMI) myocardial infarction  |
| I22.X               | Subsequent ST elevation (STEMI) and non-ST elevation (NSTEMI) myocardial infarction   |
| I23.X               | Certain current complications following ST elevation (STEMI) and non-ST elevation (NSTEMI) myocardial infarction (within the 28 day period) |
| I24.X               | Other acute ischemic heart diseases   |
| I25.X               | Chronic ischemic heart disease  |
| R07.2               | Precordial pain   |

| ICD10 Code or Range | Description   |
|---------------------|---|
| R07.8X              | Other chest pain  |
| R07.9               | Chest pain, unspecified   |
| R09.89              | Other specified symptoms and signs involving the circulatory and respiratory systems  |
| R94.3X              | Abnormal results of cardiovascular function studies                                   |
| Z13.6               | Encounter for screening for cardiovascular disorders                                  |
| Z82.4X              | Family history of ischemic heart disease and other diseases of the circulatory system |

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# FoundationOne CDx

MOL.TS.303.A  
v1.0.2020

## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedures addressed by this guideline | Procedure code |
|--|----------------|
| FoundationOne CDx                      | 0037U          |

## What are somatic mutation tests

### Definition

Somatic mutation tests are broadly defined here as any test that measures changes in DNA, RNA, or chromosomes found in tumor tissue that is used to make cancer management decisions.

- Somatic mutation tests are increasingly useful for therapy selection. Many cancer therapies are targeted at particular gene functions (therapeutic targets) and some require information about tumor genetics to use the therapies effectively (companion diagnostics). In these cases, NCCN as well as the FDA have outlined tumor testing that is recommended for specific cancers and the associated treatment implications.<sup>1-5</sup>

### Test information

- A variety of complex testing methodologies, combined with clinical information and patient preferences, are increasingly being used to inform clinical decision making among cancer patients with malignant solid tumors. One such test methodology is next-generation sequencing (NGS), frequently offered in the context of large gene panels that allow for the rapid and accurate sequencing of multiple genes at once. NGS is more frequently being used by oncologists in clinical practice to identify clinically actionable mutations that could be targeted by one or more appropriate cancer therapies. Concurrently, significant advancements in drug development have led to the introduction of specialty pharmaceuticals designed to target tumor-associated mutations identifiable by NGS. However, the extent to which the use of NGS in clinical practice, compared with routine methods (histopathology) or alternative commercial NGS tests, improves patient-important clinical outcomes is still unclear.<sup>6,7</sup>

- According to Foundation Medicine, the test manufacturer, FoundationOne CDx™ (F1CDx) is an in vitro diagnostic device that uses next generation sequencing (NGS) to detect substitutions, insertions, deletion alterations, and copy number alterations (CNAs) in 324 genes and select gene rearrangements. In addition to genomic signatures, the test also detects microsatellite instability (MSI) and tumor mutational burden (TMB) to inform treatment decisions about immunotherapies.<sup>8</sup>
- The biomarker TMB is assessed by measuring the number of somatic mutations in genes sequenced in F1CDx and extrapolating to the whole genome. F1CDx is designed to include all somatically altered genes in human solid tumors that are validated targets for therapy, “either FDA approved or in clinical trials, and/or that are unambiguous drivers of oncogenesis based on current knowledge.”<sup>8</sup>
- The manufacturer states that customized software and algorithms determine these genomic variants. Using a single DNA extraction method, patient DNA is extracted and isolated from formalin-fixed paraffin embedded (FFPE) tumor tissue specimens. The F1CDx platform uses a whole-genome shotgun library construction and hybridization-based capture of DNA extracted from tumor tissue before sequencing. Custom software is then used to determine genomic variants.
- The F1CDx report provides the following information:<sup>9</sup>
  - A listing of all alterations in tested genes that are known or likely to be cancer driver alterations and genomic signatures, some of which may also be associated with companion diagnostic information.
  - When indicated, the F1CDx report will include FDA-approved therapeutic options that may be considered based on detected alterations and tumor types for which F1CDx is approved as a companion diagnostic.
  - If an identified genomic alteration or genomic signature may be associated with treatment resistance, the F1CDx report will include a note notifying of potential resistance.
  - If no genomic alteration or genomic signatures associated with companion diagnostic–relevant information are identified, the F1CDx report will note that there are no reportable alterations with companion diagnostic claims.
  - The professional services section of the F1CDx report provides a list of potential clinical trials and investigational options to consider for identified genomic alterations or genomic signatures. Rationale, targets, and location of potential clinical trials are described in detail.

## Guidelines and evidence

- The National Comprehensive Cancer Network (NCCN) provides the following guidance:
  - NCCN Guidelines for Treatment of Cancer by Site provide detailed guidelines on the use of individual tumor markers for each cancer type addressed.<sup>5</sup>

- NCCN also makes the following recommendations specifically for using multi-gene panels in the evaluation of non-small cell lung cancer (NSCLC): “The NCCN NSCLC Guidelines Panel strongly endorses broader molecular profiling with the goal of identifying rare driver mutations for which effective drugs may already be available, or to appropriately counsel patients regarding the availability of clinical trials. Broad molecular profiling is a key component of the improvement of care of patients with NSCLC.”<sup>2</sup>
- NCCN also maintains a biomarker compendium stating “the goal of the NCCN Biomarkers Compendium is to provide essential details for those tests which have been approved by NCCN Guideline Panels and are recommended by the NCCN Guidelines.”<sup>4</sup> Biomarkers for specific cancer types that are listed in the NCCN Biomarker Compendium have a level of evidence associated with their clinical utility.
- The National Academy of Clinical Biochemistry (NACB, 2009) issued general tumor marker quality practice guidelines “to encourage more appropriate use of tumor marker tests.” They provide the following guidelines to determine if a tumor marker is useful:<sup>6</sup>
  - “The marker results are appropriate precisely for the required application (i.e., risk assessment, screening, diagnosis, prognosis, prediction, or post-treatment monitoring).”
  - “The marker results separate patients into two or more populations whose outcomes differ so strikingly that they and their caregiver would treat one group differently than another.”
  - “The estimate of the separation in outcomes for marker positive and negative is reliable.”
- On November 30, 2017, the FDA approved FoundationOne CDx panel testing as a companion diagnostic test for use in 5 disease indications 1) non-small cell lung cancer (NSCLC), 2) colorectal cancer (CRC), 3) melanoma, 4) breast cancer, and 5) ovarian cancer. Results of the F1CDx may help to inform disease management in accordance with approved drug labeling and clinical practice guidelines for particular individuals with NSCLC, melanoma, breast cancer, colorectal cancer, or ovarian cancer. See FDA document [here](#).<sup>11</sup>

## Criteria

- No previous panel testing performed on the member's tumor, AND
- Testing is being requested in order to effectively and safely prescribe a treatment or medication per an FDA label, AND
- The member has one of the following cancer types:
  - Non-small cell lung cancer, or



- Metastatic or unresectable melanoma, or
- Metastatic breast cancer, or
- Metastatic colorectal cancer, or
- Advanced ovarian cancer, or
- At least 5 tumor markers included in the panel individually meet criteria for the member's tumor type based on one of the following:
  - All criteria are met from a test-specific guideline if one is available, or
  - An oncology therapy FDA label requires results from the tumor marker test to effectively or safely use the therapy for the member's cancer type, or
  - NCCN guidelines include the tumor marker test in the management algorithm for that particular cancer type and all other requirements are met (specific pathology findings, staging, etc.); however, the tumor marker must be explicitly included in the guidelines and not simply included in a footnote as an intervention that "may be considered", or
  - The NCCN Biomarker Compendium has a level of evidence of at least 2A for the tumor marker's application to the member's specific cancer type

### **Billing and reimbursement**

This panel will only be considered for reimbursement when billed with an appropriate panel CPT code.

### **References**

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# FMR1-Related Disorders (Fragile X) Genetic Testing

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## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedures addressed by this guideline | Procedure codes |
|--|-----------------|
| FMR1 Expansion Analysis                | 81243           |
| FMR1 Methylation Analysis              | 81244           |

## What are FMR1-related disorders

### Definition

FMR1-related disorders are a group of disorders caused by mutations in the FMR1 gene. These include fragile X syndrome, fragile X-associated tremor/ataxia syndrome (FXTAS), and FMR1-related primary ovarian insufficiency (POI).<sup>1</sup>

### FMR1-related phenotypes

- Fragile X Syndrome
  - Fragile X syndrome is the most common cause of inherited intellectual disability, affecting approximately 1 in 4,000 males and 1 in 8,000 females.<sup>2,3</sup>
  - Given that the mutation is on the X-chromosome, males tend to be more severely affected than females.
  - Symptoms of Fragile X syndrome vary widely and may include the following: intellectual disability, autism, large head, long face, prominent forehead and chin, protruding ears, loose joints, large testes in postpubertal males, motor and language delays, and behavioral differences.<sup>1-3</sup>
- Fragile X-associated tremor/ataxia Syndrome
  - Fragile X-associated tremor/ataxia syndrome (FXTAS) is a neurodegenerative disorder characterized by progressive cerebellar ataxia and/or intention tremor usually presenting after age 50 in individuals with a premutation allele in the gene for fragile X (FMR1).<sup>1</sup>

- Other neurologic findings of FXTAS include short term memory loss, executive function deficits, cognitive decline, dementia, Parkinsonism, peripheral neuropathy, and lower limb proximal weakness.<sup>1</sup>
- A diagnosis of FXTAS “is confirmed by the presence of a FMR1 premutation and white matter lesions on MRI in the middle cerebellar peduncles and/or brain stem, with intention tremor and/or gait ataxia.”<sup>1</sup>
- FMR1-related premature ovarian failure
  - FMR1-related primary ovarian insufficiency occurs in women who are carriers of FMR1 premutations. “Females with premutations (usually >80 CGG repeats) are at ~20% risk for premature ovarian insufficiency (POI).”<sup>4</sup>
  - Symptoms can include irregular menstruation, elevated follicle stimulating hormone (FSH), reduced fertility, and early menopause.<sup>1</sup>

## Inheritance

- FMR1 related disorders are caused by a type of genetic mutation called a triplet repeat expansion in >99% of individuals with these conditions. A triplet repeat is a sequence of three nucleotide building blocks (CGG) that is variably repeated within the FMR1 gene. The normal allele size is up to 44 repeat units.<sup>1</sup> A premutation ranges from approximately 55 repeats to 200 repeats.<sup>1</sup> A full mutation (>200 repeats) usually causes the gene to be abnormally methylated, turning it off. The number of CGG repeat copies within the FMR1 gene can expand from one generation to the next, a property known as anticipation.<sup>1,2,4</sup>
- Predictive (carrier) testing can be performed for at-risk relatives when there is a family history of fragile X syndrome, intellectual disability of unknown etiology, or other characteristic features.<sup>3</sup>
- A woman carrying a premutation or full mutation is at risk to have a child affected with fragile X syndrome. The actual risk depends on the number of repeats in her FMR1 gene.<sup>1,3</sup> Prenatal testing is available for at-risk pregnancies.
- “Most individuals with the premutation do not show fragile X syndrome–related features; however, some with large repeat sizes (>100 repeats) have been identified with learning difficulties, emotional problems, or even intellectual disability.”<sup>4</sup>
- Both male and female premutation carriers are at risk for FXTAS. Approximately 40% of premutation carrier males over the age of 50 will develop FXTAS. The risk to female premutation carriers appears to be lower.<sup>1</sup> “The penetrance of FXTAS increases with age and with premutation repeat length.”<sup>5</sup>
- “Among females with POI [premature ovarian failure] and simplex cases of adult males with cerebellar ataxia, the FMR1 premutation is identified in 4-6% and 2%, respectively.”<sup>4</sup>

## Test information

- FMR1 CGG expansion analysis measures the number of CGG repeat copies within the FMR1 gene. Repeat number classifies results as normal, intermediate (also known as gray zone or borderline), premutation, or full mutation.<sup>2,4</sup> The same analysis can be used for diagnostic, carrier, and prenatal testing.
- FMR1 CGG methylation analysis is typically assessed in those with a premutation or full mutation.<sup>1,4</sup> Abnormal methylation, causing a disruption in FMR1 protein production, is the mechanism responsible for features of Fragile X syndrome. Non-classic clinical presentations due to size and methylation mosaicism have been reported.
- Prenatal diagnosis must be undertaken with caution. Expansion analysis is equally accurate on fetal samples from amniocentesis and chorionic villus sampling (CVS). However, methylation analysis on a CVS sample may yield an ambiguous result and amniocentesis may be needed for follow up.<sup>4</sup>
- Testing for the fragile site FXA at Xq27 is no longer an acceptable diagnostic method as test sensitivity and specificity are both insufficient. Families with a diagnosis from this method should be eligible for trinucleotide repeat expansion and/or methylation studies.<sup>2</sup>

## Guidelines and evidence

### Fragile X Syndrome

- Consensus guidelines from the American Academy of Pediatrics (AAP, 2011) that address health supervision of fragile X syndrome:
  - “Because children with fragile X syndrome may not have apparent physical features, any child who presents with developmental delay, borderline intellectual abilities, or mental retardation or has a diagnosis of autism without a specific etiology should undergo molecular testing for fragile X syndrome to determine the number of CGG repeats (Fig 1). Fragile X testing should also be considered in patients in whom there is suspected, but not molecularly proven, Sotos syndrome or Prader-Willi syndrome. On the other hand, fragile X testing, is not routinely warranted for children with isolated attention-deficit/hyperactivity disorder.”<sup>5</sup>
- Practice guidelines from the American College of Medical Genetics (ACMG, 2005) recommend diagnostic testing for fragile X syndrome for “Individuals of either sex with mental retardation, developmental delay, or autism, especially if they have (a) any physical or behavioral characteristics of fragile X syndrome, (b) a family history of fragile X syndrome, or (c) male or female relatives with undiagnosed mental retardation.”<sup>2</sup>
- Practice guidelines from the American College of Medical Genetics (ACMG, 2005)<sup>2</sup> and the American College of Obstetricians and Gynecologists (ACOG, 2017)<sup>6</sup> support carrier screening for fragile X syndrome:

- ACMG: Fragile X syndrome testing should be offered to<sup>2</sup>
  - “Individuals seeking reproductive counseling who have (a) a family history of fragile X syndrome or (b) a family history of undiagnosed mental retardation.”
- ACOG: Fragile X carrier screening should be offered to:<sup>6</sup>
  - “Fragile X premutation carrier screening is recommended for women with a family history of fragile X-related disorders or intellectual disability suggestive of fragile X syndrome and who are considering pregnancy or are currently pregnant.”
- Practice guidelines from the American College of Medical Genetics (ACMG, 2005)<sup>2</sup> and the American College of Obstetricians and Gynecologists (ACOG, 2017)<sup>7</sup> support prenatal screening for fragile X syndrome:
  - ACMG: Fragile X testing is appropriate in “Fetuses of known carrier mothers.”
  - ACOG: “Prenatal diagnostic testing for fragile X syndrome should be offered to known carriers of the fragile X premutation or full mutation gene.”

#### Fragile X-associated tremor/ataxia syndrome (FXTAS)

- Evidence-based guidelines from the European Federation of Neurological Societies (EFNS, 2014)<sup>8</sup> state:
  - “In the case of sporadic ataxia and independent from onset age, we recommend routine testing for SCA1, SCA2, SCA3, SCA6 and DRPLA (in Asian patients) (level B), the step 1 panel of the recessive ataxia work-up, i.e. mutation analysis of the FRDA gene (level B), and biochemical testing that includes cholestanol, vitamin E, cholesterol, albumin, CK and α-fetoprotein.
  - If negative and if age at onset is above 45 years, we recommend screening for the FMR1 permutation [sic] in male patients (level B).”<sup>8</sup>
- Evidence-based guidelines from the European Federation of Neurological Societies (EFNS, 2010)<sup>7</sup> state:
  - “Recommendations for FXTAS genetic testing: Genetic testing for the X-linked FXTAS is recommended when there is a clinical suspicion, and it is readily available in many laboratories (Class B).”<sup>4</sup> [Class B rating = “(probably effective, ineffective, or harmful) requires at least one convincing class II study or overwhelming class III evidence”]
- Practice guidelines from the American College of Medical Genetics and Genomics (ACMG, 2005) recommend FXTAS testing for the following individuals:
  - “Men and women who are experiencing late onset intention tremor and cerebellar ataxia of unknown origin, especially if they have (a) a family history of movement disorders, (b) a family history of fragile X syndrome, or (c) male or female relatives with undiagnosed mental retardation.”<sup>2</sup>

## Primary Ovarian Insufficiency (POI)

- Practice guidelines from the American College of Medical Genetics and Genomics (ACMG, 2005)<sup>2</sup> and the American College of Obstetricians and Gynecologists (ACOG, 2017)<sup>6</sup> support carrier screening for fragile X syndrome:
  - ACOG: Fragile X carrier screening should be offered to:<sup>6</sup>
    - "If a woman has unexplained ovarian insufficiency or failure or an elevated follicle-stimulating hormone level before age 40 years, fragile X carrier screening is recommended to determine whether she has an FMR1 premutation."
  - ACMG: Fragile X syndrome testing should be offered to<sup>2</sup>
    - "Women who are experiencing reproductive or fertility problems associated with elevated follicle stimulating hormone (FSH) levels, especially if they have (a) a family history of premature ovarian failure, (b) a family history of fragile X syndrome, or (c) male or female relatives with undiagnosed mental retardation."
- ACOG committee opinion on Primary Ovarian Insufficiency in Adolescents and Young Adults (2014, Reaffirmed 2018) states:<sup>9</sup>
  - "If a woman has a personal or family history of ovarian failure or an elevated follicle-stimulating hormone (FSH) level before age 40 years without a known cause, fragile X premutation carrier testing should be offered."

## Criteria

### Targeted mutation analysis for CGG trinucleotide repeat expansion in FMR1

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - No previous molecular genetic testing of FMR1, AND
- Diagnostic Testing for Symptomatic Individuals:
  - Males and females with unexplained speech and/or language delay, motor development delay, intellectual disability (ID), or autism, or
  - Female with premature ovarian failure (cessation of menses before age of 40 years), or
  - Males and females 50 years of age or older with progressive intention tremor and cerebellar ataxia of unknown origin, or

- Males and females 50 years of age or older with white matter lesions on MRI in the middle cerebellar peduncles and/or brain stem, or
- Males and females 50 years of age or older with FXTAS-related neurologic, cognitive, or behavioral difficulties, OR
- Prenatal Testing for At-Risk Pregnancies:
  - CGG trinucleotide repeat expansion in FMR1 identified in biologic mother,\*\* OR
- Carrier Screening and Predictive Testing for Presymptomatic/Asymptomatic At Risk Individuals:
  - Age 18 years or older, and
  - Known CGG trinucleotide repeat expansion in FMR1 in 1<sup>st</sup>, 2<sup>nd</sup>, or 3<sup>rd</sup> degree biologic relative and the individual is at risk for inheriting the familial FMR1 expansion based on an X-linked inheritance pattern, or
  - Family history of premature ovarian failure (cessation of menses before age of 40 years), or
  - Family history of movement disorder and
    - Cerebellar ataxia has been ruled out
    - Other movement disorders have been ruled out, or
  - Family history of undiagnosed intellectual disability, or
  - Prior cytogenetic test suspicious for Fragile X, AND
- Possibility of X-linked inheritance has not been ruled out by male to male transmission

### **Methylation analysis**

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - CGG expansion analysis result showing a premutation or full allele size (typically greater than 55 repeats), AND
- Diagnostic Testing for Symptomatic Individuals:
  - Males and females with speech and/or language delay, motor development delay, intellectual disability (ID), or autism, or



- Female with premature ovarian failure (cessation of menses before age of 40 years), or
  - Males and females 50 years of age or older with progressive intention tremor and cerebellar ataxia of unknown origin, or
  - Males and females 50 years of age or older with white matter lesions on MRI in the middle cerebellar peduncles and/or brain stem, or
  - Males and females 50 years of age or older with FXTAS-related neurologic, cognitive, or behavioral difficulties, OR
- Prenatal Testing for At-Risk Pregnancies:
    - CGG trinucleotide repeat expansion in FMR1 identified in biologic mother\*\*

\*\* Note: CVS must be interpreted with caution. The number of CGG repeats in the fetus can be accurately determined; however, often the methylation status of FMR1 is not yet established in chorionic villi at the time of sampling. CVS results may lead to a situation in which follow-up amniocentesis is necessary to resolve an ambiguous result.

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Fragile X

# Friedreich Ataxia Genetic Testing

**MOL.TS.309.A**  
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## Introduction

Friedreich ataxia genetic testing is addressed by this guideline.

## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedures addressed by this guideline                              | Procedure codes |
|---|-----------------|
| FXN gene analysis; evaluation to detect abnormal (expanded) alleles | 81284           |
| FXN gene analysis; characterization of alleles (eg, expanded size)  | 81285           |
| FXN gene analysis; full gene sequence                               | 81286           |
| FXN gene analysis; known familial variant(s)                        | 81289           |
| FXN gene analysis, deletion/duplication                             | 81479           |

## What is Friedreich Ataxia

### Definition

Friedreich ataxia (FRDA) is an inherited neuromuscular condition.

### Incidence and Prevalence

FRDA is the most common inherited ataxia in European, Middle Eastern, Asian Indian, and North African populations.<sup>1</sup> The prevalence is 2:100,000-4:100,000.<sup>1</sup>

### Symptoms

FRDA is characterized by progressive ataxia (lack of coordination of muscle movements) of the limbs and gait, dysarthria (difficulty articulating speech), absent lower limb reflexes, sensory loss, and muscle weakness.<sup>1,2</sup> About two-thirds of individuals with FRDA also have cardiomyopathy (weakening of the heart muscle).<sup>1</sup> Approximately 30% of individuals with FRDA have diabetes mellitus.<sup>1</sup>

Symptoms typically present before 25 years of age, and most commonly between 10 and 15 years.<sup>1,2</sup> However, about 25% of affected individuals have an atypical form with later onset and/or retained reflexes.<sup>1</sup>

## Cause

Friedreich ataxia is caused by mutations in the FXN gene. Most mutations in the FXN gene cause a section of DNA, called a GAA triplet repeat, to expand.<sup>1</sup> However, a minority of affected people have a different mutation in the FXN gene.

## Inheritance

FRDA is an autosomal recessive disorder. An affected individual must inherit FXN gene mutations from both parents.<sup>1,2</sup> Full siblings of an affected individual have a 25% risk to be affected. Individuals who inherit only one mutation are called carriers. Carriers do not show symptoms, but have a 50% chance of passing on the mutation to their children. Two carriers have a 25% chance of having a child with the disorder.

## Diagnosis

The diagnosis of FRDA is confirmed when disease-causing mutations are found in both copies of the FXN gene.<sup>1</sup> 96% of individuals with FRDA have disease-causing GAA triplet repeat expansions in both FXN genes.<sup>1</sup> About 4% have a single disease-causing GAA triplet repeat expansion and a second FXN gene mutation not in the GAA repeat region.<sup>1</sup> Different genetic testing is required to identify the second mutation.

Very few people who have been clinically diagnosed with FRDA have no GAA expansion in the FXN gene. These people may have mutations in another gene, although another disease causing gene has not yet been identified.<sup>1,3</sup>

## Treatment

Treatment of FRDA is largely supportive, and includes the use of walking aids and wheelchairs for ambulation, speech therapy, occupational therapy, and other assistive devices.<sup>1</sup>

## Survival

The survival range for FRDA varies. The mean age of death is 36.5 years, with a median age of 30 years.<sup>1</sup> Some individuals have been documented to live into their 60s and 70s. Cardiac issues are the most common cause of death among individuals with FRDA.

## Test Information

### Introduction

Testing for FRDA is performed by determining the number of GAA repeats in the FXN gene. If needed, FXN sequencing or FXN deletion/duplication analysis can be subsequently performed.

### Trinucleotide repeat expansion

The main result categories are based on the number of GAA triplet repeats:<sup>1</sup>

- 5 to 33 GAA repeats is the normal range
- 34 to 65 repeats do not usually cause typical Friedreich ataxia. However, this range may be unstable and can lead to atypical disease or an increased risk for a person's child to be affected.
- 44 to 66 repeats is considered borderline given that the "shortest repeat length associated with disease has not been clearly determined."<sup>1</sup>
- 66 or more repeats are disease-causing. Usually people with typical Friedreich ataxia have 600 to 1200 repeats. Smaller numbers of repeats may lead to later onset disease.

### Sequencing

About 4% of people with Friedreich ataxia have only one GAA expansion mutation on initial testing. For these people, subsequent FXN gene sequencing is needed to identify the second gene mutation.<sup>1</sup>

Sequence analysis detects single nucleotide substitutions and small (several nucleotide) deletions and insertions. Regions analyzed typically include the coding sequence and intron/exon boundaries. Promoter regions and intronic sequences may also be sequenced if disease-causing mutations are known to occur in these regions of a gene.

### Deletion/Duplication analysis

Single or multi-exon deletions or duplication of FXN are rare but have been reported.<sup>1</sup>

Analysis for deletions and duplications can be performed using a variety of technical platforms including exon array, MLPA, and NGS data analysis.

These assays detect gains and losses too large to be identified through sequencing technology, often single or multiple exons or whole genes.

### Known familial mutation analysis

Known familial mutation analysis is performed when a causative mutation has been identified in a close relative of the individual requesting testing.

Analysis for known familial mutations is typically performed by trinucleotide repeat expansion analysis. Some mutations may require Sanger sequencing or deletion/duplication analysis.

## Guidelines and Evidence

### Introduction

This section includes relevant guidelines and evidence pertaining to genetic testing for FRDA.

### European Federation of Neurological Sciences

- The European Federation of Neurological Sciences (EFNS, 2014) stated the following regarding testing for ataxia:<sup>3</sup>
  - “In the case of a family history compatible with an autosomal recessive cerebellar ataxia, we recommend a three-step diagnostic approach.”
    - “Step 1: mutation analysis of the FRDA gene for Friedreich’s ataxia (although one can refrain from this in the case of severe cerebellar atrophy), and biochemical testing that includes cholestanol, vitamin E, cholesterol, albumin, creatine kinase (CK) and a-fetoprotein. Also consider doing nerve conduction studies/EMG (presence versus absence of peripheral neuropathy, axonal versus demyelinating) and referral to an ophthalmologist (retinitis pigmentosa, cataract, cherry red spot etc.) (Table S2) (good practice point).”
    - “Step 2: mutation analysis of the SACS, POLG, Aprataxin (APTX) and SPG7 genes (taking into account specific phenotypes, as given in Table S2), and bio-chemical testing for white cell enzymes, phytanic acid and long chain fatty acids (good practice point).”
    - “Step 3: referral to a specialized centre, e.g. for skin or muscle biopsy targeted at diagnoses such as Niemann Pick type C, recessive ataxia with coenzyme Q deficiency [aarF domain containing kinase 3 (ADCK3)/autosomal recessive spinocerebellar ataxia 9 (SCAR9)] and mitochondrial disorders, or for extended genetic screening using gene panel diagnostics (good practice point).”
  - “In the case of sporadic ataxia and independent from onset age, we recommend routine testing for SCA1, SCA2, SCA3, SCA, and DRPLA (in Asian patients) (level B), the step one panel of the recessive ataxia workup, i.e mutation analysis of the FRDA gene (level B), and biochemical testing that includes cholestanol, vitamin E, cholesterol, albumin, CK, and alpha-fetoprotein.”
- For the diagnosis of Friedreich ataxia, guidelines from the European Federation of Neurological Societies (EFNS, 2010) created by consensus of experts members

following literature review recommend: "In cases presenting with early onset ataxia, peripheral sensory neuropathy, and absence of marked cerebellar atrophy at MRI, genetic test for FRDA mutation is recommended (Class B)."<sup>2</sup>

### **American College of Medical Genetics**

The American College of Medical Genetics (ACMG, 2013) states the following regarding testing for hereditary ataxias:<sup>4</sup>

- "Establishing the diagnosis of hereditary ataxia requires:
  - Detection on neurological examination of typical clinical signs including poorly coordinated gait and finger/hand movements, dysarthria (incoordination of speech), and eye movement abnormalities such as nystagmus, abnormal saccade movements, and ophthalmoplegia.
  - Exclusion of nongenetic causes of ataxia (see Differential Diagnosis below).
  - Documentation of the hereditary nature of the disease by finding a positive family history of ataxia, identifying an ataxia-causing mutation, or recognizing a clinical phenotype characteristic of a genetic form of ataxia."
- "Differential diagnosis of hereditary ataxia includes acquired, nongenetic causes of ataxia, such as alcoholism, vitamin deficiencies, multiple sclerosis, vascular disease, primary or metastatic tumors, and paraneoplastic diseases associated with occult carcinoma of the ovary, breast, or lung, and the idiopathic degenerative disease multiple system atrophy (spinal muscular atrophy). The possibility of an acquired cause of ataxia needs to be considered in each individual with ataxia because a specific treatment may be available."
- "Testing strategy when the family history suggests autosomal recessive inheritance
  - A family history in which only sibs are affected and/or when the parents are consanguineous suggests autosomal recessive inheritance. Because of their frequency and/or treatment potential, FRDA, A-T, AOA1, AOA2, AVED, and metabolic or lipid storage disorders such as Refsum disease and mitochondrial diseases should be considered."
- "Testing simplex cases.
  - If no acquired cause of the ataxia is identified, the probability is ~13% that the affected individual has SCA1, SCA2, SCA3, SCA6, SCA8, SCA17, or FRDA, and mutations in rare ataxia genes are even less common.
  - Other possibilities to consider are a de novo mutation in a different autosomal dominant ataxia, decreased penetrance, alternative paternity, or a single occurrence of an autosomal recessive or X-linked disorder in a family such as fragile X-associated tremor/ataxia syndrome.
  - Although the probability of a positive result from molecular genetic testing is low in an individual with ataxia who has no family history of ataxia, such testing is

usually justified to establish a specific diagnosis for the individual's medical evaluation and for genetic counseling.

- Always consider a possible nongenetic cause such as multiple system atrophy, cerebellar type in simplex cases."

## Criteria

### Introduction

Requests for FRDA testing are reviewed using these criteria.

### Known familial mutation analysis

- Pre- and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- No previous FXN gene analysis performed that would have identified the known familial mutation, AND
- Known disease-causing mutation in FXN gene identified in 1<sup>st</sup> degree relative(s), AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy

### GAA trinucleotide repeat analysis

- Genetic counseling:
  - Pre- and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing
  - No previous GAA repeat analysis of FXN performed, and
  - No known mutation identified by previous analysis, AND
- Individual has been diagnosed with cerebellar ataxia, regardless of age of onset, AND
- Family history is consistent with autosomal recessive inheritance (including simplex cases), AND
- The member does not have a known underlying cause for their ataxia (e.g. alcoholism, vitamin deficiencies, multiple sclerosis, vascular disease, tumors, known mutation), AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy



## Sequence analysis

- Genetic Counseling:
  - Pre- and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - Member does not have a known mutation in both copies of the FXN gene, and
  - No previous sequencing analysis of the FXN gene, and
  - Previous GAA trinucleotide repeat analysis was performed and revealed a GAA expansion on only one allele, and
  - Testing is needed to confirm the diagnosis of Friedreich Ataxia, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy

## Deletion/duplication analysis

- Genetic Counseling:
  - Pre- and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - Member does not have a known mutation in both copies of the FXN gene, and
  - Previous GAA trinucleotide repeat analysis was performed and revealed a GAA expansion on only one allele, and
  - Previous GAA sequencing was performed and did not identify a mutation on either FXN allele, and
  - Testing is needed to help confirm the diagnosis of Friedreich Ataxia, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy

## Exclusions and Other Considerations

For requests for multigene panels, please see the guideline *Hereditary Ataxia Multigene Panel Testing*.

## References

### Introduction

These references are cited in this guideline.

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# Gaucher Disease Testing

MOL.TS.173.A

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## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedures addressed by this guideline | Procedure codes |
|--|-----------------|
| GBA Known Familial Mutation Analysis   | 81403           |
| GBA Targeted Mutation Analysis         | 81251           |
| GBA Sequencing                         | 81479           |

## What is Gaucher Disease

### Definition

Gaucher disease is a genetic disease that affects multiple organs and tissues.

- There are several types of Gaucher disease, each with varying signs and symptoms:<sup>1,2</sup>
  - Type 1 is the most common type of Gaucher Disease. Unlike other types, type 1 does not affect the central nervous system (CNS). Symptoms include enlargement of the liver and spleen (hepatosplenomegaly), anemia, low blood platelets, lung disease, and bone abnormalities.
  - Type 2/Type 3. These types are rarer, usually more severe, and affect the brain and CNS. Common symptoms include seizures, hyperextension of the spine, and lockjaw, in addition to the symptoms listed above for type 1. Type 2 is more severe, and affected individuals usually do not survive past childhood. Type 3 affected individuals have more slowly progressing symptoms and can survive into adulthood.
  - Perinatal lethal. The most severe form of Gaucher disease has symptoms that begin during pregnancy or in early infancy, including swelling, dry/scaly skin (ichthyosis), and serious neurological problems. Affected infants usually survive only a few days after birth.
  - Cardiovascular. This type has mainly heart manifestations. Symptoms include the hardening of heart valves, eye abnormalities, bone disease, and enlarged spleen.

- These subtypes are identified through clinical symptoms and do not correlate well with the different mutations that cause Gaucher disease.<sup>2</sup>
- Gaucher disease is relatively common in Ashkenazi Jewish populations, affecting about 1 in 500 to 1 in 1,000 people.<sup>1</sup> It is much less common in the general population, affecting about 1 in 50,000 to 1 in 100,000 people.<sup>1</sup>
- Gaucher disease is caused by changes, or mutations to the GBA gene.<sup>1-3</sup> The GBA gene makes the enzyme beta-glucosylceramidase, also called acid beta-glucocerebrosidase. This enzyme helps break down fatty substances in cells. Mutations in GBA lead to a buildup of these fatty substances to toxic levels. This buildup damages tissues and organs, leading to symptoms of Gaucher disease.<sup>1-3</sup>
- Gaucher disease is an autosomal recessive disorder. An affected person inherits two GBA gene mutations -- one from each parent.<sup>1,2</sup>
  - People who have only one GBA mutation are called carriers. Carriers do not show symptoms of Gaucher disease, but have a 50% chance of passing the mutation on to their children.
  - Two carriers of Gaucher disease have a 25% chance of having a child affected with the disease.
- Clinical findings alone are insufficient for a definitive diagnosis of Gaucher disease.<sup>2</sup>
- If Gaucher disease is suspected in a symptomatic person, beta-glucosylceramidase enzyme testing should be performed first. People affected with Gaucher disease have 0-15% the normal level of beta-glucosylceramidase compared to healthy individuals. Measuring beta-glucosylceramidase levels is a reliable way to confirm a suspected case of Gaucher disease.<sup>2,4,5</sup> beta-glucosylceramidase levels within the normal range rule out Gaucher disease.
- Genetic testing can be used to identify the disease-causing mutations in an affected person diagnosed by enzyme analysis.<sup>1</sup> Identifying the causative GBA mutations can aid in prognostication. Genetic testing is recommended for reproductive purposes when parents of an affected child need to know the mutations for preimplantation genetic diagnosis or prenatal diagnosis. Mutation analysis can also confirm disease-causing mutations when a diagnosis by enzyme analysis is inconclusive.<sup>1</sup> Enzyme testing is not appropriate to identify unaffected carriers.<sup>2</sup>

## Test information

- **GBA Mutation Panel:** Clinically-available testing panels look for four or more of most common mutations in the GBA gene.
  - Four mutations (N370S, L444P, 84GG, IVS2+1) account for about 90% of mutations in the Ashkenazi Jewish population and about 50%-60% of mutations in the non-Ashkenazi Jewish population.<sup>1</sup>
  - Some laboratories include several other common mutations in their panels.

- Carrier screening by GBA mutation panel for Gaucher disease is widely available as part of an “Ashkenazi Jewish Panel” that includes several other genetic diseases that are more common in this population. (See Ashkenazi Jewish Carrier Screening for more information.)
- **GBA Sequence Analysis:** This test analyzes the entire coding region of the GBA gene and will find mutations that the GBA mutation panel could not.<sup>1</sup>
  - The detection rate of sequencing is about 99%.
  - This test is indicated in people with Gaucher disease who have one or no mutations identified by mutation panel testing.
  - This test is also indicated for reproductive partners of individuals who have a GBA mutation.
- **GBA known familial mutation testing:** When there is a family history of Gaucher disease, the family mutations should be identified prior to carrier testing in at-risk family members when possible. A mutation panel can be used if the family mutations are included in the panel. If the family mutations are not included in the panel and were identified through sequencing, then GBA known familial mutation testing is necessary.<sup>2</sup>
- **Prenatal or preimplantation genetic diagnosis:** This testing is possible in at-risk pregnancies if the parental mutations are known.

## Guidelines and evidence

- No US evidence-based diagnostic guidelines have been identified.
- A 2015 expert-authored review recommends the following testing strategy for diagnosis of an affected person:<sup>2</sup>
  - “Assay of glucosylceramidase enzyme activity in leukocytes or other nucleated cells is the confirmatory diagnostic test.”
  - “Molecular genetic testing and the identification of two disease-causing alleles provide an alternative means of confirming the diagnosis. There is broad heterogeneity in causative variants; in individuals in whom genetic testing identifies a novel GBA variant, biochemical testing to confirm the diagnosis should be considered.”
  - “Targeted analysis for pathogenic variants in a proband originally diagnosed by biochemical testing may be considered for genetic counseling purposes, primarily to identify the pathogenic variants and permit carrier detection among at-risk relatives.”
- Reviews published in peer-reviewed medical literature support this and offer some considerations for genotyping:

- Archives of Internal Medicine (1998):<sup>4</sup>
  - “The most efficient and reliable method of establishing the diagnosis of Gaucher disease is the assay of  $\beta$ -glucocerebrosidase activity.”
  - “Knowledge of the genotype may be helpful in predicting the severity and rate of progression of clinical symptoms in patients. For example, the homozygous N370S allele is usually associated with a generally less severe phenotype, although with wide clinical variability; the heterozygous state for N370S is protective against central nervous system involvement; and the L444P allele in the homozygous state is associated with early neurologic symptoms common in the types 2 and 3 clinical classifications.”
- The Brazilian Study Group on Gaucher Disease (2009):<sup>5</sup>
  - “Definitive diagnosis of [Gaucher disease] requires confirmation by the acid  $\beta$ -glucosidase enzyme assay in leukocytes or fibroblasts.”
  - “N370S homozygotes generally present with a less severe phenotype, whereas L444P and D409H homozygosity confers neurologic involvement. Despite these general genotype-phenotype correlations, disease severity, and clinical outcomes cannot be predicted on the basis of genotype.”
- Professional guidelines generally support Gaucher disease carrier screening for those at increased risk.<sup>6,7</sup>
- Consensus guidelines from the American College of Obstetricians and Gynecologists (ACOG, 2009) address carrier screening and prenatal diagnosis for Gaucher disease:<sup>6</sup>
  - “Individuals with a positive family history of one of these disorders [including Gaucher disease] should be offered carrier screening for the specific disorder and may benefit from genetic counseling.”
  - Carrier screening for Ashkenazi Jewish people is routinely recommended for some disorders (i.e., Tay-Sachs, Canavan, cystic fibrosis, familial dysautonomia). However, for testing of a group of other disorders more common in this population (including Gaucher disease), ACOG simply states: “Individuals of Ashkenazi Jewish descent may inquire about the availability of carrier screening for other disorders.”
  - “If it is determined that this individual [an Ashkenazi Jewish descent partner] is a carrier, the other partner should be offered screening.”
  - “When both partners are carriers of one of these disorders, they should be referred for genetic counseling and offered prenatal diagnosis.”
- Consensus guidelines from the American College of Medical Genetics (2008) recommend routine carrier screening for a group of disorders that includes Gaucher disease when at least one member of the couple is Ashkenazi Jewish and that couple is pregnant or planning pregnancy.<sup>7</sup>

## Criteria

### Carrier Testing

#### GBA Known Familial Mutation Analysis

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - No previous molecular genetic testing of GBA, AND
- Carrier Screening:
  - GBA mutation(s) identified in 1st, 2nd, or 3rd degree biologic relative(s), OR
- Prenatal Testing for At-Risk Pregnancies:
  - GBA mutation(s) identified in both biologic parents.

#### GBA Targeted Mutation Analysis for Ashkenazi Mutations (Four Mutations)

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - No previous GBA genetic testing, including Ashkenazi Jewish screening panels containing targeted mutation analysis for Gaucher disease, AND
- Carrier Screening:
  - Ashkenazi Jewish descent, regardless of disease status and results of glucosylceramidase assay, and
  - Intention to reproduce

### Diagnostic and Expanded Carrier Testing

#### GBA Sequencing

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - No previous GBA full sequencing analysis, and

- If Ashkenazi Jewish, testing for 4 common mutations is negative, AND
- Diagnostic Testing for Symptomatic Individuals:
  - Glucosylceramidase enzyme activity in peripheral blood leukocytes is 0-15% of normal activity, and
  - Characteristic bone changes including osteopenia, focal lytic or sclerotic bone lesions or osteonecrosis, or
  - Hepatosplenomegaly and hematologic changes including anemia or thrombocytopenia, or
  - Primary neurologic disease which could include one or more of the following: cognitive impairment, bulbar signs, pyramidal signs, oculomotor apraxia, or seizures (progressive myoclonic epilepsy), OR
- Diagnostic Testing for Asymptomatic Carriers:
  - One mutation detected by targeted mutation analysis, and
  - Glucosylceramidase enzyme activity in peripheral blood leukocytes is 0-15% of normal activity, OR
- Testing for Individuals with Family History or Partners of Carriers:
  - 1<sup>st</sup>, 2<sup>nd</sup>, or 3<sup>rd</sup> degree biologic relative with Gaucher disease clinical diagnosis, family mutation unknown and testing unavailable, or
  - Partner is monoallelic or biallelic for GBA mutation, and has the potential and intention to reproduce with this partner.

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# Genetic Testing for Autism

**MOL.TS.269.A**  
v1.0.2020

## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedures covered by this guideline   | Procedure codes |
|--|-----------------|
| Autism spectrum disorder known familial mutation analysis                                  | 81403           |
| AFF2 gene analysis; evaluation to detect abnormal (eg, expanded) alleles                   | 81171           |
| AFF2 gene analysis; characterization of alleles (eg, expanded size and methylation status) | 81172           |
| Miscellaneous autism spectrum disorder gene tests  | 81400           |
| Miscellaneous autism spectrum disorder gene tests  | 81401           |
| Miscellaneous autism spectrum disorder gene tests  | 81402           |
| Miscellaneous autism spectrum disorder gene tests  | 81403           |
| Miscellaneous autism spectrum disorder gene tests  | 81404           |
| Miscellaneous autism spectrum disorder gene tests  | 81405           |
| Miscellaneous autism spectrum disorder gene tests  | 81406           |
| Miscellaneous autism spectrum disorder gene tests  | 81407           |
| Miscellaneous autism spectrum disorder gene tests  | 81408           |
| Miscellaneous autism spectrum disorder gene tests  | 81479           |

## What is Autism Spectrum Disorder

### Definition

Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by persistent deficits in communication and social interaction, as well as restricted, repetitive patterns of behavior, interests, or activities.

### Incidence and Prevalence

ASD affects approximately 1/59 children and is 3-4 times more common in males.<sup>1,2</sup>

### Symptoms

ASD was previously divided into categories that included autistic disorder, Asperger's disorder, childhood disintegrative disorder, and pervasive developmental disorder not otherwise specified (PDDNOS). With current diagnostic criteria, these categories were subsumed under the diagnosis of ASD.

Symptom onset is in early childhood (typically before 3 years of age).<sup>2</sup> There are both syndromic and non-syndromic forms of inherited autism. The constellation of associated findings is highly dependent on the cause of autism.

ASD is often accompanied by intellectual disability, behavioral difficulties, and sensory abnormalities.

Clinical information (e.g. presence of specific congenital malformations, dysmorphic features, and other symptoms) may be used in some cases to help narrow down the suspected cause. In these cases, it may be possible to identify a narrow subset of genes that may be responsible for a person's ASD.

### Cause

ASD has multiple causes. These include, but are not limited to, acquired causes such as head injury, endocrine disorders (e.g. hypothyroidism), toxic exposure (e.g. fetal alcohol syndrome), inborn errors of metabolism (e.g. phenylketonuria), and central nervous system infection.<sup>2</sup>

There are also many known genetic conditions which are associated with an increased risk for ASD. A thorough clinical genetics evaluation is estimated to result in an identified cause in 30–40% of affected individuals.<sup>3</sup> Chromosome microarray analysis has the highest diagnostic yield of any single test for ASD, with an estimated detection rate of at least 10%.<sup>2,3</sup>

### Inheritance

Inheritance patterns differ between the various syndromes associated with ASD. Inherited forms of autism can show autosomal dominant, autosomal recessive, or X-linked patterns of inheritance.

## Diagnosis

Autism Spectrum Disorder is diagnosed through evaluation of an individual's development and behaviors by an appropriate specialist (such as neurodevelopmental pediatrician or developmental-behavioral pediatrician). Medical tests such as hearing screening, vision screening, and neurological evaluations may also be performed.<sup>1</sup>

## Treatment

Treatment for ASD includes behavioral interventions such as applied behavioral analysis (ABA) therapy, structured educational interventions, and in some cases, pharmacotherapy.<sup>2</sup> In a limited number of cases (mostly metabolic disorders), knowing the genetic mutation that is responsible for the ASD can help to guide treatment. Identifying a genetic syndrome may also alert the healthcare team to potential comorbidities for which evaluation and surveillance may be needed.

## Survival

Life expectancy in autism is reduced. This is often secondary to accidents such as drowning.<sup>4</sup> Comorbid conditions can also affect survival.

## Test information

### Introduction

Testing for Autism Spectrum Disorder may include chromosomal microarray analysis, known familial mutation analysis, single gene sequence analysis, single gene deletion/duplication analysis, or multi-gene panels of various sizes.

### Sequence Analysis

- Until recently, most sequencing tests used the Sanger sequencing methodology that was originally developed in the 1970s. Sanger sequencing is labor intensive and did not lend itself to high-throughput applications.
- Next generation sequencing (NGS), which is also sometimes called massively parallel sequencing, was developed in 2005 to allow larger scale and more efficient gene sequencing. NGS relies on sequencing many copies of small pieces of DNA simultaneously and using bioinformatics to assemble the sequence. NGS may not perform as well as Sanger sequencing in some applications.
- NGS tests vary in technical specifications (e.g., depth of coverage, extent of intron/exon boundary analysis, methodology of large deletion/duplication analysis).
- Sequence analysis detects single nucleotide substitutions and small (several nucleotide) deletions and insertions. Regions analyzed typically include the coding sequence and intron/exon boundaries. Promoter regions and intronic sequences

may also be sequenced if disease-causing mutations are known to occur in these regions of a gene.

- The efficiency of NGS has led to an increasing number of large, multi-gene testing panels. NGS panels that test several genes at once are particularly well-suited to conditions caused by more than one gene or where there is considerable clinical overlap between conditions.
- Results may be obtained that cannot be adequately interpreted based on the current knowledge base. When a sequence variation is identified that has not been previously characterized or shown to cause the disorder in question, it is called a variant of uncertain significance (VUS). VUSs are relatively common findings when sequencing large amounts of DNA with NGS.
- Under certain circumstances, technologies used in multi-gene testing may fail to identify mutations that might be identifiable through single-gene testing. If high clinical suspicion exists for a particular syndrome testing for that syndrome should be performed instead of a broad multi-gene panel.
- Since genes can be easily added or removed from multi-gene tests over time by a given lab, medical records must document which genes were included in the specific multi-gene test used and in which labs they were performed.
- Additionally, tests should be chosen to
  - maximize the likelihood of identifying mutations in the genes of interest
  - contribute to alterations in patient management
  - minimize the chance of finding variants of uncertain clinical significance.
- Autism spectrum disorder multi-gene panels include a wide variety of genes: from a few to hundreds or even thousands.
- Multi-gene ASD panels may also include genes believed to be associated with disease (e.g. “autism susceptibility” genes), but with a lower impact on risk than recognized syndromes. Results for such genes are of less clear value because there often are not clear management recommendations for mutation-positive individuals.

### **Deletion/Duplication Analysis**

- Analysis for deletions and duplications can be performed using a variety of technical platforms including exon array, MLPA, and NGS data analysis.
- Deletion/duplication panels may be billed separately from sequencing panels.
- These assays detect gains and losses too large to be identified through sequencing technology, often single or multiple exons or whole genes.

## Known Familial Mutation Analysis

- Analysis for known familial mutations is typically performed by Sanger sequencing, but if a targeted mutation panel is available, this may be more efficient and cost effective technology to use.
- Known familial mutation analysis is performed when a causative mutation has been identified in a close relative of the individual being tested.

## Guidelines and evidence

### Introduction

The following section includes relevant guidelines and evidence pertaining to Autism Spectrum Disorder testing.

### American Academy of Child and Adolescent Psychiatry

The American Academy of Child and Adolescent Psychiatry (AACAP, 2014) states that as a clinical standard, clinicians should coordinate an appropriate multidisciplinary assessment of children with ASD. This includes the following:<sup>2</sup>

- "All children with ASD should have a medical assessment, which typically includes physical examination, a hearing screen, a Wood's lamp examination for signs of tuberous sclerosis, and genetic testing, which may include G-banded karyotype, fragile X testing, or chromosomal microarray."
- "Unusual features in the child (e.g., history of regression, dysmorphology, staring spells, family history) should prompt additional evaluations... Genetic or neurologic consultation, neuroimaging, EEG, and additional laboratory tests should be obtained when relevant, based on examination or history (e.g., testing for the MECP2 gene in cases of possible Rett's disorder)."

### The American College of Medical Genetics and Genomics

The American College of Medical Genetics and Genomics (ACMG, 2013) recommends a genetic evaluation, with a tiered approach, for all individuals with diagnosed ASD:<sup>3</sup>

- "Several well-described single-gene disorders have been reported for which ASDs can be seen as part of the expanded phenotype associated with changes in that gene...For a selected few of such conditions, there is adequate evidence to suggest testing for changes in these genes in patients with ASDs with no other identifiable etiology. These would include fragile X syndrome, methyl-CPG-binding protein 2 (MECP2) spectrum disorders, and phosphatase and tensin homolog (PTEN)-related conditions."
- First tier

- Three-generation family history with pedigree analysis.
- Initial evaluation to identify known syndromes or associated conditions
  - Examination with special attention to dysmorphic features
  - If specific syndromic diagnosis is suspected, proceed with targeted testing
  - If appropriate clinical indicators present, perform metabolic and/ or mitochondrial testing (alternatively, consider a referral to a metabolic specialist)
- Chromosomal microarray: oligonucleotide array-comparative genomic hybridization or single-nucleotide polymorphism array.
- DNA testing for fragile X (to be performed routinely for male patients and in females if indicators are present - e.g., family history and phenotype).
- Second tier
  - MECP2 sequencing to be performed for all females with ASDs
  - MECP2 duplication testing in males, if phenotype is suggestive
  - PTEN testing only if the head circumference is >2.5 SD above the mean
  - Brain magnetic resonance imaging only in the the presence of specific indicators (e.g., microcephaly, regression, seizures, and history of stupor/coma)
- The following are genetic tests “that have been suggested in the etiologic evaluation of ASDs, but currently with insufficient evidence to recommend routine testing:” CDKL5 testing, NSD1 testing, chromosome 15 methylation/UBE3A gene testing, methylation/epigenetic testing, mitochondrial gene sequencing/oligoarray, and metabolic studies.

### **The National Institute for Health and Clinical Excellence**

The National Institute for Health and Clinical Excellence (NICE, 2011) states the following regarding medical investigations following diagnosis of an ASD: “Do not routinely perform any medical investigations as part of an autism diagnostic assessment, but consider the following in individual circumstances and based on physical examination, clinical judgment and the child or young person's profile:<sup>5</sup>

- Genetic tests, as recommended by your regional genetics center, if there are specific dysmorphic features, congenital anomalies and/or evidence of intellectual disability
- Electroencephalography if there is suspicion of epilepsy.”

**Kalsner et al., 2017**

A peer reviewed 2017 article assessed the clinical utility of a targeted gene panel (101-237 genes) in 100 well-phenotyped patients with ASD, and found<sup>6</sup>

- 12% diagnostic yield for chromosomal microarray
- 0% diagnostic yield for targeted gene panel (11 pathogenic variants identified; all assessed as non-causative by clinicians based on clinical evaluation of patient, allele frequency in the study population, or conflicting data in the literature on causation)
- If patient does not fit a syndromic diagnosis, the authors suggest ACMG recommended tests followed by whole exome sequencing in patients with ASD plus
  - Severe disability
  - Congenital abnormalities
  - Co-morbid conditions (eg: seizure disorder)
  - Abnormal head size

**Criteria****Introduction**

Requests for Autism Spectrum Disorder testing are reviewed using the following clinical criteria.

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**Note** This guideline does not address chromosomal microarray testing. Please see the following test specific guideline for additional information: *Chromosomal Microarray Testing For Developmental Disorders*.

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**Known Familial Mutation Testing**

- Genetic counseling:
  - Pre- and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - No previous genetic testing for the known familial mutation, AND
- Diagnostic Testing for Symptomatic Individuals:
  - Known family mutation in a causative gene in 1st, 2nd, or 3rd degree biologic relative, OR



- Prenatal Testing for At-Risk Pregnancies:
  - Known familial disease-causing mutation identified in both biologic parents (if recessive), or a single biologic parent or an affected sibling of the pregnancy (if dominant).

### **Autism Single Gene Diagnostic Tests (Sequencing and Deletion/Duplication)**

- The member has a formal diagnosis of ASD/autism as made by an appropriate health care professional, AND
- The member has a condition that will benefit from information provided by the requested autism gene testing based on the following:
  - The member displays at least one clinical feature (in addition to autism) of the suspected condition for which testing is being requested, AND
    - The member's medical management would be significantly altered by the genetic diagnosis, or
    - A particular treatment is being considered for the member that requires a genetic diagnosis, OR
  - The member meets all criteria in a test-specific guideline, if available (see the Table below for a list of genes, associated conditions, and applicable guideline), AND
- The member does not have a known underlying cause for their autism (e.g. known genetic condition), AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

### **Autism Multi-Gene Panels**

This test is considered investigational and/or experimental.

- Investigational and experimental (I&E) molecular and genomic (MolGen) tests refer to assays involving chromosomes, DNA, RNA, or gene products that have insufficient data to determine the net health impact, which typically means there is insufficient data to support that a test accurately assesses the outcome of interest (analytical and clinical validity), significantly improves health outcomes (clinical utility), and/or performs better than an existing standard of care medical management option. Such tests are also not generally accepted as standard of care in the evaluation or management of a particular condition.
- In the case of MolGen testing, FDA clearance is not a reliable standard given the number of laboratory developed tests that currently fall outside of FDA oversight and FDA clearance often does not assess clinical utility.

**Note** Multi-gene panels for individuals with a primary medical diagnosis of ASD have not demonstrated a high diagnostic yield and are not likely to lead to a change in treatment. Comprehensive ASD panels, regardless of panel size, are not medically necessary and therefore, not reimbursable. However, separate clinical guidelines may apply to panel testing for members who having findings in addition to ASD, such as seizures or multiple congenital anomalies.

### Billing and reimbursement considerations

- The billed amount should not exceed the list price of the test.
- Autism Spectrum Disorder panels, regardless of how they are billed, are not medically necessary and, therefore, are not reimbursable.
- Genetic testing is only necessary once per lifetime. Therefore, a single gene included in a panel or a multi-gene panel may not be reimbursed if testing has been performed previously. Exceptions may be considered if technical advances in testing demonstrate significant advantages that would support a medical need to retest.
- This guideline may not apply to genetic testing for indications that are addressed in test-specific guidelines. Please see the test-specific list of guidelines for a complete list of test-specific panel guidelines.

This list is not all-inclusive.

### ***Common autism genes, associate conditions, and applicable guidelines***

| Gene    | CPT         | Condition                                | Applicable guideline name                                | Applicable guideline number |
|---------|-------------|--|--|-----------------------------|
| 15q11.2 | 81331       | Prader-Willi Syndrome, Angelman Syndrome | Prader-Willi Syndrome Testing; Angelman Syndrome Testing | MOL.TS.217; MOL.TS.126      |
| AFF2    | 81171 81172 | Fragile X Syndrome 2 (FRAXE)             | Genetic Testing for Autism                               | MOL.TS.269                  |
| ARX     | 81404       | ARX-Related Neurodevelopmental Disorders | Genetic Testing for Autism                               | MOL.TS.269                  |

| Gene    | CPT         | Condition  | Applicable guideline name  | Applicable guideline number |
|---------|-------------|--|----------------------------|-----------------------------|
| BRAF    | 81406       | Noonan Syndrome, Cardiofaciocutaneous Syndrome                     | Genetic Testing for Autism | MOL.TS.269                  |
| CACNA1C | 81479       | Timothy Syndrome   | Genetic Testing for Autism | MOL.TS.269                  |
| CDKL5   | 81406       | Infantile Spasms; Early Seizure Variant Rett Syndrome              | Genetic Testing for Autism | MOL.TS.269                  |
| CHD7    | 81407       | CHARGE Syndrome  | Genetic Testing for Autism | MOL.TS.269                  |
| CNTNAP2 | 81406       | Autosomal Recessive Intellectual Disability, Pitt-Hopkins-Like     | Genetic Testing for Autism | MOL.TS.269                  |
| DHCR7   | 81405       | Smith-Lemli-Opitz Syndrome   | Genetic Testing for Autism | MOL.TS.269                  |
| FGD1    | 81479       | Aarskog-Scott Syndrome, Syndromic X-Linked Intellectual Disability | Genetic Testing for Autism | MOL.TS.269                  |
| FMR1    | 81243 81244 | Fragile X Syndrome   | Fragile X Syndrome Testing | MOL.TS.172                  |
| FOLR1   | 81479       | Cerebral Folate Transport Deficiency                               | Genetic Testing for Autism | MOL.TS.269                  |
| FOXP1   | 81404       | Congenital Variant Rett Syndrome                                   | Genetic Testing for Autism | MOL.TS.269                  |

| Gene   | CPT   | Condition   | Applicable guideline name  | Applicable guideline number |
|--------|-------|---|----------------------------|-----------------------------|
| FOXP1  | 81479 | Intellectual Disability with Language Impairment, with or without Autistic Features | Genetic Testing for Autism | MOL.TS.269                  |
| FOXP2  | 81479 | FOXP2-Related Speech-Language Disorders   | Genetic Testing for Autism | MOL.TS.269                  |
| FTSJ1  | 81406 | X-Linked Intellectual Disability  | Genetic Testing for Autism | MOL.TS.269                  |
| GRIN2A | 81479 | GRIN2A-Related Speech Disorders & Epilepsy (Landau-Kleffner included)               | Genetic Testing for Autism | MOL.TS.269                  |
| KDM5C  | 81407 | X-Linked Intellectual Disability  | Genetic Testing for Autism | MOL.TS.269                  |
| L1CAM  | 81407 | Hydrocephalus with Aqueductal Stenosis, MASA Syndrome                               | Genetic Testing for Autism | MOL.TS.269                  |
| MBD5   | 81479 | MBD5 Haploinsufficiency   | Genetic Testing for Autism | MOL.TS.269                  |

| Gene   | CPT         | Condition  | Applicable guideline name  | Applicable guideline number |
|--------|-------------|--|----------------------------|-----------------------------|
| MECP2  | 81302       | Classic Rett Syndrome, Preserved Speech Variant Rett Syndrome, MECP2-Related Epileptic Encephalopathy (males), X-Linked ID | Rett Syndrome Testing      | MOL.TS.224                  |
| MED12  | 81401 81479 | MED12-Related Disorders  | Genetic Testing for Autism | MOL.TS.269                  |
| MEF2C  | 81479       | Intellectual disability, Stereotypic Movements, Epilepsy, and/or Cerebral Malformations                                    | Genetic Testing for Autism | MOL.TS.269                  |
| NF1    | 81408       | Neurofibromatosis 1  | Genetic Testing for Autism | MOL.TS.269                  |
| NIPBL  | 81479       | Cornelia de Lange Syndrome   | Genetic Testing for Autism | MOL.TS.269                  |
| NLGN3  | 81405       | Susceptibility to Autism/Asperger's  | Genetic Testing for Autism | MOL.TS.269                  |
| NLGN4X | 81405       | X-Linked Intellectual Disability, Susceptibility to Autism   | Genetic Testing for Autism | MOL.TS.269                  |
| NRXN1  | 81479       | Autosomal Recessive Intellectual Disability, Pitt-Hopkins-Like   | Genetic Testing for Autism | MOL.TS.269                  |

| Gene       | CPT         | Condition   | Applicable guideline name             | Applicable guideline number |
|------------|-------------|---|---------------------------------------|-----------------------------|
| PCDH19     | 81405       | Epilepsy & Intellectual Disability Limited to Females | Genetic Testing for Autism            | MOL.TS.269                  |
| PTCHD1     | 81479       | Susceptibility to Autism                              | Genetic Testing for Autism            | MOL.TS.269                  |
| PTEN       | 81321       | PTEN Hamartoma Tumor Syndromes                        | PTEN Hamartoma Tumor Syndrome Testing | MOL.TS.223                  |
| PTPN11     | 81406       | Noonan Syndrome                                       | Genetic Testing for Autism            | MOL.TS.269                  |
| RAI1       | 81405       | Smith-Magenis Syndrome                                | Genetic Testing for Autism            | MOL.TS.269                  |
| SHANK2     | 81479       | Susceptibility to Autism                              | Genetic Testing for Autism            | MOL.TS.269                  |
| SHANK3     | 81479       | Phelan-McDermid Syndrome                              | Genetic Testing for Autism            | MOL.TS.269                  |
| SLC2A1     | 81405       | GLUT1 Deficiency                                      | Genetic Testing for Autism            | MOL.TS.269                  |
| SLC6A8     | 81479       | Creatine Transporter Deficiency                       | Genetic Testing for Autism            | MOL.TS.269                  |
| SLC9A6     | 81406       | Christianson Syndrome                                 | Genetic Testing for Autism            | MOL.TS.269                  |
| SMC1A      | 81479       | Cornelia de Lange Syndrome                            | Genetic Testing for Autism            | MOL.TS.269                  |
| TCF4       | 81406       | Pitt-Hopkins Syndrome                                 | Genetic Testing for Autism            | MOL.TS.269                  |
| TSC1, TCS2 | 81406 81407 | Tuberous Sclerosis                                    | Genetic Testing for Autism            | MOL.TS.269                  |

| Gene  | CPT   | Condition                        | Applicable guideline name  | Applicable guideline number |
|-------|-------|----------------------------------|----------------------------|-----------------------------|
| UBE3A | 81406 | Angelman Syndrome                | Angelman Syndrome Testing  | MOL.TS.126                  |
| ZEB2  | 81405 | Mowat-Wilson Syndrome            | Genetic Testing for Autism | MOL.TS.269                  |
| ZNF4  | 81404 | X-Linked Intellectual Disability | Genetic Testing for Autism | MOL.TS.269                  |

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# Genetic Testing for Epilepsy

MOL.TS.257.A

v1.0.2020

## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedures covered by this guideline                      | Procedure codes |
|---|-----------------|
| CSTB Full Gene Analysis                                   | 81189           |
| CSTB Gene Analysis; evaluation to detect abnormal alleles | 81188           |
| CSTB Known Familial Mutation Analysis                     | 81190           |
| Miscellaneous epilepsy gene tests                         | 81400           |
| Miscellaneous epilepsy gene tests                         | 81401           |
| Miscellaneous epilepsy gene tests                         | 81402           |
| Miscellaneous epilepsy gene tests                         | 81403           |
| Miscellaneous epilepsy gene tests                         | 81404           |
| Miscellaneous epilepsy gene tests                         | 81405           |
| Miscellaneous epilepsy gene tests                         | 81406           |
| Miscellaneous epilepsy gene tests                         | 81407           |
| Miscellaneous epilepsy gene tests                         | 81408           |
| Miscellaneous epilepsy gene tests                         | 81479           |

## What is epilepsy

### Definition

Epilepsy is a neurological condition that causes seizures. It affects approximately 1% of children and 1.8% of adults.<sup>1</sup>

- Epilepsy can manifest in different ways, including different types of seizures or with multiple neurodevelopmental and medical complications besides seizures. Seizure types include generalized seizures (absence seizures, tonic-clonic seizures) and focal seizures (simple focal seizures, complex focal seizures, secondary generalized seizures, among others).
- An electroencephalography (EEG) can be used to help diagnose epilepsy and possibly give information as to the seizure type. A brain magnetic resonance



imaging (MRI) scan can further help define whether epilepsy is caused by a structural brain abnormality or help determine the origin of epilepsy.

- Epilepsy has multiple causes. These include, but are not limited to, acquired causes such as stroke, brain tumor, head injury, and central nervous system infection.<sup>1</sup> There are also numerous genetic conditions associated with epilepsy. It is estimated that approximately 40% of individuals with seizures have an underlying genetic basis for their condition (see Table 1 for a list of common genetic causes).<sup>2</sup>
- Inheritance patterns differ between various epilepsy syndromes including dominant, X-linked, recessive, mitochondrial and recessive causes, in addition to epilepsy caused by de novo (or new) genetic mutations. Clinical heterogeneity is also seen in these conditions.
- Epileptic encephalopathy is a group of disorders in which seizures are accompanied by developmental delays, cognitive impairment, or a host of other neurological issues such as feeding difficulties, sleep dysregulation, and behavioral problems.<sup>3</sup> Knowledge regarding the genetic basis of these disorders has increased significantly in the last decade due to the advent of high throughput Next Generation Sequencing methods, resulting in wider availability of multi-gene panel testing. The following are examples of epileptic encephalopathies:
  - Ohtahara syndrome (Early Infantile Epileptic Encephalopathy)
    - “Characterized by early onset intractable tonic spasms, suppression-burst pattern on interictal EEG, and poor prognosis.”<sup>4</sup>
    - “To date, various genes, which have essential roles in the brain’s neuronal and interneuronal functions, have been reported to be associated with Ohtahara syndrome. For instance, syntaxin binding protein 1 (STXBP1) regulates synaptic vesicle release<sup>11</sup>; aristaless-related homeobox (ARX) acts as a regulator of proliferation and differentiation of neuronal progenitors<sup>12</sup>; solute carrier family 25 member 22 (SLC25A22) encodes a mitochondrial glutamate transporter<sup>13</sup>; and potassium voltage-gated channel, KQT-like subfamily, member 2 (KCNQ2) plays a key role in a cell’s ability to generate and transmit electrical signals.”<sup>5</sup>
  - Dravet Syndrome (Severe Myoclonic Epilepsy of Infancy)
    - “Clinical cardinal features include febrile or afebrile generalized or hemiconvulsions starting in the first year of life, seizure evolution to a mixture of intractable generalized (myoclonic or atonic seizures, atypical absences) and focal seizures, normal early development, subsequent psychomotor retardation, and normal brain imaging at onset.”<sup>4</sup>
    - “In most of the cases with Dravet syndrome, one single gene has been involved, in contrast to other epileptic encephalopathy syndromes. SCN1A mutations have been shown in at least 80% of patients with Dravet syndrome.”<sup>5</sup>
  - Infantile Spasms (West Syndrome and X-linked Infantile Spasms)

- “West syndrome is characterized by a specific seizure type, i.e., epileptic spasms, a unique interictal EEG pattern termed hypsarrhythmia, and psychomotor retardation. Spasms start within the first year of life, mainly between 4 and 6 months of age.”<sup>4</sup>
- “There are multiple genetic determinants of infantile spasms, which are usually explained by mutations in distinct genes. Genetic analysis of children with unexplained infantile spasms have demonstrated mutations on the X chromosome in genes such as ARX, cyclin-dependent kinase-like 5 (CDKL5), and UDP-N-acetylglucosaminyltransferase subunit (ALG13) as well as de novo mutations in autosomal genes, including membrane-associated guanylate kinase, WW and PDZ domain containing protein 2 (MAGI2), STXBP1, sodium channel alpha 1 subunit (SCN1A), sodium channel protein type 2 subunit alpha (SCN2A), g-aminobutyric acid (GABA) A receptor, beta 3 (GABRB3), and dynamin 1 (DNM1).”<sup>5</sup>
- Epilepsy and Intellectual Disability Limited to Females
  - “Epilepsy and intellectual disability limited to females (EFMR) is an underrecognized disorder with X-linked inheritance but surprisingly only affecting females while sparing transmitting males. Seizure, cognitive, and psychiatric phenotypes show heterogeneity. Seizures start from the age of 6 to 36 months and may be precipitated by fever. Seizure types include GTCS, myoclonic and tonic seizures, absences, and focal seizures.”<sup>4</sup>
  - “Different mutations of PCDH19 (protocadherin 19), including missense, nonsense, and frameshift mutations, have been reported as the cause of EFMR.”<sup>4</sup>
- Genetic testing for epilepsy is complicated by many factors. Epilepsy syndromes frequently have overlapping features, such as the types of seizures involved and/or additional clinical findings. Many (if not most) epilepsy syndromes, including epileptic encephalopathy, are genetically heterogeneous, and can be caused by mutations in a number of different genes. Sometimes, the inheritance pattern or the presence of pathognomonic features makes the underlying syndrome clear. However, in many cases, it can be difficult to reliably diagnose an epilepsy syndrome based on clinical and family history alone.
  - Clinical information (e.g. age of onset, seizure type, EEG results, etc.) may be used in some cases to help narrow down the suspected cause. In these cases, it may be possible to identify a narrow subset of genes that may be responsible for a person’s epilepsy.
- Treatment for epilepsy ranges from antiepileptic drugs (AEDs) to the ketogenic diet to vagal nerve stimulation to epilepsy surgery in the most severe situations. Not all treatments will work for everyone and often, it takes multiple treatment trials to find a regimen that is successful. In a rapidly growing number of epilepsy disorders, knowing the genetic mutation that is responsible for the epilepsy has been shown to help guide management and provide more disease-specific treatment.<sup>6,7</sup>

## Test information

- There are various methods used to test for mutations in genes which can cause epilepsy.
  - Chromosomal microarray analysis
  - Single gene analysis
  - Multi-gene panels
  - Exome sequencing
- Chromosomal microarray (CMA) testing is used to detect missing (deletions) or extra (duplications) segments of DNA. Whole-genome screening for CNVs identifies potentially pathogenic deletions or duplications in ~5% of patients with a range of epilepsy phenotypes, including focal epilepsy, generalized epilepsies, epileptic encephalopathies, fever-associated epilepsy syndromes, and patients with neurodevelopmental disorders and epilepsy.<sup>8</sup>
- Until recently, most single or multi-gene sequencing used the Sanger sequencing methodology that was originally developed in the 1970s. Sanger sequencing is labor intensive and did not lend itself to high-throughput applications.
- Next generation sequencing (NGS), also known as high throughput or massively parallel sequencing, allows larger scale and more efficient gene sequencing. NGS relies on sequencing many copies of small pieces of DNA simultaneously and using bioinformatics analysis to assemble and interpret the identified genetic sequence changes. The diagnostic yield of NGS in patients with epileptic encephalopathies ranges is estimated to be 20-30%.<sup>9,10</sup>
- The efficiency of NGS has led to an increasing number of large, multi-gene testing panels. NGS panels that test several genes at once are particularly well-suited to conditions caused by more than one gene or where there is considerable clinical overlap between conditions, making it difficult to reliably narrow down likely causes based on history and clinical examination. As a result, many laboratories have developed large multi-gene panels that overcome these limitations. NGS based testing has been shown to dramatically improve the diagnostic rate for children and adults with epilepsy, as well as significantly shorten the time from assessment to diagnosis.<sup>11,12</sup>
- Under certain circumstances, technologies used in multi-gene testing may fail to identify mutations that might be identifiable through single-gene testing. If high clinical suspicion remains for a particular syndrome after negative multi-gene test results, consultation with the testing lab and/or additional targeted genetic testing may be warranted. Further, multi-gene panels may fail to detect small deletions or duplications of genes included on the panel in which case, single gene deletion/duplication testing may be needed.
- Multi-gene tests vary in technical specifications (e.g., depth of coverage, extent of intron/exon boundary analysis, methodology of large deletion/duplication analysis). Therefore, selection of the appropriate panel takes into consideration

several factors including technical specifications, clinical differential diagnosis, and desired turn-around time.

- Since genes can be easily added or removed from multi-gene tests over time by a given lab, medical records must document which genes were included in the specific multi-gene test used from each patient, the labs in which they were performed, and the year of testing.
- Additionally, tests should be chosen that maximize the likelihood of identifying mutations in the genes of interest and that will alter the patient's medical management and treatment.

## Guidelines and evidence

- No current U.S guidelines address the use of multi-gene panels in epilepsy.
- In 2016, a peer reviewed article on genetic testing for epileptic encephalopathy stated the following:
  - “Second line investigations: Targeted next generation sequencing panels of epileptic encephalopathy genes for individuals with epileptic encephalopathy.”<sup>3</sup>
- In 2016, a peer reviewed article on genetic causes of early-onset epileptic encephalopathy stated the following:
  - “Molecular-based studies on early-onset epileptic encephalopathies should be performed, necessitating programmed genetical algorithms. If the phenotype could be determined with clinical findings, specific gene testing would be helpful in diagnosis. However, if the phenotype could not be determined because of overlapping phenotypes of different syndromes and the spectrum of phenotypes seen in different mutations, the use of gene panels for epilepsy would increase the probability of correct diagnosis. In a recent study, the rate of diagnosis with targeted single gene sequencing has been reported as 15.4%, whereas the rate has increased to 46.2% with the utility of epilepsy gene panels.”<sup>5</sup>
- A Task Force for the ILAE Commission of Pediatrics (2015) published recommendations for the management of infantile seizures. These recommendations included the following on treatments:<sup>13</sup>
  - “for Dravet syndrome, strong evidence supports that stiripentol is effective (in combination with valproate and clobazam), whereas weak evidence supports that topiramate, zonisamide, valproate, bromide, and the ketogenic diet are possibly effective; and for Ohtahara syndrome, there is weak evidence that most antiepileptic drugs are poorly effective.”<sup>13</sup>
  - “Genetic evaluation for Dravet syndrome and other infantile-onset epileptic encephalopathies should be available at tertiary and quaternary levels of care (optimal intervention would permit an extended genetic evaluation) (level of evidence—weak recommendation, level C)”<sup>13</sup>

- “Early diagnosis of some mitochondrial conditions may alter long-term outcome, but whether screening at quaternary level is beneficial is unknown (level of evidence U)”<sup>13</sup>
- Multiple peer-reviewed articles have shown that epilepsy multi-gene panels have a significant diagnostic yield when seizure onset is in infancy or early childhood.<sup>14-16</sup>

## Criteria

This policy applies to all epilepsy testing, including single gene analysis and multi-gene panels, which are defined as assays that simultaneously test for more than one epilepsy gene. Medical necessity coverage generally relies on criteria established for testing individual genes.

Coverage criteria differ based on the type of testing being performed (i.e., individual epilepsy genes separately chosen versus pre-defined panels of epilepsy genes) and how that testing will be billed (one or more individual epilepsy gene procedure codes, specific panel procedure codes, or unlisted procedure codes).

### Epilepsy single gene tests

Epilepsy single gene tests will be covered when the following criteria are met:

- The member has a condition that will benefit from information provided by the requested epilepsy gene testing based on at least one of the following criteria:
  - The member displays clinical features of the condition for which testing is being requested and a particular treatment is being considered for the member that requires a genetic diagnosis, OR
  - A particular AED is being considered for the member and the AED is contraindicated for individuals with mutations in that gene, defined by ONE of the following criteria:
    - A neurology therapy FDA label requires results from the genetic test to effectively or safely use or avoidance of the therapy for the member’s epilepsy type and the member has not previously had a trial of the therapy, OR
    - An American neurological society specifically recommends the testing for the safe and effective use or avoidance of a therapy and the member has not previously had a trial of the therapy, OR
  - The member meets all criteria in a test-specific guideline, if available (see Table 1 for a list of genes, associated conditions, and applicable policy), AND
- The member does not have a known underlying cause for their seizures (e.g. tumor, head trauma, known genetic condition), AND

- Rendering laboratory is a qualified provider of service per the Health Plan policy.

### **Epilepsy multi-gene panels**

When separate procedure codes will be billed for individual epilepsy genes (e.g., Tier 1 MoPath codes 81200-81355 or Tier 2 MoPath codes 81400-81408), each individually billed test will be evaluated separately. The following criteria will be applied:

- The member has a condition that will benefit from information provided by the requested epilepsy gene testing based on at least one of the following criteria:
  - The member displays clinical features of the condition for which testing is being requested and a particular treatment is being considered for the member that requires a genetic diagnosis, OR
  - A particular AED is being considered for the member and the AED is contraindicated for individuals with mutations in that gene by ONE of the following:
    - A neurology therapy FDA label requires results from the genetic test to effectively or safely use or avoidance the therapy for the member's epilepsy type and the member has not previously had a trial of the therapy, or
    - An American neurological society specifically recommends the testing for the safe and effective use or avoidance of a therapy and the member has not previously had a trial of the therapy, OR
  - The member meets all criteria in a test-specific guideline, if available, (see Table 1 for a list of genes, associated conditions, and applicable policy), AND
- The member does not have an known underlying cause for their seizures (e.g. tumor, head trauma, known genetic condition), AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

If the member meets the following criteria, the entire panel will be approved. However, the laboratory will be redirected to use a panel CPT code for billing purposes (e.g. 81479):

- The member has a diagnosis of early infantile epileptic encephalopathy, OR
- The member has a diagnosis of infantile spasms, OR
- The member has a diagnosis of intractable, neonatal seizures, OR
- The member has a diagnosis of febrile seizures with at least one episode of status epilepticus, OR
- The member has a progressive neurological disease defined by the following:
  - Member has epilepsy with persistent loss of developmental milestones, and

- Member's seizures are worsening in severity and/or frequency despite treatment, OR
- A particular AED is being considered for the member and there are 2 or more genes on the panel for which the AED is contraindicated for individuals with mutations in that gene by ONE of the following:
  - A neurology therapy FDA label requires results from the genetic test to effectively or safely use or avoidance the therapy for the member's epilepsy type and the member has not previously had a trial of the therapy, or
  - An American neurological society specifically recommends the testing for the safe and effective use or avoidance of a therapy and the member has not previously had a trial of the therapy, AND
- The member does not display clinical features of a specific condition for which testing is available (e.g. Tuberous Sclerosis, Angelman Syndrome, Rett Syndrome, etc.), AND
- The member does not have a known underlying cause for their seizures (e.g. tumor, head trauma, known genetic condition), AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

When a multi-gene panel is being requested and will be billed with a single panel CPT code (e.g. 81479), the panel will be considered medically necessary when the following criteria are met:

- The member has a diagnosis of early infantile epileptic encephalopathy, OR
- The member has a diagnosis of infantile spasms, OR
- The member has a diagnosis of intractable, neonatal seizures, OR
- The member has a diagnosis of febrile seizures with at least one episode of status epilepticus, OR
- The member has a progressive neurological disease defined by the following:
  - Member has epilepsy with persistent loss of developmental milestones, and
  - Member's seizures are worsening in severity and/or frequency despite treatment, OR
- A particular AED is being considered for the member and there are 2 or more genes on the panel for which the AED is contraindicated for individuals with mutations in that gene by ONE of the following:
  - A neurology therapy FDA label requires results from the genetic test to effectively or safely use or avoidance the therapy for the member's epilepsy type and the member has not previously had a trial of the therapy, or

- An American neurological society specifically recommends the testing for the safe and effective use or avoidance of a therapy and the member has not previously had a trial of the therapy, AND
- The member does not display clinical features of a specific condition for which testing is available (e.g. Tuberous Sclerosis, Angelman Syndrome, Rett Syndrome, etc.), AND
- The member does not have a known underlying cause for their seizures (e.g. tumor, head trauma, known genetic condition), AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

### **Billing and reimbursement considerations**

- The billed amount should not exceed the list price of the test.
- Large epilepsy panels may not be medically necessary when smaller panels are available and are more appropriate based on the clinical findings.
- Genetic testing for a specific gene may be necessary only once per lifetime. Therefore, a single gene included in a panel or a multi-gene panel may not be reimbursed if testing has been performed previously. Exceptions may be considered if technical advances in testing demonstrate significant advantages that would support a medical need to retest. Further, given rapidly advancing knowledge regarding genetic variations in epilepsy and in normal or healthy populations, re-analysis of genetic tests may be warranted at regular intervals.
- This guideline may not apply to genetic testing for indications that are addressed in test-specific guidelines. Please see the test-specific list of guidelines for a complete list of test-specific panel guidelines.
- If a panel was previously performed and an updated, larger panel is being requested, only testing for the medically necessary, previously untested genes will be reimbursable. Therefore, only the most appropriate procedure codes for those additional genes will be considered for reimbursement.
- If the laboratory will not accept redirection to a single code, the medical necessity of each billed component procedure will be assessed independently using the criteria above for single gene testing. Only the individual panel components that meet medical necessity criteria as a first tier of testing will be reimbursed. The remaining individual components will not be reimbursable.

### **Table 1: Common epilepsy genes, associated conditions and applicable guidelines**

This is a representative list of known epilepsy genes and is not all inclusive:



| Gene    | CPT   | Condition   | Applicable guideline name    | Applicable guideline number |
|---------|-------|---|------------------------------|-----------------------------|
| ALDH7A1 | 81406 | Pyridoxine-Dependent Epilepsy                             | Genetic Testing for Epilepsy | MOL.TS.257                  |
| ARX     | 81404 | ARX-Related Neurodevelopmental Disorders                  | Genetic Testing for Epilepsy | MOL.TS.257                  |
| ATP1A2  | 81406 | Familial Hemiplegic Migraine                              | Genetic Testing for Epilepsy | MOL.TS.257                  |
| ARGHEF9 | 81479 | ARGHEF9-Related Epilepsy (EOEE included)                  | Genetic Testing for Epilepsy | MOL.TS.257                  |
| CACNA1A | 81185 | Familial Hemiplegic Migraine, Episodic Ataxia             | Genetic Testing for Epilepsy | MOL.TS.257                  |
| CDKL5   | 81406 | Infantile Spasms; Early Seizure Variant Rett Syndrome     | Genetic Testing for Epilepsy | MOL.TS.257                  |
| CHD2    | 81479 | CHD2-Related Neurodevelopmental Disorders (EOEE included) | Genetic Testing for Epilepsy | MOL.TS.257                  |
| CHRNA2  | 81479 | ADNFLE  | Genetic Testing for Epilepsy | MOL.TS.257                  |
| CHRNA4  | 81405 | ADNFLE  | Genetic Testing for Epilepsy | MOL.TS.257                  |
| CHRN2   | 81405 | ADNFLE  | MOL.TS.257                   | MOL.TS.257                  |
| CLN3    | 81479 | Neuronal Ceroid Lipofuscinosis                            | MOL.TS.257                   | MOL.TS.257                  |
| CLN5    | 81479 | Neuronal Ceroid Lipofuscinosis                            | Genetic Testing for Epilepsy | MOL.TS.257                  |

| Gene    | CPT                     | Condition   | Applicable guideline name    | Applicable guideline number |
|---------|-------------------------|---|------------------------------|-----------------------------|
| CLN8    | 81479                   | Neuronal Ceroid Lipofuscinosis                            | MOL.TS.257                   | MOL.TS.257                  |
| CNTNAP2 | 81406                   | Pitt-Hopkins-Like Syndrome                                | MOL.TS.257                   | MOL.TS.257                  |
| CSTB*   | 81188<br>81189<br>81190 | PME (Unverricht-Lundborg)                                 | MOL.TS.257                   | MOL.TS.257                  |
| DEPDC5  | 81479                   | DEPDC5-Related Epilepsy                                   | Genetic Testing for Epilepsy | MOL.TS.257                  |
| EFHC1   | 81406                   | Susceptibility to Juvenile Absence & Myoclonic Epilepsies | Genetic Testing for Epilepsy | MOL.TS.257                  |
| EPM2A   | 81404                   | PME (Lafora Disease)                                      | Genetic Testing for Epilepsy | MOL.TS.257                  |
| FOLR1   | 81479                   | Cerebral Folate Transport Deficiency                      | Genetic Testing for Epilepsy | MOL.TS.257                  |
| FOXG1   | 81404                   | Congenital Variant Rett Syndrome                          | Genetic Testing for Epilepsy | MOL.TS.257                  |
| GABRA1  | 81479                   | GABRA1-Related Epilepsy (EOEE included)                   | Genetic Testing for Epilepsy | MOL.TS.257                  |
| GABRB3  | 81479                   | GABRB3-Related Epilepsy (EOEE included)                   | Genetic Testing for Epilepsy | MOL.TS.257                  |
| GABRG2  | 81405                   | GABRG2-Related Epilepsy (GEFS+ included)                  | Genetic Testing for Epilepsy | MOL.TS.257                  |

| Gene   | CPT   | Condition   | Applicable guideline name    | Applicable guideline number |
|--------|-------|---|------------------------------|-----------------------------|
| GAMT   | 81479 | Creatine Deficiency Syndromes   | Genetic Testing for Epilepsy | MOL.TS.257                  |
| GATM   | 81479 | Creatine Deficiency Syndromes   | Genetic Testing for Epilepsy | MOL.TS.257                  |
| GRIN2A | 81479 | GRIN2A-Related Speech Disorders & Epilepsy (Landau-Kleffner included) | Genetic Testing for Epilepsy | MOL.TS.257                  |
| KCNJ10 | 81404 | EAST/SeSAME Syndrome  | Genetic Testing for Epilepsy | MOL.TS.257                  |
| KCNQ2  | 81406 | KCNQ2-Related Disorders (BFNS & EOOE included)                        | Genetic Testing for Epilepsy | MOL.TS.257                  |
| KCNQ3  | 81479 | KCNQ3-Related Disorders (BFNS included)                               | Genetic Testing for Epilepsy | MOL.TS.257                  |
| KCNT1  | 81479 | KCNT1-Related Disorders (ADNFLE & EOOE included)                      | Genetic Testing for Epilepsy | MOL.TS.257                  |
| KCTD7  | 81479 | PME With or Without Inclusions, Neuronal Ceroid Lipofuscinosis        | Genetic Testing for Epilepsy | MOL.TS.257                  |
| LG11   | 81479 | Autosomal Dominant Partial Epilepsy with Auditory Features            | Genetic Testing for Epilepsy | MOL.TS.257                  |

| Gene     | CPT   | Condition   | Applicable guideline name    | Applicable guideline number |
|----------|-------|---|------------------------------|-----------------------------|
| MBD5     | 81479 | MBD5 Haploinsufficiency   | Genetic Testing for Epilepsy | MOL.TS.257                  |
| MECP2    | 81302 | Classic Rett Syndrome; MECP2-Related Epileptic Encephalopathy (males)                   | Rett Syndrome Testing        | MOL.TS.224                  |
| MEF2C    | 81479 | Intellectual disability, Stereotypic Movements, Epilepsy, and/or Cerebral Malformations | Genetic Testing for Epilepsy | MOL.TS.257                  |
| NHLRC1   | 81403 | PME (Lafora Disease)  | Genetic Testing for Epilepsy | MOL.TS.257                  |
| NRXN1    | 81479 | Pitt-Hopkins-Like Syndrome  | Genetic Testing for Epilepsy | MOL.TS.257                  |
| PCDH19   | 81405 | Epilepsy & Intellectual Disability Limited to Females                                   | Genetic Testing for Epilepsy | MOL.TS.257                  |
| PNKP     | 81479 | PNKP-Related Epilepsy (EOEE included)   | Genetic Testing for Epilepsy | MOL.TS.257                  |
| PNPO     | 81479 | Pyridoxamine 5'-Phosphate Oxidase Deficiency  | Genetic Testing for Epilepsy | MOL.TS.257                  |
| POLG     | 81406 | POLG-Related Disorders (Alpers Syndrome included)                                       | Genetic Testing for Epilepsy | MOL.TS.257                  |
| PRICKLE1 | 81479 | PME   | Genetic Testing for Epilepsy | MOL.TS.257                  |

| Gene     | CPT   | Condition  | Applicable guideline name    | Applicable guideline number |
|----------|-------|--|------------------------------|-----------------------------|
| PPT1     | 81479 | Neuronal Ceroid Lipofuscinosis                             | Genetic Testing for Epilepsy | MOL.TS.257                  |
| PRRT2    | 81479 | PRRT2-Related Disorders                                    | Genetic Testing for Epilepsy | MOL.TS.257                  |
| SCARB2   | 81479 | Action Myoclonus-Renal Failure Syndrome; PME               | Genetic Testing for Epilepsy | MOL.TS.257                  |
| SCN1A    | 81407 | SCN1A-Related Disorders (Dravet syndrome & GEFS+ included) | Genetic Testing for Epilepsy | MOL.TS.257                  |
| SCN1B    | 81404 | SCN1B-Related Disorders (GEFS+ & EOEE included)            | Genetic Testing for Epilepsy | MOL.TS.257                  |
| SCN2A    | 81479 | SCN2A-Related Disorders (BFIS & EOEE included)             | Genetic Testing for Epilepsy | MOL.TS.257                  |
| SCN8A    | 81479 | SCN8A-Related Disorders (BFIS & EOEE Included)             | Genetic Testing for Epilepsy | MOL.TS.257                  |
| SLC19A3  | 81479 | Biotin-Thiamine-Responsive Basal Ganglia Disease           | Genetic Testing for Epilepsy | MOL.TS.257                  |
| SLC2A1   | 81405 | GLUT1 Deficiency   | Genetic Testing for Epilepsy | MOL.TS.257                  |
| SLC25A22 | 81479 | SLC25A22-Related Epilepsy (EOEE included)                  | Genetic Testing for Epilepsy | MOL.TS.257                  |

| Gene    | CPT   | Condition                                 | Applicable guideline name    | Applicable guideline number |
|---------|-------|---|------------------------------|-----------------------------|
| SLC9A6  | 81406 | Christianson Syndrome                     | Genetic Testing for Epilepsy | MOL.TS.257                  |
| SPTAN1  | 81479 | SPTAN1-Related Epilepsy (EOEE included)   | Genetic Testing for Epilepsy | MOL.TS.257                  |
| STXBP1  | 81406 | STXBP1-Related Disorders (EOEE included)  | Genetic Testing for Epilepsy | MOL.TS.257                  |
| TBC1D24 | 81479 | TBC1D24-Related Disorders (EOEE included) | Genetic Testing for Epilepsy | MOL.TS.257                  |
| TCF4    | 81406 | Pitt-Hopkins Syndrome                     | Genetic Testing for Epilepsy | MOL.TS.257                  |
| TSC1    | 81406 | Tuberous Sclerosis                        | Genetic Testing for Epilepsy | MOL.TS.257                  |
| TSC2    | 81407 | Tuberous Sclerosis                        | Genetic Testing for Epilepsy | MOL.TS.257                  |
| TPP1    | 81479 | Neuronal Ceroid Lipofuscinosis            | Genetic Testing for Epilepsy | MOL.TS.257                  |
| UBE3A   | 81406 | Angelman Syndrome                         | Angelman Syndrome Testing    | MOL.TS.126                  |
| ZEB2    | 81405 | Mowat-Wilson Syndrome                     | Genetic Testing for Epilepsy | MOL.TS.257                  |

**Note** \*90% of Unverricht-Lundborg syndrome is due to a repeat expansion in CSTB that may not be detected using next-generation sequencing and requires specific testing for repeat expansions.

ADNFLE = Autosomal Dominant Frontal Lobe Epilepsy; BFIS = Benign Familial Infantile Seizures; BFNS = Benign Familial Neonatal Seizures; EOEE = Early-Onset Epileptic Encephalopathy; GEFS+ = Generalized Epilepsy with Febrile Seizures Plus; PME = Progressive Myoclonic Epilepsy

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# Genetic Testing for Nonsyndromic Hearing Loss and Deafness

MOL.TS.273.A

v1.0.2020

## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedures addressed by this guideline   | Procedure codes |
|--|-----------------|
| GJB2 Sequencing  | 81252           |
| GJB6 Common Variant Analysis   | 81254           |
| MT-RNR1 Targeted Mutation Analysis   | 81401           |
| MT-TS1, MT-RNR1 Targeted Mutation Analysis   | 81401           |
| MT-RNR1 Sequencing   | 81403           |
| MT-TS1 Sequencing  | 81403           |
| GJB2 Known Familial Mutation Analysis  | 81253           |
| Hearing loss (e.g., nonsyndromic hearing loss, Usher syndrome, Pendred syndrome); genomic sequence analysis panel, must include sequencing of at least 60 genes, including CDH23, CLRN1, GJB2, GPR98, MTRNR1, MYO7A, MYO15A, PCDH15, OTOF, SLC26A4, TMC1, TMPRSS3, USH1C, USH1G, USH2A, and WFS1 | 81430           |
| Hearing loss (e.g, nonsyndromic hearing loss, Usher syndrome, Pendred syndrome); duplication/deletion analysis panel, must include copy number analyses for STRC and DFNB1 deletions in GJB2 and GJB6 genes  | 81431           |
| Miscellaneous hearing loss and deafness gene tests   | 81400           |
| Miscellaneous hearing loss and deafness gene tests   | 81401           |

| Procedures addressed by this guideline             | Procedure codes |
|--|-----------------|
| Miscellaneous hearing loss and deafness gene tests | 81403           |
| Miscellaneous hearing loss and deafness gene tests | 81404           |
| Miscellaneous hearing loss and deafness gene tests | 81405           |
| Miscellaneous hearing loss and deafness gene tests | 81406           |
| Miscellaneous hearing loss and deafness gene tests | 81407           |
| Miscellaneous hearing loss and deafness gene tests | 81408           |
| Miscellaneous hearing loss and deafness gene tests | 81479           |

## What is nonsyndromic hearing loss and deafness

### Definition

Prelingual hearing loss affects about 1 out of every 500 individuals.<sup>1</sup> Approximately 20% of cases are attributed to environmental causes, including viral (cytomegalovirus) or bacterial (meningitis) infection, trauma, prenatal exposure to certain drugs, and other environmental factors.<sup>1</sup> The remaining 80% of cases are thought to be genetic, either as part of a recognized genetic syndrome, or as isolated, nonsyndromic hearing loss (NSHL).<sup>1</sup>

- 95% of congenital hearing loss is detected by newborn screening. Diagnosis of hearing loss may involve physiologic testing (including auditory brainstem response or ABR/BAER) and/or audiometry.<sup>1</sup>
- 70-80% of genetic hearing loss is nonsyndromic, with no related systemic findings.<sup>1,2</sup> Some syndromic forms of hearing loss and deafness may masquerade as nonsyndromic in infancy and early childhood, before additional symptoms emerge. For example, goiter does not develop until puberty or adulthood in Pendred syndrome; retinitis pigmentosa emerges in adolescence in Usher syndrome; and males with Deafness-Dystonia-Optic Neuronopathy (Mohr-Tranebjaerg) Syndrome begin having progressive neurological symptoms in their teens.<sup>3</sup>
- Many inheritance patterns are possible in NSHL; 80% is autosomal recessive, 15-19% is autosomal dominant, and ~1% is mitochondrial or X-linked.<sup>1,2</sup>

- A study of 440 individuals with genetic hearing loss found mutations in ~40% of cases tested with a multigene panel. The only feature with an adverse effect on test yield was unilateral hearing loss, for which the panel only identified mutations in 1% of cases.<sup>3</sup> In another study, the mutation detection rate was ~60% via multigene panel; multigene panel testing was noted to be more cost-effective than single gene testing.<sup>4</sup>
- While the most common cause of severe-to-profound autosomal recessive NSHL in most populations is mutation of GJB2 (DFNB1 locus), there is ethnic variability.<sup>1,3,5</sup> Approximately 1% of DFNB1 is due to compound heterozygous mutations in GJB2 and GJB6.<sup>3</sup> The most common cause of mild-to-moderate autosomal recessive hearing loss is mutations of STRC.<sup>1</sup>
- Mitochondrial NSHL is caused by mutations in MT-RNR1 (~71%), MT-TS1 (~29%), and rarely by mutations in other mitochondrial encoded genes (less than 1%).<sup>5,6</sup> MT-RNR1 pathogenic variants, particularly the m.1555A>G allele, are associated with a predisposition to aminoglycoside ototoxicity, with ~100% penetrance after exposure to aminoglycosides.<sup>2,6</sup> Without aminoglycoside exposure, penetrance is ~80% by age 65 years.<sup>6</sup>
- Management of congenital hearing loss or deafness may include hearing aids, cochlear implants, and appropriate educational interventions<sup>1</sup>. Uncovering the genetic etiology of the hearing loss may also identify (or allay concerns about) comorbidities that may require referral for specialty care.<sup>1,2</sup>

## Test information

- There are various methods used to test for mutations in genes which can cause hearing loss and deafness.
  - Single gene analysis
  - Panel testing using next generation sequencing
- Until recently, most sequencing tests used the Sanger sequencing methodology that was originally developed in the 1970s. Sanger sequencing is labor intensive and did not lend itself to high-throughput applications.
- Next generation sequencing (NGS), which is also sometimes called massively parallel sequencing, has been developing since about 2005 to allow larger scale and more efficient gene sequencing. NGS relies on sequencing many copies of small pieces of DNA simultaneously and using bioinformatics to assemble the sequence.
- The efficiency of NGS has led to an increasing number of large, multi-gene testing panels. NGS panels that test several genes at once are particularly well-suited to conditions caused by more than one gene or where there is considerable clinical overlap between conditions, making it difficult to reliably narrow down likely causes. As a result, several laboratories have begun to combine genes involved in certain

conditions, which often have both of those characteristics. However, NGS may not perform as well as Sanger sequencing in some applications. Results may also be obtained that cannot be adequately interpreted based on the current knowledgebase. When a sequence variation is identified that has not been previously characterized or shown to cause the disorder in question, it is called a variant of uncertain significance (VUS). VUSs are relatively common findings when sequencing large amounts of DNA with NGS.

- Nonsyndromic hearing loss and deafness multi-gene panels include a wide variety of genes associated with nonsyndromic hearing loss and deafness. Multi-gene nonsyndromic hearing loss and deafness panels may also include genes for syndromes that mimic nonsyndromic hearing loss (e.g. Usher syndrome Pendred syndrome, Jervell and Lange-Nielsen syndrome, etc.).
- Under certain circumstances, technologies used in multi-gene testing may fail to identify mutations that might be identifiable through single-gene testing. If high clinical suspicion remains for a particular syndrome after negative multi-gene test results, consultation with the testing lab and/or additional targeted genetic testing may be warranted.
- Multi-gene tests vary in technical specifications (e.g., depth of coverage, extent of intron/exon boundary analysis, methodology of large deletion/duplication analysis).
- Since genes can be easily added or removed from multi-gene tests over time by a given lab, medical records must document which genes were included in the specific multi-gene test used from each patient, and in which labs they were performed.
- Additionally, tests should be chosen that maximize the likelihood of identifying mutations in the genes of interest and that will alter patient management.

## Guidelines and evidence

- In 2016, the International Pediatric Otolaryngology Group (IPOG) stated:<sup>7</sup>
  - “In the setting of unilateral hearing loss, genetic testing has a limited role unless syndromic hearing loss is suspected.”
  - “After an audiogram and physical exam, comprehensive genetic testing (CGT) that relies on next generation sequencing (NGS) methodologies should guide subsequent workup in children with bilateral sensorineural hearing loss.”
  - “Diagnostic rates for single gene testing for GJB2/GJB6 vary significantly based on the patient’s ethnicity, and do not outperform the diagnostic rates for comprehensive genetic testing. In cases where CGT is unavailable, single gene testing can be directed by the audiometric phenotype and ethnicity.”
  - The general consensus of the authors was that temporal bone imaging “should not be a routine part of the diagnostic algorithm for bilateral symmetric sensorineural hearing loss.”

- In 2014, the American College of Medical Genetics and Genomics (ACMG) made the following recommendations:<sup>2</sup>
  - A genetic evaluation is recommended for all cases of congenital deafness or hearing loss with onset in childhood or early adulthood. While the usefulness of ancillary testing (e.g. electrocardiogram, renal ultrasound, temporal bone imaging and ophthalmology examination) was mentioned, it was acknowledged that genetic testing via NGS panels would soon become more cost-effective. Cytomegalovirus (CMV) testing is important for cases of congenital hearing loss, but only accurate in the first 6 weeks of life.
  - Genetic testing to confirm a diagnosis of suspected syndromic hearing loss is recommended based on clinical findings. For apparently nonsyndromic hearing loss, a tiered approach was recommended: If the personal and family history is suggestive of a particular gene, single gene testing should be performed first. For simplex cases and cases with apparent autosomal recessive inheritance, the next step should be testing of GJB2 and GJB6. If single-gene testing is not diagnostic, testing via NGS panels, whole exome sequencing, or whole genome sequencing should be considered.
  - The statement stopped short of endorsing the use of NGS panels as a first-tier test, but noted they are “rapidly replacing” sequencing of the GJB2 and GJB6 loci and would soon be a more cost-effective alternative.
- An expert-authored review of nonsyndromic hearing loss states:<sup>3</sup>
  - “A comprehensive deafness-specific genetic panel that includes all genes implicated in nonsyndromic hearing loss and nonsyndromic hearing loss mimics is recommended as the initial genetic test.”
  - “Performing sequence analysis of GJB2 alone is not cost-effective unless it is limited to persons with severe-to-profound congenital nonsyndromic hearing loss. Offering single-gene testing of GJB2 reflexively to everyone with congenital hearing loss without regard to the degree of hearing loss is not evidence based and not cost effective.”
- An expert-authored review on hereditary hearing loss and deafness<sup>1</sup> likewise states that a multi-gene test is recommended for apparent nonsyndromic hearing loss, while individuals with features of syndromic hearing loss should be diagnosed with targeted genetic testing. Ancillary cardiac, ophthalmologic and renal evaluations are only recommended on the basis of genetic test results or clinical findings.
- An expert-authored review on mitochondrial NSHL<sup>6</sup> states that the diagnosis should be suspected in individuals with moderate-to-profound hearing loss and a family history suggestive of maternal inheritance (e.g. no transmission through a male), or onset of hearing loss after exposure to an aminoglycoside antibiotic.
  - To confirm the diagnosis: MT-RNR1 testing, beginning with targeted analysis for the m.1555A>G mutation, is recommended following aminoglycoside exposure. In the absence of aminoglycoside exposure, testing of both MT-RNR1 and MT-

TS1 is recommended. If these tests fail to confirm a diagnosis, mitochondrial genome sequencing can be considered.

- An alternative strategy is to perform a multi-gene panel that includes both MT-RNR1 and MT-TS1, plus other genes of interest.

## Criteria

### Known Familial Mutation Analysis

- Previous testing:
  - Member has not previously had testing for the requested mutation(s), AND
- Member has a 1<sup>st</sup>, 2<sup>nd</sup>, or 3<sup>rd</sup> degree biologic relative with a pathogenic mutation(s) in a gene associated with nonsyndromic hereditary hearing loss or deafness, AND
- Member is at risk of inheriting the pathogenic mutation based on the family history and the inheritance pattern associated with the mutation, AND
- Diagnostic testing:
  - Member has nonsyndromic hearing loss or deafness that is consistent with the mutation in the family, OR
- Carrier screening:
  - Member is of reproductive age, and
  - Member has ability and intention to reproduce, or
  - Member is currently pregnant.

### GJB2 Sequencing

- Previous testing:
  - Member has not previously had GJB2 sequencing, and
  - No known pathogenic hearing loss/deafness gene variants in a biologic relative, AND
- Diagnostic Testing:
  - Member has a diagnosis of bilateral sensorineural hearing loss, and
  - Prelingual onset of hearing loss (prior to speech development), and
  - No known cause for the member's hearing loss (e.g., prenatal exposure to ototoxic medication or TORCH infection, known genetic disorder), and

- Absence of significant dysmorphism, congenital anomalies or other signs of syndromic hearing loss, and
- Member's family history is consistent with autosomal recessive inheritance (including simplex cases), OR
- Carrier screening
  - Member is of reproductive age, and
  - Has potential and intention to reproduce, and
  - Has a reproductive partner who is a carrier of a GJB2/GJB6 mutation, or
  - Has a reproductive partner with GJB2/GJB6-related deafness.

### **GJB6 Common Variant Analysis for 309kb and 232kb Deletions**

- Previous testing:
  - Member has not previously had GJB6 common variant analysis or deletion/duplication analysis, AND
- Diagnostic Testing:
  - Member meets criteria for GJB2 sequencing, and
  - No mutation or only one mutation identified on GJB2 sequencing, OR
- Carrier screening
  - Member is of reproductive age, and
  - Has potential and intention to reproduce, and
  - Has a 1<sup>st</sup>, 2<sup>nd</sup>, or 3<sup>rd</sup>-degree biologic relative with a GJB6 variant, or
  - Member meets criteria for GJB2 sequencing, and
  - No mutation identified on GJB2 sequencing.

### **MT-RNR1 Targeted Mutation Analysis for m.1555A>G Mutation**

- Previous testing:
  - Member has not previously had MT-RNR1 targeted mutation analysis, and
  - No known pathogenic hearing loss/deafness gene variants in a biologic relative, AND
- Diagnostic Testing:
  - Member has a diagnosis of bilateral sensorineural hearing loss, and

- No known cause for the member's hearing loss (e.g., prenatal exposure to ototoxic medication or TORCH infection, known genetic disorder), and
- Absence of significant dysmorphism, congenital anomalies or other signs of syndromic hearing loss, and
- Member has one of the following risk factors for MT-RNR1 related deafness:
  - History of aminoglycoside antibiotic exposure (gentamycin, tobramycin, amikacin, kanamycin, or streptomycin), or
  - Member's family history is strongly suggestive of mitochondrial inheritance (no transmission through a male).

### MT-RNR1 Sequencing

- Previous testing:
  - Member has not previously had MT-RNR1 sequencing, and
  - No mutations detected in any previous MT-RNR1 testing (targeted m.1555A>G mutation analysis), and
  - No known pathogenic hearing loss/deafness gene variants in a biologic relative, AND
- Diagnostic Testing:
  - Member has a diagnosis of bilateral sensorineural hearing loss, and
  - No known cause for the member's hearing loss (e.g., prenatal exposure to ototoxic medication or TORCH infection, known genetic disorder), and
  - Absence of significant dysmorphism, congenital anomalies or other signs of syndromic hearing loss, and
  - Member has one of the following risk factors for MT-RNR1 related deafness:
    - Aminoglycoside antibiotic exposure (gentamycin, tobramycin, amikacin, kanamycin, or streptomycin) prior to hearing loss onset, or
    - Member's family history is strongly suggestive of mitochondrial inheritance (no transmission through a male).

### MT-TS1 Sequencing

- Previous testing:
  - Member has not previously had MT-TS1 analysis, and
  - No mutations detected in any previous MT-TS1 testing (targeted variant analysis), and



- No known pathogenic hearing loss/deafness gene variants in a biologic relative, AND
- Diagnostic Testing:
  - Member has a formal diagnosis of bilateral sensorineural hearing loss, and
  - No known cause for the member's hearing loss (e.g., prenatal exposure to ototoxic medication or TORCH infection, known genetic disorder), and
  - Absence of significant dysmorphism, congenital anomalies, or other signs of syndromic hearing loss, and
  - Member's family history is strongly suggestive of mitochondrial inheritance (no transmission through a male).

### **Hearing Loss and Deafness Multigene Panel Testing**

When a multi-gene panel is being requested and will be billed with a panel CPT code (e.g. 81430, 81431, 81479), the panel will be considered medically necessary when the following criteria are met:

- Previous testing:
  - Member has not previously had a hearing loss panel, and
  - No known pathogenic hearing loss/deafness gene variants in a biologic relative, AND
- Diagnostic Testing:
  - Member has a diagnosis of bilateral sensorineural hearing loss, and
  - No known cause for the member's hearing loss (e.g., prenatal exposure to ototoxic medication or TORCH infection, known genetic disorder), and
  - Absence of significant dysmorphism, congenital anomalies or other signs of syndromic hearing loss.

When separate procedure codes will be billed for individual hearing loss genes (e.g., Tier 1 MoPath codes 81200-81355 or Tier 2 MoPath codes 81400-81408), the entire panel will be approved if the above criteria are met. However, the laboratory will be redirected to use an appropriate panel CPT code for billing purposes (e.g. 81430, 81431, 81479).

### **Billing and reimbursement considerations**

- The billed amount should not exceed the list price of the test.
- Broad hearing loss and deafness panels may not be medically necessary when a narrower panel is available and more appropriate based on the clinical findings.

- Genetic testing is only necessary once per lifetime. Therefore, a single gene included in a panel or a multi-gene panel may not be reimbursed if testing has been performed previously. Exceptions may be considered if technical advances in testing demonstrate significant advantages that would support a medical need to retest.
- If a panel was previously performed and an updated, larger panel is being requested, only testing for the medically necessary, previously untested genes will be reimbursable. Therefore, only the most appropriate procedure codes for those additional genes will be considered for reimbursement.
- If the laboratory will not accept redirection to a single code, the medical necessity of each billed component procedure will be assessed independently, and only the individual panel components that meet medical necessity criteria as a first tier of testing will be reimbursed. The remaining individual components will not be reimbursable.
  - If appropriate first-tier tests cannot be determined on the basis of clinical and family histories, only the following genes may be considered for reimbursement: GJB2, STRC, SLC26A4, TECTA, MYO15A, MYO7A.
- If a single hearing loss/deafness gene test is billed simultaneously with a panel code (e.g. 81430), only the billed procedure that meets medical necessity criteria as a first tier of testing will be reimbursed.
  - Panel testing will generally be the most appropriate first-tier test, except when the history is strongly suggestive of the individual genetic disorder requested (e.g. congenital, severe-to-profound deafness for GJB2 analysis or history of aminoglycoside exposure for MT-RNR1 analysis).

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# Genitourinary Conditions Molecular Testing

**MOL.CS.106.A**  
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## Introduction

Molecular testing for genitourinary conditions is addressed by this guideline.

## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedures addressed by this guideline   | Procedure codes         |
|--|-------------------------|
| Chlamydia trachomatis  | 87490<br>87491<br>87492 |
| Neisseria gonorrhoeae  | 87590<br>87591<br>87592 |
| Trichomonas vaginalis  | 87660<br>87661          |
| Candida species  | 87480<br>87481<br>87482 |
| Candida species panel (C. albicans, C. glabrata, C. parapsilosis, C. kruseii, C. tropicalis, and C. auris), amplified probe technique with qualitative report of the presence or absence of each species | 0068U                   |
| Gardnerella  | 87510<br>87511<br>87512 |
| Human Papillomavirus (HPV), low-risk types (for example, 6, 11, 42, 43, 44)  | 87623                   |

| Procedures addressed by this guideline  | Procedure codes         |
|---|-------------------------|
| Human Papillomavirus (HPV), high-risk types (for example, 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68)   | 87624<br>G0476          |
| Human papillomavirus (HPV), high-risk types (ie, 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68), male urine  | 0096U                   |
| Human Papillomavirus (HPV), types 16 and 18 only, includes type 45, if performed  | 87625                   |
| Herpes simplex virus  | 87528<br>87529<br>87530 |
| Infectious agent detection by nucleic acid (DNA or RNA), not otherwise specified; direct probe technique, each organism   | 87797                   |
| Infectious agent detection by nucleic acid (DNA or RNA), not otherwise specified; amplified probe technique, each organism  | 87798                   |
| Infectious agent detection by nucleic acid (DNA or RNA), not otherwise specified; quantification, each organism   | 87799                   |
| Infectious agent detection by nucleic acid (DNA or RNA), human papillomavirus (HPV) for five or more separately reported highrisk HPV types (eg, 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 59, 68) (ie, genotyping) | 0500T                   |

## Test information

### Introduction

Molecular testing for genitourinary conditions may include nucleic acid testing, flow cytometry, immunohistochemistry, or other specialized molecular studies.

## Guidelines and evidence

### Introduction

This section includes relevant guidelines and evidence pertaining to molecular testing

for genitourinary conditions.

## **Chlamydia trachomatis**

### **Centers for Disease Control and Prevention**

The Centers for Disease Control and Prevention (CDC, 2015) Sexually Transmitted Diseases Treatment Guidelines recommended the following in regard to chlamydia trachomatis testing:<sup>1</sup>

- “Annual screening of all sexually active women aged <25 years is recommended, as is screening of older women at increased risk for infection (e.g., those who have a new sex partner, more than one sex partner, a sex partner with concurrent partners, or a sex partner who has a sexually transmitted infection. Although CT incidence might be higher in some women aged ≥25 years in some communities, overall the largest burden of infection is among women aged <25 years.”
- “Although evidence is insufficient to recommend routine screening for C. trachomatis in sexually active young men because of several factors (e.g., feasibility, efficacy, and cost-effectiveness), the screening of sexually active young men should be considered in clinical settings with a high prevalence of chlamydia (e.g., adolescent clinics, correctional facilities, and STD clinics) or in populations with high burden of infection (e.g., MSM).”

### **U.S. Preventive Services Task Force**

The U.S. Preventive Services Task Force (USPSTF) further recommended:<sup>2</sup>

- “For sexually active women, the USPSTF recommends screening for chlamydia in sexually active women age 24 years and younger and in older women who are at increased risk for infection.”
- “For sexually active men, the USPSTF concludes that the current evidence is insufficient to assess the balance of benefits and harms of screening for chlamydia.”

## **Neisseria gonorrhoeae**

### **Centers for Disease Control and Prevention**

The Centers for Disease Control and Prevention (CDC, 2015) Sexually Transmitted Diseases Treatment Guidelines recommended the following in regard to neisseria gonorrhoeae testing:<sup>1</sup>

- “Routine screening for N. gonorrhoeae on an annual basis is recommended for all sexually active females <25 years of age.”

### **U.S. Preventive Services Task Force**

The U S. Preventive Services Task Force (USPSTF) further recommended:<sup>2,3,4</sup>

- “All pregnant women aged <25 years and older women at increased risk for gonorrhea (e.g., those with a new sex partner, more than one sex partner, a sex partner with concurrent partners, or a sex partner who has a sexually transmitted infection) should be screened for *N. gonorrhoeae* at the first prenatal visit.”

### **Trichomonas vaginalis**

#### **Centers for Disease Control and Prevention**

The Centers for Disease Control and Prevention (CDC, 2015) Sexually Transmitted Diseases Treatment Guidelines recommended the following in regard to trichomonas vaginalis testing:<sup>1</sup>

- “Evidence does not support routine screening for *Trichomonas vaginalis* in asymptomatic pregnant women. Women who report symptoms should be evaluated and treated appropriately.”
- “Women with HIV infection should also be screened for trichomonas at the initial visit and annually thereafter.”

### **Candida species**

#### **Centers for Disease Control and Prevention**

The Centers for Disease Control and Prevention (CDC, 2015) Sexually Transmitted Diseases Treatment Guidelines recommended the following in regard to candida species testing:<sup>1</sup>

- “Identifying *Candida* by culture in the absence of symptoms or signs is not an indication for treatment, because approximately 10%–20% of women harbor *Candida* sp. and other yeasts in the vagina.”
- “In settings where pH paper, KOH, and microscopy are not available, alternative commercially available point-of-care tests or clinical laboratory testing can be used to diagnose vaginitis.”

### **Gardnerella vaginalis**

#### **Centers for Disease Control and Prevention**

The Centers for Disease Control and Prevention (CDC, 2015) Sexually Transmitted Diseases Treatment Guidelines recommended the following in regard to gardnerella vaginalis testing:<sup>1</sup>

- “A Gram stain (considered the gold standard laboratory method for diagnosing BV) is used to determine the relative concentration of lactobacilli (i.e., long Gram-positive rods), Gram- negative and Gram-variable rods and cocci (i.e., *G. vaginalis*, *Prevotella*, *Porphyromonas*, and peptostreptococci), and curved Gram negative rods (i.e., *Mobiluncus*) characteristic of BV.”
- “In settings where pH paper, KOH, and microscopy are not available, alternative commercially available point-of-care tests or clinical laboratory testing can be used to diagnose vaginitis.”

## Herpes simplex virus

### Centers for Disease Control and Prevention

The Centers for Disease Control and Prevention (CDC, 2015) Sexually Transmitted Diseases Treatment Guidelines recommended the following in regard to herpes simplex virus testing:<sup>1</sup>

- “Routine HSV-2 serologic screening of pregnant women is not recommended.”

### U.S. Preventive Services Task Force

The USPSTF genital herpes screening guidelines further recommended:<sup>5,6</sup>

- Since serologic screening for genital herpes is associated with a high rate of false-positive test results and potential psychosocial harms, routine serologic screening for genital herpes simplex virus (HSV) infection in asymptomatic adolescents and adults, including those who are pregnant, is not warranted.

### American Academy of Family Physicians

The American Academy of Family Physicians stated:

- Because “it is unclear how to counsel patients with a positive serologic test result but no history of genital herpes symptoms”, the AAFP concurs with the USPSTF and CDC recommendations against serologic screening for genital herpes.<sup>7</sup>

## Human papillomavirus

### Centers for Disease Control and Prevention

The Centers for Disease Control and Prevention (CDC, 2015) Sexually Transmitted Diseases Treatment Guidelines recommended the following in regard to human papillomavirus testing:<sup>1</sup>

- No HPV test can determine which HPV infection will clear and which will progress. However, in certain circumstances, HPV tests can determine whether a woman is at increased risk for cervical cancer. These tests are not for



detecting other HPV-related problems, nor are they useful in women aged <25 years or men of any age.

- “The role of testing for non-oncogenic HPV types (e.g., 6 and 11) is unclear and is not recommended.”

#### **U.S. Preventive Services Task Force**

The U.S. Preventive Services Task Force (USPSTF) further recommended:

- The USPSTF recommends screening by cervical cytology alone in women aged 21-29 years. HPV testing for screening purposes is not recommended in this age group.<sup>8</sup>

#### **American Cancer Society, American Society for Colposcopy and Cervical Pathology, and American Society for Clinical Pathology**

The American Cancer Society, American Society for Colposcopy and Cervical Pathology, and American Society for Clinical Pathology recommend the following in regard to human papillomavirus testing:

- “Women ages 30–65 years should be screened with cytology and HPV testing (“cotesting”) every 5 years (preferred) or cytology alone every 3 years (acceptable). There is insufficient evidence to change screening intervals in this age group following a history of negative screens.”<sup>9</sup>

#### **American College of Obstetricians and Gynecologists**

The American College of Obstetricians and Gynecologists (ACOG) stated:

- The American College of Obstetricians and Gynecologists cervical cancer clinical management guidelines recommend that HPV testing should be performed to detect only high-risk types of HPV.<sup>10</sup>

#### **American Society for Colposcopy and Cervical Pathology**

The American Society for Colposcopy and Cervical Pathology further stated:

- The American Society for Colposcopy and Cervical Pathology guidelines state that “testing for low-risk (nononcogenic) HPV types has no role in the evaluation of women with abnormal cervical cytologic results”.<sup>11</sup>

### **Bacterial vaginosis**

#### **American College of Obstetricians and Gynecologists**

The American College of Obstetricians and Gynecologists (ACOG) Practice Bulletin on Vaginitis (2006, reaffirmed 2017)<sup>12</sup> stated that because bacterial vaginosis (BV) is polymicrobial and the associated microorganisms are part of the normal flora, therefore “the mere presence of the organisms, especially *G. vaginalis*, on a culture does not mean that the patient has bacterial vaginosis”.

ACOG further stated that a clinical diagnosis for bacterial vaginosis is based on the Amsel criteria and requires the presence of three out of four clinical criteria (abnormal discharge, pH >4.5, positive amine odor when KOH added to vaginal sample, and presence of more than 20% clue cells on microscopy).

### Centers for Disease Control and Prevention

The Centers for Disease Control and Prevention (CDC, 2015) Sexually Transmitted Diseases Treatment Guidelines stated that:<sup>1</sup>

- “BV can be diagnosed by the use of clinical criteria (i.e Amsel's Diagnostic Criteria or Gram stain. A Gram stain (considered the gold standard laboratory method for diagnosing BV) is used to determine the relative concentration of lactobacilli (i.e., long Gram-positive rods), Gram- negative and Gram-variable rods and cocci (i.e., *G. vaginalis*, *Prevotella*, *Porphyromonas*, and peptostreptococci), and curved Gram negative rods (i.e., *Mobiluncus*) characteristic of BV.”
- “Detection of specific organisms might be predictive of BV by PCR. Additional validation is needed before these tests can be recommended to diagnose BV.”

### Society of Obstetricians and Gynecologists of Canada

The Society of Obstetricians and Gynecologists of Canada (SOGC) has published guidelines for the screening and management of bacterial vaginosis (2015)<sup>13</sup> and additional guidelines for screening and management during pregnancy (2017) that state the following: <sup>14</sup>

- “Bacterial vaginosis should be diagnosed using either clinical (Amsel’s) or laboratory (Gram stain with objective scoring system) criteria. (II-2A)”

## Criteria

### Introduction

Requests for molecular testing for genitourinary conditions are reviewed using these criteria.

### Chlamydia trachomatis

| Test name   | Procedure code | Reimbursement  |
|---|----------------|--|
| Chlamydia Trachomatis Detection, Direct Method    | 87490          | Procedure code is eligible for reimbursement when criteria are met |
| Chlamydia Trachomatis Detection, Amplified Method | 87491          | Procedure code is eligible for reimbursement when criteria are met |

| Test name  | Procedure code | Reimbursement  |
|--|----------------|--|
| Chlamydia Trachomatis Detection, Quantification Method | 87492          | Procedure code is not eligible for reimbursement under any circumstances |

### Direct or amplified Chlamydia trachomatis detection

#### Medical necessity requirements:

Nucleic acid amplification testing (NAAT) for Chlamydia trachomatis through either direct (CPT 87490) or amplified (CPT 87491) probe studies is considered medically necessary for individuals with clinical indications as outlined here.

Indications for testing in asymptomatic individuals:

- Routine annual screening of all sexually active women aged less than or equal to 25 years<sup>1</sup>
- Screening of sexually active women greater than 25 years of age with risk factors (e.g., those who have a new sex partner or multiple sex partners)<sup>1</sup>
- Routine screening for all pregnant women during one of the first prenatal visits<sup>1</sup>
- Retesting of all pregnant women aged less than or equal to 25 years performed during the third trimester<sup>1</sup>
- Retesting of all pregnant women over age 25 during the third trimester when at increased risk for Chlamydia (e.g., women who have a new or multiple sex partners, women with a history of a previous STI, high risk behavior such as inconsistent condom use, sex work)<sup>1</sup>
- Screening of sexually active men with risk factors (e.g., men in correctional facilities, presenting to STI clinics, or who have infected partner)<sup>1</sup>

Indications for testing in symptomatic individuals:<sup>1</sup>

- Cervicitis
- Urethritis

Test frequency:

Repeat testing to document eradication of infection after completing an appropriate treatment regimen is recommended only in the following settings: patient is pregnant, symptoms persist, re-infection is suspected, or compliance with therapy is in question. Routine test of cure is not recommended.<sup>1,2</sup>

- Non-pregnant recently infected women should be retested 3 to 12 months after treatment.<sup>1,2</sup>

- Based on guidelines for initial and repeat testing, no more than five screenings in a year should be necessary regardless of pregnancy or other risk factors.

**Billing and reimbursement:**

When testing is medically necessary, the following limitations apply:

- NAAT may be performed on urine, vaginal, or cervical samples.<sup>1</sup> It is usually sufficient to test one site. When necessary to test more than one site, no more than 3 units of 87490 or 87491 for chlamydia trachomatis molecular testing may be billed for the same date of service.
- More than one type of molecular test for the same organism will not be reimbursed for the same date of service (e.g., 87490 and 87491 may not be billed together).
- Based on guidelines for initial and repeat testing, no more than five screenings in a year should be necessary regardless of pregnancy or other risk factors.

**Quantitative Chlamydia trachomatis testing**

**Medical necessity requirements:**

Medical necessity of quantitative testing for chlamydia trachomatis (CPT 87492) has not been demonstrated, and is therefore determined to be investigational and experimental. This procedure code is not eligible for reimbursement for any clinical indications.

**Billing and reimbursement:**

If the laboratory's testing platform consists of direct or amplified and quantitative testing methodologies, yet only direct or amplified testing is considered medically necessary based on these criteria, the lab may request reimbursement for only the medically necessary components of the test by using a procedure code that does not represent all testing methodologies performed.

**Neisseria gonorrhoeae**

| Test name                               | Procedure code | Reimbursement  |
|---|----------------|--|
| Neisseria Gonorrhoeae, Direct Method    | 87590          | Procedure code is eligible for reimbursement when criteria are met |
| Neisseria Gonorrhoeae, Amplified Method | 87591          | Procedure code is eligible for reimbursement when criteria are met |

| Test name                                    | Procedure code | Reimbursement  |
|--|----------------|--|
| Neisseria Gonorrhoeae, Quantification Method | 87592          | Procedure code is not eligible for reimbursement under any circumstances |

### Direct or amplified *Neisseria gonorrhoeae* detection

#### Medical necessity requirements:

Nucleic acid amplification testing (NAAT) for *Neisseria gonorrhoeae* through either direct (CPT 87590) or amplified (CPT 87591) probe studies is considered medically necessary for individuals with clinical indications as outlined here.

Indications for testing in asymptomatic individuals:

- Annual screening of all sexually active women aged less than or equal to 25 years.<sup>1</sup>
- Screening of women greater than 25 years who are at increased risk for infection (e.g., women with previous gonorrhea infection, other STIs, new or multiple sex partners, and inconsistent condom use, sex workers, or women living in communities with a high prevalence of disease).<sup>3</sup>
- All pregnant women at increased risk for gonorrhea (as defined in in the above criteria) should be screened at the first prenatal visit for *N. gonorrhoeae*.<sup>4</sup>
- Uninfected pregnant women who remain at high risk for gonococcal infection also should be retested during the third trimester.<sup>1</sup>
- Screening of sexually active individuals who have an infected partner.<sup>1</sup>

Indications for testing in symptomatic individuals<sup>1</sup>

- Cervicitis
- Urethritis

Test frequency:

When indicated, repeat testing to document eradication should not be performed until 3-4 weeks after the positive result.<sup>1</sup> Pregnant women diagnosed with gonococcal infection during the first trimester should be retested within approximately 3–6 months<sup>2</sup>, preferably in the third trimester. Recently infected women should be retested 3 to 12 months after treatment. When repeat testing is indicated, the following limitations apply:

- Repeat testing will not be reimbursed if performed within three weeks (less than 21 days) from a previous test.

- Based on guidelines for initial and repeat testing, no more than five screenings in a year should be necessary regardless of pregnancy or other risk factors.

#### Billing and reimbursement

When testing is medically necessary, the following limitations apply:

- Nucleic acid amplification test (NAAT) may be performed on urine, vaginal, or cervical samples<sup>1</sup>. It is usually sufficient to test one site. When necessary to test more than one site, no more than 3 units of 87590 or 87591 for *Neisseria gonorrhoeae* molecular testing may be billed for the same date of service.
- More than one type of molecular test for the same organism will not be reimbursed for the same date of service (e.g., 87590 and 87591 may not be billed together).

#### Quantitative *Neisseria gonorrhoeae* testing

##### Medical necessity requirements:

Medical necessity of quantitative testing for *Neisseria gonorrhoeae* (CPT 87592) has not been demonstrated, and is therefore determined to be investigational and experimental. This procedure code is not eligible for reimbursement for any clinical indications.

##### Billing and reimbursement:

If the laboratory's testing platform consists of direct or amplified and quantitative testing methodologies, yet only direct or amplified testing is considered medically necessary based on these criteria, the lab may request reimbursement for only the medically necessary components of the test by using a procedure code that does not represent all testing methodologies performed.

#### *Trichomonas vaginalis*

| Test name   | Procedure code | Reimbursement  |
|---|----------------|--|
| Trichomonas Vaginalis Detection, Direct Method    | 87660          | Procedure code is eligible for reimbursement when criteria are met |
| Trichomonas Vaginalis Detection, Amplified Method | 87661          | Procedure code is eligible for reimbursement when criteria are met |

## Direct or amplified *Trichomonas vaginalis* detection

### Medical necessity requirements:

Nucleic acid amplification testing (NAAT) for *Trichomonas vaginalis* through either direct (CPT 87660) or amplified (CPT 87661) probe studies is considered medically necessary for individuals with clinical indications as outlined here.

Indications for testing in asymptomatic individuals:<sup>1</sup>

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- Evidence does not support routine screening for *Trichomonas vaginalis* in asymptomatic women (pregnant or non-pregnant) or men who are not at high risk for infection.
- Screening can be considered in those at increased risk for *Trichomonas vaginalis* infection for reasons such as new or multiple sex partners, history of STIs, sex work, or drug use.
- Screening should also be performed in sexually active women who are HIV-positive at entry into care and then at least annually.

Indications for testing in symptomatic individuals:<sup>1</sup>

- Vaginitis, abnormal vaginal discharge, cervicitis, nongonococcal urethritis, vulvar pruritis, or pelvic inflammatory disease.
- Sexually active women with trichomoniasis may be rescreened for *Trichomonas vaginalis* at 3 months following initial infection.
- Screening of sexually active individuals who have an infected partner.

Test frequency:

- Repeat testing should not be necessary more frequently than every three months.
- Based on guidelines for initial and repeat testing, no more than five screenings in a year should be necessary regardless of pregnancy or other risk factors.

### Billing and reimbursement:

The medical necessity of testing will be determined based on the following claims data:

- When testing asymptomatic individuals, an ICD code that supports increased risk, infected partner, or positive HIV status must be submitted on the claim. For guidance, see Tables: *ICD Codes Indicating High Risk Indications*, *ICD Codes Indicating Infected Partner*, and *ICD Codes Indicating HIV Positive Status*.

- When testing symptomatic individuals, an ICD code that describes the common symptoms, as defined in Table: *ICD Codes Indicating Symptoms of Genitourinary Conditions*, must be submitted on the claim.

When testing is medically necessary, the following limitations apply:

- Nucleic acid amplification test (NAAT) may be performed on urine, vaginal, or cervical samples.<sup>1</sup> It is usually sufficient to test one site. When necessary to test more than one site:
  - Additional units must be billed with modifier 59.
  - No more than 3 units of 87660 or 87661 for *Trichomonas vaginalis* molecular testing may be billed for the same date of service.
- More than one type of molecular test for the same organism will not be reimbursed for the same date of service (e.g., 87660 and 87661 may not be billed together).

### Candida species

| Test name                                | Procedure code | Reimbursement   |
|--|----------------|---|
| Candida Detection, Direct Method         | 87480          | Procedure code is eligible for reimbursement when criteria are met.       |
| Candida Detection, Amplified Method      | 87481          | Procedure code is eligible for reimbursement when criteria are met.       |
| Candida Detection, Quantification Method | 87482          | Procedure code is not eligible for reimbursement under any circumstances. |
| Candida Species Panel, Amplified Method  | 0068U          | Procedure code is not eligible for reimbursement under any circumstances. |

### Direct or amplified Candida detection

#### Medical necessity requirements:

Nucleic acid amplification testing (NAAT) for *Candida albicans* through either direct (CPT 87480) or amplified (CPT 87481) probe studies is considered medically necessary for individuals with clinical indications as outlined here.

Indications for asymptomatic individuals:



- Evidence does not support routine screening for *Candida* species in asymptomatic pregnant women, non-pregnant women, or men unless HIV status is positive.<sup>1</sup>

Indications for symptomatic individuals:<sup>1</sup>

- *Candida* testing is generally diagnosed by non-molecular methods (clinical criteria, microscopy, culture, etc.). Molecular testing for *Candida* should rarely be necessary.
- However, guidelines do support molecular testing for *Candida* in symptomatic females when microscopy is not available.
  - Post-service medical necessity review may be employed to ensure appropriate non-molecular methods have been utilized or were unavailable.

**Billing and reimbursement:**

The medial necessity of testing will be determined based on the following claims data:

- When testing asymptomatic males or females, an ICD code that supports positive HIV status must be submitted on the claim (see Table: *ICD Codes Indicating HIV Positive Status*). Note that testing for males is only indicated when HIV positive (i.e., no symptomatic or other testing indications).
- When testing symptomatic females, an ICD code that describes the common symptoms must be submitted on the claim (see Table: *ICD Codes Indicating Symptoms of Genitourinary Conditions*).
- When testing is medically necessary, the following limitations apply:
  - It should only be necessary to test one site. Therefore, only one unit per date of service is reimbursable.
  - Subtyping for *Candida glabrata* and other nonalbicans *Candida* species is not routinely medically necessary, so only one unit will be routinely reimbursed. Exceptions may be considered if complicated vulvovaginal candidiasis (VVC) is diagnosed. Complicated VVC may include<sup>1</sup>:
    - Recurrent VVC (defined as 4 or more episodes of symptomatic VVC within 1 year), or
    - Severe VVC (i.e. extensive vulvar erythema, edema, excoriation, and fissure formation)
- More than one type of molecular test for the same organism will not be reimbursed for the same date of service (e.g., 87480 and 87481 may not be billed together).

## Quantitative Candida testing

### Medical necessity requirements:

Medical necessity of quantitative testing for *Candida albicans* (CPT 87482) has not been demonstrated, and is therefore determined to be investigational and experimental. This procedure code is not eligible for reimbursement for any clinical indications.

### Billing and reimbursement:

If the laboratory's testing platform consists of direct or amplified and quantitative testing methodologies, yet only direct or amplified testing is considered medically necessary based on these criteria, the lab may request reimbursement for only the medically necessary components of the test by using a procedure code that does not represent all testing methodologies performed.

## Gardnerella vaginalis

| Test name   | Procedure code | Reimbursement  |
|---|----------------|--|
| Gardnerella Vaginalis Detection, Direct Method    | 87510          | Procedure code is eligible for reimbursement when criteria are met.      |
| Gardnerella Vaginalis Detection, Amplified Method | 87511          | Procedure code is eligible for reimbursement when criteria are met.      |
| Gardnerella Vaginalis Detection, Quantification   | 87512          | Procedure code is not eligible for reimbursement under any circumstances |

### Direct or amplified Gardnerella vaginalis detection

#### Medical necessity requirements:

Nucleic acid amplification testing (NAAT) for *Gardnerella vaginalis* through either direct (CPT 87510) or amplified (CPT 87511) probe studies is considered medically necessary for individuals with clinical indications as outlined here.

Indications for asymptomatic individuals:<sup>1</sup>

- Evidence does not support routine screening for *Gardnerella vaginalis* in asymptomatic pregnant women, non-pregnant women, or men for any indications.

Indications for symptomatic individuals:<sup>1</sup>

- Gardnerella vaginalis testing is generally diagnosed by non-molecular methods (clinical criteria and microscopy.). Molecular testing for Gardnerella vaginalis should rarely be necessary.
- However, guidelines do support molecular testing for Gardnerella vaginalis in symptomatic females only when microscopy is not available.
  - Post-service medical necessity review may be employed to ensure appropriate non-molecular methods have been utilized or were unavailable.

**Billing and reimbursement:**

The medical necessity of testing will be determined based on the following claims data:

- When testing symptomatic females, an ICD code that describes the common symptoms must be submitted on the claim (see Table: *ICD Codes Indicating Symptoms of Genitourinary Conditions*).
- Note that there are no medically necessary indications for testing in males
- When testing is medically necessary, the following limitations apply:
  - Medical necessity of quantitative testing for Gardnerella vaginalis (CPT 87512) has not been demonstrated for any indication, and is therefore determined to be investigational and experimental.
  - It should only be necessary to test one site. Therefore, only one unit per date of service is reimbursable.
  - More than one type of molecular test for the same organism will not be reimbursed for the same date of service (e.g., 87510 and 87511 may not be billed together).

**Quantitative Gardnerella vaginalis testing****Medical necessity requirements:**

Medical necessity of quantitative testing for Gardnerella vaginalis (CPT 87512) has not been demonstrated, and is therefore determined to be investigational and experimental. This procedure code is not eligible for reimbursement for any clinical indications.

**Billing and reimbursement:**

If the laboratory's testing platform consists of direct or amplified and quantitative testing methodologies, yet only direct or amplified testing is considered medically necessary based on these criteria, the lab may request reimbursement for only the medically necessary components of the test by using a procedure code that does not represent all testing methodologies performed.

**Herpes simplex virus (HSV)**

| Test name   | Procedure code | Reimbursement   |
|---|----------------|---|
| Herpes Simplex Virus Detection, Direct Method         | 87528          | Procedure code is eligible for reimbursement when criteria are met. |
| Herpes Simplex Virus Detection, Amplified Method      | 87529          | Procedure code is eligible for reimbursement when criteria are met. |
| Herpes Simplex Virus Detection, Quantification Method | 87530          | Procedure code is eligible for reimbursement when criteria are met. |

**Direct or amplified Herpes simplex virus (HSV) detection****Medical necessity requirements:**

Nucleic acid amplification testing (NAAT) for Herpes simplex virus (HSV) through either direct (CPT 87528) or amplified (CPT 87529) probe studies is considered medically necessary for individuals with clinical indications as outlined here.

Indications for testing in asymptomatic Individuals: <sup>1,5,6,7</sup>

- Current guidelines explicitly recommend against testing asymptomatic individuals for HSV.

Indications for testing in symptomatic Individuals: <sup>1,5,6</sup>

- New or recurrent vesicular and/or ulcerative lesions, vesicles or ulcers on or around the genitals, rectum, buttocks, thighs
- Recurrent genital symptoms or atypical symptoms and negative HSV cultures

**Billing and reimbursement:**

When testing is medically necessary, the following limitations apply:

- It should only be necessary to test one site. Therefore, only one unit per date of service is reimbursable.
- More than one type of molecular test for the same organism will not be reimbursed for the same date of service (e.g., 87528 and 87529 may not be billed together).

## Quantitative Herpes simplex virus testing

### Medical necessity requirements:

Quantitative testing for Herpes simplex virus (HSV) (CPT 87530) may be reasonable for monitoring disease in some circumstances.

Therefore, quantitative HSV testing will be reimbursable when a diagnosis has been established and the need for monitoring is documented in the medical record. Quantitative HSV testing should not be used for the primary diagnosis of HSV.

### Billing and reimbursement:

If the laboratory's testing platform consists of direct or amplified and quantitative testing methodologies, yet only direct or amplified testing is considered medically necessary based on these criteria, the lab may request reimbursement for only the medically necessary components of the test by using a procedure code that does not represent all testing methodologies performed.

## Human Papillomavirus (HPV)

| Test name   | Procedure code | Reimbursement   |
|---|----------------|---|
| Human Papillomavirus (HPV) Detection, Low-Risk Types  | 87623          | Procedure code is not eligible for reimbursement under any circumstances. |
| Human Papillomavirus (HPV) Detection, High-Risk Types | 87624          | Procedure code is eligible for reimbursement when criteria are met.       |
| Human Papillomavirus (HPV) Detection, Types 16 and 18 | 87625          | Procedure code is eligible for reimbursement when criteria are met.       |
| Human Papillomavirus (HPV) Detection, High-Risk Types | G0476          | Procedure code is eligible for reimbursement when criteria are met.       |

## Low-risk genotyping for human papillomavirus (HPV)

### Medical necessity requirements:

Medical necessity of testing for low-risk (non-oncogenic) types of HPV (CPT 87623) has not been demonstrated, and is therefore determined to be investigational and experimental. This procedure code is not eligible for reimbursement for any clinical indications.

- Per American College of Obstetrics and Gynecology cervical cancer clinical management guidelines, HPV testing should be performed only to detect high-risk (oncogenic) types of HPV.<sup>10</sup>
- As stated in American Society for Colposcopy and Cervical Pathology (ASCCP) guidelines, “testing for low-risk (nononcogenic) HPV types has no role in the evaluation of women with abnormal cervical cytologic results.”<sup>11</sup>
- Per Centers for Disease Control and Prevention (CDC) Sexually Transmitted Diseases guidelines, “HPV testing is not recommended for anogenital wart diagnosis, because test results are not confirmatory and do not guide genital wart management.”<sup>1</sup>

### High-risk genotyping for human papillomavirus (HPV)

#### Medical necessity requirements:

Nucleic acid amplification testing (NAAT) for human papillomavirus high-risk genotypes (87624, 87625, G0476) is considered medically necessary for individuals with clinical indications as outlined here.

Indications for testing in asymptomatic individuals:<sup>8-11</sup>

- Screening for cervical cancer is recommended every 3 years by cervical cytology alone. HPV testing to screen for cervical cancer should not be performed in women under age 30.<sup>8</sup>
- Among women age 30-65:<sup>8</sup>
  - high risk HPV testing alone may be performed every 5 years, or
  - high risk HPV testing may be performed every 5 years in combination with pap smear (co-testing) for routine screening.
- Women aged 30 years and older who are HPV positive but cytology negative may:<sup>9,11</sup>
  - Test again by co-testing in one year, or
  - Be tested by HPV high risk oncogenic subtype genotyping
- Women aged 30 years and older with cytology reported as negative and with absent or insufficient endocervical/transformation zone (EC/TZ) component and no or unknown HPV test result

Indications for testing in symptomatic individuals:<sup>1,9-11</sup>

- Reflex to HPV testing for management of women with atypical squamous cells of undetermined significance (ASC-US) cervical cytology results starting at age 21.

- Co-testing at 1 year post cervical intraepithelial neoplasia grade 1 (CIN1) or no lesion preceded by HPV-16 or HPV-18 positivity, persistent untyped oncogenic HPV, ASC-US, and low grade squamous intraepithelial lesion (LSIL) starting at age 25.
- For women treated for cervical intraepithelial neoplasia (CIN 2, CIN 3, or CIN 2, 3), co-testing at 12 months and 24 months is recommended.
- Post-menopausal women with LSIL.

**Exclusions:**

Medical necessity of the following methods for HPV detection has not been demonstrated and is therefore determined to be investigational and experimental.

- Flow cytometry (e.g., HPV OncoTect) (CPT 88184, 88185, and/or 88187).

**Billing and reimbursement:**

Based on medical necessity criteria, HPV testing will not be reimbursed when billed for individuals with the following age restrictions:

- Testing in asymptomatic individuals is not medically necessary before age 30.
- Testing is not medically necessary before age 21 for any indication (asymptomatic or symptomatic).

When testing is medically necessary, the following imitations apply:

- Nucleic acid amplification test (NAAT) may be performed on endocervical samples.<sup>9,11</sup> It is usually sufficient to test one specimen.
- Therefore, no more than 1 unit of CPT 87624 or 87625 for human papillomavirus molecular testing may be billed for the same date of service.
- More than one type of molecular test for the same organism will not be reimbursed for the same date of service. For example, nucleic acid detection of high risk subtypes HPV-16 and HPV-18 by two methodologies (CPT 87624 and 87625) cannot be billed together, and nucleic acid detection by either of these methodologies cannot be billed with a test using another molecular methodology (e.g., in situ hybridization, CPT 88365).

**Infectious agent, not otherwise specified**

| Test name   | Procedure code | Reimbursement  |
|---|----------------|--|
| Miscellaneous Infectious Agent Detection, Direct Molecular Method       | 87797          | Procedure code is not eligible for reimbursement for indications addressed by this policy. |
| Miscellaneous Infectious Agent Detection, Amplified Molecular Method    | 87798          | Procedure code is not eligible for reimbursement for indications addressed by this policy. |
| Miscellaneous Infectious Agent Detection, Quantitative Molecular Method | 87799          | Procedure code is not eligible for reimbursement for indications addressed by this policy. |

**Miscellaneous infectious agent detection****Medical necessity requirements:**

- Molecular testing to detect a variety of organisms that do not have organism-specific procedure codes may be billed under the infectious agents not otherwise specified (NOS) codes (CPT 87797, 87798, 87799). This guideline only addresses some organisms and clinical settings. It does not apply to all testing performed under these codes.
- The genitourinary organisms for which molecular testing is supported by guidelines are represented by organism-specific CPT codes. There are no clinical indications for any infectious agents billed under not otherwise (NOS) specified procedure codes that are supported by current evidence for the evaluation or management of genitourinary conditions, including bacterial vaginosis. Therefore, testing for organisms NOS is considered investigational and experimental in the setting of screening for genitourinary conditions, including but not limited to the following tests:
  - NuSwab<sup>®</sup> , Vaginitis Plus (VG+) (A vaginae, BVAB-2, Megasphaera Type 1)<sup>15</sup>
  - OneSwab<sup>®</sup> (A vaginae, Megasphaera Type 1 and 2, BVAB-2)<sup>16</sup>
  - SureSwab<sup>®</sup> Vaginosis, Vaginitis Plus (G vaginalis, A vaginae, Megasphaera species)<sup>17</sup>



**Billing and reimbursement:**

The following criteria are used to determine if testing for infectious agents NOS is being performed in the setting of genitourinary condition detection or management, including bacterial vaginosis:

- When billed with any ICD code included in Table: *ICD Codes Indicating Testing Done for Genitourinary Conditions* below.
- When billed on the same date of service with any other organism-specific CPT code referenced in this policy.

**ICD10 Codes**

- ICD10 codes in this section may be used to support medical necessity as described in the above guidelines.

**ICD Codes Indicating High Risk Indications**

| ICD10 Code or Range | Description  |
|---------------------|--|
| F10.X               | Alcohol related disorders  |
| F11.X               | Opioid related disorders   |
| F12.X               | Cannabis related disorders                                       |
| F13.X               | Sedative, hypnotic, or anxiolytic related disorders              |
| F14.X               | Cocaine related disorders  |
| F15.X               | Other stimulant related disorders                                |
| F16.X               | Hallucinogen related disorders                                   |
| F18.X               | Inhalant related disorders                                       |
| F19.X               | Other psychoactive substance related disorders                   |
| O99.32X             | Drug use complicating pregnancy, childbirth, and the puerperium  |
| Z72.5X              | High risk sexual behavior  |
| Z77.9               | Other contact with and (suspected) exposures hazardous to health |

**ICD Codes Indicating Infected Partner**

| ICD10 Code or Range | Description  |
|---------------------|--|
| Z20.2               | Contact with and (suspected) exposure to infections with a predominantly sexual mode of transmission |
| Z20.6               | Contact with and (suspected) exposure to human immunodeficiency virus [HIV]                          |
| Z20.8X              | Contact with and (suspected) exposure to other communicable diseases                                 |
| Z20.9               | Contact with and (suspected) exposure to unspecified communicable disease                            |

**ICD Codes Indicating HIV Positive Status**

| ICD10 Code or Range | Description  |
|---------------------|--|
| B20                 | Human immunodeficiency virus [HIV] disease   |
| B97.35              | Human immunodeficiency virus, type 2 [HIV-2]   |
| O98.7X              | Human immunodeficiency virus [HIV] disease complicating pregnancy, childbirth and the puerperium |
| R75                 | Inconclusive laboratory evidence of human immunodeficiency virus [HIV]                           |
| Z21                 | Asymptomatic human immunodeficiency virus [HIV] infection status                                 |

**ICD Codes Indicating Symptoms of Genitourinary Conditions**

| ICD10 Code or Range | Description                                    |
|---------------------|--|
| A56.X               | Other sexually transmitted chlamydial diseases |
| A59.X               | Urogenital trichomoniasis                      |
| B37.3               | Candidiasis of vulva and vagina                |
| B37.4X              | Candidiasis of other urogenital sites          |
| L29.X               | Pruritus                                       |
| N34.X               | Urethritis and urethral syndrome               |

| ICD10 Code or Range | Description   |
|---------------------|---|
| N35.1X              | Postinfective urethral stricture, not elsewhere classified                |
| N37                 | Urethral disorders in diseases classified elsewhere                       |
| N72                 | Inflammatory disease of cervix uteri                                      |
| N73.X               | Other female pelvic inflammatory diseases                                 |
| N75.X               | Diseases of Bartholin's gland   |
| N76.X               | Other inflammation of vagina and vulva                                    |
| N77.X               | Vulvovaginal ulceration and inflammation in diseases classified elsewhere |
| N89.8               | Other specified noninflammatory disorders of vagina                       |
| N89.9               | Noninflammatory disorder of vagina, unspecified                           |
| N94.1               | Dyspareunia   |
| N95.2               | Postmenopausal atrophic vaginitis   |
| O23.X               | Infections of genitourinary tract in pregnancy                            |
| O86.X               | Other puerperal infections  |
| R10.2               | Pelvic and perineal pain  |

#### ***ICD Codes Indicating Testing Done for Genitourinary Conditions***

| ICD10 Code or Range | Description                                    |
|---------------------|--|
| A50.X               | Congenital syphilis                            |
| A51.X               | Early syphilis                                 |
| A52.X               | Late syphilis                                  |
| A53.X               | Other and unspecified syphilis                 |
| A54.X               | Gonococcal infection                           |
| A55                 | Chlamydial lymphogranuloma (venereum)          |
| A56.X               | Other sexually transmitted chlamydial diseases |
| A57                 | Chancroid                                      |
| A58                 | Granuloma inguinale                            |

| ICD10 Code or Range | Description   |
|---------------------|---|
| A59.X               | Trichomoniasis  |
| A60.X               | Anogenital herpesviral [herpes simplex] infections                          |
| A63.X               | Other predominantly sexually transmitted diseases, not elsewhere classified |
| A64                 | Unspecified sexually transmitted disease                                    |
| A74.89              | Other chlamydial diseases   |
| A74.9               | Chlamydial infection, unspecified (includes childbirth and postpartum)      |
| B37.3               | Candidiasis of vulva and vagina   |
| B37.4X              | Candidiasis of other urogenital sites                                       |
| B97.7               | Papillomavirus as the cause of diseases classified elsewhere                |
| L29.X               | Pruritus  |
| M02.30              | Reiter's disease, unspecified site  |
| N34.X               | Urethritis and urethral syndrome  |
| N35.X               | Urethral stricture  |
| N37                 | Urethral disorders in diseases classified elsewhere                         |
| N39.0               | Urinary tract infection, site not specified                                 |
| N39.9               | Disorder of urinary system, unspecified                                     |
| N70.X               | Salpingitis and oophoritis  |
| N71.X               | Inflammatory disease of uterus, except cervix                               |
| N72                 | Inflammatory disease of cervix uteri  |
| N73.X               | Other female pelvic inflammatory diseases                                   |
| N74                 | Female pelvic inflammatory disorders in diseases classified elsewhere       |
| N75.X               | Diseases of Bartholin's gland   |
| N76.X               | Other inflammation of vagina and vulva                                      |
| N77.X               | Vulvovaginal ulceration and inflammation in diseases classified elsewhere   |
| N87.X               | Dysplasia of cervix uteri   |

| ICD10 Code or Range | Description  |
|---------------------|--|
| N94.1               | Dyspareunia  |
| O09.X               | Supervision of high risk pregnancy   |
| O23.X               | Infections of genitourinary tract in pregnancy   |
| O86.X               | Other puerperal infections   |
| R87.5               | Abnormal microbiological findings in specimens from female genital organs                            |
| R87.6X              | Abnormal cytological findings in specimens from female genital organs                                |
| R87.8X              | Other abnormal findings in specimens from female genital organs                                      |
| Z00.00              | Encounter for general adult medical examination without abnormal findings                            |
| Z00.8               | Encounter for other general examination  |
| Z01.4X              | Encounter for gynecological examination  |
| Z11.3               | Encounter for screening for infections with a predominantly sexual mode of transmission              |
| Z11.51              | Encounter for screening for human papillomavirus (HPV)   |
| Z11.59              | Encounter for screening for other viral diseases   |
| Z11.8               | Encounter for screening for other infectious and parasitic diseases                                  |
| Z11.9               | Encounter for screening for infectious and parasitic diseases, unspecified                           |
| Z12.4               | Encounter for screening for malignant neoplasm of cervix   |
| Z20.2               | Contact with and (suspected) exposure to infections with a predominantly sexual mode of transmission |
| Z20.6               | Contact with and (suspected) exposure to human immunodeficiency virus [HIV]                          |
| Z20.8X              | Contact with and (suspected) exposure to communicable diseases                                       |
| Z20.9               | Contact with and (suspected) exposure to unspecified communicable disease                            |

| ICD10 Code or Range | Description   |
|---------------------|---|
| Z30.X               | Encounter for contraceptive management                                |
| Z31.X               | Encounter for procreative management                                  |
| Z32.X               | Encounter for pregnancy test and childbirth and childcare instruction |
| Z33.X               | Pregnant state  |
| Z34.X               | Encounter for supervision of normal pregnancy                         |
| Z36                 | Encounter for antenatal screening of mother                           |
| Z39.X               | Encounter for maternal postpartum care and examination                |
| Z64.0               | Problems related to unwanted pregnancy                                |
| Z64.1               | Problems related to multiparity                                       |
| Z71.7               | Human immunodeficiency virus [HIV] counseling                         |
| Z72.5X              | High risk sexual behavior   |
| Z77.9               | Other contact with and (suspected) exposures hazardous to health      |
| Z97.5               | Presence of (intrauterine) contraceptive device                       |

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# GPS Cancer (NantHealth)

MOL.TS.241.A

v1.0.2020

## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedure addressed by this guideline | Procedure code |
|---------------------------------------|----------------|
| GPS Cancer                            | 81479          |

## What is GPS Cancer

### Definition

NantHealth GPS Cancer™ is a molecular profiling suite used to create personalized treatment plans for cancer patients. The test is for use in patients with solid tumors and utilizes whole DNA genome sequencing of 20,000 genes, whole RNA transcriptome sequencing of over 200,000 transcripts, analysis of proteins for drug sensitivity or resistance, analysis of antigens for monoclonal antibody therapy, quantitative protein analysis, as well as a comparison of tumor DNA to the patient's normal DNA. These analyses are done to give the health care provider and patient a better understanding of the pathology as well as inform treatment decisions.<sup>1</sup>

- Molecular profiling tests used in cancer diagnosis and prognostic applications are generally limited in scope to specific genes or specific proteins. Comprehensive tests that give a full molecular picture of the patient's tumor may aid in clinical decisions.<sup>1</sup>

### Test information

- The test suite uses a tissue block sample of the highest carcinoma grade of the patient's tumor; in some cases, slides can be used. In addition, a sample of the patient's blood is also sent to NantHealth to compare the patient's normal DNA to the tumor DNA.<sup>2</sup>
- The GPS Cancer report includes DNA and RNA mutations/alterations, protein levels, any available therapies (FDA approved), therapies to which the tumor may be resistant, and any clinical trials for which the patient may be eligible. Information from the GPS Cancer report should be interpreted alongside the patient's medical history, since all potential therapies listed on the report may not be recommended for individuals with certain comorbidities or characteristics.<sup>2,3</sup>

## Guidelines and evidence

- No specific evidence-based U.S. testing guidelines were identified.
- There are no published studies evaluating the analytical and clinical validity and clinical utility of this test. Additional clinical studies are necessary to assess the use of the GPS Cancer test in improving patient health outcomes in patients with cancer.

## Criteria

- This test is considered investigational and/or experimental.
  - Investigational and experimental (I&E) molecular and genomic (MolGen) tests refer to assays involving chromosomes, DNA, RNA, or gene products that have insufficient data to determine the net health impact, which typically means there is insufficient data to support that a test accurately assesses the outcome of interest (analytical and clinical validity), significantly improves health outcomes (clinical utility), and/or performs better than an existing standard of care medical management option. Such tests are also not generally accepted as standard of care in the evaluation or management of a particular condition.
  - In the case of MolGen testing, FDA clearance is not a reliable standard given the number of laboratory developed tests that currently fall outside of FDA oversight and FDA clearance often does not assess clinical utility.

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# Hemoglobinopathies Genetic Testing

MOL.TS.308.A

v1.0.2020

## Introduction

Testing for hemoglobinopathies is addressed by this guideline.

## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedures addressed by this guideline     | Procedure codes |
|--|-----------------|
| HBA1/HBA2 Targeted Mutation Analysis       | 81257           |
| HBA1/HBA2 Known Familial Mutation Analysis | 81258           |
| HBA1/HBA2 Sequencing                       | 81259           |
| HBA1/HBA2 Deletion/Duplication Analysis    | 81269           |
| HBB Targeted Mutation Analysis             | 81361           |
| HBB Known Familial Mutation Analysis       | 81362           |
| HBB Sequencing                             | 81364           |
| HBB Deletion/Duplication Analysis          | 81363           |

## What are Hemoglobinopathies

### Definition

Hemoglobinopathies are a group of genetic disorders involving abnormal production or structure of the hemoglobin protein.<sup>1</sup>

Hemoglobin is found in red blood cells and is responsible for delivering oxygen throughout the body. It is composed of four polypeptide sub-units (globin chains) that normally associate with each other in one of the following forms:

- Hemoglobin A (HbA), composed of two alpha and two beta chains, makes up about 95-98% of adult hemoglobin.
- Hemoglobin A<sub>2</sub> (HbA<sub>2</sub>), composed of two alpha and two delta chains, makes up about 2-3% of adult hemoglobin.

- Hemoglobin F (HbF, fetal hemoglobin), composed of two alpha and two gamma chains, makes up about 1-2% of adult hemoglobin.

While there is only one beta globin gene (HBB), there are 2 different genes that code for alpha globin: HBA1 and HBA2. Thus, humans have 4 alpha globin gene copies (two from each parent) and 2 beta globin gene copies (one from each parent).

More than one thousand hemoglobin variants have been discovered to date.<sup>1</sup> Although most do not cause disease, some variants affect the size, shape, and efficacy of red blood cells.<sup>2</sup>

### Incidence and Prevalence

Hemoglobinopathy, in all of its forms, constitutes the most common Mendelian disease in the world. Approximately 7% of the world's population carries a mutation associated with a hemoglobinopathy.<sup>3</sup> Ethnic-specific carrier rates for various hemoglobinopathies appear in the table below (adapted from March of Dimes Genetic Screening Pocket Facts)<sup>4,5</sup> Although hemoglobinopathies are more common in certain ethnic groups, they have been described in populations worldwide.

#### *Ethnic-specific carrier rates for various hemoglobinopathies*

| Ethnicity                           | Beta Thalassemia trait | Alpha thalassemia trait (cis vs trans)* | Sickle cell trait |
|-------------------------------------|------------------------|---|-------------------|
| Mediterranean                       | 1/20 - 1/30            | 1/30 - 1/50 (trans)                     | 1/30 - 1/50       |
| African American                    | 1/75                   | 1/30 (trans)                            | 1/12              |
| Non-Hispanic Caribbean, West Indian | 1/50 - 1/75            | 1/30 (trans)                            | 1/12              |
| West African                        | 1/50                   | 1/30 (trans)                            | 1/6               |
| Hispanic Caribbean                  | 1/75                   | Variable                                | 1/30              |
| Hispanic, Mexican, Central American | 1/30 - 1/50            | Variable                                | 1/30 - 1/200      |
| Asian                               | 1/50                   | 1/20 (cis)                              | Rare              |
| Southeast Asian                     | 1/30                   | >1/20 (cis)                             | Rare              |

**Note** \* The clinically significant carrier state of alpha thalassemia is defined as the absence or dysfunction of two copies of the HBA genes. If both non-working copies are on the same chromosome, the mutations are referred to as being in 'cis'. If there is one gene from each chromosome affected, the mutations are referred to as being in 'trans'.

## Symptoms

Most cases of hemoglobinopathies in the US are diagnosed through newborn screening prior to symptom onset.<sup>2</sup> The exception is the severe form of alpha thalassemia (Hb Bart syndrome), which has prenatal onset and can cause fetal or neonatal death.

Apha thalassemia: Hb Bart syndrome (absence of all 4 alpha globin genes) presents as general fetal edema, pleural and pericardial effusion, and severe anemia.<sup>4</sup> HbH disease (absence of 3 of the 4 alpha globin genes) presents postnatally with anemia, enlarged spleen, and mild jaundice.<sup>6</sup>

Beta thalassemia: Untreated severe beta-thalassemia (beta<sup>0</sup>-thalassemia, or beta thalassemia major) can present as failure to thrive with an enlarged liver and spleen.<sup>7,8</sup> Milder forms of the disease (Beta<sup>+</sup> thalassemia or beta thalassemia intermedia) present later in life with milder anemia.<sup>7</sup> Very mild forms of beta thalassemia can be clinically asymptomatic.

Sickle cell disease: Untreated sickle cell disease presents as hemolytic anemia, vaso-occlusive events, and swelling of the hands and feet.<sup>9</sup>

Carriers of hemoglobinopathies are usually clinically asymptomatic but typically have subclinical microcytic anemia (abnormal blood indices).

## Cause

Thalassemias are typically caused by mutations in globin chain genes that result in reduced or absent synthesis of a normal protein product. Structural hemoglobin variants are caused by mutations in globin chain genes that result in synthesis of normal quantities of an abnormal protein product.

Alpha thalassemia is caused by loss of function mutations in the HBA1 or HBA2 genes. Gene deletions are the most common causative mutations.<sup>6</sup> Of non-deletion mutations, the point mutation Hb Constant Spring (HbCS) is the most common and may be clinically more severe than a deletion mutation; this mutation is most common in Southeast Asians.<sup>6</sup> Symptoms occur when 3 or 4 of the 4 alpha globin genes are dysfunctional or absent. If 1 or 2 genes are dysfunctional or absent, the individual is asymptomatic and considered a carrier of alpha thalassemia.

Beta thalassemia is caused by loss of function mutations in the HBB gene. Nonsense, small frameshift, and splice site mutations are the most common causative mutations, and gene deletions are rare. In general, beta<sup>0</sup> thalassemias are due to complete loss of the beta globin protein, while beta<sup>+</sup> thalassemias are due to decreased production of beta globin.<sup>7</sup>

HbS (sickle hemoglobin) is caused by a single HBB mutation (p.Glu6Val).<sup>6</sup> Similarly, HbC (p.Glu6Lys) and HbE (p.Glu26Lys) are also caused by single HBB mutations. Other structural hemoglobin variants are grouped according to electrophoretic properties (HbD, HbG) but have multiple subtypes caused by different mutations, potentially in different hemoglobin chain-coding genes.<sup>1</sup>

Structural hemoglobin variants can be co-inherited with one another or with alpha or beta thalassemia deletions/mutations. These combinations can result in a wide range of phenotypes, dependent upon both the specific structural variant and thalassemia mutation.<sup>8</sup>

## Inheritance

Most hemoglobinopathies are inherited in an autosomal recessive manner.<sup>6,8</sup> If both parents are carriers, there is a 25% chance with each pregnancy to have an affected child.<sup>4,6</sup>

Carriers of beta thalassemia mutations and the HbS structural variant are often referred to as having thalassemia trait or sickle cell trait. As there are 2 different alpha globin genes (HBA1 and HBA2), the absence or dysfunction of two of the four genes is required to be considered a carrier (alpha thalassemia trait). The absence or dysfunction of 1 of the 4 alpha globin genes is often referred to as silent carrier state.

## Diagnosis

Hemoglobinopathies are diagnosed based on clinical presentation and/or hematologic laboratory analysis.<sup>10</sup> These tests include:

- Complete blood count (CBC): mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) are measures of red cell size, and diagnose microcytic anemia. MCV and MCH are usually decreased in thalassemias.
- Hemoglobin electrophoresis, isoelectric focusing (IEF) and high performance liquid chromatography (HPLC) detect and quantify hemoglobin subtypes, identifying structural hemoglobin variants and detecting abnormal levels of normal adult hemoglobin.<sup>1</sup> In beta thalassemia, HbA<sub>2</sub> and HbF are usually increased.<sup>8</sup>
- Exclusion of iron deficiency as a cause of anemia via serum iron concentration, ferritin, transferrin and/or total iron binding capacity assessment.

Molecular testing is not generally required for diagnosis or management purposes, but may be indicated if the hematologic results are inconclusive, when molecular findings would impact medical management, or to identify familial mutations for reproductive planning purposes.<sup>6,8</sup>

## Treatment

Hemoglobinopathies are treated with packed red cell transfusions as needed.<sup>6,8,9</sup> Iron chelation therapy helps prevent iron overload in individuals receiving regular transfusions.<sup>6,8</sup> Individuals with sickle cell disease may also be treated with hydroxyurea to increase production of fetal hemoglobin.<sup>9</sup>

## Test information

### Introduction

Diagnostic testing for hemoglobinopathies is generally based on clinical findings, red blood cell indices (MCV and MCH) and results of quantitative hemoglobin electrophoresis and other protein-based analyses of hemoglobin.

### HBA1 and HBA2 genetic testing

Genetic testing for hemoglobinopathies caused by variants of alpha globin genes HBA1 and HBA2 may include common mutation panel, gene sequencing, deletion/duplication analysis, or known familial mutation analysis.

#### HBA1 and HBA2 targeted mutation analysis

About 90% of pathogenic HBA1 and HBA2 variants can be identified by a targeted panel. Detection rates depend on ethnicity.<sup>4</sup> The most common deletions are - $\alpha^{3.7}$ , - $\alpha^{4.2}$ , and - $\alpha^{20.5}$  (single gene deletions), and -SEA, -MED, -FIL, and -THAI (double gene deletions). These are the deletions most commonly found in the Southeast Asian, African, Middle Eastern, West Indian, and Mediterranean populations.<sup>6</sup> Some common mutation panel tests also include Hb Constant Spring.

#### HBA1 and HBA2 sequencing

If common deletion testing for HBA1 and HBA2 is negative or does not find the expected number of mutations, sequencing of the HBA1 and HBA2 genes can be used to identify variants caused by point mutation.<sup>6</sup>

#### HBA1 and HBA2 deletion/duplication analysis

Fewer than 5% of pathogenic HBA1 and HBA2 variants have been detected by deletion/duplication analysis of the HBA1/HBA2 locus.<sup>6</sup> Some labs perform this type of assessment instead of a targeted deletion panel. Deletion/duplication analysis is performed if a gene triplication or other copy number variation is suspected based on phenotype.

#### HBA1 and HBA2 known familial mutation analysis

This test looks specifically for known mutation(s) previously identified in the family. This may be accomplished through a targeted assessment of the specific familial mutation or a common deletion panel.

### HBB genetic testing

Genetic testing for hemoglobinopathies caused by variants of beta globin gene HBB may include targeted mutation analysis, gene sequencing, deletion/duplication analysis, or known familial mutation analysis.

### **HBB targeted mutation analysis**

Targeted HBB mutation panels can consist of a few of the most common structural hemoglobin variants and beta thalassemia associated mutations or dozens of reported mutations across ethnicities.<sup>7,8</sup> Clinical sensitivity of a panel depends on patient ethnicity, hematologic test results, and the mutations included on the panel.

### **HBB sequencing**

Full HBB gene sequencing identifies >99% of mutations in the coding region, including the common HbS and beta thalassemia mutations.<sup>8,9</sup>

### **HBB deletion/duplication analysis**

Beta thalassemia caused by pathogenic HBB deletion or duplication is rare, but has been reported.<sup>8</sup>

### **HBB known familial mutation analysis**

This test looks specifically for known mutation previously identified in the family. This may be accomplished through a targeted assessment of the specific familial mutation(s) or a common mutation panel.

## **Guidelines and evidence**

### **Introduction**

This section includes relevant guidelines and evidence pertaining to hemoglobinopathy testing.

### **American College of Obstetricians and Gynecologists (ACOG)**

Evidence-based guidelines from ACOG (2007) recommend that couples at risk of having a child with a hemoglobinopathy be offered prenatal diagnostic options including amniocentesis or chorionic villus sampling (CVS) (level A recommendation: based on "good and consistent scientific evidence").<sup>11</sup> Identification of parental mutations should be performed before prenatal diagnosis to inform interpretation of prenatal results.<sup>11</sup>

ACOG Committee Opinion 690 (2017) states that all patients considering pregnancy or already pregnant, regardless of screening strategy and ethnicity, should be offered complete blood count and screening for hemoglobinopathy.<sup>12</sup>

ACOG Committee Opinion 691 (2017) expands on recommended screening methodology: All pregnant women should have a complete blood count with red cell indices. For women of high risk ethnicity (African, Mediterranean, Middle Eastern, Southeast Asian and West Indian), hemoglobin electrophoresis should also be performed. For all other women, hemoglobin electrophoresis is recommended only if red cell indices indicate low mean MCH or MCV.<sup>13</sup>



## The Society of Obstetricians and Gynaecologists of Canada (SOGC)

Evidence-based guidelines from SOGC (2016) state the following regarding screening for thalassemia and other hemoglobinopathies.<sup>14</sup>

- “Carrier screening for hemoglobinopathies should be offered to women/families from ethnic backgrounds with a reported increased carrier frequency, when red blood cell indices reveal a mean cellular volume < 80 fl, or electrophoresis reveals an abnormal hemoglobin type. However, the use of ethnicity alone in the carrier risk identification process may create screening inconsistency and missed opportunity for carrier identification, with both obstetrical and fetal implications. High clinical suspicion is required as well. Screening should be done in the pre-conception period or as early into the pregnancy as possible. (II-2A) (GRADE moderate/moderate)”
- “Carrier screening for thalassemia/hemoglobinopathies should be offered by the most responsible health care provider or reproductive genetic provider and include:”
  - “Complete blood count”
  - “Hemoglobin (Hb) electrophoresis (HE) or Hb high performance liquid chromatography (HHPLC)”
  - “Quantification of Hb alpha 2 and fetal Hb”
  - “Serum ferritin/H bodies (blood smear stain using brilliant cresyl blue) if microcytosis (mean cellular volume < 80 fl) and/or hypochromia (mean cellular Hb < 27 pg) in the presence of a normal HE or HHPLC assessment. (II-2A) (GRADE moderate/moderate)”
- “If the female thalassemia screening results are abnormal, a hemoglobinopathy screening protocol should be undertaken for the male partner. (III-A) (GRADE low/moderate)”
- “If both reproductive partners are found to be carriers of thalassemia or a combination of thalassemia and hemoglobin variant, they should be referred for formal genetic counselling (reproductive risks, recommended prenatal testing, and diagnostic management). (II3A) (GRADE moderate/moderate)”

## British Committee for Standards in Haematology (BCSH)

The BCSH issued a comprehensive guideline for postnatal screening and diagnosis of hemoglobinopathies.<sup>15</sup> The guideline includes indications for DNA analysis, noting that “the identification of haemoglobins is often presumptive, based on electrophoretic mobility or other characteristics in an individual of appropriate family origin. Presumptive identification should be based on a minimum of two techniques based on different principles. Definitive identification usually requires DNA analysis, mass spectrometry or protein sequencing.”

“The majority of couples at risk of having a child affected with b-thalassaemia or SCD should be identified initially by routine laboratory techniques through the antenatal

screening programme. The diagnosis of a-thalassaemia is more complicated because DNA analysis is the only accurate way to distinguish between a<sup>+</sup> and a<sup>0</sup> thalassaemia. However it is not practical to seek to confirm all potential cases of a-thalassaemia by DNA analysis because the a<sup>+</sup> form is too common and not usually clinically important.”

## Criteria

### Introduction

Requests for hemoglobinopathy testing are reviewed using these criteria.

### HBA1 and HBA2 Targeted Mutation Analysis

- Genetic Counseling:
  - Pre- and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - No previous HBA1 or HBA2 targeted mutation testing has been performed, AND
- Diagnostic Testing for Symptomatic Individuals:
  - Results of hematologic tests examining MCV, MCH, iron deficiency, and hemoglobin electrophoresis do not conclusively diagnose or rule out alpha thalassemia, and
  - Documentation from ordering provider indicates how test results will be used to directly impact medical care for the individual (e.g. change in surveillance or treatment plan), OR
- Carrier Testing:
  - Member is pregnant or of reproductive age with intention to reproduce, and
    - Both member and partner meet the following criteria:
      - MCV and/or MCH lower than reference range of testing lab, and
      - Hemoglobin electrophoresis is not consistent with beta chain abnormality, and
      - Iron deficiency anemia has been ruled out, or
  - Member is currently pregnant and meets above criteria and the father of the pregnancy is not available for testing but believed to be from a high risk ethnic population, and
  - Identification of pathogenic familial mutations is required for prenatal diagnosis or pregnancy planning, AND

- Rendering laboratory is a qualified provider of service per the Health Plan policy.

### **HBA1 and HBA2 Deletion Analysis**

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - Previous common mutation panel genetic testing for HBA1 or HBA2 mutations (if performed) was negative, AND
- Diagnostic Testing for Symptomatic Individuals:
  - Results of hematologic tests examining MCV, MCH, iron deficiency, and hemoglobin electrophoresis do not conclusively diagnose or rule out alpha thalassemia, and
  - Documentation from ordering provider indicates how test results will be used to directly impact medical care for the individual (e.g. change in surveillance or treatment plan), OR
- Carrier Testing:
  - Member is pregnant or of reproductive age with intention to reproduce, and
    - Both member and partner meet the following criteria:
      - MCV and/or MCH lower than reference range of testing lab, and
      - Hemoglobin electrophoresis is not consistent with beta chain abnormality, and
      - Iron deficiency anemia has been ruled out, or
  - Member is currently pregnant and meets above criteria and the father of the pregnancy is not available for testing but believed to be from a high risk ethnic population, and
  - Identification of pathogenic familial mutations is required for prenatal diagnosis or pregnancy planning, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

### **HBA1 and HBA2 Sequencing**

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND

- Previous Genetic Testing
  - Previous common mutation panel or deletion/duplication genetic testing for HBA1 or HBA2 was negative, AND
- Diagnostic Testing for Symptomatic Individuals:
  - Results of hematologic tests examining MCV, MCH, iron deficiency, and hemoglobin electrophoresis do not conclusively diagnose or rule out alpha thalassemia, and
  - Documentation from ordering provider indicates how test results will be used to directly impact medical care for the individual (e.g. change in surveillance or treatment plan), OR
- Carrier Testing:
  - Member is pregnant or of reproductive age with intention to reproduce, and
    - Both member and partner meet the following criteria:
      - MCV and/or MCH lower than reference range of testing lab, and
      - Hemoglobin electrophoresis is not consistent with beta chain abnormality, and
      - Iron deficiency anemia has been ruled out, or
  - Member is pregnant and meets above criteria and the father of the pregnancy is not available for testing but believed to be from a high risk ethnic population, and
  - Identification of pathogenic familial mutations is required for prenatal diagnosis or pregnancy planning, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

### **HBA1 and HBA2 Known Familial Mutation Analysis**

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - Known familial mutation in HBA1 and/or HBA2 identified in a close blood relative, and
  - No previous genetic testing for known HBA1 or HBA2 family mutation, AND
- Diagnostic Testing for Symptomatic Individuals:

- Results of hematologic tests examining MCV, MCH, iron deficiency, and hemoglobin electrophoresis do not conclusively diagnose or rule out alpha thalassemia, and
- Documentation from ordering provider indicates how test results will be used to directly impact medical care for the individual (e.g. change in surveillance or treatment plan), OR
- Carrier Screening:
  - Member is pregnant or of reproductive age with intention to reproduce, and
    - Both member and partner meet the following criteria:
      - MCV and/or MCH lower than reference range of testing lab, and
      - Hemoglobin electrophoresis is not consistent with beta chain abnormality, and
      - Iron deficiency anemia has been ruled out, or
  - Member is pregnant and meets above criteria and the father of the pregnancy is not available for testing but believed to be from a high risk ethnic population, and
  - Identification of pathogenic familial mutations is required for prenatal diagnosis or pregnancy planning, OR
- Prenatal Testing:
  - Both biological parents carry HBA1/HBA2 mutations that put the pregnancy at risk for a clinically significant anemia, or
  - The pregnant member carries HBA1/HBA2 mutations that put the pregnancy at risk for a clinically significant anemia and the father of the pregnancy is unavailable but believed to be from a high risk ethnic population, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

### **HBB Targeted Mutation Analysis**

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - No previous genetic testing for HBB mutation, AND
- Diagnostic Testing for Symptomatic Individuals:

- Results of hematologic tests examining MCV, MCH, iron deficiency, and hemoglobin electrophoresis do not conclusively diagnose or rule out beta thalassemia, or
- Hemoglobin electrophoresis shows common structural variant caused by mutation contained on the requested panel (HbS, HbC, HbE, etc), and
- Documentation from ordering provider indicates how test results will be used to directly impact medical care for the individual (e.g. change in surveillance or treatment plan), OR
- Carrier Testing:
  - Member is pregnant or of reproductive age with intention to reproduce, and
    - Both member and partner meet the following criteria:
      - MCV and/or MCH lower than reference range of testing lab, and
      - Iron deficiency anemia has been ruled out, and
      - Hemoglobin electrophoresis shows
        - elevated Hb A<sub>2</sub> (based on reference range of the testing lab) consistent with beta thalassemia, or
        - common structural variant caused by mutation contained on the requested panel (HbS, HbC, HbE, etc), or
  - Member is pregnant and meets above criteria and the father of the pregnancy is not available for testing but believed to be from a high risk ethnic population, and
  - Identification of pathogenic familial mutations is required for prenatal diagnosis or pregnancy planning, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

### **HBB Sequencing**

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - Previous HBB targeted mutation analysis (if performed) was negative, or
  - Individual is not of a high risk ethnicity for which HBB targeted mutation analysis is available and of high sensitivity, AND
- Diagnostic Testing for Symptomatic Individuals:

- Results of hematologic tests examining MCV, MCH, iron deficiency, and hemoglobin electrophoresis do not conclusively diagnose or rule out beta thalassemia, or
- Hemoglobin electrophoresis shows uncommon structural variant caused by one of several possible HBB mutations, and
- Documentation from ordering provider indicates how test results will be used to directly impact medical care for the individual (e.g. change in surveillance or treatment plan), OR
- Carrier Testing:
  - Member is pregnant or of reproductive age with intention to reproduce, and
  - Both member and partner meet the following criteria:
    - Results of hematologic tests examining MCV, MCH, iron deficiency, and hemoglobin electrophoresis do not conclusively rule out beta thalassemia, or
    - Hemoglobin electrophoresis shows uncommon structural variant caused by one of several possible HBB mutations, or
  - The pregnant member meets above criteria for HBB carrier testing and the father of the pregnancy is unavailable but believed to be from a high risk ethnic population, and
  - Identification of pathogenic familial mutations is required for prenatal diagnosis or pregnancy planning, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

### **HBB Deletion/Duplication Analysis**

- Genetic Counseling:
  - Pre- and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - Previous testing via either HBB targeted mutation analysis or HBB full sequencing performed and negative, AND
- Diagnostic Testing for Symptomatic Individuals:
  - Results of hematologic tests examining MCV, MCH, iron deficiency, and hemoglobin electrophoresis do not conclusively diagnose or rule out beta thalassemia, and

- Documentation from ordering provider indicates how test results will be used to directly impact medical care for the individual (e.g. change in surveillance or treatment plan), OR
- Carrier Testing:
  - Member is pregnant or of reproductive age with intention to reproduce, AND
  - Both member and partner have
    - Results of hematologic tests examining MCV, MCH, iron deficiency, and hemoglobin electrophoresis do not conclusively rule out beta thalassemia, or
    - Hemoglobin electrophoresis shows uncommon structural variant caused by one of several possible HBB mutations, or
  - The pregnant member meets above criteria for HBB carrier testing and the father of the pregnancy is unavailable but believed to be descended from a high risk ethnic population, and
  - Identification of pathogenic familial mutations is required for prenatal diagnosis or pregnancy planning, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy

### **HBB Known Familial Mutation Analysis**

- Genetic Counseling:
  - Pre- and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - Known familial mutation in HBB identified in a close blood relative, and
  - No previous genetic testing for known HBB family mutation, AND
- Diagnostic Testing for Symptomatic Individuals:
  - Results of hematologic tests examining MCV, MCH, iron deficiency, and hemoglobin electrophoresis do not conclusively rule out beta thalassemia, and
  - Documentation from ordering provider indicates how test results will be used to directly impact medical care for the individual (e.g. change in surveillance or treatment plan), OR
- Carrier Testing:
  - Member is pregnant or of reproductive age with intention to reproduce, AND
  - Both member and partner have



- Results of hematologic tests examining MCV, MCH, iron deficiency, and hemoglobin electrophoresis do not conclusively rule out beta thalassemia, or
- Hemoglobin electrophoresis shows uncommon structural variant caused by one of several possible HBB mutations, or
- The pregnant member meets above criteria for HBB carrier testing and the father of the pregnancy is unavailable but believed to be descended from a high risk ethnic population, and
- Identification of pathogenic familial mutations is required for prenatal diagnosis or pregnancy planning, AND
- Prenatal Testing
  - Both biological parents carry HBB mutations that put the pregnancy at risk for a clinically significant anemia, or
  - The pregnant member carries an HBB mutation that puts the pregnancy at risk for a clinically significant anemia and the father of the pregnancy is unavailable but believed to be from a high risk ethnic population, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

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# Hereditary Ataxia Multigene Panel Testing

MOL.TS.310.A  
v1.0.2020

## Introduction

Hereditary Ataxia Multigene Panel testing is addressed by this guideline.

## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedures addressed by this guideline  | Procedure codes |
|---|-----------------|
| Hereditary Ataxia Multigene Panel (including sequencing of at least 15 genes) | 81443           |
| Unlisted molecular pathology procedure  | 81479           |

## What are hereditary ataxias

### Definition

The hereditary ataxias are a group of genetic disorders. They are characterized by slowly progressive uncoordinated, unsteady movement and gait, and often poor coordination of hands, eye movements, and speech. Cerebellar atrophy is also frequently seen.<sup>1</sup>

### Incidence and prevalence

Prevalence estimates vary. The prevalence of autosomal dominant ataxias is approximately 1-5:100,000.<sup>1</sup> One study in Norway estimated the prevalence of hereditary ataxia at 6.5 per 100,000 people.<sup>2</sup>

### Symptoms

Although hereditary ataxias are made up of multiple different conditions, they are characterized by slowly progressive uncoordinated, unsteady movement and gait, and often poor coordination of hands, eye movements, and speech. Cerebellar atrophy is also frequently seen.<sup>1</sup>

## Cause

Hereditary ataxias are caused by mutations in one of numerous genes. The following genes are associated with hereditary ataxia; however, this list is not intended to be all inclusive: ATN1, ATXN1, ATXN2, ATXN3, CACNA1A, ATXN7, TBP, and FMR1.

## Inheritance

Most hereditary ataxias, including the spinocerebellar ataxias (SCA), dentatorubral-pallidoluysian atrophy (DRPLA), and episodic ataxia (EA) types 1 and 2, are inherited in an autosomal dominant manner. Children of an affected person have a 50% chance of inheriting the mutation.

A few of the hereditary ataxias, including Friedreich ataxia and ataxia telangiectasia, are inherited in an autosomal recessive manner. Two carrier parents have a 25% chance with each pregnancy to have an affected child.

In the absence of a family history, it can be difficult to differentiate the type or subtype of hereditary ataxia based on clinical features.<sup>1</sup> One study found that in approximately 13% of apparently sporadic ataxias, a causative genetic change was identified.<sup>3</sup>

## Diagnosis

The diagnosis of hereditary ataxia is suspected based on clinical and family history, neurological exam, and neuroimaging studies.<sup>1</sup> Acquired causes of ataxia — including alcoholism, vitamin deficiencies, multiple sclerosis, vascular disease, and tumors — should be ruled out.<sup>1</sup>

Molecular genetic testing can be used to establish a specific diagnosis.

## Treatment

Treatment of ataxia is largely supportive, and includes the use of canes and walkers for ambulation, speech therapy, and other assistive devices.<sup>1</sup>

## Survival

The survival range of the hereditary ataxias varies across the multiple conditions included in this group.

## Test Information

### Introduction

Testing for hereditary ataxias may include known familial mutation analysis, single gene testing, or multi-gene panel testing.

## Expansion analysis

Several of the ataxias are caused by triplet repeat expansions. Testing for these conditions is performed by expansion analysis to identify the number of repeats. Expansion analysis can be performed for diagnostic testing, presymptomatic testing, as well as prenatal testing.

## Multigene panel testing

Until recently, most sequencing tests used the Sanger sequencing methodology that was originally developed in the 1970s. Sanger sequencing is labor intensive and did not lend itself to high-throughput applications.

Next generation sequencing (NGS), which is also sometimes called massively parallel sequencing, was developed in 2005 to allow larger scale and more efficient gene sequencing. NGS relies on sequencing many copies of small pieces of DNA simultaneously and using bioinformatics to assemble the sequence. NGS may not perform as well as Sanger sequencing in some applications.

NGS tests vary in technical specifications (e.g., depth of coverage, extent of intron/exon boundary analysis, methodology of large deletion/duplication analysis).

Sequence analysis detects single nucleotide substitutions and small (several nucleotide) deletions and insertions. Regions analyzed typically include the coding sequence and intron/exon boundaries. Promoter regions and intronic sequences may also be sequenced if disease-causing mutations are known to occur in these regions of a gene.

The efficiency of NGS has led to an increasing number of large, multi-gene testing panels. NGS panels that test several genes at once are particularly well-suited to conditions caused by more than one gene or where there is considerable clinical overlap between conditions.

Results may be obtained that cannot be adequately interpreted based on the current knowledgebase. When a sequence variation is identified that has not been previously characterized or shown to cause the disorder in question, it is called a variant of uncertain significance (VUS). VUSs are relatively common findings when sequencing large amounts of DNA with NGS.

Under certain circumstances, technologies used in multi-gene testing may fail to identify mutations that might be identifiable through single-gene testing. If high clinical suspicion exists for a particular syndrome testing for that syndrome should be performed instead of a broad multi-gene panel.

## Known familial mutation analysis

Known familial mutation analysis is performed when a causative mutation has been identified in a close relative of the individual requesting testing.

Analysis for known familial mutations is performed by trinucleotide repeat expansion analysis, Sanger sequencing or deletion/duplication analysis.

## Guidelines and Evidence

### Introduction

This section includes relevant guidelines and evidence pertaining to hereditary ataxia testing.

### American College of Medical Genetics

The American College of Medical Genetics (ACMG, 2013) states the following regarding testing for hereditary ataxia:<sup>4</sup>

- “Establishing the diagnosis of hereditary ataxia requires:
  - Detection on neurological examination of typical clinical signs including poorly coordinated gait and finger/hand movements, dysarthria (incoordination of speech), and eye movement abnormalities such as nystagmus, abnormal saccade movements, and ophthalmoplegia.
  - Exclusion of nongenetic causes of ataxia (see Differential Diagnosis below).
  - Documentation of the hereditary nature of the disease by finding a positive family history of ataxia, identifying an ataxia-causing mutation, or recognizing a clinical phenotype characteristic of a genetic form of ataxia.”
- “Differential diagnosis of hereditary ataxia includes acquired, nongenetic causes of ataxia, such as alcoholism, vitamin deficiencies, multiple sclerosis, vascular disease, primary or metastatic tumors, and paraneoplastic diseases associated with occult carcinoma of the ovary, breast, or lung, and the idiopathic degenerative disease multiple system atrophy (spinal muscular atrophy). The possibility of an acquired cause of ataxia needs to be considered in each individual with ataxia because a specific treatment may be available.”
- “Testing strategy when family history suggests autosomal dominant inheritance”
  - “An estimated 50–60% of the dominant hereditary ataxias can be identified with highly accurate and specific molecular genetic testing for SCA1, SCA2, SCA3, SCA6, SCA7, SCA8, SCA10, SCA12, SCA17, and DRPLA; all have nucleotide repeat expansions in the pertinent genes.
  - Because of the broad clinical overlap, most laboratories that test for the hereditary ataxias have a battery of tests including testing for SCA1, SCA2, SCA3, SCA6, SCA7, SCA10, SCA12, SCA14, and SCA17. Many laboratories offer them as two groups in stepwise fashion based on population frequency, testing first for the more common ataxias, SCA1, SCA2, SCA3, SCA6, and SCA7. Although pursuing multiple genes simultaneously may seem less optimal than serial genetic testing, it is important to recognize that the cost of the battery of ataxia tests often is equivalent to that of an MRI. Positive results from the molecular genetic testing are more specific than MRI findings in the hereditary ataxias.

- Testing for the less common hereditary ataxias should be individualized and may depend on factors such as ethnic background (SCA3 in the Portuguese, SCA10 in the Native American population with some exceptions); seizures (SCA10); presence of tremor (SCA12, fragile X-associated tremor/ataxia syndrome); presence of psychiatric disease or chorea (SCA17); or uncomplicated ataxia with long duration (SCA6, SCA8, and SCA14). Dysphonia and palatal myoclonus are associated with calcification of the dentate nucleus of cerebellum (SCA20).
- If a strong clinical indication of a specific diagnosis exists based on the affected individual's examination (e.g., the presence of retinopathy, which suggests SCA7) or if family history is positive for a known type, testing can be performed for a single disease."
- "Testing strategy when the family history suggests autosomal recessive inheritance
  - A family history in which only sibs are affected and/or when the parents are consanguineous suggests autosomal recessive inheritance. Because of their frequency and/or treatment potential, FRDA, A-T, AOA1, AOA2, AVED, and metabolic or lipid storage disorders such as Refsum disease and mitochondrial diseases should be considered."
- "Testing simplex cases.
  - If no acquired cause of the ataxia is identified, the probability is ~13% that the affected individual has SCA1, SCA2, SCA3, SCA6, SCA8, SCA17, or FRDA, and mutations in rare ataxia genes are even less common.
  - Other possibilities to consider are a de novo mutation in a different autosomal dominant ataxia, decreased penetrance, alternative paternity, or a single occurrence of an autosomal recessive or X-linked disorder in a family such as fragile X-associated tremor/ataxia syndrome.
  - Although the probability of a positive result from molecular genetic testing is low in an individual with ataxia who has no family history of ataxia, such testing is usually justified to establish a specific diagnosis for the individual's medical evaluation and for genetic counseling.
  - Always consider a possible nongenetic cause such as multiple system atrophy, cerebellar type in simplex cases."

### European Federation of Neurological Sciences

The European Federation of Neurological Sciences (EFNS, 2014) states the following regarding testing for hereditary ataxias:<sup>5</sup>

- "In the case of a family history that is compatible with an autosomal dominant cerebellar ataxia, screening for SCA1, SCA2, SCA3, SCA6, SCA7, and SCA17 is recommended (Level B). In Asian patients, DRPLA should also be tested for."

- “If mutation analysis is negative, we recommend contact with or referral to a specialized clinic for reviewing the phenotype and further genetic testing (good practice point).”
- “In the case of sporadic ataxia and independent from onset age, we recommend routine testing for SCA1, SCA2, SCA3, SCA6, and DRPLA (in Asian patients) (level B), the step one panel of the recessive ataxia workup, i.e. mutation analysis of the FRDA gene (level B), and biochemical testing that includes cholestanol, vitamin E, cholesterol, albumin, CK, and alpha-fetoprotein.”

### Peer reviewed literature

Hadjivassiliou M, Martindale J, Shanmugarajah P, et al (2017) stated the following with regard to testing for hereditary ataxias:

- “We have shown that patients with early onset idiopathic ataxia (irrespective of family history) are much more likely to have a genetic aetiology (81%) than those with late onset idiopathic ataxia (55%). One possible selection criterion for genetic testing is early onset ataxia. Additional selection criteria may include the presence of other clinical features, for example, 1% of patients with histologically suspected/genetically confirmed mitochondrial disease had ataxia with other clinical features (eg, deafness, diabetes, myoclonus, etc) and only 9% pure ataxia.”<sup>3</sup>
- “Furthermore, the presence of severe cerebellar atrophy without any clinical correlation and with well-preserved spectroscopy of the cerebellum often suggests that the ataxia is long standing (maybe even early onset) and slowly progressive. Patients should therefore be offered genetic testing. The pattern of cerebellar involvement on MR spectroscopy may also direct to a particular diagnosis. Most genetic ataxias involve both the hemispheres and the vermis while the majority of immune-mediated acquired ataxias (eg, gluten ataxia, anti-GAD ataxia and primary autoimmune cerebellar ataxia) have a predilection for the vermis.”<sup>3</sup>

## Criteria

### Introduction

Requests for hereditary ataxia multigene panel testing are reviewed using these criteria.

### Multi-gene panel testing

- Genetic counseling:
  - Pre- and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing



- No previous testing of requested genes, and
- No known mutation identified by previous analysis, and
- No known familial mutation in a gene known to cause ataxia, AND
- Diagnostic Testing for Symptomatic Individuals
  - Individual has been diagnosed with cerebellar ataxia, regardless of age of onset, AND
- Documentation from ordering provider indicating how test results will be used to directly impact medical care for the individual (e.g. change in surveillance or treatment plan), AND
- The member does not have a known underlying cause for their ataxia (e.g. alcoholism, vitamin deficiencies, multiple sclerosis, vascular disease, tumors, known mutation, etc), AND
- Family and medical history do not point to a specific genetic diagnosis or pattern of inheritance for which a more focused test or panel would be appropriate, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy

### **Billing and reimbursement considerations**

- Gene panels that are specific to hereditary ataxias will only be considered for reimbursement. This testing will only be considered for reimbursement when billed with an appropriate panel CPT code: 81443 or 81479. Analysis of individual genes will not be reimbursed separately.
- For focused spinocerebellar ataxia (SCA) panel test requests, see *Spinocerebellar Ataxia Genetic Testing* guideline

## **References**

### **Introduction**

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# Hereditary Cancer Syndrome Multigene Panels

**MOL.TS.182.A**  
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## Introduction

Hereditary cancer syndrome multigene panel testing is addressed by this guideline.

## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedures addressed by this guideline       | Procedure codes |
|--|-----------------|
| APC Sequencing                               | 81201           |
| APC Deletion/Duplication Analysis            | 81203           |
| ATM Sequencing                               | 81408           |
| BRCA1/2 Sequencing                           | 81163           |
| BRCA1/2 Deletion/Duplication Analysis        | 81164           |
| BRCA1 Sequencing                             | 81165           |
| BRCA1 Deletion/Duplication Analysis          | 81166           |
| BRCA2 Sequencing                             | 81216           |
| BRCA2 Deletion/Duplication Analysis          | 81167           |
| Chromosomal Microarray [BAC], Constitutional | 81228           |
| Chromosomal Microarray [SNP], Constitutional | 81229           |
| MLH1 Sequencing                              | 81292           |
| MLH1 Deletion/Duplication Analysis           | 81294           |
| MSH2 Sequencing                              | 81295           |
| MSH2 Deletion/Duplication Analysis           | 81297           |
| MSH6 Sequencing                              | 81298           |
| MSH6 Deletion/Duplication Analysis           | 81300           |
| PMS2 Sequencing                              | 81317           |
| PMS2 Deletion/Duplication Analysis           | 81319           |

| Procedures addressed by this guideline  | Procedure codes |
|---|-----------------|
| PTEN Sequencing   | 81321           |
| PTEN Deletion/Duplication Analysis  | 81323           |
| Hereditary breast cancer-related disorders (eg, hereditary breast cancer, hereditary ovarian cancer, hereditary endometrial cancer); genomic sequence analysis panel, must include sequencing of at least 10 genes, always including BRCA1, BRCA2, CDH1, MLH1, MSH2, MSH6, PALB2, PTEN, STK11, and TP53 | 81432           |
| Hereditary breast cancer-related disorders (eg, hereditary breast cancer, hereditary ovarian cancer, hereditary endometrial cancer); duplication/deletion analysis panel, must include analyses for BRCA1, BRCA2, MLH1, MSH2, and STK11   | 81433           |
| Hereditary colon cancer disorders (eg, Lynch syndrome, PTEN hamartoma syndrome, Cowden syndrome, familial adenomatosis polyposis); genomic sequence analysis panel, must include sequencing of at least 10 genes, including APC, BMPR1A, CDH1, MLH1, MSH2, MSH6, MUTYH, PTEN, SMAD4, and STK11          | 81435           |
| Hereditary colon cancer disorders (eg, Lynch syndrome, PTEN hamartoma syndrome, Cowden syndrome, familial adenomatosis polyposis); duplication/deletion analysis panel, must include analysis of at least 5 genes, including MLH1, MSH2, EPCAM, SMAD4, and STK11  | 81436           |
| Hereditary neuroendocrine tumor disorders (eg, medullary thyroid carcinoma, parathyroid carcinoma, malignant pheochromocytoma or paraganglioma); genomic sequence analysis panel, must include sequencing of at least 6 genes, including MAX, SDHB, SDHC, SDHD, TMEM127, and VHL                        | 81437           |

| Procedures addressed by this guideline  | Procedure codes |
|---|-----------------|
| Hereditary neuroendocrine tumor disorders (eg, medullary thyroid carcinoma, parathyroid carcinoma, malignant pheochromocytoma or paraganglioma); duplication/deletion analysis panel, must include analyses for SDHB, SDHC, SDHD, and VHL | 81438           |
| BRCAplus , Ambry Genetics   | 0129U           |
| BreastNext, Ambry Genetics, Ambry Genetics  | 0102U           |
| ColoNext, Ambry Genetics, Ambry Genetics  | 0101U           |
| OvaNext, Ambry Genetics, Ambry Genetics   | 0103U           |
| Miscellaneous hereditary cancer syndrome gene tests   | 81400           |
| Miscellaneous hereditary cancer syndrome gene tests   | 81401           |
| Miscellaneous hereditary cancer syndrome gene tests   | 81402           |
| Miscellaneous hereditary cancer syndrome gene tests   | 81403           |
| Miscellaneous hereditary cancer syndrome gene tests   | 81404           |
| Miscellaneous hereditary cancer syndrome gene tests   | 81405           |
| Miscellaneous hereditary cancer syndrome gene tests   | 81406           |
| Miscellaneous hereditary cancer syndrome gene tests   | 81407           |
| Miscellaneous hereditary cancer syndrome gene tests   | 81408           |
| Miscellaneous hereditary cancer syndrome gene tests   | 81479           |

## What are hereditary cancer syndromes

### Definition

A hereditary cancer syndrome is when a mutation in a single gene causes a significantly increased risk for certain cancers. Hereditary cancer syndromes are usually characterized by a pattern of specific cancer types occurring together in the same family, younger ages of cancer diagnosis than usual, or other co-existing non-cancer conditions.

### Prevalence

Most cancer is sporadic and believed to be caused by a mix of behavioral or lifestyle, environmental, and inherited risk factors. However, about 5-10% of cancers are believed to have a major inherited component.<sup>1</sup>

### Hereditary cancer syndromes

There are at least 50 hereditary cancer syndromes.<sup>1</sup> This table lists some of the most common along with associated cancers.<sup>2</sup>

| Syndrome   | Associated cancers   |
|--|--|
| Hereditary breast and ovarian cancer syndrome (HBOC) | <ul style="list-style-type: none"> <li>• breast</li> <li>• ovarian, fallopian tube, or primary peritoneal cancer</li> <li>• pancreatic</li> <li>• prostate</li> </ul>  |
| Lynch syndrome                                       | <ul style="list-style-type: none"> <li>• colorectal</li> <li>• endometrial</li> <li>• small bowel</li> <li>• stomach</li> <li>• ovarian</li> <li>• pancreatic</li> <li>• ureteral and renal pelvis</li> <li>• biliary tract</li> <li>• brain</li> <li>• sebaceous adenoma</li> <li>• keratoacanthoma tumors</li> </ul> |

| Syndrome                       | Associated cancers  |
|--------------------------------|---|
| Familial adenomatous polyposis | <ul style="list-style-type: none"> <li>• colorectal and other gastrointestinal cancers</li> <li>• gastrointestinal tract polyps such as adenomas and fundic gland</li> <li>• osteomas</li> <li>• desmoids</li> <li>• thyroid cancer</li> <li>• hepatoblastoma</li> </ul>          |
| MUTYH-associated polyposis     | <ul style="list-style-type: none"> <li>• colorectal and other gastrointestinal cancers</li> <li>• adenomas</li> <li>• hyperplastic polyps</li> </ul>  |
| Cowden syndrome                | <ul style="list-style-type: none"> <li>• benign and malignant tumors of the breast, endometrium, and thyroid</li> <li>• cancer and polyps (hamartomas) in the colon and rectum</li> </ul>   |
| Li Fraumeni syndrome           | <ul style="list-style-type: none"> <li>• soft tissue sarcoma</li> <li>• osteosarcoma</li> <li>• leukemia</li> <li>• melanoma</li> <li>• breast</li> <li>• pancreas</li> <li>• colon</li> <li>• adrenal cortex</li> <li>• stomach</li> <li>• esophagus</li> <li>• brain</li> </ul> |

| Syndrome               | Associated cancers  |
|------------------------|---|
| Peutz-Jeghers syndrome | <ul style="list-style-type: none"> <li>• polyps (hamartomas) in the stomach</li> <li>• small intestine and colon</li> <li>• pancreas</li> <li>• lung</li> <li>• breast</li> <li>• uterine</li> <li>• ovarian</li> </ul> |

### Overlapping clinical findings

Many hereditary cancer syndromes can include the same types of cancer and therefore have overlapping clinical findings. For example, breast cancer is a feature of HBOC caused by BRCA mutations, Li Fraumeni syndrome, Cowden syndrome, and others. Sometimes, the pattern of cancers in the family or pathognomonic features makes the underlying syndrome clear. However, in many cases it can be difficult to reliably diagnose hereditary cancer syndromes based on clinical and family history alone.

## Test information

### Introduction

Testing for hereditary cancer syndromes may include multigene panel testing.

### Sanger Sequencing

Until recently, most sequencing tests used the Sanger sequencing methodology that was originally developed in the 1970s. Sanger sequencing is labor intensive and did not lend itself to high-throughput applications.

### Next-generation sequencing (NGS)

NGS, which is also sometimes called massively parallel sequencing, has been developing since about 2005 to allow larger scale and more efficient gene sequencing. NGS relies on sequencing many copies of small pieces of DNA simultaneously and using bioinformatics to assemble the sequence.

The efficiency of NGS has led to an increasing number of large, multi-gene testing panels. NGS panels that test several genes at once are particularly well-suited to conditions caused by more than one gene or where there is considerable clinical overlap between conditions making it difficult to reliably narrow down likely causes. As



a result, several laboratories have begun to combine genes involved in causing various hereditary cancer syndromes, which often have both of those characteristics.

### **Detection rate of NGS**

NGS may not perform as well as Sanger sequencing in some applications. Results may also be obtained that cannot be adequately interpreted based on the current knowledgebase.

- When a sequence variation is identified that has not been previously characterized or shown to cause the disorder in question, it is called a variant of uncertain significance (VUS). VUSs are relatively common findings when sequencing large amounts of DNA with NGS.
- Under certain circumstances, technologies used in multi-gene testing may fail to identify mutations that might be identifiable through single-gene testing. If high clinical suspicion remains for a particular syndrome after negative multi-gene test results, consultation with the testing lab, additional targeted genetic testing, or both may be warranted.

### **Hereditary cancer syndrome multi-gene panels**

Hereditary cancer syndrome multi-gene panels include a wide variety of genes and may be focused on the genetic causes of a particular cancer type or broad detection of common hereditary cancer syndromes.

Multi-gene tests vary in technical specifications. For example, different labs may have different depth of coverage, extent of Intron/Exon Boundary analysis, or methodology of large Deletion/Duplication Analysis.

Because genes can be easily added or removed from multi-gene tests over time by a given lab, medical records must document

- the genes included in the specific multi-gene test used from each patient, and
- the labs that performed the panels.

### **Moderate risk genes**

Panels may also include genes believed to be associated with cancer, but with a more modest impact on risk than recognized hereditary cancer syndromes. Results for such genes are of less clear value because there often are not clear management recommendation for mutation-positive individuals.

## **Guidelines and evidence**

### **Introduction**

This section includes relevant guidelines and evidence pertaining to hereditary cancer

syndrome panel testing.

### **National Comprehensive Cancer Network (NCCN)**

The National Comprehensive Cancer Network (NCCN) makes the following general recommendations for using multi-gene panels in evaluating risk for breast and ovarian cancer and now includes this option in some management algorithms:<sup>3,4</sup>

“Because of their complexity, multi-gene testing is ideally offered in the context of professional genetic expertise for pre- and post-test counseling.”

“Testing of an individual without a cancer diagnosis should only be considered when an appropriate affected family member is unavailable for testing.”

“When more than one gene can explain an inherited cancer syndrome, then multi-gene testing may be more efficient and/or cost effective. As commercially available tests differ in the specific genes analyzed (as well as classification of variants and many other factors), choosing the specific laboratory and test panel is important. Multi-gene testing can include ‘intermediate’ penetrant (moderate-risk) genes. For many of these genes, there is limited data on the degree of cancer risk and there are no clear guidelines on risk management for carriers of mutations. Not all genes included on available multi-gene tests are necessarily clinically actionable.” If a moderate risk gene mutation is identified, “gene carriers should be encouraged to participate in clinical trials or genetic registries.”

“As is the case with high-risk genes, it is possible that the risks associated with moderate-risk genes may not be entirely due to that gene alone, but may be influenced by gene/gene or gene/environment interactions. In addition, certain pathogenic/likely pathogenic variant in genes may post higher or lower risk than other pathogenic/likely pathogenic variants in that same gene. Therefore, it may be difficult to use a known pathogenic/likely pathogenic variant alone to assign risk for relatives.”

“Mutations in many breast cancer susceptibility genes involved in DNA repair may be associated with the rare autosomal recessive condition, Fanconi anemia.” Therefore, multi-gene testing may unexpectedly reveal that an individual and their family are at an increased risk for this condition.

“There is an increased likelihood of finding variants of unknown significance when testing for mutations in multiple genes.”

### **American Society of Breast Surgeons**

The American Society of Breast Surgeons (2019) published a consensus guideline on genetic testing for hereditary breast cancer. They state the following:

- “Breast surgeons, genetic counselors, and other medical professionals knowledgeable in genetic testing can provide patient education and counseling and make recommendations to their patients regarding genetic testing and arrange testing. When the patient’s history and/or test results are complex, referral to a certified genetic counselor or genetics professional may be useful. Genetic testing

is increasingly provided through multi-gene panels. There are a wide variety of panels available, with different genes on different panels. There is a lack of consensus among experts regarding which genes should be tested in different clinical scenarios. There is also variation in the degree of consensus regarding the understanding of risk and appropriate clinical management of mutations in some genes.”

- “Genetic testing should be made available to all patients with a personal history of breast cancer. Recent data support that genetic testing should be offered to each patient with breast cancer (newly diagnosed or with a personal history). If genetic testing is performed, such testing should include BRCA1/BRCA2 and PALB2, with other genes as appropriate for the clinical scenario and family history. For patients with newly diagnosed breast cancer, identification of a mutation may impact local treatment recommendations (surgery and potentially radiation) and systemic therapy. Additionally, family members may subsequently be offered testing and tailored risk reduction strategies.”
- “Genetic testing should be made available to all patients with a personal history of breast cancer. Every patient being seen by a breast surgeon, who had genetic testing in the past and no pathogenic variant was identified, should be re-evaluated and updated testing considered. In particular, a patient who had negative germline BRCA1 and 2 testing, who is from a family with no pathogenic variants, should be considered for additional testing.<sup>1</sup> Genetic testing performed prior to 2014 most likely would not have had PALB2 or other potentially relevant genes included and may not have included testing for large genomic rearrangements in BRCA1 or BRCA2.”
- “Genetic testing should be made available to patients without a history of breast cancer who meet NCCN guidelines. Unaffected patients should be informed that testing an affected relative first, whenever possible, is more informative than undergoing testing themselves. When it is not feasible to test the affected relative first, then the unaffected family member should be considered for testing if they are interested, with careful pre-test counseling to explain the limited value of “uninformative negative” results. It is also reasonable to order a multi-gene panel if the family history is incomplete (i.e., a case of adoption, patient is uncertain of exact type of cancer affecting family members, among others) or other cancers are found in the family history, as described above.”

### American College of Medical Genetics

The American College of Medical Genetics has a policy statement that offers general guidance on the clinical application of large-scale sequencing focusing primarily on whole exome and whole genome testing. However, some of the recommendations regarding counseling around unexpected results, variants of unknown significance, and minimum requirements for reporting apply to many NGS applications.<sup>5</sup>

## Criteria

### Introduction

Requests for hereditary cancer syndrome panel testing are reviewed using these criteria.

### Criteria

This guideline applies to all hereditary cancer syndrome panels, which are defined as assays that simultaneously test for more than one hereditary cancer syndrome. This guideline does not apply when testing more than one gene related to the same hereditary cancer syndrome (e.g., Lynch syndrome).

Medical necessity coverage generally relies on criteria established for testing individual hereditary cancer syndromes. See the Coverage Guidance table for examples of genes known to be included in currently available hereditary cancer syndrome multi-gene panels with coverage guidance. This is not intended to be a complete list of available genes as these panels are evolving rapidly.

However, this guideline takes into account the efficiency gains from simultaneously testing multiple candidate genes. Therefore, coverage requirements rely to some degree on how the panel will be billed. Panels may be billed in a variety of ways:

- Gene sequencing portion:
  - A separate CPT code for sequencing each gene studied or a subset (e.g., 81201, 81294, 81297, etc.)
  - A single CPT code developed specifically for a particular type of panel (e.g., 81432, 81435, 81437)
  - A single unlisted CPT code (e.g., 81479)
- Deletion/duplication analysis portion:
  - A separate CPT code for deletion/duplication analysis of each gene studied or a subset (e.g., 81203, 81292, 81294, 81404, 81479, etc.)
  - A single CPT code developed specifically for a particular type of panel (e.g., 81433, 81436, 81438)
  - Microarray analysis (e.g., 81228 or 81229)
  - Part of a single unlisted CPT code for the sequencing and deletion/duplication portions of the panel (e.g., 81479)

Hereditary cancer syndrome multi-gene panels will be reimbursed when the following criteria are met:

- Panel will be billed with separate procedure codes for each gene analyzed (however, please note that the billed amount should not exceed the list price of the test).
  - The medical necessity of each billed procedure will be assessed independently. See the Coverage Guidance table for gene-specific policy guidance.
    - When a patient meets medical necessity criteria for any hereditary cancer syndrome gene(s) included in a multi-gene panel, genetic testing for the clinically indicated gene(s) will be reimbursed. This includes the sequencing and deletion/duplication<sup>†</sup> components.
    - Any genes that are included in a multi-gene panel but do NOT meet medical necessity criteria will NOT be reimbursed. It will be at the laboratory, provider, and patient's discretion to determine if a multi-gene panel remains the preferred testing option.
  - Sequencing and/or deletion/duplication analysis<sup>†</sup> of any hereditary cancer syndrome gene(s) should only be performed once per lifetime and will therefore only be reimbursed once per lifetime. If gene testing was previously performed, and is now being included in a panel, such testing will not be separately reimbursable regardless of whether clinical coverage criteria are met, OR
- Panel will be billed with a single procedure code to represent all genes being sequenced, with or without another single procedure code representing the deletion/duplication analysis<sup>†</sup> portion. Code(s) may be specific to that panel or an unlisted code, such as 81479.
  - No previous hereditary cancer syndrome testing has been performed
    - Medical necessity must be established for at least two conditions included in the panel (e.g., hereditary breast and ovarian cancer and Li Fraumeni syndrome). Note that this is two conditions and not two genes (i.e., meeting criteria for only Lynch syndrome, which is caused by mutations in at least 5 genes, would not fulfill criteria alone).

Although not a complete list, the following are considered separate conditions:

      - Hereditary breast cancer - this includes both BRCA1/2 and PALB2.
      - Lynch syndrome
      - Li-Fraumeni
      - Familial adenomatous polyposis
      - Cowden syndrome
  - Testing for one condition was performed and billed separately. A multi-gene panel is now being considered as a reflex and will be billed at a rate comparable to single syndrome pricing (e.g., myRisk update).

- Medical necessity must be established for at least one condition included in the panel in addition to the already tested condition (e.g., hereditary breast and ovarian cancer was already performed, but Lynch syndrome criteria are also met).
- Note that if BRCA1/2 testing was already performed and PALB2 criteria are now met, PALB2 testing alone would be reimbursable and not a reflex panel test (e.g. myRisk Update).

† When deletion/duplication testing is not part of a single panel CPT code being billed, deletion/duplication testing should be billed in only one of the following ways:

- A separate CPT code for deletion/duplication analysis of each individual gene (may include non-specific molecular pathology tier 2 codes or unlisted code 81479), or
- A single CPT code specific to the performed deletion/duplication analysis panel, or
- A single microarray procedure

Procedure codes representing multiple methods for deletion/duplication testing will not be reimbursable for the same panel (e.g., test-specific deletion/duplication procedure codes and microarray will not both be reimbursable for the same panel).

### Coverage guidance

The following table describes coverage guidance for genes associated with hereditary cancer syndromes.

#### **Coverage Guidance for Genes Included in Hereditary Cancer Syndrome Multi-Gene Panels**

| Condition                                      | Test Name                          | CPT   | Claim Code | Reimbursement |
|--|------------------------------------|-------|------------|---------------|
| Birt-Hogg-Dube syndrome                        | FLCN Sequencing                    | 81479 | FLCNSEQ    | MOL.CU.109    |
|  | FLCN Deletion/Duplication Analysis | 81479 | FLCNDD     | MOL.CU.109    |
| Cowden syndrome, PTEN hamartoma tumor syndrome | PTEN Deletion/Duplication Analysis | 81323 | NONE       | MOL.TS.223    |
|  | PTEN Sequencing                    | 81321 | NONE       | MOL.TS.223    |
| Cutaneous malignant melanoma                   | CDK4 Deletion/Duplication Analysis | 81479 | CDK4DD     | MOL.TS.170    |

| Condition                             | Test Name                            | CPT   | Claim Code | Reimbursement  |
|---------------------------------------|--------------------------------------|-------|------------|----------------|
|                                       | CDK4 Exon 2 Sequencing               | 81479 | CDK4EX2    | MOL.TS.170     |
|                                       | CDKN2A Deletion/Duplication Analysis | 81479 | CDKN2A     | MOL.TS.170     |
|                                       | CDKN2A Sequencing                    | 81404 | CDKN2A     | MOL.TS.170     |
| Familial adenomatous polyposis        | APC Deletion/Duplication Analysis    | 81203 | NONE       | MOL.TS.168     |
|                                       | APC Sequencing                       | 81201 | NONE       | MOL.TS.168     |
| Familial breast and/or ovarian cancer | AKT1 Deletion/Duplication Analysis   | 81479 | AKT1DD     | Not reimbursed |
|                                       | AKT1 Sequencing                      | 81479 | AKT1SEQ    | Not reimbursed |
|                                       | ATM Deletion/Duplication Analysis    | 81479 | ATM        | Not reimbursed |
|                                       | ATM Sequencing                       | 81408 | ATM        | Not reimbursed |
|                                       | BARD1 Deletion/Duplication Analysis  | 81479 | BARD1DD    | Not reimbursed |
|                                       | BARD1 Sequencing                     | 81479 | BARD1SEQ   | Not reimbursed |
|                                       | BRIP1 Deletion/Duplication Analysis  | 81479 | BRIP1DD    | Not reimbursed |
|                                       | BRIP1 Sequencing                     | 81479 | BRIP1      | Not reimbursed |
|                                       | CHEK2 Deletion/Duplication Analysis  | 81479 | CHEK2DD    | Not reimbursed |
|                                       | CHEK2 Sequencing                     | 81479 | CHEK2SEQ   | Not reimbursed |

| Condition | Test Name                                | CPT   | Claim Code | Reimbursement  |
|-----------|--|-------|------------|----------------|
|           | FAM175A<br>Deletion/Duplication Analysis | 81479 | FAM175ADD  | Not reimbursed |
|           | FAM175A<br>Sequencing                    | 81479 | FAM175ASEQ | Not reimbursed |
|           | GEN1<br>Deletion/Duplication Analysis    | 81479 | GEN1DD     | Not reimbursed |
|           | GEN1<br>Sequencing                       | 81479 | GEN1SEQ    | Not reimbursed |
|           | MRE11A<br>Deletion/Duplication Analysis  | 81479 | MRE11ADD   | Not reimbursed |
|           | MRE11A<br>Sequencing                     | 81479 | MRE11ASEQ  | Not reimbursed |
|           | NBN<br>Deletion/Duplication Analysis     | 81479 | NBNDD      | Not reimbursed |
|           | NBN<br>Sequencing                        | 81479 | NBNSEQ     | Not reimbursed |
|           | RAD50<br>Deletion/Duplication Analysis   | 81479 | RAD50DD    | Not reimbursed |
|           | RAD50<br>Sequencing                      | 81479 | RAD50SEQ   | Not reimbursed |
|           | RAD51<br>Deletion/Duplication Analysis   | 81479 | RAD51DD    | Not reimbursed |
|           | RAD51<br>Sequencing                      | 81479 | RAD51SEQ   | Not reimbursed |
|           | RAD51C<br>Deletion/Duplication Analysis  | 81479 | RAD51CDD   | Not reimbursed |
|           | RAD51C<br>Sequencing                     | 81479 | RAD51CSEQ  | Not reimbursed |
|           | RAD51D<br>Deletion/Duplication Analysis  | 81479 | RAD51DDD   | Not reimbursed |

Hereditary Cancer Syndrome



| Condition                  | Test Name                             | CPT   | Claim Code | Reimbursement  |
|----------------------------|---------------------------------------|-------|------------|----------------|
|                            | RAD51D Sequencing                     | 81479 | RAD51DSEQ  | Not reimbursed |
|                            | SMARCA4 Deletion/Duplication Analysis | 81479 | SMARCA4DD  | Not reimbursed |
|                            | SMARCA4 Sequencing                    | 81479 | SMARCA4SEQ | Not reimbursed |
|                            | XRCC2 Deletion/Duplication Analysis   | 81479 | XRCC2DD    | Not reimbursed |
|                            | XRCC2 Sequencing                      | 81479 | XRCC2SEQ   | Not reimbursed |
|                            | XRCC3 Deletion/Duplication Analysis   | 81479 | XRCC3DD    | Not reimbursed |
|                            | XRCC3 Sequencing                      | 81479 | XRCC3SEQ   | Not reimbursed |
| Familial colorectal cancer | AXIN2 Deletion/Duplication Analysis   | 81479 | AXIN2DD    | Not reimbursed |
|                            | AXIN2 Sequencing                      | 81479 | AXIN2SEQ   | Not reimbursed |
|                            | GALNT12 Deletion/Duplication Analysis | 81479 | GALNT12DD  | Not reimbursed |
|                            | GALNT12 Sequencing                    | 81479 | GALNT12SEQ | Not reimbursed |
|                            | MLH3 Deletion/Duplication Analysis    | 81479 | MLH3DD     | Not reimbursed |
|                            | MLH3 Sequencing                       | 81479 | MLH3SEQ    | Not reimbursed |
|                            | POLE Sequencing                       | 81479 | POLESEQ    | Not reimbursed |
|                            | POLD1 Sequencing                      | 81479 | POLD1SEQ   | Not reimbursed |

| Condition   | Test Name                             | CPT   | Claim Code | Reimbursement  |
|---|---------------------------------------|-------|------------|----------------|
| Familial cutaneous telangiectasia and cancer syndrome | ATR Deletion/Duplication Analysis     | 81479 | ATRDD      | Not reimbursed |
|   | ATR Sequencing                        | 81479 | ATRSEQ     | Not reimbursed |
| Familial pancreatic cancer                            | PRSS1 Deletion/Duplication Analysis   | 81479 | PRSS1      | Not reimbursed |
|   | PRSS1 Sequencing                      | 81404 | PRSS1      | Not reimbursed |
| Familial prostate cancer                              | HOXB13 Deletion/Duplication Analysis  | 81479 | HOXB13DD   | Not reimbursed |
|   | HOXB13 Sequencing                     | 81479 | HOXB13SEQ  | Not reimbursed |
| Familial renal cell carcinoma                         | MITF Sequencing                       | 81479 | MITFSEQ    | Not reimbursed |
|   | MITF Deletion/Duplication Analysis    | 81479 | MITFDD     | Not reimbursed |
| Familial Wilms tumor                                  | WT1 Sequencing                        | 81405 | WT1        | MOL.CU.109     |
| Hereditary breast and ovarian cancer                  | BRCA1/2 Sequencing                    | 81163 | NONE       | MOL.TS.238     |
|   | BRCA1/2 Deletion/Duplication Analysis | 81164 | NONE       | MOL.TS.238     |
|   | BRCA1 Sequencing                      | 81165 | NONE       | MOL.TS.238     |
|   | BRCA1 Deletion/Duplication Analysis   | 81166 | NONE       | MOL.TS.238     |
|   | BRCA2 Sequencing                      | 81216 | NONE       | MOL.TS.238     |
|   | BRCA2 Deletion/Duplication Analysis   | 81167 | NONE       | MOL.TS.238     |

| Condition  | Test Name                            | CPT   | Claim Code | Reimbursement  |
|--|--------------------------------------|-------|------------|----------------|
| Hereditary breast and pancreatic cancer                          | PALB2 Sequencing                     | 81406 | PALB2      | MOL.TS.251     |
| Hereditary diffuse gastric cancer                                | CDH1 Sequencing                      | 81406 | CDH1       | MOL.CU.109     |
|  | CTNNA1 Deletion/Duplication Analysis | 81479 | CTNNA1DD   | MOL.CU.109     |
|  | CTNNA1 Sequencing                    | 81479 | CTNNA1SEQ  | MOL.CU.109     |
| Hereditary leiomyomatosis with renal cell cancer                 | FH Sequencing                        | 81405 | FH         | MOL.CU.109     |
| Hereditary mixed polyposis syndrome                              | GREM1 Deletion/Duplication Analysis  | 81479 | GREM1DD    | Not reimbursed |
|  | GREM1 Sequencing                     | 81479 | GREM1SEQ   | Not reimbursed |
| Hereditary mixed polyposis syndrome, Juvenile polyposis syndrome | BMPR1A Deletion/Duplication Analysis | 81479 | BMPR1ADD   | MOL.CU.109     |
|  | BMPR1A Sequencing                    | 81479 | BMPR1ASEQ  | MOL.CU.109     |
| Hereditary papillary renal cell carcinoma                        | MET Sequencing                       | 81479 | METSEQ     | MOL.CU.109     |
|  | MET Deletion/Duplication Analysis    | 81479 | METDD      | MOL.CU.109     |
| Hereditary paraganglioma-pheochromocytoma syndromes              | SDHA Sequencing                      | 81406 | SDHA       | MOL.CU.109     |
|  | SDHB Deletion/Duplication Analysis   | 81479 | SDHB       | MOL.CU.109     |
|  | SDHB Sequencing                      | 81405 | SDHB       | MOL.CU.109     |

## Hereditary Cancer Syndrome

| Condition                   | Test Name                             | CPT   | Claim Code | Reimbursement |
|-----------------------------|---------------------------------------|-------|------------|---------------|
|                             | SDHC Deletion/Duplication Analysis    | 81404 | SDHC       | MOL.CU.109    |
|                             | SDHC Sequencing                       | 81405 | SDHC       | MOL.CU.109    |
|                             | SDHD Deletion/Duplication Analysis    | 81479 | SDHD       | MOL.CU.109    |
|                             | SDHD Sequencing                       | 81404 | SDHD       | MOL.CU.109    |
|                             | MAX Sequencing                        | 81479 | MAXSEQ     | MOL.CU.109    |
|                             | MAX Deletion/Duplication Analysis     | 81479 | MAXDD      | MOL.CU.109    |
|                             | SDHAF2 Sequencing                     | 81479 | SDHAF2SEQ  | MOL.CU.109    |
|                             | SDHAF2 Deletion/Duplication Analysis  | 81479 | SDHAF2DD   | MOL.CU.109    |
|                             | TMEM127 Sequencing                    | 81479 | TMEM127SEQ | MOL.CU.109    |
|                             | TMEM127 Deletion/Duplication Analysis | 81479 | TMEM127DD  | MOL.CU.109    |
| Juvenile polyposis syndrome | SMAD4 Deletion/Duplication Analysis   | 81405 | SMAD4      | MOL.CU.109    |
|                             | SMAD4 Sequencing                      | 81406 | SMAD4      | MOL.CU.109    |
| Li-Fraumeni syndrome        | TP53 Deletion/Duplication Analysis    | 81479 | TP53       | MOL.TS.193    |
|                             | TP53 Sequencing                       | 81405 | TP53       | MOL.TS.193    |
|                             | TP53 Targeted Sequencing              | 81404 | TP53       | MOL.TS.193    |

| Condition                             | Test Name                           | CPT   | Claim Code | Reimbursement |
|---------------------------------------|-------------------------------------|-------|------------|---------------|
| Lynch syndrome                        | EPCAM Deletion/Duplication Analysis | 81403 | EPCAM      | MOL.TS.197    |
|                                       | MLH1 Deletion/Duplication Analysis  | 81294 | NONE       | MOL.TS.197    |
|                                       | MLH1 Sequencing                     | 81292 | NONE       | MOL.TS.197    |
|                                       | MSH2 Deletion/Duplication Analysis  | 81297 | NONE       | MOL.TS.197    |
|                                       | MSH2 Sequencing                     | 81295 | NONE       | MOL.TS.197    |
|                                       | MSH6 Deletion/Duplication Analysis  | 81300 | NONE       | MOL.TS.197    |
|                                       | MSH6 Sequencing                     | 81298 | NONE       | MOL.TS.197    |
|                                       | PMS2 Deletion/Duplication Analysis  | 81319 | NONE       | MOL.TS.197    |
|                                       | PMS2 Sequencing                     | 81317 | NONE       | MOL.TS.197    |
| Multiple endocrine neoplasia type 1   | MEN1 Deletion/Duplication Analysis  | 81404 | MEN1       | MOL.CU.109    |
|                                       | MEN1 Sequencing                     | 81405 | MEN1       | MOL.CU.109    |
| Multiple endocrine neoplasia, type 2A | RET Sequencing                      | 81406 | RET        | MOL.CU.109    |
|                                       | RET Targeted Sequencing             | 81405 | RET        | MOL.CU.109    |
| MUTYH-associated polyposis            | MUTYH Deletion/Duplication Analysis | 81479 | MUTYH      | MOL.TS.206    |
|                                       | MUTYH Sequencing                    | 81406 | MUTYH      | MOL.TS.206    |

| Condition                     | Test Name                            | CPT   | Claim Code | Reimbursement  |
|-------------------------------|--------------------------------------|-------|------------|----------------|
| Neurofibromatosis type 1      | NF1 Deletion/Duplication Analysis    | 81479 | NF1        | MOL.CU.109     |
|                               | NF1 Sequencing                       | 81408 | NF1        | MOL.CU.109     |
| Peutz-Jeghers syndrome        | STK11 Deletion/Duplication Analysis  | 81404 | STK11      | MOL.TS.216     |
|                               | STK11 Sequencing                     | 81405 | STK11      | MOL.TS.216     |
| Tumor predisposition syndrome | BAP1 Deletion/Duplication Analysis   | 81479 | BAP1DD     | Not reimbursed |
|                               | BAP1 Sequencing                      | 81479 | BAP1SEQ    | Not reimbursed |
| Unknown phenotype             | CHEK1 Deletion/Duplication Analysis  | 81479 | CHEK1DD    | Not reimbursed |
|                               | CHEK1 Sequencing                     | 81479 | CHEK1SEQ   | Not reimbursed |
| von Hippel-Lindau syndrome    | RAD51B Deletion/Duplication Analysis | 81479 | RAD51BDD   | Not reimbursed |
|                               | RAD51B Sequencing                    | 81479 | RAD51BSEQ  | Not reimbursed |
|                               | VHL Deletion/Duplication Analysis    | 81403 | VHL        | MOL.TS.233     |
|                               | VHL Sequencing                       | 81404 | VHL        | MOL.TS.233     |

### Not reimbursed

Gene testing is not reimbursed strictly for hereditary cancer indications. In general, this category applies to genes that have only a low to moderate impact on cancer risk (compared to high penetrance cancer syndrome-causing genes) and no clear management guidelines associated with identifying a mutation.

## References

### Introduction

These references are cited in this guideline.

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# Hereditary Connective Tissue Disorder Testing

MOL.TS.268.A  
v1.0.2020

## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedures addressed by this guideline                             | Procedure codes |
|--|-----------------|
| Aortic Dysfunction or Dilation Genomic Sequencing Analysis Panel   | 81410           |
| Aortic Dysfunction or Dilation Duplication/Deletion Analysis Panel | 81411           |
| Miscellaneous hereditary connective tissue disorder gene tests     | 81400           |
| Miscellaneous hereditary connective tissue disorder gene tests     | 81401           |
| Miscellaneous hereditary connective tissue disorder gene tests     | 81402           |
| Miscellaneous hereditary connective tissue disorder gene tests     | 81403           |
| Miscellaneous hereditary connective tissue disorder gene tests     | 81404           |
| Miscellaneous hereditary connective tissue disorder gene tests     | 81405           |
| Miscellaneous hereditary connective tissue disorder gene tests     | 81406           |
| Miscellaneous hereditary connective tissue disorder gene tests     | 81407           |
| Miscellaneous hereditary connective tissue disorder gene tests     | 81408           |
| Miscellaneous hereditary connective tissue disorder gene tests     | 81479           |



## What are hereditary connective tissue disorders

### Definition

Hereditary connective tissue disorders (HCTDs) are a group of disorders that affect the connective tissues that support the skin, bones, joints, heart, blood vessels, eyes, and other organs.<sup>1</sup>

- While specific features vary by type, an unusually large range of joint movement (hypermobility) and cardiovascular disease (such as thoracic aortic aneurysms and dissections, or TAAD) are features that are present in many HCTDs. Medical management may differ based on the underlying genetic etiology.
- In many cases, a careful clinical examination by a specialist familiar with clinical features of these conditions can help to point toward one condition or group of conditions. In these cases, testing for gene(s) associated with a single condition or group of conditions would be most appropriate. However, in some cases, it can be difficult to reliably diagnose an HCTD based on clinical and family history alone.
- More than a half million people in the United States are estimated to have an HCTD.<sup>1</sup>
- There are more than 200 HCTDs.<sup>1</sup> Some of the most common types are summarized below:
  - **Arterial tortuosity syndrome (ATS)** — An autosomal recessive disorder associated with severe and widespread tortuosity of the aorta and middle-sized arteries, with an increased risk of aneurysms and dissections. Other features include stenosis of the aorta and/or pulmonary arteries, characteristic facies with high palate and dental crowding, and soft/doughy skin. Additional connective tissue disorder features that may be present include skeletal findings (scoliosis, pectus anomalies, joint laxity), hernias, hypotonia, and ocular involvement (myopia, keratoconus). SLC2A10 is the only gene known to be associated with ATS. Sequence variants are the most common; exon deletions have been reported in a couple cases.<sup>2</sup>
  - **Congenital contractural arachnodactyly (Beals syndrome)** — An autosomal dominant disorder characterized by a Marfan-like appearance (tall, slender habitus in which arm span exceeds height) and long, slender fingers and toes (arachnodactyly). Most affected individuals have a “crumpled” appearance to their ears and most have contractures of major joints (knees and ankles) at birth. Hip contractures, adducted thumbs, and club foot may occur. The majority of affected individuals have muscular hypoplasia. Kyphosis/scoliosis is present in about half of all affected individuals. Dilatation of the aorta is occasionally present. “FBN2 is the only gene in which mutation is known to cause congenital contractural arachnodactyly.”<sup>3</sup>
  - **Cutis laxa** — A group of disorders characterized by lax, sagging skin that often hangs in loose folds, causing the face and other parts of the body to have a droopy appearance. Extremely wrinkled skin may be particularly noticeable on

the neck and in the armpits and groin. Other features may include arterial aneurysm and dissection, emphysema, and inguinal or umbilical hernia. There are autosomal dominant, autosomal recessive, and X-linked forms. Causative autosomal genes include ELN, FBLN5, ATP6V0A2, EFEMP2, and LTBP4.<sup>4,5</sup> The X-linked form is due to mutations in ATP7A (see also Occipital Horn Syndrome).<sup>4</sup>

- **Ehlers Danlos syndromes (EDS)** — A heterogeneous group of disorders, the majority of which share the features of joint hypermobility and skin involvement. There are 13 types: classical, classical-like, cardiac-valvular, vascular, hypermobile (includes “joint hypermobility syndrome”), arthrochalasia, dermatosparaxis, kyphoscoliotic, spondylodysplastic, musculocontractural, myopathic, periodontal, and brittle cornea syndrome. Some types have autosomal dominant inheritance, while others are autosomal recessive. Hypermobile type is the most common, but its genetic etiology is currently unknown. Genetic testing is available for the other EDS types (see Table 1 below for a list of genes).<sup>6,7</sup>
- **Homocystinuria due to cystathionine beta-synthase deficiency** — An autosomal recessive metabolic disorder in which affected individuals have markedly elevated plasma total homocysteine and methionine. Clinical features include involvement of the eye (ectopia lentis and/or severe myopia), skeletal system (excessive height, long limbs, scoliosis, and pectus excavatum), and vascular system (thromboembolism). Many have developmental delay/intellectual disability. Treatment involves maintenance of normal or near-normal plasma homocysteine concentrations using a specialized diet and vitamin supplementation. The diagnosis can be substantiated by detection of biallelic pathogenic mutations in the CBS gene. Sequence analysis detects 95-98% of mutations, while deletion/duplication analysis detects <5%.<sup>8</sup>
- **Loeys-Dietz syndrome (LDS)** — LDS is an autosomal dominant disorder that affects many parts of the body.<sup>9</sup> LDS is caused by mutations in six genes: TGFBR2 (55-60%), TGFBR1 (20-25%), SMAD3 (5-10%), TGFB2 (5-10%), TGFB3 (1-5%), or SMAD2 (1-5%). Major manifestations of this condition include “vascular findings (dilatation or dissection of the aorta, other arterial aneurysms or tortuosity), skeletal findings (pectus excavatum or pectus carinatum, scoliosis, joint laxity or contracture, long thin fingers and toes, cervical spine malformation and/or instability), craniofacial findings (widely spaced eyes, bifid uvula/cleft palate, craniosynostosis), and cutaneous findings (translucent skin, easy bruising, dystrophic scars).”<sup>9</sup> Given that there is no clinical diagnostic criteria established for LDS, genetic testing, either through serial single-gene testing or use of a multigene panel, can establish the diagnosis.<sup>9</sup>
- **Marfan syndrome (MFS)** — MFS is an autosomal dominant disorder that affects connective tissue in many parts of the body.<sup>9</sup> MFS is caused by mutations in the FBN1 gene. Up to 93% of people meeting diagnostic criteria for MFS will have a mutation in this gene. Diagnostic criteria, called the Ghent criteria, exists for MFS. Major manifestations of the disease include aortic enlargement and ectopia lentis. Other features include, but are not limited to, bone overgrowth and joint laxity, long arms and legs, scoliosis, sternum

deformity (pectus excavatum or carinatum), long thin fingers and toes, dural ectasia (stretching of the dural sac), hernias, stretch marks on the skin, and lung bullae. Symptoms can present in males or females at any age. Symptoms typically worsen over time. Infants who present with symptoms typically have the most severe disease course.<sup>10</sup>

- **NOTCH1-related aortic valve disease** — NOTCH1 variants can be associated with autosomal dominant congenital heart defects affecting the left ventricular outflow tract (LVOT), most commonly bicuspid aortic valve (BAV). Adult-onset aortic valve calcification is a frequent feature. NOTCH1 variants have also been identified in 4.2% of individuals with sporadic BAV and much less frequently with other LVOT malformations. Mutations in this gene are also associated with Adams-Oliver syndrome, which is characterized by aplasia cutis congenita of the scalp and malformations of the limbs, brain, and cardiovascular system.<sup>11</sup>
- **Osteogenesis imperfecta (OI)** — A group of disorders associated with a propensity to fractures with little or no trauma. Additional features may include skeletal anomalies, short stature, hearing loss, and blue/gray sclera. The severity is highly variable, ranging from a mild form with few fractures and normal life expectancy, to severe forms with neonatal lethality. OI types I-IV account for the majority of cases, and are caused by heterozygous mutations in the COL1A1 and COL1A2 genes. Inheritance is autosomal dominant. Autosomal recessive forms of OI are rare, and can be associated with mutations in a number of different genes.<sup>12</sup>
- **Periventricular nodular heterotopia (PVNH)** — An X-linked condition, which is prenatally or neonatally lethal in most males. Therefore, most affected individuals are female. In addition to PVNH, some individuals have connective tissue anomalies such as joint hypermobility, aortic dilation, and other vascular anomalies. 93% of individuals with FLNA-related PVNH have a sequence variant; genomic rearrangements have been reported in a few cases.<sup>13</sup>
- **Stickler syndrome** — A disorder characterized by ocular findings (myopia, cataract and retinal detachment), hearing loss, craniofacial findings (midfacial underdevelopment and cleft palate), mild spondyloepiphyseal dysplasia and/or early-onset arthritis. Clinical diagnostic criteria are available. >90% of cases are due to mutations in COL2A1 or COL11A1. Mutations in these genes, and COL11A2, are inherited in an autosomal dominant pattern. Mutations in COL9A1, COL9A2, and COL9A3 are rare, and inherited in an autosomal recessive pattern.<sup>14</sup>
- **Thoracic Aortic Aneurysm and Dissection (TAAD)** — Familial TAAD is defined as dilatation and/or dissection of the thoracic aorta, absence of clinical features of MFS, LDS or vascular EDS, and a positive family history of TAAD. Approximately 30% of families with heritable thoracic aortic disease (HTAD) who do not have a clinical diagnosis of MFS or another syndrome have a causative mutation in one of 14 known HTAD-related genes (see Table 1 below).<sup>15</sup>

## Test information

- Clinical genetic testing is available for many HCTDs. However, hypermobile EDS (hEDS), joint hypermobility syndrome, and isolated joint hypermobility, including “hypermobility spectrum disorders”, continue to require a clinical diagnosis, since the genetic etiology of these disorders is not yet known.<sup>7</sup>
- Prior to the widespread availability of next generation sequencing (NGS), most sequencing tests used the Sanger sequencing methodology that was originally developed in the 1970s. Sanger sequencing is labor intensive and did not lend itself to high-throughput applications.
- NGS, which is also sometimes called massively parallel sequencing, has been developing since about 2005 to allow larger scale and more efficient gene sequencing. NGS relies on sequencing many copies of small pieces of DNA simultaneously and using bioinformatics to assemble the sequence.
- The efficiency of NGS has led to an increasing number of large, multi-gene testing panels. NGS panels that test several genes at once are particularly well-suited to conditions caused by more than one gene or where there is considerable clinical overlap between conditions, making it difficult to reliably narrow down likely causes. As a result, several laboratories have begun to combine genes involved in certain conditions, which often have both of those characteristics. However, NGS may not perform as well as Sanger sequencing in some applications. Results may also be obtained that cannot be adequately interpreted based on the current knowledgebase. When a sequence variation is identified that has not been previously characterized or shown to cause the disorder in question, it is called a variant of uncertain significance (VUS). VUSs are relatively common findings when sequencing large amounts of DNA.
- HCTD multi-gene panels include a wide variety of genes associated with connective tissue disorders. Multi-gene panels may also include genes believed to be associated with HCTDs, but with a lower impact on risk than recognized syndromes. Results for such genes are of less clear value because there often are not clear management recommendations for mutation-positive individuals.
- Under certain circumstances, technologies used in multi-gene testing may fail to identify mutations that might be identifiable through single-gene testing. If high clinical suspicion remains for a particular syndrome after negative multi-gene test results, consultation with the testing lab and/or additional targeted genetic testing may be warranted.
- Multi-gene tests vary in technical specifications (e.g., depth of coverage, extent of intron/exon boundary analysis, methodology of large deletion/duplication analysis).
- Since genes can be easily added or removed from multi-gene tests over time by a given lab, medical records must document which genes were included in the specific multi-gene test used from each patient, and in which labs they were performed.
- Additionally, tests should be chosen that maximize the likelihood of identifying mutations in the genes of interest and that will alter patient management.

## Guidelines and evidence

- No current U.S guidelines address the use of multi-gene panels in HCTDs.
- An expert-authored review (updated in 2016)<sup>15</sup> states the following regarding hEDS: “If a patient’s personal or family history is suggestive of one of the other types of EDS or another hereditary disorder of connective tissue or arterial fragility syndrome, analysis of an associated gene or multi-gene connective tissue disease panel may be appropriate. Failure to identify a pathogenic variant with such multiple gene testing reduces the likelihood of an arterial fragility syndrome, but does not completely rule it out, especially in the setting of a positive personal or family history of arterial fragility. Negative testing for an arterial fragility syndrome also does not confirm a diagnosis of EDS, hypermobility type. Therefore, such testing is not recommended in the absence of specific suggestive signs, symptoms, or family history.”
- According to the International Consortium on the Ehlers-Danlos Syndromes (2017):<sup>6</sup>
  - “In view of the vast genetic heterogeneity and phenotypic variability of the EDS subtypes, and the clinical overlap between many of these subtypes, but also with other HCTDs, the definite diagnosis relies for all subtypes, except hEDS, on molecular confirmation with identification of (a) causative variant(s) in the respective gene.”
  - “Molecular diagnostic strategies should rely on NGS technologies, which offer the potential for parallel sequencing of multiple genes. Targeted resequencing of a panel of genes...is a time- and cost-effective approach for the molecular diagnosis of the genetically heterogeneous EDS. When no mutation (or in case of an autosomal recessive condition only one mutation) is identified, this approach should be complemented with a copy number variant (CNV) detection strategy to identify large deletions or duplications, for example Multiplex Ligation-dependent Probe Amplification (MLPA), qPCR, or targeted array analysis.”
  - “The diagnosis of hEDS remains clinical as there is yet no reliable or appreciable genetic etiology to test for in the vast majority of patients.”

## Criteria

This guideline applies to hereditary connective tissue disorder testing, including single genes as well as multi-gene panels, which are defined as assays that simultaneously test for more than one hereditary connective tissue disorder gene. Medical necessity determination generally relies on criteria established for testing individual genes.

Medical necessity criteria differ based on the type of testing being performed (i.e., individual hereditary connective tissue disorder genes separately chosen versus pre-defined panels of genes) and how that testing will be billed (one or more individual gene procedure codes, specific panel procedure codes, or unlisted procedure codes).

***Hereditary Connective Tissue Disorder single gene tests will be reimbursed when the following criteria are met:***

- The member has or is suspected to have a condition that will benefit from information provided by the requested hereditary connective tissue disorder gene testing based on at least one of the following:
  - The member displays clinical features of the condition for which testing is being requested and a genetic diagnosis would result in changes to the member's medical management, OR
  - The member meets all criteria in a test-specific guideline, if available (see *table: Common hereditary connective tissue disorder genes, associated conditions, and applicable guidelines*), AND
- The member does not have a known underlying cause for their symptoms (e.g. known genetic condition), AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

***Hereditary Connective Tissue Disorder multi-gene panels will be reimbursed when the following criteria are met:***

When separate procedure codes will be billed for individual hereditary connective tissue disorder genes (e.g., Tier 1 MoPath codes 81200-81355 or Tier 2 MoPath codes 81400-81408), each individually billed test will be evaluated separately. The following criteria will be applied:

- The member has or is suspected to have a condition that will benefit from information provided by the requested hereditary connective tissue disorder gene testing based on at least one of the following:
  - The member displays clinical features of the condition for which testing is being requested and a genetic diagnosis would result in changes to the member's medical management, OR
  - The member meets all criteria in a test-specific guideline, if available, (see *Common hereditary connective tissue disorder genes, associated conditions, and applicable guidelines* table for a list of genes, associated conditions, and applicable guideline), AND
- The member does not have a known underlying cause for their symptoms (e.g. known genetic condition), AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

When a patient meets medical necessity criteria for any hereditary connective tissue disorder gene(s) included in the panel, genetic testing for the clinically indicated gene(s) will be reimbursed. This includes the sequencing and deletion/duplication<sup>†</sup> components. Any genes that are included in a multi-gene panel but do NOT meet medical necessity criteria will NOT be a reimbursable service. It will be at the

laboratory, provider, and patient's discretion to determine if a multi-gene panel remains the preferred testing option.

When a multi-gene panel is being requested and will be billed with a single panel CPT code (e.g. 81410, 81479) to represent all genes being sequenced, with or without another single procedure code representing the deletion/duplication<sup>†</sup> analysis portion, the panel will be considered medically necessary when the following criteria are met:

- Medical necessity must be established for at least TWO conditions included in the panel based on the following:
  - The member displays clinical features of the condition for which testing is being requested and a genetic diagnosis would result in changes to the member's medical management, OR
  - The member meets all criteria in a test-specific guideline, if available, (see *table: Common hereditary connective tissue disorder genes, associated conditions, and applicable guidelines*), AND
- The member does not have a known underlying cause for their symptoms (e.g. known genetic condition), AND
- Clinical features are not sufficiently specific to suggest a single causative gene, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

<sup>†</sup>When deletion/duplication testing is not part of a single panel CPT code being billed, deletion/duplication testing should be billed in only one of the following ways:

- A separate CPT code for deletion/duplication analysis of each individual gene (may include non-specific molecular pathology tier 2 codes and/or unlisted code 81479), or
- A single CPT code specific to the performed deletion/duplication analysis panel (e.g. 81411, 81479), or
- A single microarray procedure (e.g. 81228 or 81229).

Procedure codes representing multiple methods for deletion/duplication testing will not be reimbursable for the same panel (e.g., test-specific deletion/duplication procedure codes and microarray will not both be reimbursable for the same panel).

### Exceptions

The following are specifically non-reimbursed indications for Hereditary Connective Tissue Disorder testing:

- Members personal and/or family history are suggestive of hypermobile EDS or the related clinical entity, "joint hypermobility syndrome"

- Isolated joint hypermobility, including both asymptomatic and symptomatic forms (e.g., “hypermobility spectrum disorders”)

### Billing and reimbursement considerations

- The billed amount should not exceed the list price of the test.
- Broad connective tissue disorder panels may not be medically necessary when a narrower panel is available and more appropriate based on the clinical findings.
- Genetic testing is only necessary once per lifetime. Therefore, a single gene included in a panel or a multi-gene panel may not be reimbursed if testing has been performed previously. Exceptions may be considered if technical advances in testing demonstrate significant advantages that would support a medical need to retest.
- This guideline may not apply to genetic testing for indications that are addressed in test-specific guidelines. Please see the test-specific list of guidelines for a complete list of test-specific panel guidelines.
- If a panel was previously performed and an updated, larger panel is being requested, only testing for the medically necessary, previously untested genes will be reimbursable. Therefore, only the most appropriate procedure codes for those additional genes will be considered for reimbursement.

### Common hereditary connective tissue disorder genes, associated conditions, and applicable guidelines

#### *Common hereditary connective tissue disorder genes, associated conditions, and applicable guidelines*

| Condition                              | Gene     | CPT   | Applicable guideline |
|--|----------|-------|----------------------|
| Arterial tortuosity syndrome           | SLC2A10  | 81479 | MOL.TS.268           |
| Congenital contractural arachnodactyly | FBN2     | 81479 | MOL.TS.268           |
| Cutis laxa                             | ALDH18A1 | 81479 | MOL.TS.268           |
|  | ATP6V0A2 | 81479 | MOL.TS.268           |
|  | EFEMP2   | 81479 | MOL.TS.268           |
|  | ELN      | 81479 | MOL.TS.268           |



| Condition                    | Gene    | CPT   | Applicable guideline |
|------------------------------|---------|-------|----------------------|
|                              | FBLN5   | 81479 | MOL.TS.268           |
|                              | LTBP4   | 81479 | MOL.TS.268           |
|                              | PYCR1   | 81479 | MOL.TS.268           |
| Ehlers-Danlos syndrome (EDS) | ADAMTS2 | 81479 | MOL.TS.267           |
|                              | B3GALT6 | 81479 | MOL.TS.267           |
|                              | B4GALT7 | 81479 | MOL.TS.267           |
|                              | C1R     | 81479 | MOL.TS.267           |
|                              | C1S     | 81479 | MOL.TS.267           |
|                              | CHST14  | 81479 | MOL.TS.267           |
|                              | COL1A1  | 81408 | MOL.TS.267           |
|                              | COL1A2  | 81408 | MOL.TS.267           |
|                              | COL12A1 | 81479 | MOL.TS.267           |
|                              | COL3A1  | 81479 | MOL.TS.267           |
|                              | COL5A1  | 81479 | MOL.TS.267           |
|                              | COL5A2  | 81479 | MOL.TS.267           |
|                              | DSE     | 81479 | MOL.TS.267           |
|                              | FKBP14  | 81479 | MOL.TS.267           |
|                              | PLOD1   | 81479 | MOL.TS.267           |
|                              | PRDM5   | 81479 | MOL.TS.267           |

| Condition   | Gene     | CPT         | Applicable guideline |
|---|----------|-------------|----------------------|
|   | SLC39A13 | 81479       | MOL.TS.267           |
|   | TNXB     | 81479       | MOL.TS.267           |
|   | ZNF469   | 81479       | MOL.TS.267           |
| Homocystinuria (cystathionine beta-synthase deficiency)   | CBS      | 81401 81406 | MOL.TS.268           |
| Juvenile polyposis/hereditary hemorrhagic telangiectasia  | SMAD4    | 81406       | MOL.TS.268           |
|   | SMAD4    | 81405       | MOL.TS.268           |
| Loeys-Dietz syndrome                                      | SMAD3    | 81479       | MOL.TS.268           |
|   | SMAD2    | 81479       | MOL.TS.268           |
|   | TGFB2    | 81479       | MOL.TS.268           |
|   | TGFB3    | 81479       | MOL.TS.268           |
|   | TGFBR1   | 81405       | MOL.TS.268           |
|   | TGFBR2   | 81405       | MOL.TS.268           |
| MED12-related disorders                                   | MED12    | 81401 81479 | MOL.TS.268           |
| Marfan syndrome   | FBN1     | 81408       | MOL.TS.202           |
|   | TGFBR1   | 81405       | MOL.TS.202           |
|   | TGFBR2   | 81405       | MOL.TS.202           |
| NOTCH1-related aortic valve disease/Adams-Oliver syndrome | NOTCH1   | 81407       | MOL.TS.268           |

| Condition                                      | Gene    | CPT   | Applicable guideline |
|--|---------|-------|----------------------|
| Occipital horn syndrome/Menkes                 | ATP7A   | 81479 | MOL.TS.268           |
| Osteogenesis imperfecta                        | COL1A1  | 81408 | MOL.TS.268           |
|  | COL1A2  | 81408 | MOL.TS.268           |
| Periventricular nodular heterotopia            | FLNA    | 81479 | MOL.TS.268           |
| Pseudoxanthoma elasticum                       | ABCC6   | 81479 | MOL.TS.268           |
| Shprintzen-Goldberg syndrome                   | SKI     | 81479 | MOL.TS.268           |
| Stickler syndrome                              | COL11A1 | 81479 | MOL.TS.268           |
|  | COL11A2 | 81479 | MOL.TS.268           |
|  | COL2A1  | 81479 | MOL.TS.268           |
|  | COL9A1  | 81479 | MOL.TS.268           |
|  | COL9A2  | 81479 | MOL.TS.268           |
|  | COL9A3  | 81479 | MOL.TS.268           |
| Thoracic aortic aneurysm and dissection (TAAD) | ACTA2   | 81405 | MOL.TS.227           |
|  | BGN     | 81479 | MOL.TS.227           |
|  | COL3A1  | 81479 | MOL.TS.227           |
|  | FBN1    | 81408 | MOL.TS.227           |
|  | MAT2A   | 81479 | MOL.TS.227           |
|  | MFAP5   | 81479 | MOL.TS.227           |

| Condition | Gene   | CPT   | Applicable guideline |
|-----------|--------|-------|----------------------|
|           | MYH11  | 81408 | MOL.TS.227           |
|           | MYLK   | 81479 | MOL.TS.227           |
|           | PRKG1  | 81479 | MOL.TS.227           |
|           | SMAD3  | 81479 | MOL.TS.227           |
|           | TGFB2  | 81479 | MOL.TS.227           |
|           | TGFB3  | 81479 | MOL.TS.227           |
|           | TGFBR1 | 81405 | MOL.TS.227           |
|           | TGFBR2 | 81405 | MOL.TS.227           |

**Note** Several genes in this table are associated with multiple genetic disorders, including some not listed above. The test should be reviewed for the appropriate condition/indication.

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# HFE Hemochromatosis Testing

MOL.TS.183.A

v1.0.2020

## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedure addressed by this guideline            | Procedure code |
|--|----------------|
| HFE Targeted Mutation Analysis (common variants) | 81256          |
| HFE Sequence Analysis                            | 81479          |
| HFE Deletion/Duplication Analysis                | 81479          |

## What is HFE hemochromatosis

### Definition

HFE hemochromatosis is characterized by inappropriately high absorption of iron by the small intestinal mucosa.

There is a phenotypic spectrum of HFE hemochromatosis which includes:

- Clinical HFE hemochromatosis, where individuals manifest end-organ damage secondary to iron overload;
- Biochemical HFE hemochromatosis, where transferrin-iron saturation is increased and the only evidence of iron overload is increased serum ferritin concentration; and
- Non-expressing C282Y homozygotes, where individuals have neither clinical manifestations of HFE hemochromatosis nor iron overload.

Clinical HFE hemochromatosis leads to excess iron absorption and storage in the liver, heart, pancreas, and other organs.<sup>1</sup> Individuals who are untreated may experience the following symptoms: abdominal pain, weakness, lethargy, weight loss, arthralgias, diabetes mellitus, and increased risk of cirrhosis when the serum ferritin is higher than 1,000 ng/mL. Other findings may include progressive increase in skin pigmentation, congestive heart failure, and/or arrhythmias, arthritis, and hypogonadism. Clinical HFE hemochromatosis is more common in men than women.<sup>1</sup>

HFE hemochromatosis is caused by mutations in the HFE gene and is inherited in an autosomal recessive manner.<sup>1</sup> About 1 in 200 to 1 in 400 non-Hispanic whites in North America are affected with HFE hemochromatosis.<sup>2</sup>

Among individuals of northern European ancestry, the prevalence of individuals homozygous for HFE C282Y variant is 2:1,000 to 5:1,000. In non-Hispanic whites in North America, the prevalence of HFE C282Y homozygotes is 1:200 to 1:400. The disorder is less common among African Americans, Hispanics, and Asians.<sup>1</sup>

HFE hemochromatosis can be effectively treated in most people. Phlebotomy therapy can alleviate almost all symptoms of iron overload if initiated before organ damage occurs.<sup>3</sup>

When HFE hemochromatosis is suspected, serum iron studies, including transferrin saturation (TS) or unsaturated iron-binding capacity, are the first step in establishing a diagnosis. HFE genetic testing is recommended if TS greater than or equal to 45%.<sup>4</sup>

Current guidelines support HFE genetic testing in people with:<sup>2,4-5</sup>

- Serologic evidence of iron overload, considered to be a transferrin saturation greater than or equal to 45% and elevated ferritin
- A known family history of hemochromatosis
- A known family mutation in the HFE gene in a first degree relative

## Test information

- HFE Mutation Analysis
  - Common changes in the HFE gene associated with HFE hemochromatosis are C282Y, H63D, and S65C.<sup>1</sup>
  - C282Y and H63D are the most common and account for 87% of hereditary hemochromatosis in European populations.<sup>1</sup> The next most common cause are individually rare mutations.<sup>6</sup> Many labs do not test for S65C because it accounts for <1% of HFE hemochromatosis.<sup>1</sup> There is controversy over whether the H63D variant causes clinical disease<sup>2</sup>. The combination of these mutations determines both the chances of symptoms occurring and their severity.
- HFE sequencing and deletion/duplication analysis is also available and may be necessary for individuals who do not have northern European ancestry.<sup>1</sup>

## Guidelines and evidence

- The American Association for the Study of Liver Diseases (AASLD) Practice Guidelines (2011):<sup>6</sup>
  - “In a patient with suggestive symptoms, physical findings, or family history, a combination of transferrin saturation (TS) and ferritin should be obtained rather than relying on a single test. (1B) If either is abnormal (TS ≥45% or ferritin above the upper limit of normal), then HFE mutation analysis should be performed. (1B)”



- “The guideline developers recommend screening (iron studies and HFE mutation analysis) of first-degree relatives of patients with HFE-related HH to detect early disease and prevent complications”
- Screening for Hereditary Hemochromatosis: A Clinical Practice Guideline from the American College of Physicians (2005):<sup>3</sup>
  - “Physicians should discuss the risks, benefits, and limitations of genetic testing in patients with a positive family history of hereditary hemochromatosis or those with elevated serum ferritin level or transferrin saturation. Before genetic testing, individuals should be made aware of the benefits and risks of genetic testing. This should include discussing available treatment and its efficacy; costs involved; and social issues, such as impact of disease labeling, insurability and psychological well-being, and the possibility of as-yet-unknown genotypes associated with hereditary hemochromatosis.”

## Criteria

### HFE known familial mutation testing

- Clinical Consultation:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - No previous genetic testing of the HFE gene, AND
- Presymptomatic/Asymptomatic Genetic Testing:
  - HFE mutation identified in 1<sup>st</sup> degree biological relative, OR
- Diagnostic Testing:
  - Serologic evidence of iron overload, defined as transferrin saturation greater than or equal to 45% and/or elevated ferritin, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

### HFE targeted mutation testing

- Clinical Consultation:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - No previous genetic testing of the HFE gene, AND

- Presymptomatic/Asymptomatic Genetic Testing:
  - Documented family history of first-degree relative with HFE hemochromatosis, OR
- Diagnostic Testing:
  - Serologic evidence of iron overload, defined as transferrin saturation greater than or equal to 45% and/or elevated ferritin, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

### **HFE gene sequence analysis**

- Clinical Consultation:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - No previous sequencing of the HFE gene, and
  - Previous targeted HFE genetic testing performed and zero or one mutation identified, AND
- Diagnostic Testing:
  - Serologic evidence of iron overload, defined as transferrin saturation greater than or equal to 45% and/or elevated ferritin, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

### **HFE deletion/duplication analysis**

- Clinical Consultation:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - No previous deletion/duplication analysis of the HFE gene, and
  - Previous HFE sequencing performed and zero or one mutation identified, AND
- Diagnostic Testing:
  - Serologic evidence of iron overload, defined as transferrin saturation greater than or equal to 45% and/or elevated ferritin, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

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# Genetic Testing for Hereditary Pancreatitis

MOL.TS.287.A

v1.0.2020

## Introduction

Genetic testing for hereditary pancreatitis is addressed by this guideline.

## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedures addressed by this guideline  | Procedure codes |
|---|-----------------|
| CASR Sequencing                         | 81405           |
| CFTR Deletion/Duplication Analysis      | 81222           |
| CFTR Known Familial Mutation Analysis   | 81221           |
| CFTR Sequencing                         | 81223           |
| CFTR Targeted Mutation Analysis         | 81479           |
| CLDN2 Sequencing                        | 81479           |
| CPA1 Sequencing                         | 81479           |
| CTRC Known Familial Mutation Analysis   | 81403           |
| CTRC Sequencing                         | 81405           |
| PRSS1 Deletion/Duplication Analysis     | 81479           |
| PRSS1 Known Familial Mutation Analysis  | 81403           |
| PRSS1 Sequencing                        | 81404           |
| PRSS1 Targeted Mutation Analysis        | 81401           |
| SPINK1 Deletion/Duplication Analysis    | 81479           |
| SPINK1 Known Familial Mutation Analysis | 81403           |
| SPINK1 Sequencing                       | 81404           |
| Hereditary Pancreatitis Multigene Panel | 81479           |

## What is pancreatitis

### Definition

Pancreatitis is inflammation of the pancreas that may be acute, acute recurrent, or chronic.<sup>1</sup>

Acute pancreatitis is defined as two of the three following findings:<sup>2</sup>

- Abdominal pain
- Elevated serum amylase or lipase (greater than 3x the upper limit of normal)
- Findings consistent with pancreatic inflammation on abdominal imaging

Acute recurrent pancreatitis is defined as multiple (2 or more), discrete episodes of acute pancreatitis without any evidence of chronic pancreatitis. There must be complete resolution of clinical and laboratory findings between episodes.

Chronic pancreatitis is defined as an irreversible fibro-inflammatory process which leads to permanent changes in the pancreatic parenchyma and function. It may be documented by one of the following:<sup>1,2</sup>

- Abdominal imaging
- Functional studies (e.g. pancreatic exocrine insufficiency or pancreatic endocrine insufficiency with diabetes mellitus)
- Histology

Idiopathic sporadic pancreatitis is when a single individual in a family is affected, and the etiology is unknown despite comprehensive investigations.

Familial pancreatitis is pancreatitis of any cause (genetic or non-genetic) that occurs in a family with a greater incidence than would be expected by chance alone.<sup>1</sup>

Hereditary pancreatitis (HP) is a rare cause of acute, acute recurrent, and chronic pancreatitis. It is defined as a personal history of pancreatitis and pancreatitis diagnosed in two first-degree relatives or in three second degree relatives spanning at least two generations. Beginning with the first report of PRSS1 mutation in a family with HP, it has been shown that multiple genetic risk factors are associated with this disease.<sup>3</sup>

Mutations in the following genes contribute to the development of acute recurrent and chronic pancreatitis:<sup>1</sup>

- PRSS1 mutations are the most common cause of hereditary pancreatitis.<sup>1,2</sup> They follow autosomal dominant inheritance and have a penetrance of approximately 80%. Since 1996, more than 35 mutations in PRSS1 have been found to be associated with hereditary pancreatitis.<sup>4</sup>
- SPINK1 mutations have been associated with a risk for autosomal recessive HP. There is evidence that heterozygous SPINK1 mutations increase the severity of

acute recurrent and chronic pancreatitis due to mutations in PRSS1, CFTR, CASR, or CTRC.<sup>1,4</sup>

- CFTR mutations follow autosomal recessive inheritance, and individuals with biallelic CFTR pathogenic variants may have atypical cystic fibrosis (CF), putting them at risk for additional manifestations such as lung disease, male infertility, and chronic sinusitis. All CFTR mutations that cause CF are also risk factors for pancreatitis; however, mutations that do not cause classic CF may still be risk factors for pancreatitis.<sup>1</sup>
- CTRC mutations have been identified in individuals with acute recurrent and chronic pancreatitis. These variants were initially thought to be modifier genes, however they have been shown to be sufficient to cause disease without other identifiable genetic or environmental risk factors.<sup>5</sup>
- CASR mutations may be a predisposing genetic factor for pancreatitis either in isolation or as modifying risk when other genetic causes are present.<sup>6</sup>
- CLDN2, CPA1, and GGT1 variants have been implicated as risk factors or modifiers for chronic pancreatitis, but less is known about the utility of screening for these mutations compared to the others mentioned above.
- While single pathogenic variants in SPINK1, CFTR, and CTRC may be associated with an increased risk of pancreatitis, additional unidentified modifying factors may contribute to the disease. Double heterozygotes appear to have a further increased risk.<sup>1</sup>
- Rare disorders that include pancreatitis/pancreatic insufficiency as part of a more complex syndrome include Schwachman-Diamond syndrome (SBDS), mitochondrial DNA deletions, CEL-associated maturity-onset diabetes of the young (MODY), and Johanson-Blizzard syndrome (UBR1).<sup>1</sup>

Treatment of HP focuses on longitudinal monitoring of endocrine and exocrine pancreatic function, enzyme and nutritional supplementation, pain management and monitoring for complications (such as decreased bone mineral density and fat soluble vitamin deficiencies). Endoscopic and surgical therapies may be necessary in some cases. Affected people are discouraged from smoking and drinking alcohol.

Up to 5% of patients with chronic pancreatitis may develop pancreatic cancer. The efficacy of pancreatic cancer screening has not been proven, and this screening is typically recommended to take place in a research setting.<sup>7</sup>

## Test information

### Introduction

Gene mutations and variants have been detected in the CFTR, CTRC, PRSS1, and SPINK1 genes in people with hereditary pancreatitis (HP).<sup>1</sup> Most testing laboratories perform sequence analysis using next generation sequencing (NGS).

The mutation detection rate for PRSS1 sequencing is 60-100%, and deletion/duplication analysis is at least 6%.<sup>2</sup> N29I (p.Asn29Ile) and R122H (p.Arg122His) variants account for approximately 90% of cases of pathogenic variants observed in PRSS1-related HP.<sup>2</sup> The majority of SPINK1 mutations are sequence variants, with deletions having been reported in a very small number of cases.<sup>1</sup> The frequency of CFTR deletions in HP has not been investigated; however they occur rarely in cystic fibrosis (approximately 1%).<sup>1</sup>

Test results particularly for the PRSS1 gene, may offer prognostic information since the risk of pancreatic cancer in those with chronic pancreatitis is significantly increased. However, genetic testing cannot predict the age of onset or disease severity.<sup>1,7</sup>

Identifying a mutation in an affected individual can be used to test at-risk family members with familial mutation analysis.

## Guidelines and evidence

### Introduction

The following section includes relevant guidelines and evidence pertaining to genetic testing for hereditary pancreatitis.

### United European Gastroenterology

United European Gastroenterology (2018) guidelines on chronic pancreatitis state:<sup>8</sup>

- “A diagnosis of cystic fibrosis needs to be ruled out in all patients with CP onset before the age of 20 years as well as in patients with so-called ‘idiopathic’ CP (regardless of the age of onset). (GRADE 1B, strong agreement)”
- “All patients with a family history or early onset disease (less than 20 years) should be offered genetic testing for associated variants. (GRADE 2C, strong agreement)”
- “Genetic testing was recommended to include PRSS1, SPINK1, CPA1, CTRC, CEL, and “may include screening for variants in CFTR. (GRADE 2C, strong agreement)”

### 2017 Expert authored review

A 2017 expert authored review on pediatric acute recurrent and chronic pancreatitis concluded that:<sup>9</sup>

- “The search for a genetic cause of ARP or CP should include a sweat chloride test (even if newborn screening for cystic fibrosis (CF) is negative) and PRSS1 gene mutation testing. Genetic testing for CF should be considered if a sweat test is unable to be performed.” (Strong consensus, definitely yes)
- “Mutation analysis of the genes SPINK1, CFTR and CTRC may identify risk factors for ARP or CP.” (Strong consensus, definitely yes).

## 2016 Expert authored review

A 2016 expert-authored review on hereditary pancreatitis states:<sup>10</sup>

- “[...] targeted genetic testing of members of an established HP family may be considered in cases of unexplained recurrent acute pancreatitis or chronic pancreatitis, an affected individual with a first or second-degree relative with pancreatitis, unexplained pancreatitis in a child requiring hospitalization and/or when there is a known mutation in the family.”
- “[...] next generation sequencing approaches such as whole exome sequencing or whole genome sequencing should not be used for PRSS1 testing because of challenges in sequence alignment. If a mutation is not identified from sequencing or targeted mutation analysis, deletion/duplication analysis can be considered.”
- “In families where a deleterious variant has been identified, predictive genetic testing may be considered in close family members...Genetic testing of asymptomatic family members in a family without an identifiable mutation is uninformative.”
- “Genetic testing may be indicated in a child with diagnosed or suspected pancreatitis...Predictive genetic testing for asymptomatic patients less than 16 years of age is not recommended and does not have clear benefits.”

## American College of Gastroenterology

American College of Gastroenterology (ACG, 2015) guidelines on genetic testing for hereditary gastrointestinal cancer syndromes state that having a history of hereditary pancreatitis is a risk factor for familial pancreatic adenocarcinoma, and genetic testing for pancreatitis-associated genes should be considered for pancreatic cancer patients with “a personal history of at least 2 acute attacks of acute pancreatitis of unknown etiology, a family history of pancreatitis, or early-age onset chronic pancreatitis.”<sup>11</sup>

## American Pancreatic Association

American Pancreatic Association (2014) guidelines state “Several genetic variations have been associated with pancreatitis including PRSS1, PRSS2, SPINK1, CTSC, CASR and CFTR. The role of these gene mutations in CP is becoming increasingly recognized and better understood.” It is also noted that “knowledge of gene, gene-environment interactions may translate into new diagnostic and treatment paradigms” (Strong recommendation, level of evidence – moderate)<sup>12</sup>

## 2014 Expert authored review

A 2014 expert-authored review on pancreatitis recommends molecular genetic testing in a proband with pancreatitis and at least of one of the following:<sup>1</sup>

- “An unexplained documented episode of acute pancreatitis in childhood”
- “Recurrent acute attacks of pancreatitis of unknown cause”



- “Chronic pancreatitis of unknown cause, particularly with onset before age 25 years”
- “A history of at least one relative with recurrent acute pancreatitis, chronic pancreatitis of unknown cause, or childhood pancreatitis of unknown cause”
- PRSS1 sequencing is recommended, followed by deletion/duplication analysis if sequencing is negative. Alternatively, a multi-gene panel that includes PRSS1, SPINK1, CFTR, and CTRC may be appropriate.

### **American College of Gastroenterology**

The American College of Gastroenterology (ACG, 2013) guideline on management of acute pancreatitis states: “Genetic testing may be considered in young patients (<30 years old) if no cause is evident and a family history of pancreatic disease is present (conditional recommendation, low quality of evidence).”<sup>13</sup>

### **2010 Expert authored review**

A 2010 expert-authored review on genetic testing in pancreatitis states:<sup>14</sup>

- “Because of the high penetrance (80%) of the more common PRSS1 mutations, especially R112H and N29I, testing is generally accepted for diagnostic purposes in symptomatic individuals. The confirmation of a genetic etiology of pancreatitis provides a valid explanation for both symptoms and/or disease, and may be helpful to predict a lack of efficacy with various endoscopic or operative procedures.”
- “[T]here is currently no clinical indication for the routine use of SPINK1 mutation testing for either diagnostic or screening purposes and has no implications in altering the management of patients with pancreatitis.”
- “[T]he CTRC gene that is the most recently identified pancreatitis susceptibility gene, should be approached in a similar fashion to SPINK1 as it is also associated with a very low penetrance.”
- Regarding testing for CFTR mutations, “In subjects presenting with pancreatitis, the overwhelming rationale for further testing is to exclude or confirm the diagnosis of CF [cystic fibrosis]. The traditional sweat test remains the primary diagnostic test for CF disease in the genomic age. In any symptomatic individual, diagnostic testing should include sweat testing as the primary test and referral to a CF clinic made if sweat chloride concentration is borderline (40-59 mmol/L) or abnormal (>60 mmol/L). CFTR mutation analysis in isolation, as the first-line clinical diagnostic test, is unlikely to change management but may instead give false reassurance of the absence of CF if CFTR genotyping fails to identify mutations or alternatively be inappropriately thought to be diagnostic of CF... [T]here is currently no rationale for CFTR mutation screening for risk of pancreatitis alone.”

## Fourth International Symposium of Inherited Diseases of the Pancreas

The Fourth International Symposium of Inherited Diseases of the Pancreas (2007) recommended that symptomatic patients be referred for genetic counseling to consider PRSS1 testing when at least one of the following conditions are met, in order to determine if they may be candidates for pancreatic cancer surveillance:<sup>15</sup>

- “≥2 attacks of acute pancreatitis of unknown etiology”
- “Idiopathic chronic pancreatitis, particularly if disease onset occurs <25 years of age”
- “One first-degree or second-degree relative with pancreatitis”
- “Unexplained documented episode of childhood pancreatitis that required hospitalization and where there is concern that HP should be excluded.”
- “Asymptomatic people should be referred for genetic counseling to consider testing for a PRSS1 mutation when the patient has one first-degree relative with a defined HP gene mutation.”

### 2007 Expert authored review

A 2007 expert-authored guideline on nonsyndromic pancreatitis states that genetic testing should be considered when an affected patient fulfills at least one of the following criteria:<sup>16</sup>

- “A family history of recurrent acute pancreatitis, idiopathic chronic pancreatitis, or childhood pancreatitis without a known cause”
- “Relatives known to carry mutations associated with pancreatitis”
- “A series of recurrent acute attacks of pancreatitis for which there is no other explanation”
- “An unexplained documented episode of pancreatitis as a child”
- “Idiopathic chronic pancreatitis (especially when onset of pancreatitis precedes age 25)”
- “Patients eligible for approved research protocols”
- “[...] symptomatic family members at risk of inheriting a PRSS1 mutation may wish to be tested after a mutation has been identified in the family... Testing asymptomatic individuals for CFTR and SPINK1 mutations is not recommended because a large fraction of those who carry mutations in these genes never develop pancreatitis. CFTR carrier testing should be offered to unaffected relatives of a CFTR mutation that is capable of causing classic CF.”

## 2007 Expert authored review

A 2007 expert-authored review on hereditary pancreatitis recommends PRSS1 and SPINK1 mutation testing in symptomatic patients with one of the following:<sup>17</sup>

- “recurrent unexplained attacks of acute pancreatitis and positive family history”
- “unexplained chronic pancreatitis and a positive family history”
- “unexplained chronic pancreatitis without a positive family history after exclusion of other causes”
- “unexplained pancreatitis episode in children”

## CLDN2, CEL, and PNLIP genes

Pathogenic variants in the CLDN2, CEL, and PNLIP genes may result in an increased risk of developing pancreatitis as mutations in these genes are enriched in chronic pancreatitis patient populations. However, current data remains limited and the clinical utility of screening for these genetic variants is uncertain.

## Criteria

### Introduction

Requests for genetic testing for hereditary pancreatitis are reviewed using the following criteria.

### Known Familial Mutation Analysis

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - No previous genetic testing for known familial mutation, and
  - Pathogenic pancreatitis-associated mutation(s) in a 1st degree biologic relative, AND
- Diagnostic Testing in Symptomatic Individuals:
  - Member is symptomatic (at least one documented episode of acute pancreatitis or a diagnosis of acute recurrent or chronic pancreatitis), OR
- Predisposition Testing for Presymptomatic/Asymptomatic Individuals:
  - Age 16 years or older

## PRSS1 Analysis

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Testing:
  - No previous PRSS1 analysis, AND
- Diagnostic Testing for Symptomatic Individuals:
  - An unexplained, documented episode of acute pancreatitis in an individual less than 18 years of age, or
  - Acute recurrent pancreatitis (2 or more documented episodes) or chronic pancreatitis, and
    - Symptom onset prior to age 25 years, and/or
    - A first degree biologic relative with recurrent acute pancreatitis, idiopathic chronic pancreatitis, or childhood pancreatitis (less than 18 years of age) without a known cause, and
  - No known etiology for the member's pancreatitis (e.g. alcoholism, gallstones, known genetic disorder), and
  - Absence of extra-pancreatic features suggestive of a complex genetic syndrome or cystic fibrosis (e.g. chronic sinopulmonary disease, failure-to-thrive, obstructive azoospermia due to congenital absence of the vas deferens, etc.), AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

## Pancreatitis Multigene Panel

When a multi-gene panel is being requested and will be billed with the appropriate CPT panel code, the panel will be considered medically necessary when the following criteria are met:

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Testing:
  - No previous multi-gene analysis, and
  - PRSS1 analysis, if previously performed, was negative, AND
- Diagnostic Testing for Symptomatic Individuals:

- An unexplained, documented episode of acute pancreatitis in an individual less than 18 years of age, or
  - Acute recurrent pancreatitis (2 or more documented episodes) or chronic pancreatitis, and
    - Symptom onset prior to age 25 years, or
    - A first degree biologic relative with recurrent acute pancreatitis, idiopathic chronic pancreatitis, or childhood pancreatitis (less than 18 years of age) without a known cause, and
  - No known etiology for the member's pancreatitis (e.g., alcoholism, gallstones, known genetic disorder), and
  - Absence of extra-pancreatic features suggestive of a complex genetic syndrome or cystic fibrosis (e.g., chronic sinopulmonary disease, failure-to-thrive, obstructive azoospermia due to congenital absence of the vas deferens, etc.), AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

### **CLDN2, PNLIP, and CEL Analysis**

These tests are considered investigational and/or experimental.

- Investigational and experimental (I&E) molecular and genomic (MolGen) tests refer to assays involving chromosomes, DNA, RNA, or gene products that have insufficient data to determine the net health impact, which typically means there is insufficient data to support that a test accurately assesses the outcome of interest (analytical and clinical validity), significantly improves health outcomes (clinical utility), and/or performs better than an existing standard of care medical management option. Such tests are also not generally accepted as standard of care in the evaluation or management of a particular condition.
- In the case of MolGen testing, FDA clearance is not a reliable standard given the number of laboratory developed tests that currently fall outside of FDA oversight and FDA clearance often does not assess clinical utility.

### **Billing and Reimbursement Considerations**

#### Paragraph

- When separate procedure codes will be billed for individual pancreatitis-associated genes (e.g., Tier 1 MoPath codes 81200-81355 or Tier 2 MoPath codes 81400-81408), the entire panel will be approved if the above criteria are met. However, the laboratory will be redirected to the use of an appropriate panel CPT code for billing purposes.
- The billed amount should not exceed the list price of the test.

- Broad gastrointestinal disease panels may not be medically necessary when a narrower panel is available and more appropriate based on the clinical findings.
- Genetic testing is only necessary once per lifetime. Therefore, a single gene included in a panel or a multi-gene panel may not be reimbursed if testing has been performed previously. Exceptions may be considered if technical advances in testing demonstrate significant advantages that would support a medical need to retest.
- If a panel was previously performed and an updated, larger panel is being requested, only testing for the medically necessary, previously untested genes will be reimbursable. Therefore, only the most appropriate procedure codes for those additional genes will be considered for reimbursement.
- If the laboratory will not accept redirection to a panel code, the medical necessity of each billed component procedure will be assessed independently.
  - In general only a limited number of panel components that are most likely to explain the member's presentation will be reimbursable. The remaining panel components will not be reimbursable.
  - When the test is billed with multiple stacked codes, only the following genes may be considered for reimbursement in a tiered fashion:
    - PRSS1
    - SPINK1
    - CFTR
    - CTSC

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**Note** This guideline applies to testing for nonsyndromic hereditary pancreatitis. This guideline does not apply to genetic testing for syndromes that may include pancreatitis as part of a more complex phenotype (e.g. Schwachman-Diamond syndrome, CEL-related MODY, mitochondrial DNA deletion disorders, Johanson-Blizzard syndrome). Testing for those disorders should be guided by an test-specific guidelines, if available (e.g. Maturity-Onset Diabetes of the Young (MODY) Testing and Mitochondrial DNA Deletion Syndromes), or applicable clinical use guidelines. This guideline does not address CFTR analysis for individuals suspected of having Cystic Fibrosis. For this indication, see the guideline Cystic Fibrosis Testing.

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## Introduction

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# HIV Tropism Testing for Maraviroc Response

MOL.TS.185.A

v1.0.2020

## Introduction

HIV tropism testing for maraviroc response is addressed by this guideline.

## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedures addressed by this guideline | Procedure codes |
|--|-----------------|
| HIV-1 Tropism Phenotyping              | 87999           |
| HIV-1 Tropism Genotyping, Common       | 87901           |
| HIV-1 Tropism Genotyping, Other        | 87906           |

## What is HIV tropism testing for Maraviroc response

### Definition

HIV tropism testing is used to help determine an individual's response to maraviroc (Selzentry®). Maraviroc is only effective against CCR5-tropic HIV-1.

### Human immunodeficiency virus (HIV)

HIV replicates itself in humans by infecting T-cells with CD4 receptors (often called CD4 cells). HIV-1 enters the CD4 cell by binding one of two cell surface co-receptors: CCR5 or CXCR4.<sup>1,2</sup>

#### Tropism classifications

Tropism is the ability of HIV-1 virus to use one or both of these co-receptors. There are three main tropism classifications:<sup>3</sup>

- **CCR5 tropism (R5-tropic)** — HIV-1 virus that only infects cells with the CCR5 co-receptor.
- **CXCR4 tropism (X4-tropic)** — HIV-1 virus that only infects cells with the CXCR4 co-receptor.

- **Dual or mixed tropism (D/M-tropic)** — HIV-1 virus populations that can use either co-receptor to infect cells.

### **Tropism classification changes**

CCR5-tropic virus predominates in early infection and treatment naïve patients.<sup>1-3</sup>

CXCR4 tropism increases both as the disease progresses and with treatment.<sup>1</sup> In later infection, CXCR4 tropism emerges in about 20% of treatment naïve patients.<sup>3</sup>

Treatment experienced patients have up to a 50% chance for the presence of CXCR4-tropic virus.<sup>1</sup>

### **Treatment**

Maraviroc is an antiretroviral drug that selectively binds to the CCR5 co-receptor. This blocks CCR5-tropic HIV-1 from binding to the co-receptor and entering the cell.<sup>4</sup>

#### **Contraindication**

Maraviroc is effective only against CCR5-tropic HIV-1. Patients with viruses using both the CXCR4 and CCR5 receptors (dual/mixed tropic) do not respond virologically to Maraviroc.<sup>4,5</sup> Therefore, Maraviroc is not indicated for CXCR4-tropic or dual or mixed-tropic HIV-1 infections.<sup>4</sup>

#### **Clinical resistance**

Virologic failure on Maraviroc can result from outgrowth of undetected CXCR4 virus as a result of Maraviroc treatment.<sup>4</sup>

### **Test information**

#### **Introduction**

HIV tropism testing may include phenotype testing or genotyping assays.

#### **When to test**

HIV tropism testing should be performed before Maraviroc therapy is initiated. Maraviroc should only be used in adults with CCR5-tropic HIV-1 infections based on those results.<sup>2,4</sup>

Testing may also be considered for patients with treatment failure on Maraviroc. Treatment failure is often associated with a switch to CXCR4 tropism.<sup>6</sup>

#### **Phenotype testing (Trofile® )**

Phenotype testing was the first method available and is most widely recommended.<sup>2,7</sup> Phenotyping works by exposing cell lines with CCR5 or CXCR4 co-receptors to virus

made with a patient's HIV-1 genes that control tropism. The virus' ability to infect each cell line is assessed based on the expression of a reporter gene.<sup>2,8</sup> The Trofile website states the assay is "100% sensitive at detecting 0.3% CXR4-using minor variant."<sup>8</sup> Patients enrolled in Maraviroc clinical trials were screened using the Trofile phenotype assay.<sup>8,9</sup> A newer, more sensitive version of the assay was subsequently released.<sup>2</sup>

## Genotyping assays

There are two genotypic assays used for tropism.

- The first assesses the V3-coding region of the HIV-1 envelope gene (the third variable loop, V3) which is the primary determinant of tropism. Quest Diagnostics' website states that sensitivity to detect X4 virus in 90% of dual-mixed samples is 18% X4 at a viral load of 25,000 copies/mL and 6% X4 at a viral load of 100,000 copies/mL. The genotyping assay assesses part of the HIV-1 envelope gene (the third variable loop, V3) that is the primary determinant of tropism. Quest Diagnostics' website states that sensitivity is 5% at a viral load of 10,000 HIV-1 copies/mL.<sup>10</sup>
- The second, HIV-1 proviral DNA genotypic tropism testing, is available for patients with HIV RNA <1,000 copies/mL. These assays evaluate HIV-1 proviral DNA integrated within infected cells for CXCR4-utilizing viral strains.<sup>11</sup>

## Guidelines and evidence

### Introduction

This section includes relevant guidelines and evidence pertaining to HIV tropism testing for maraviroc response.

### Department of Health and Human Services Panel

A Department of Health and Human Services Panel on Antiretroviral Guidelines for Adults and Adolescents (2018) recommends:<sup>2</sup>

- "Coreceptor tropism assay should be performed whenever the use of a CCR5 inhibitor is being considered." [Evidence level AI]
- "Coreceptor tropism testing is recommended in patients who exhibit virologic failure on a CCR5 inhibitor." [Evidence level BIII]
- "A phenotypic tropism assay is preferred to determine HIV-1 co-receptor usage." [Evidence level AI]
- "A genotypic tropism assay should be considered as an alternative test to predict HIV-1 co-receptor usage." [Evidence level BII]

- “A proviral DNA tropism assay can be utilized for patients with undetectable HIV-1 RNA when a CCR5 antagonist is considered for use in a new regimen (e.g., as part of a regimen switch or simplification).” [Evidence level BII]
- “Compared to genotypic testing, phenotypic testing has more evidence supporting its utility. Therefore, a phenotypic test for co-receptor usage is generally preferred [Evidence level AI]. However, because phenotypic testing is more expensive, requires more time to perform, and may have logistic challenges, a genotypic test to predict HIV-1 co-receptor usage should be considered as an alternative test” [Evidence level BII]

### **Infectious Diseases Society of America**

The Infectious Diseases Society of America (IDSA, 2013) guidelines agree that tropism testing should be done before starting any CCR5 antagonist. IDSA also states patients who exhibit virologic failure while taking a CCR5 antagonist may also be considered for tropism testing.<sup>7</sup>

### **Maraviroc**

Maraviroc (Selzentry<sup>®</sup>) has been approved for use in treatment-experienced patients 16 years of age and older with only CCR5-tropic HIV-1 virus and evidence of replication despite the use of several other antiretroviral therapies.<sup>4</sup> Regarding tropism testing, Maraviroc product labeling states that:<sup>4</sup>

- “Tropism testing must be conducted with a highly sensitive tropism assay that has demonstrated the ability to identify patients appropriate for SELZENTRY use.”
- “Use of SELZENTRY is not recommended in subjects with dual/mixed or CXCR4-tropic HIV-1 as efficacy was not demonstrated in a phase 2 study of this patient group.”

### **Criteria**

CCR5 tropism testing is considered medically necessary for the following individuals:

- Individuals with HIV-1 infection considering a CCR5 inhibitor, OR
- Individuals taking a CCR5 inhibitor who experience treatment failure

### **References**

### **Introduction**

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# HLA-B\*1502 Variant Analysis for Carbamazepine Response

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## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedure addressed by this guideline | Procedure code |
|---------------------------------------|----------------|
| HLA-B*1502 Genotyping                 | 81381          |

## What is HLA-B\*1502

### Definition

Variation in the HLA-B gene is associated with increased risk for adverse reactions to certain drugs. Testing positive for either one or two HLA-B\*1502 alleles increases a person's risk for a serious adverse skin reaction to carbamazepine.<sup>1,2</sup>

- Carbamazepine (Tegretol<sup>®</sup>, Tegretol XR<sup>®</sup>, Equetro<sup>®</sup>, Eptol<sup>®</sup>, Carbatrol<sup>®</sup>) is an antiepileptic agent used in the treatment of seizure disorders, psychiatric disorders, and pain from trigeminal neuralgia.
- A strong association between the risk of developing Stevens-Johnson syndrome (SJS) and/ or toxic epidermal necrolysis (TEN) with carbamazepine treatment and the presence of the inherited variant of the HLA-B gene, HLA-B\*1502, has been demonstrated in studies involving patients of Chinese ancestry. For this population, the risk of having a serious reaction is 10 times higher than the risk in Caucasians for which 1 to 6 per 10,000 new users of carbamazepine have a serious reaction to the drug.<sup>2-6</sup>
- Across Asian populations, notable variation exists in the prevalence of HLA-B\*1502. Individuals at highest risk are those of Han Chinese descent, followed by those in Vietnam, Cambodia, the Reunion Islands, Thailand, India (specifically Hindus), Malaysia, and Hong Kong. The frequency of HLA-B\*15:02 is very low in other populations.<sup>1</sup>
- Testing for HLA-B\*1502 should be performed prior to initiating carbamazepine treatment for most patients of Asian ancestry. Over 90% of carbamazepine treated patients who will experience SJS/TEN have this reaction within the first few months of treatment and providers should consider this in determining the need for screening at-risk patients who are currently on therapy.<sup>1</sup>

- Having HLA-B\*1502 is not abnormal, and there is no other known risk from having it.<sup>1</sup>

## Test information

- HLA-B\*1502 testing is performed using DNA extracted from whole blood or cheek cells. The test is positive if either one or two HLA-B\*1502 alleles are detected and negative if no HLA-B\*1502 alleles are detected.<sup>2</sup>

## Guidelines and evidence

- The Clinical Pharmacogenetic Implementation Consortium (2013) published guidelines on the use of HLA-B\*1502 testing for patients prescribed carbamazepine:<sup>1</sup>
  - “HLA-B\*1502 has a very distinct ethnic and regional distribution that is important to consider when evaluating population risk...The frequency of HLA-B\*1502 is highest in Han Chinese...estimates...have been as high as 36%. In general, rates in China range from 1 to 12%. Rates in Singapore and Hong Kong have also been estimated at 10–12%. Rates in Malaysia and Thailand are estimated at 6–8%, whereas in different regions of India, the rates range from 2 to 6%. Korea and Japan have low frequencies of the allele at 0.5 and 0.1%, respectively. The allele is also quite rare in African populations (not observed) and Europeans (0–0.02%).”
  - “HLA-B\*1502 is specific for SJS and TEN; there is no evidence that it predisposes to MPEs or hypersensitivity syndrome.”
  - “Much of the evidence linking HLA-B\*1502 to SJS/TEN was generated in both children and adults.”
  - “Carbamazepine-induced SJS/TEN usually develops within the first 3 months of therapy; therefore, patients who have been taking carbamazepine for longer than 3 months without developing cutaneous reactions are at low risk (but not zero) of carbamazepine-induced adverse events in the future, regardless of HLA-B\*1502 status.”
- A very early study has demonstrated a potential relationship between two other members of the HLA-B75 serotype commonly found in Southeast Asian populations and carbamazepine-induced SJS/TEN. There was a significant association with SJS/TEN found for Southeast Asian individuals with HLA-B\*1521 and HLA-B\*1511 who were prescribed carbamazepine. It was discovered that all HLA-B75 serotype molecules shared a similar capability to bind carbamazepine. More studies must be performed to further delineate this association.<sup>8</sup>
- Product labeling for carbamazepine (Tegretol XR® ) warns for the potential of developing a serious dermatological reaction from treatment with carbamazepine in HLA-B\*1502 positive individuals.<sup>1</sup>

- Carbamazepine should not be used in patients positive for HLA-B\*1502 unless the benefits clearly outweigh the risks. Patients who test negative for the allele have a low risk of SJS/TEN, but should have routine monitoring for toxicity.<sup>1</sup>
- Carbamazepine should be discontinued at the first sign of a rash, unless the rash is clearly not drug-related. If signs or symptoms suggest SJS/TEN, carbamazepine should not be resumed and alternative therapy should be considered.<sup>1</sup>

## Criteria

HLA-B\*1502 variant testing is indicated in individuals with Asian ancestry prior to initiation of or during the first nine months of treatment with carbamazepine therapy.

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# HLA-B\*5701 Genotyping for Abacavir Hypersensitivity

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## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedure addressed by this guideline | Procedure code |
|---------------------------------------|----------------|
| HLA-B*5701 Genotyping                 | 81381          |

## What is HLA-B\*5701

### Definition

Abacavir is used in the treatment of patients with human immunodeficiency virus (HIV).

- The most important adverse effect limiting the use of abacavir is a hypersensitivity reaction (HSR) which occurs in approximately 5-8% of patients.<sup>1</sup>
  - The abacavir HSR includes a combination of rash, fever, GI symptoms (such as nausea, vomiting, diarrhea, or abdominal cramping), constitutional symptoms (tachycardia, hypotension, myalgia, fatigue, pain, malaise, dizziness and headache) and respiratory symptoms.<sup>1</sup>
  - Symptoms usually appear within the first six weeks of abacavir therapy, but can happen at any time.<sup>1-3</sup>
- People with a positive HLA-B\*5701 test are at risk for abacavir HSR. Not all HLA-B\*5701 carriers will have immunologic-confirmed HSR.<sup>2</sup> In studies of people who have experienced an immunologically-confirmed HSR, about half (47.9%) test positive for the HLA-B\*5701 allele.<sup>1</sup>
- People with a negative HLA-B\*5701 are at low risk for abacavir HSR. A negative HLA-B\*5701 test result does not completely rule out the possibility of an HSR. Those who test negative should be monitored carefully for signs of toxicity, especially in the first six weeks of treatment.<sup>4</sup>
- Demographic risk factors for abacavir HSR show a higher risk in white and Hispanic populations (5-8%) compared to 2-3% in the black population.<sup>4,5</sup> The frequency in Asian populations is very low.<sup>2</sup>
- Screening HIV-1 patients for HLA-B\*5701 prior to starting abacavir can reduce the rate of clinically suspected HSR by approximately 60%.<sup>1</sup>

## Test information

- HLA-B\*5701 testing is performed on a blood or cheek swab sample. The test can be performed in different ways by different labs. Some labs will test for specific gene variants associated with the B\*5701 haplotype, where other labs may sequence the DNA in the HLA-B region.
- In general, results can be interpreted as:
  - HLA-B\*5701 positive – person is at high risk for developing abacavir HSR; abacavir-containing drugs should be avoided.
  - HLA-B\*5701 negative – person is at lower risk for developing abacavir HSR; if abacavir treatment is used, this person should be monitored for toxicity.

## Guidelines and evidence

- The Infectious Disease Society of America (2013)<sup>6</sup> and the Department of Health and Human Services' (DHHS) Panel on Antiretroviral Guidelines for Adults and Adolescents (2016)<sup>4</sup> HIV guidelines recommend that:
  - HLA-B\*5701 genotyping should be performed in all patients prior to initiating abacavir therapy.
  - HLA-B\*5701 positive patients should not be prescribed abacavir; however, the guidelines state that if abacavir is used in HLA-B\*5701 positive patients, careful monitoring for HSR is warranted.
  - A negative test result does not rule out the possibility of an HSR but makes the chance of HSR less likely.
  - Patients should be counseled about the potential for experiencing HSR before being treated with abacavir-containing drugs, regardless of HLA-B\*5701 test results.
  - HLA-B\*5701 positive status should be recorded as an abacavir allergy in the patient's medical record.
- The DHHS's Panel on Antiretroviral Therapy and Medical Management of HIV-Infected Children (2016) recommends against the use of abacavir in children who test positive for HLA-B\*5701.<sup>7</sup>
- The Clinical Pharmacogenetics Implementation Consortium (CPIC, 2014) published an update to their *Guidelines on HLA-B Genotype and Abacavir Dosing*.<sup>8</sup> A focused literature review found no new evidence to change their original (2012) recommendations, which include:
  - "HLA-B\*5701 screening should be performed in all abacavir-naive individuals before initiation of abacavir-containing therapy."

HLA-B\*5701

- “In abacavir-naive individuals who are HLA-B\*5701-positive, abacavir is not recommended and should be considered only under exceptional circumstances when the potential benefit, based on resistance patterns and treatment history, outweighs the risk.”
- “There is some debate among clinicians regarding whether HLA-B\*5701 testing is necessary in patients who had previously tolerated abacavir chronically, discontinued its use for reasons other than HSR, and are now planning to resume abacavir. The presence of HLA-B\*5701 has a positive predictive value of ~50% for immunologically confirmed hypersensitivity, indicating that some HLA-B\*5701-positive individuals can be, and have been, safely treated with abacavir. However, we were unable to find any data to show that HLA-B\*5701-positive individuals with previous, safe exposure to abacavir had zero risk of HSR upon re-exposure.”
- Product labeling for abacavir-containing drugs recommends:<sup>9-11</sup>
  - HLA-B\*5701 testing prior to initiating treatment with abacavir and prior to reinitiating abacavir when HLA-B\*5701 status is unknown even if the patient has previously tolerated treatment with abacavir.
  - For HLA-B\*5701-positive patients, treatment with an abacavir-containing regimen is not recommended and should be considered only with close medical supervision and under exceptional circumstances when the potential benefit outweighs the risk.
  - Abacavir is contraindicated in patients with previous hypersensitivity to abacavir.
  - Discontinue abacavir at the first sign of a suspected hypersensitivity reaction.
- Careful monitoring for adverse effects is recommended during the first six weeks of abacavir therapy, when an HSR is most likely to happen. However, an HSR can occur at any time during treatment with abacavir.<sup>1,2,9-11</sup>

## Criteria

HLA-B\*5701 testing is indicated in individuals with HIV-1 prior to the initiation of any abacavir-containing therapy.

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HLA-B\*5701

# Huntington Disease

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## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedure addressed by this guideline                                   | Procedure code |
|---|----------------|
| HTT Gene Analysis; evaluation to detect abnormal (eg, expanded) alleles | 81271          |
| HTT Gene Analysis; characterization of alleles (eg expanded size)       | 81274          |

## What is Huntington disease

### Definition

Huntington disease (HD) is an autosomal dominant neurodegenerative disorder causing progressive cognitive, motor, and psychiatric disturbances.<sup>1</sup>

### Prevalence

The prevalence of HD ranges from 9.71 to 17 per 100,000 people in populations of European descent.<sup>1</sup>

It is less frequent in individuals of Chinese, Japanese, Korean, Finnish or indigenous South African descent. The prevalence of HD is believed to be highest in individuals living in the Lake Maracaibo region of Venezuela.<sup>1</sup>

### Cause

HD is caused by expansion of a CAG trinucleotide repeat mutation in the HTT gene. The number of CAG repeats is typically associated with the severity of disease.

| When a person has this number of CAG repeats ... | Then the person ...  |
|--|--|
| 26 or fewer                                      | is unaffected.   |
| 27 to 35   | is in the intermediate range and is typically not affected with HD. However, any offspring are at risk for HD, because the repeat number can expand over generations. <sup>1</sup> |

| When a person has this number of CAG repeats ... | Then the person ...  |
|--|--|
| 36 to 39   | is at risk for HD but may not develop symptoms. <sup>1</sup> |
| 40 or more                                       | will develop HD symptoms. <sup>1</sup>                       |

### Onset

The mean age of onset of symptoms is 35-44 years of age.<sup>1</sup> 1 Approximately 5-10% of individuals with HD have onset of symptoms before 20 years of age.<sup>1</sup> This is known as juvenile HD. Juvenile HD most commonly results from paternally inherited HD mutations with larger CAG repeats.

### Survival

Median survival time is 15-18 years after onset.<sup>1</sup>

### Inheritance

HD is an autosomal dominant condition. When a parent has HD, each offspring has a 50% risk of inheriting the mutation. Typically, as the disease passes through generations

- severity of HD symptoms increases, and
- age of onset decreases.

This is seen more often when inherited through a male. This phenomenon is known as anticipation.<sup>1</sup>

### Treatment

There is no cure for HD. Some pharmacologic treatments may be effective in decreasing some of the associated symptoms, such as chorea, rigidity and psychiatric disturbances.<sup>1</sup>

### Test information

#### Introduction

Testing for Huntington disease includes analysis to determine the number of CAG repeats.

## CAG repeat testing

Testing for Huntington disease is performed by determining the number of CAG repeats in the HTT gene.<sup>1</sup> CAG repeat analysis has a mutation detection rate greater than 99%.<sup>1</sup>

### Diagnostic testing

Symptomatic HD testing is appropriate for individuals who have a known or suspected diagnosis of HD based on clinical symptoms.<sup>2</sup>

### Predictive testing

Predictive HD testing is appropriate for adults who have a known family history of HD, and wish to know their HD mutation status. Predictive testing should be performed in the context of thorough counseling (described below in Guidelines/Evidence).<sup>2-4</sup> Predictive HD testing is generally not recommended for minors or for testing of pregnancies.<sup>2-8</sup> Predictive testing for HD cannot accurately predict progression of behavioral symptoms.<sup>1</sup> However, an estimate of age of onset is possible based on the number of CAG repeats detected.<sup>9</sup> Additionally, the number of CAG repeats may be helpful to predict age of death (but not the duration of symptoms) and the rate of cognitive, motor, and functional decline.<sup>10,11</sup>

## Guidelines and evidence

### Introduction

This section includes relevant guidelines and evidence pertaining to Huntington disease testing.

### United States Huntington's Disease Genetic Testing Group

The United States Huntington's Disease Genetic Testing Group (2016)<sup>2</sup> has guidelines regarding genetic testing for Huntington disease.

#### Symptomatic testing

“Confirmatory testing by analysis of the HD gene is offered at or after the time of the clinical diagnosis of HD. The presence of a CAG repeat expansion in a person with HD symptoms confirms the clinical impression and supports a diagnosis of HD.”

#### Predictive testing

Asymptomatic (predictive) testing is supported in the context of a predictive testing protocol that includes

- optional neurological exam
- mental health assessment,

- pre- and post-test counseling regarding implications of positive and negative test results, and
- documented informed consent.

### **Predictive testing protocol support**

The predictive testing protocol is also supported by guidelines from

- the International Huntington Association and the World Federation of Neurology Research Group on Huntington's Chorea (1994),<sup>3</sup>
- the American Society of Human Genetics,<sup>8</sup>
- the American College of Medical Genetics and Genomics,<sup>5</sup> and
- the National Society of Genetic Counselors.<sup>5- 6</sup>

## **Criteria**

### **Introduction**

Requests for Huntington disease testing are reviewed using these criteria.

### **Criteria**

- Clinical Consultation:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), and
  - Examination by a geneticist or physician familiar with genetic movement disorders, AND
- Previous Genetic Testing:
  - No previous genetic testing of HTT, AND
- Diagnostic Testing for Symptomatic Individuals:
  - For individuals 18 years of age or older, at least 2 of the following must be present:
    - Progressive motor disability featuring involuntary movements (chorea) and gait disturbance, and/or
    - Behavioral disturbances including:
      - Personality change
      - Depression



- Cognitive decline, and/or
  - Family history of Huntington disease
- For individuals 17 years of age or younger, at least 2 of the following must be present:
  - Progressive motor disability featuring involuntary movements (chorea) and gait disturbance, and/or
  - Cognitive decline, and/or
  - Stiffness or rigidity, and/or
  - Epilepsy/myoclonus and tremor, and/or
  - Family history of Huntington disease, OR
- Predictive Testing for Presymptomatic/Asymptomatic At-Risk Individuals:
  - For individuals 18 years of age or older:
    - Known CAG trinucleotide repeat expansion in HTT in 1<sup>st</sup>, 2<sup>nd</sup>, or 3<sup>rd</sup> degree biologic relative, or
    - One or more 1<sup>st</sup> degree biologic relative(s) with clinical diagnosis of HD and mutation unknown/not yet tested

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### Introduction

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# Hypertrophic Cardiomyopathy Testing

MOL.TS.189.A

v1.0.2020

## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedures addressed by this guideline  | Procedure codes |
|---|-----------------|
| Hypertrophic Cardiomyopathy Genetic Testing Panel (at least 5 cardiomyopathy-related genes) | 81439           |
| Hypertrophic Cardiomyopathy Known Familial Mutation Analysis                                | 81403           |
| Hypertrophic Cardiomyopathy Genetic Testing Panel   | S3865           |
| Hypertrophic Cardiomyopathy Known Familial Mutation Analysis                                | S3866           |
| MYBPC3 Sequencing   | 81407           |
| MYH7 Sequencing   | 81407           |
| TNNI3 Sequencing  | 81405           |
| TNNT2 Sequencing  | 81406           |

## What is hypertrophic cardiomyopathy

### Definition

Hypertrophic cardiomyopathy (HCM) is a genetic condition associated with unexplained thickening of the walls of the left ventricle (called left ventricular hypertrophy or LVH).<sup>1,2</sup>

- A clinical diagnosis is suggested by a nondilated left ventricle with a wall thickness of 13-15mm or more in adults,<sup>3,4</sup> or  $\geq 2$  standard deviations in children.<sup>5</sup> However, some individuals with HCM have smaller LV measurements and variable patterns of LVH may be observed.<sup>4,5</sup>
- Other causes of LVH should be ruled out, including underlying cardiac disease (e.g., chronic hypertension, aortic stenosis), extreme physiologic hypertrophy (“athlete’s heart”), and other multisystem disorders that may have LVH as a feature

(e.g., Fabry disease, Friedreich's ataxia, Noonan syndrome, LEOPARD syndrome, Danon disease, Barth syndrome, Pompe syndrome).<sup>4,6</sup>

- Signs and symptoms are variable ranging from a lifelong asymptomatic course to progressive heart failure and sudden cardiac death.<sup>1,2</sup>
- HCM affects about 1 in 500 people, and is the most common cause of sudden cardiac death among young people under 35 - especially athletes.<sup>4</sup>
- HCM is an autosomal dominant condition. First-degree relatives (parents/siblings/children) of someone with HCM have up to a 50% chance of also being affected. Longitudinal clinical screening is recommended for at-risk relatives.<sup>2,5,7</sup>
- HCM is caused by a mutation in one of at least 14 genes.<sup>2</sup> Genetic testing can be useful to confirm a diagnosis of inherited HCM in a person with unexplained LVH. A family history of LVH, heart failure, or sudden cardiac death supports the diagnosis of HCM but is not required to make a diagnosis. The severity and likelihood of cardiac death may be associated with the gene mutation that causes HCM.<sup>4</sup>
- Identifying a gene mutation does not significantly change management for someone diagnosed with HCM.<sup>6</sup> However, once the disease-causing mutation is identified, at-risk relatives can have reliable genetic testing to define their risk and screening needs.<sup>7</sup>

## Test information

- HCM Sequencing Panels vary by laboratory but most laboratories test at least the eight genes most commonly linked to HCM. Mutations in the MYH7 and MYB3 genes are most common.<sup>1</sup> About 35-60% of people clinically diagnosed with HCM will have a mutation in one of the genes commonly tested.<sup>1,5</sup>
- Once a mutation is identified in a family member, the mutation can be specifically identified with >99% accuracy in asymptomatic family members, or those with equivocal symptoms.<sup>2</sup>

## Guidelines and evidence

### Diagnostic testing

- Evidence-based practice guidelines for the genetic evaluation of cardiomyopathies, including HCM, from the Heart Failure Society of America (HFSA, 2018) state:<sup>6</sup>
  - Genetic testing is recommended for the most clearly affected family member (Level of evidence A)
    - Genetic testing is recommended to determine if a pathogenic variant can be identified to facilitate patient management and family screening

- The level of evidence for testing in HCM is based on studies showing a high diagnostic yield of genetic testing in children and adults and prognostic value of genotype status
- In addition to routine newborn screening tests, specialized evaluation of infants with cardiomyopathy is recommended, and genetic testing should be considered.
- The American College of Medical Genetics and Genomics (ACMG, 2018) published a practice resource on genetic testing for cardiomyopathies. This practice resource is an abbreviated version of the Heart Failure Society Guidelines above, on which ACMG collaborated. They state the following:<sup>8,9</sup>
  - “Recommendation 1. Genetic testing is recommended for patients with cardiomyopathy.”
  - “(a) Genetic testing is recommended for the most clearly affected family member.”
  - “(c) In addition to routine newborn screening tests, specialized evaluation of infants with cardiomyopathy is recommended, and genetic testing should be considered.”
- Evidence-based guidelines from the European Society of Cardiology published in 2014<sup>10</sup> state:
  - “Genetic testing is recommended in patients fulfilling diagnostic criteria for HCM, when it enables cascade genetic screening of their relatives.” (Class 1, Level B)
  - “It is recommended that genetic testing be performed in certified diagnostic laboratories with expertise in the interpretation of cardiomyopathy-related mutations.” (Class 1, Level C)
  - “In the presence of symptoms and signs of disease suggestive of specific causes of HCM, genetic testing is recommended to confirm the diagnosis.” (Class 1, Level B)
  - “Genetic testing in patients with a borderline diagnosis of HCM should be performed only after detailed assessment by specialist teams.” (Class IIa, Level C)
  - “Post-mortem genetic analysis of stored tissue or DNA should be considered in deceased patients with pathologically confirmed HCM, to enable cascade genetic screening of their relatives.” (Class IIa, Level C)
- The Cardiac Society of Australia and New Zealand (2013) made the following recommendation regarding the use of diagnostic testing for HCM:<sup>11</sup>
  - “Genetic testing may also help to discriminate between HCM and other causes of left ventricular hypertrophy, including hypertension and ‘athlete’s heart’.”

- A 2011 expert consensus statement from the Heart Rhythm Society (HRS) and the European Heart Rhythm Association (EHRA)<sup>12</sup> makes Class 1 recommendation that:
  - “Comprehensive or targeted (MYBPC3, MYH7, TNNI3, TNNT2, TPM1) HCM genetic testing is recommended for any patient in whom a cardiologist has established a clinical diagnosis of HCM based on examination of the patient’s clinical history, family history, and electrocardiographic/ echocardiographic phenotype.”
- Evidence-based guidelines from the American College of Cardiology Foundation (ACCF) and the American Heart Association (AHA) published in 2011 state:
  - “Genetic testing for HCM and other genetic causes of unexplained cardiac hypertrophy is recommended in patients with an atypical clinical presentation of HCM or when another genetic condition is suspected to be the cause.” (Class 1, Level of evidence B).<sup>5</sup>
  - “Genetic testing is reasonable in the index patient to facilitate the identification of first-degree family members at risk for developing HCM.” (Class IIa, Level of Evidence B).<sup>5</sup>

### **Predictive testing**

- Evidence-based practice guidelines for the genetic evaluation of cardiomyopathies, including HCM, from the Heart Failure Society of America (HFSA, 2018) state:<sup>6</sup>
  - Cascade genetic testing of at-risk family members is recommended for pathogenic and likely pathogenic variants (Level of evidence A).
- The American College of Medical Genetics and Genomics (ACMG, 2018) published a practice resource on genetic testing for cardiomyopathies. This practice resource is an abbreviated version of the Heart Failure Society Guidelines above, on which ACMG collaborated. They state the following:<sup>8,9</sup>
  - “(b) Cascade genetic testing of at-risk family members is recommended for pathogenic and likely pathogenic variants.”
- Evidence-based guidelines from the European Society of Cardiology published in 2014 state:<sup>10</sup>
  - “It is recommended that genetic testing be performed in certified diagnostic laboratories with expertise in the interpretation of cardiomyopathy-related mutations.” (Class 1, Level C)
  - “Cascade genetic screening, after pre-test counseling, is recommended in first-degree adult relatives of patients with a definite disease-causing mutation.” (Class I, Level B)

- “Clinical evaluation, employing ECG and echocardiography and long-term follow-up, is recommended in first-degree relatives who have the same definite disease-causing mutation as the proband.” (Class 1, Level C)
- “First-degree relatives who do not have the same definite disease-causing mutation as the proband should be discharged from further follow-up but advised to seek re-assessment if they develop symptoms or when new clinically relevant data emerge in the family.” (Class IIa, Level B)
- The Cardiac Society of Australia and New Zealand (2013) made the following recommendation regarding the use of predictive testing for HCM:<sup>11</sup>
  - “Identifying the disease-causing gene mutation can be very valuable for a family, as it can allow earlier management of at-risk members and avoid unnecessary screening of non-carriers.”
- A 2011 expert consensus statement from the Heart Rhythm Society (HRS) and the European Heart Rhythm Association (EHRA)<sup>12</sup> makes Class 1 recommendation that:
  - “Mutation-specific genetic testing is recommended for family members and appropriate relatives following the identification of the HCM-causative mutation in an index case.”
- Evidence-based guidelines from the American College of Cardiology Foundation (ACCF) and the American Heart Association (AHA) published in 2011 make the following Class I recommendations:
  - “Screening (clinical, with or without genetic testing) is recommended in first-degree relatives of patients with HCM.” (Level of Evidence: B)<sup>5</sup>
  - “In individuals with pathogenic mutations who do not express the HCM phenotype, it is recommended to perform serial electrocardiogram (ECG), transthoracic echocardiogram (TTE), and clinical assessment at periodic intervals (12 to 18 months in children and adolescents and about every 5 years in adults), based on the patient's age and change in clinical status.” (Level of Evidence: B)<sup>5</sup>

## Criteria

### Known Familial Mutation(s) for Hypertrophic Cardiomyopathy

- Genetic Counseling:
  - Pre- and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:

- No previous HCM-associated genetic testing inclusive of known family mutation, AND
- Diagnostic/Predisposition Testing for Presymptomatic/Asymptomatic Individuals:\*\*
  - HCM known family mutation in 1<sup>st</sup> or 2<sup>nd</sup> degree biologic relative, OR
- Diagnostic Testing for Symptomatic Individuals:
  - HCM known family mutation in 1<sup>st</sup> or 2<sup>nd</sup> degree biologic relative
  - Echocardiogram demonstrating LVH without obvious cause (valvular disease, hypertension, infiltrative or neuromuscular disorder), and
  - Myocardial wall thickening of greater than or equal to 15mm (1.5cm), or
  - Presence of pathognomonic histopathologic features of HCM
    - Myocyte disarray
    - Hypertrophy
    - Increased myocardial fibrosis, and
  - The results of the test will directly impact the diagnostic and treatment options that are recommended for the patient, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

**\*\*NOTE:** Since symptoms may occur in childhood, testing of children who are at-risk for a pathogenic mutation may be appropriate, but requires genetic counseling and careful consideration of ethical issues related to genetic testing in minors.<sup>3</sup>

### Hypertrophic Cardiomyopathy Genetic Testing Panel

- Genetic Counseling:
  - Pre- and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Testing:
  - No previous genetic testing for HCM, AND
- Diagnostic Testing for Symptomatic Individuals:
  - Echocardiogram demonstrating LVH without obvious cause (valvular disease, hypertension, infiltrative or neuromuscular disorder), and
  - Myocardial wall thickening of greater than or equal to 15mm (1.5cm), or
  - Presence of pathognomonic histopathologic features of HCM
    - Myocyte disarray



- Hypertrophy
- Increased myocardial fibrosis, and
- The results of the test will directly impact the diagnostic and treatment options that are recommended for the patient, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

### **Billing and reimbursement considerations**

- When multiple CPT codes are billed for components of a panel and there is a more appropriate CPT code representing the panel, eviCore will redirect to the panel code(s).
- If the laboratory will not accept redirection to a panel code, the medical necessity of each billed component procedure will be assessed independently.
  - In general, only a limited number of panel components that are most likely to explain the member's presentation will be reimbursable. The remaining panel components will not be reimbursable.
  - When the test is billed with multiple stacked codes, only the following genes may be considered for reimbursement:
    - MYH7
    - MYBPC3
    - TNNT2
    - TNNI3

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<http://europace.oxfordjournals.org/content/europace/13/8/1077.full.pdf>

# Immunohistochemistry (IHC)

MOL.CS.104.A

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## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedures addressed by this guideline   | Procedure codes |
|--|-----------------|
| Immunohistochemistry or immunocytochemistry, per specimen; each additional single antibody stain procedure (List separately in addition to code for primary procedure) | 88341           |
| Immunohistochemistry or immunocytochemistry, per specimen; initial single antibody stain procedure   | 88342           |
| Immunohistochemistry or immunocytochemistry, per specimen; each multiplex antibody stain procedure   | 88344           |

## What is immunohistochemistry

### Definition

Immunohistochemistry (IHC) is a method used to determine the expression of biomarkers in tissue. Antibodies that detect specific antigens (proteins, biomarkers) are applied to tissue and attach to their target antigen. The antibodies are tagged with a visible label that allows the pattern or distribution of the antigen in the tissue to be directly visualized under a microscope.

### Test information

Immunohistochemistry is used widely in pathology for diagnosis, sub-typing, and increasingly to identify therapeutic targets. It has also dramatically changed the approach to evaluating cancer of unknown primary. There are numerous other applications and some of the most common are outlined below:<sup>1-3</sup>

- Diagnostic
  - Initial detection of malignancy, tumor classification (including cancers of unknown origin), and sub-typing

- Identification of infections (e.g., H. pylori induced gastritis or CMV infection)
- Neurodegenerative disorder classification
- Therapy Selection/Management
  - Identification of specific therapeutic targets (e.g., HER2 IHC to determine response to trastuzumab)
  - Monitoring therapeutic response
  - Further characterization of prognosis to gauge treatment aggressiveness
- Genetic Disorder Evaluation
  - Altered gene expression predictive of an underlying genetic disorder (e.g., loss of expression of the mismatch repair genes associated with Lynch syndrome)
  - Skeletal muscle biopsy protein abnormalities that help establish a specific muscular dystrophy diagnosis

## Criteria

This guideline addresses common issues in billing for immunohistochemistry-based tests using CPT codes 88341-88344. It is not intended to encompass immunohistochemistry-based tests billed using more specific CPT codes (e.g., 88360, 88361).

## Medical necessity requirements

Given the numerous applications of IHC, it is not practical or desirable to create policy to address the specific use of stains in various settings. That said, it is possible to determine a maximum number of stains that should be reasonable for the vast majority of applications.

Several studies have included data on the average number of immunohistochemical (IHC) stains used per case in various settings. In a cost-effectiveness of IHC study, Raab (2000) modeled the analysis on a 5-antibody panel, which was the average number of antibodies ordered per case in that hospital system. No data was provided on the upper and lower limits of that range.<sup>5</sup>

A study published by Shah, et al. (2012) looked at the use of IHC stains among different pathology practice settings, which included academic, private and commercial practices. The study concluded that regardless of where IHC was performed, the average number of stains ordered per case was similar among all groups although ranges varied considerably. Pathologists from private groups performed an average of 4 stains (range 1-13), whereas those in commercial laboratories used an average of 3 stains per case (range 1-7 for both). When broken down by organ system, the highest average was 6 stains per case for head and neck tissue.<sup>6</sup>

The National Comprehensive Cancer Network (NCCN) Guidelines for Treatment of Cancer by Site provide detailed guidelines on the use of individual IHC stains in the diagnosis and management of each cancer type addressed.<sup>7</sup>

National Comprehensive Cancer Network (NCCN, 2018) Guidelines on Occult Primary (Cancer of Unknown Primary [CUP]) state the following: “In patients with occult primary tumors, immunohistochemical studies are useful for the characterization of poorly differentiated or undifferentiated tumors and for cell-type determination and pathologic diagnosis. However, exhaustive IHC studies (in excess of 10-12 stains) have not been shown to increase the diagnostic accuracy in identifying the putative primary sites.” NCCN recommends a tiered approach as follows: first tier determines tissue lineage, second tier can suggest putative primary sites, and an optional third tier to identify therapeutic targets in select patients.<sup>8</sup>

Based on these findings, most immunohistochemistry applications should rarely require more than 12 IHC stains per unique specimen. Therefore the following criteria will be applied when processing claims for IHC procedures:

- In addition to the first stain billed with one unit of 88342, reimbursement will routinely be limited to 11 units of CPT 88341 per specimen.
- Any claim for IHC, regardless of the number of units billed, may be subject to post-service medical necessity review if excess units are suggested based on the available clinical information.

### **Billing and reimbursement considerations**

There are currently three codes for reporting qualitative IHC stains: 88341, 88342, and 88344. IHC stains are now reported per unique specimen instead of per block (paraffin-embedded tissue). Examples of unique specimens that may be evaluated on the same date of service are separate colon polyps or skin biopsies from different lesions.

- Current codes:
  - Codes 88342 and 88341 are reported for a single antibody stain procedure.
  - Code 88344 is used to report a multiplex staining procedure\* (e.g., PIN-4, ADH-5, Uro-3 triple stain).
- Retired codes:
  - Code 88343 was deleted in 2015.
  - The HCPCS codes G0461 and G0462 are also no longer reportable.

\*Multiplex staining refers to the use of two or more different antibodies mixed together (“cocktails”) that demonstrate different staining characteristics on a single slide. Multiplex does not refer to antibody cocktails such as Cytokeratin AE1/AE3 that do not show distinct color changes between antibodies. There are a limited, although

expanding, number of multiplex stains with PIN4 being among the most frequently utilized (evaluation of prostate biopsies).<sup>4</sup>

Therefore, the following limitations will be applied when processing claims for IHC procedures:

- Qualitative immunohistochemistry procedure codes 88341-88344 should only be used when other, more specific, procedure codes are not available to describe the performed test, AND
- For single antibody stains:
  - One unit of 88342 should be used for the first single antibody applied to a unique specimen. Additional stains applied to that same specimen are billed with one unit of 88341 per stain to an allowable maximum (see Allowable Units policy below). 88341 should therefore not be billed without 88342 on the same date of service, and
  - It is generally unnecessary to test more than three separate specimens on the same date of service. Therefore, no more than three units of 88342 are routinely reimbursable on a single date of service, and
  - When more than one specimen is studied, the units of 88341 applicable to each unique specimen should be entered separately on the claim and each entry should have a corresponding unit of 88342 billed, OR
- For multiplex antibody stains:
  - One unit of 88344 may be used for a multiplex stain applied to a unique specimen. A multiplex stain is defined as a combination of antibodies that yield separately identifiable staining characteristics on a single slide, and
  - No more than one multiplex stain on one specimen should be necessary on a single date of service, AND
- Modifiers 26 and TC can be used to split codes 88341, 88342, and 88344 into their technical and professional components. When split, one unit of the technical component of a code and one unit of the professional component of a code will be viewed as the equivalent of one unit when calculating maximum allowable units for any code. Alternatively, the sum of units billed with the same modifier (e.g. TC) can fulfill the maximum allowable units regardless of whether the units for the other modifier (e.g., 26) are ever billed.

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**Note** \*Multiplex staining refers to the use of two or more different antibodies mixed together (“cocktails”) that demonstrate different staining characteristics on a single slide. Multiplex does not refer to antibody cocktails such as Cytokeratin AE1/AE3 that do not show distinct color changes between antibodies. There are a limited, although expanding, number of multiplex stains with PIN4 being among the most frequently utilized (evaluation of prostate biopsies).<sup>4</sup>

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# KRAS Testing for Anti-EGFR Response in Metastatic Colorectal Cancer

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## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedures addressed by this guideline               | Procedure codes |
|--|-----------------|
| KRAS Exon 2 Targeted Mutation Analysis               | 81275           |
| KRAS Targeted Mutation Analysis, Additional Variants | 81276           |
| KRAS Sequencing                                      | 81405           |

## What is KRAS mutation analysis

### Definition

KRAS mutation analysis on metastatic colorectal cancer (mCRC) tissue helps identify patients who are most likely to respond to EGFR-targeted therapy (Erbix<sup>®</sup> and Vectibix<sup>®</sup>).<sup>1-4</sup>

- EGFR-targeted therapies usually bind EGFR, block its signaling to KRAS, and inhibit cellular proliferation, angiogenesis, and metastasis.<sup>3</sup>
- Approximately 40% of mCRC tumors have an activating KRAS mutation.<sup>3</sup>
- Anti-EGFR therapy is ineffective for treating mCRC tumors with an activating KRAS mutation because EGFR no longer controls KRAS activation.
- Thus, testing identifies the subset of patients who are resistant to anti-EGFR treatment, avoiding unnecessary drug toxicity and cost.<sup>3,5,6</sup> In addition, some patients with KRAS mutant tumors were found to have an inferior outcome when treated with EGFR-targeted therapy.<sup>3,7</sup>

### Test information

- **KRAS Targeted Mutation Analysis** identifies specific KRAS gene mutations — usually including at least the seven most common mutations in codons 12 and 13 of exon 2 that account for most activating mutations.<sup>3,8</sup> Expanded targeted-mutation

panels will detect additional activating mutations in exons 3 and 4 of the KRAS gene, including mutations in codons 59, 61, 117, and 146.

- **KRAS Gene Sequencing Analysis** identifies most clinically significant mutations in the KRAS gene, including the common activating mutations detected by the targeted mutation assays described above. It has the broadest coverage in KRAS testing, looking at most, if not all, coding areas within the gene. Sequencing may be performed on the KRAS gene alone, or on the KRAS gene as part of a multi-gene panel typically performed by next-generation sequencing.

## Guidelines and evidence

- The National Comprehensive Cancer Network states (NCCN, 2019):<sup>2</sup>
  - “All patients with metastatic colorectal cancer should have tumor tissue genotyped for RAS (KRAS and NRAS) and BRAF mutations individually or as part of a next-generation sequencing (NGS) panel. Patients with any known KRAS mutation (exon 2, 3, 4) or NRAS mutation (exon 2, 3, 4) should not be treated with either cetuximab or panitumumab.”
- Evidence based guidelines from the American Society of Clinical Oncology (ASCO, 2017) state: “Patients with CRC being considered for anti-EGFR therapy must receive RAS mutational testing. Mutational analysis should include KRAS and NRAS codons 12 and 13 of exon 2, 59 and 61 of exon 3, and 117 and 146 of exon 4.”<sup>1</sup>
- These guidelines do not recommend a specific test methodology.

## Criteria

KRAS mutation testing is indicated in individuals with metastatic colorectal cancer.

## References

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# Laboratory Claim Reimbursement

MOL.CS.105.K  
v1.0.2020

## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedures addressed by this guideline  | Procedure codes   |
|---|---|
| Molecular Pathology   | 81105 - 81479   |
| Multianalyte Assays with Algorithmic Analyses (MAAA)                                  | 81490 - 81599; 0002M - 0013M                                    |
| Proprietary Laboratory Analyses (PLA)   | Molecular tests* within range (currently 0001U-0138U)           |
| Molecular Infectious Testing  | Molecular tests* within range 87149 – 87912<br>0500T<br>G0476   |
| Molecular Cytopathology Procedures (Flow Cytometry, In Situ Hybridization)            | 88120 - 88121<br>88182 - 88199                                  |
| Cytogenetics  | 88230 - 88299   |
| Molecular Surgical Pathology Procedures (Immunohistochemistry, In Situ Hybridization) | 88341 - 88344<br>88360 - 88361<br>88364 –88377<br>88380 - 88388 |
| Other Molecular Codes   | 84999<br>86152<br>86153   |
| Molecular HCPCS Codes   | S3800 - S3870<br>G0452, G9143                                   |

**Note** \*Generally defined as codes that include “DNA”, “RNA”, “nucleic acid”, “genotype”, “phenotype” or related language in the code description.

## Description

eviCore manages claims payment for our subscribing Health Plans. Procedure codes (CPT, HCPCS) are adjudicated against claims review and payment rules. Payment and coverage adjustments may be made in addition to those outlined in this policy.

The following claim reimbursement policies provide general guidance on what forms of review may be employed. They are intended to augment other clinical and administrative policies and do not represent all possible claim treatments.

## Criteria: Claims Reimbursement

### Introduction

All procedure codes included in the Molecular and Genomic Testing Laboratory Program (as outlined in the table at the top of this document) may be subject to claims review and payment policies. The following policies define many, but not all, of the most commonly applied claim edits performed under this program.

### Authorization Check

#### Required Authorization

Procedure codes that require medical necessity authorization are defined in published Plan procedure code management lists, which are generally available on the Health Plan's website. All claims will be reviewed for the presence of any procedure code that requires authorization. When required, the following process is employed:

- The procedure code(s) requiring authorization will be checked against an authorization database.
- The procedure code will be released for further adjudication if an approved authorization is on file for all units of that procedure code and the code is billed with any stipulated additional information (e.g., modifier, unique test identifier).
- If any of the following are true, the Plan's authorization requirements will be enforced.
  - A denied authorization is on file for the billed procedure code, or
  - An authorization is not on file for all units of the billed procedure code, or
  - The stipulated modifier is not appended to the code, or
  - The authorization is not valid for the date of service.

### **Pre-Service Review of Procedures That Do Not Require Prior Authorization**

When a prior authorization request is submitted for a group of procedure codes and at least one procedure code requires prior authorization, all submitted procedure codes that are under management by the Program (in any form) will be reviewed regardless of the authorization requirements for each code. The determinations for each reviewed procedure code, approved or denied, will be fully enforced during claim adjudication.

### **Substitutable Codes**

An authorization for a procedure code may be used to approve coverage for a DIFFERENT billed procedure code that is substantially similar in clinical intent and coverage requirements to the authorized code (e.g., an authorization to perform CPT 81228 is substitutable if CPT 81229 is billed). Clinically reasonable substitution rules are automatically applied through the claims adjudication process. When substitution rules are invoked, the billed procedure code is the paid procedure code. The companion Procedure Code Substitution table includes additional details. (See the Supporting Documents section at the end of this guideline.)

### **Post-Service Medical Necessity Determination**

Many lab tests that are in scope for the Molecular and Genomic Lab Management Program are not managed through mandatory authorization requirements. Appropriate billing or medical necessity may be assessed upon claim submission (post-service) prior to payment as follows:

- All procedure codes managed under this program may be subject to post-service medical necessity review.
- Any and all available claims data (e.g., ICD code, age, gender, historical or co-existing procedures, etc.) may be used to determine medical necessity or identify cases requiring further review.
  - Claims data may be sufficient to determine medical necessity without additional clinical information. When medical necessity is determined based on claims data alone, the claims information that will either support or refute medical necessity is defined in the clinical policy (e.g., submitted ICD codes do not support medical necessity for a procedure).
  - When a case is identified for additional post-service medical necessity review, communication is sent to at least the rendering provider requesting additional information with the following possible outcomes:
    - If the required clinical information is provided and fulfills criteria, the procedure is approved and the claim is released for further adjudication.
    - If the required clinical information is provided and does not fulfill criteria, the procedure is denied for lack of medical necessity.

- If the required clinical information is not provided within the specified time frame, the procedure is denied for failure to comply with the post-service review process.
- The factors that may prompt post-service medical necessity review include, but are not limited to:
  - ICD codes that support clinical criteria are not reported on the claim.
  - A billed amount threshold is exceeded.
  - A particular procedure code is billed with other procedure codes (bundled testing whether defined by the laboratory as a panel or not).
  - The claim is submitted by a provider (participating and non-participating) selected for focused review.
  - Billing portrait demonstrates billing patterns selected for focused review.
- There are multiple sources of the rules established by this policy including CMS documents, published code definitions, specialty guidelines, peer reviewed literature, expert opinion, and claims experience with codes or providers.

### Gender Nondiscrimination

Gender reported on a claim is one element used to determine medical necessity. In situations where the reported gender may not be consistent with the medical needs based on biological sex (e.g., transgender, transsexual, intersex individuals), the KX modifier should be appended to each billed procedure code that may have gender-related policy. The KX modifier will allow automated gender-specific edits to be bypassed.

### Lifetime Maximums

In general, the same or similar tests performed on heritable DNA should not need to be performed more than once on the same person in that person's lifetime (e.g., gene sequencing or a similar mutation panel on a gene should not need to be repeated). Rarely, a procedure code may be billed twice for the same female member when subsequent instances represent testing on the female member's fetus. It is the provider's responsibility to determine if any contemplated genetic testing has already been performed for the member and to avoid unnecessary repeat testing.

Lifetime maximum rules will be applied for procedure codes that involve genetic testing of heritable DNA in the following manner:

- The companion *Lifetime Maximums* table includes a list of procedure codes subject to the lifetime maximum policy. (See the Supporting Documents section at the end of this guideline.)
  - Only a single date of service will be reimbursed for any procedure code with a lifetime maximum for a single individual.

- While most procedure codes have a lifetime maximum of one unit, some have a limit of 2 (e.g. known familial mutations for recessive conditions).
- Procedure codes representing tests that may reasonably be performed on a fetus through prenatal diagnosis are covered services more than once per lifetime. When applicable, claims should include the following ICD code to indicate prenatal diagnosis: O35.2X.
- All claims submitted for procedure codes subject to lifetime maximums will be checked for previous payment in historical claims data.
- Testing more than once per lifetime is not medically necessary and such claims will be denied for reimbursement if:
  - The procedure code is known to have already been paid for that member, and
    - The member is a male, or
    - The member is a female, and
      - The code does not allow a prenatal diagnosis override, or
      - No ICD code suggesting prenatal diagnosis is submitted for a code that does allow a prenatal diagnosis override

### Frequency Rules

Tests that do not involve unchanging, inherited DNA may be repeated for medically necessary reasons. Any limits to the frequency at which such tests should be repeated is defined in the applicable clinical policy. These frequency limits will be assessed at claim submission based on available historical claims data.

### Laboratory Certification Check

The Clinical Laboratory Improvement Amendments (CLIA) was established to ensure the accuracy and reliability of laboratory testing.

All laboratories that perform any clinical (not research) testing on humans in the United States – including hospital, doctor's office, and independent labs – are subject to CLIA regulations and must have a CLIA certificate.

Several organizations are approved to accredit laboratories under CLIA (e.g., College of American Pathologists, COLA, Joint Commission, etc.). Laboratories in two states, Washington and New York, are subject to State CMS-approved laboratory programs but not a separate CLIA certification process. Laboratories located in New York must hold a New York State Clinical Laboratory permit, which meets CLIA requirements and a CLIA certificate is provided. Laboratories in Washington State are subject to the Medical Test Site (MTS) Licensing process, and when successful, an MTS license and a CLIA certificate number are both issued without a separate CLIA application process.



Lab tests are categorized by the Food and Drug Administration (FDA) based on complexity: waived, moderate (which includes the provider-performed microscopy procedures sub-category) and high complexity. Clinical laboratories must obtain a certificate that corresponds with the highest complexity of tests performed at a particular location.

Details on the categorization of tests can be found on the CMS website:

[http://www.cms.gov/Regulations-and-Guidance/Legislation/CLIA/Categorization\\_of\\_Tests.html](http://www.cms.gov/Regulations-and-Guidance/Legislation/CLIA/Categorization_of_Tests.html).

Any laboratory that submits a claim that includes any procedure code under the management of the Molecular and Genomic Testing Laboratory Program is subject to quality assessment based on the following principles.

- CLIA edits will be applied to all applicable procedure codes (as defined by CMS) that are under the Molecular and Genomic Testing Lab Program management.
- Laboratories billing procedure codes subject to CLIA edits must:
  - Hold a valid, current CLIA certificate of a type that supports the billed test complexity, and
  - Include the 10-digit CLIA certification number for the specific site where the test was performed on the submitted claim (Item 23 of the HCFA 1500 form or loop 2300 or 2400, REF/X4, 02 for electronic claims)
- Provider claims data will be cross-checked with the procedure code and CLIA certificate data from the CMS Provider of Services file to determine certification to provide the service.
- If the billing provider does not have an appropriate CLIA certificate, the service will not be eligible for reimbursement.

## Supporting Documents

Supporting documents for this Laboratory Claim Reimbursement guideline are available on request.

## References

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# Leber Hereditary Optic Neuropathy (LHON) Genetic Testing

MOL.TS.192.A

v1.0.2020

## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedures addressed by this guideline    | Procedure codes |
|---|-----------------|
| LHON Known Familial Mutation Analysis     | 81403           |
| MT-ND4, MT-ND6 Targeted Mutation Analysis | 81401           |
| Whole Mitochondrial Genome                | 81460           |

## What is Leber Hereditary Optic Neuropathy

### Definition

Leber Hereditary Optic Neuropathy (LHON) is a mitochondrial disorder that mainly affects the eye. It is characterized by bilateral painless subacute vision loss that begins in the second and third decades of life. It usually has onset between 15-30 years of age, and leads to rapid, progressive blindness. Visual acuity usually deteriorates to 20/200 or worse.<sup>1-3</sup>

- The primary cell type that is lost in LHON is the retinal ganglion cell, which is highly susceptible to disrupted ATP production and oxidative stress.<sup>4</sup>
- A diagnosis of LHON can be made clinically. “The pathologic hallmark of LHON is the selective degeneration of the retinal ganglion cell layer and optic nerve.”<sup>1</sup>
- LHON has three phases:<sup>1</sup>
  - Presymptomatic/subacute phase: Mild abnormalities in the fundus may be present. Additionally, color vision, contrast, and electroretinogram may be mildly affected.
  - Acute phase: Onset features blurred or clouded central vision usually starting in one eye, followed by other eye within weeks to months. Onset involves both eyes simultaneously in about 25% of cases. The vision loss gets progressively worse with the blurred central field enlarging (called a scotoma). Evaluation of the fundus in 80% of affected patients will show disk swelling, edema of the

- peripapillary nerve fiber layer, retinal telangiectasia, and increased vascular tortuosity without corresponding leakage on fluorescein angiography.
- Atrophic phase: Optic atrophy and worsening central scotoma will progress to severe impairment over the course of six weeks. Once the atrophic phase begins, visual acuity rarely recovers. Most individuals become legally blind.
  - Within 1 year, 97% of those affected have involvement of the second eye, such that a patient presenting with a unilateral optic neuropathy for longer than 1 year is highly unlikely to suffer from LHON-related vision loss.<sup>4</sup>
  - Other neurologic features may include: tremor, peripheral neuropathy, myopathy, and/or movement disorders. Additionally, women may develop a multiple sclerosis-like progressive disease.<sup>1</sup>
  - Unaffected LHON point mutation carriers can display subclinical signs of disease on fundus examination, including peripapillary microangiopathy, zones of mild disc pseudoedema, and telangiectasia.
  - Some clinicians treat children presymptomatically with antioxidants when their genetic status is known.
  - People who have a pathogenic variant consistent with LHON should avoid alcohol and smoking.<sup>1</sup>
  - The prevalence of LHON in most populations is unknown. In Caucasian populations estimates range from 1 in 31,000 to 1 in 50,000. Men are about 4-5 times more likely to develop LHON than women.<sup>1,2</sup>
  - LHON is caused by point mutations in the mitochondrial genome which is separate from nuclear DNA.
  - Several mtDNA mutations have been reported to cause LHON. However, 90% of affected individuals have one of three common mitochondrial mutations: G3460A (13%), G11778A (70%) and T14484C (14%).<sup>4</sup>
  - A 2016 expert-authored review stated the following regarding genotype-phenotype correlations:<sup>1</sup>
    - The mtDNA mutation T14484C is associated with a partial recovery rate of 37%–58%, while the G11778A mutation has the lowest partial recovery rate of 4%. Patients with the G3460A mutation have an intermediate prognosis, with an approximate 20% partial recovery rate.<sup>1</sup>
    - “m.3460G>A is associated with the worst impairment in visual function. m.11778G>A has an intermediate phenotype. Although published reports would appear to indicate otherwise, the m.3460G>A pathogenic variant is generally accepted among experts as having the worst visual recovery rate.”<sup>1</sup>
  - Earlier age of onset (younger than 20 years), a subacute time course of vision loss, and larger optic discs are all associated with a better visual prognosis.

- Mitochondrial DNA (mtDNA) is passed from the maternal gamete (oocyte) to the developing fetus, therefore mitochondrial diseases like LHON are inherited through the maternal lineage. Since sperm do not contribute mitochondria (and mtDNA) to a fetus, men cannot pass on any mitochondrial mutations they may carry.
  - About 60% of people with LHON have an identifiable maternal family history of disease. In the remaining 40%, the family history may be incomplete or the affected individual could have a new (de novo) mutation but this is rare.<sup>1,2</sup>
  - Not all people with an LHON disease-causing mtDNA mutation will develop symptoms. Only about 50% of males and 10% of females who have a known disease-causing LHON mutation will develop blindness.<sup>2</sup> There must be other genetic and environmental factors that explain the variable appearance of symptoms and the gender differences.<sup>1,2</sup>
- Diseases like LHON that are attributed to mtDNA mutations have unique patterns of inheritance and penetrance governed by the principles of maternal inheritance, heteroplasmy, replicative segregation, and the critical threshold. Heteroplasmy and replicative segregation contribute to the heterogeneity of mitochondrial disease phenotypes, even among related individuals. Critical threshold is reached when the wild-type mtDNA cannot compensate for the mutant mtDNA in a cell or tissue. This accounts for targeted tissue involvement and age dependent onset. Even more variability is present because tissue-specific segregation of mutant mtDNA is stochastic during embryogenesis.<sup>4</sup>

## Test information

- An ophthalmological evaluation can confirm the diagnosis of LHON:<sup>1,2</sup>
  - Eye testing may include fundus exam, visual field testing, and imaging. Other testing, including angiography and electrophysiology, are sometimes warranted. This testing may reveal characteristic findings of LHON or rule out other causes of acute vision loss.
  - In cases where a diagnosis can't be confirmed by eye findings alone, molecular genetic testing may be useful.
- The LHON three mtDNA mutation panel involves targeted testing of three common mutations in mtDNA (G3460A, G11778A and T14484C).<sup>1-3</sup> These three mutations account for over 90% of mtDNA mutations found in people with LHON.<sup>1</sup>
- Full sequencing of the entire mitochondrial genome can be done to identify the remaining 10% of mtDNA mutation in individuals affected with LHON. Since the mitochondrial genome is highly polymorphic, this is not routinely offered unless clinical suspicion is very high and paternal transmission has been ruled out.<sup>1</sup> If the status of heteroplasmy is of concern, next generation testing with high read depth may be preferable.<sup>5</sup>

LHON

- The three LHON mutations are also included on a number of more general mitochondrial targeted mutation panels (in conjunction with genes for MELAS, MERFF and Leigh syndrome).
- A number of large panels sequence the mitochondrial genome in conjunction with nuclear-encoded mitochondrial genes for a broad approach to testing.
- DNA testing can be performed on a blood specimen. Muscle biopsy is generally not necessary, but some labs accept blood, saliva and muscle samples.<sup>6</sup>

## Guidelines and evidence

- No evidence-based U.S. testing guidelines were identified for LHON.
- Although not specific to genetic testing for LHON, the Mitochondrial Medicine Society (2015)<sup>7</sup> developed consensus recommendations for the diagnosis and management of mitochondrial disease. Testing strategies, including strategies for genetic testing, were discussed.
  - Recommendations for DNA testing include the following:
    - “Massively parallel sequencing/NGS of the mtDNA genome is the preferred methodology when testing mtDNA and should be performed in cases of suspected mitochondrial disease instead of testing for a limited number of pathogenic point mutations.”
    - “Patients with a strong likelihood of mitochondrial disease because of a mtDNA mutation and negative testing in blood, should have mtDNA assessed in another tissue to avoid the possibility of missing tissue-specific mutations or low levels of heteroplasmy in blood; tissue-based testing also helps assess the risk of other organ involvement and heterogeneity in family members and to guide genetic counseling.”
    - “Heteroplasmy analysis in urine can selectively be more informative and accurate than testing in blood alone, especially in cases of MELAS due to the common m.3243 A>G mutation.”
    - “When considering nuclear gene testing in patients with likely primary mitochondrial disease, NGS methodologies providing complete coverage of known mitochondrial disease gene is preferred. Single-gene testing should usually be avoided because mutations in different genes can produce the same phenotype. If no mutation is identified via known NGS panels, then whole exome sequencing should be considered.”
- The European Federation of Neurological Sciences (2009)<sup>8</sup> provide consensus-based guidelines for LHON genetic testing: “If the phenotype suggests syndromic mitochondrial disorder due to mtDNA point mutations (MELAS, MERRF, NARP, LHON), DNA-microarrays using allele-specific oligonucleotide hybridization, real-time-PCR or single-gene sequencing are indicated.”

- The Clinical Molecular Genetics Society of the United Kingdom (2008)<sup>6</sup> provided practice-based guidelines for the molecular diagnosis of mitochondrial disease: “Investigation for the G3460A, G11778A and T14484C mutations are indicated for all LHON referrals. Secondary mutations should not be investigated as their significance is unknown. Presymptomatic testing for LHON should be undertaken cautiously and homoplasmy/heteroplasmy should be stated on the report.”
- A 2016 expert-authored review suggests the following testing strategy for those with a known or suspected diagnosis of LHON:<sup>1</sup>
  - “Three common mtDNA pathogenic variants account for 90%-95% of LHON. Targeted analysis for one of these three variants should be performed first.”
  - “A multi-gene panel that includes the mitochondrial genes that encode subunits of NADH dehydrogenase, MT-ND1, MT-ND2, MT-ND4, MT-ND4L, MT-ND5, and MT-ND6, which are known to cause LHON and other genes of interest may also be considered.”
  - “Complete mtDNA sequencing may be considered if use of targeted testing and/or a multi-gene panel did not identify a pathogenic variant, clinical suspicion remains high, and there is no evidence of paternal transmission.”
- For those seeking predictive testing (e.g. they are not currently affected), this review states:<sup>1</sup>
  - “Testing of at-risk asymptomatic adults for LHON is possible ... Such testing is not useful in predicting age of onset, severity, or rate of progression of visual loss in asymptomatic individuals.”
  - “Testing of asymptomatic individuals younger than age 18 years who are at risk for adult-onset disorders for which no treatment exists is not considered appropriate.”

## Criteria

### LHON known familial mutation testing

- Genetic Counseling:
  - Pre and post-test counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - No previous genetic testing for LHON, and
  - LHON causing mutation identified in 1<sup>st</sup> degree biological maternal relative, AND
- Predictive Testing for Asymptomatic Individual:

- 18 years of age or older, or
- Under the age of 18 years, and
  - Presymptomatic treatment with antioxidants is being considered, OR
- Diagnostic Testing for Symptomatic individuals:
  - Ophthalmology examination is suggestive, but not confirmatory, of a diagnosis of LHON, OR
- Prenatal Testing for At-Risk Pregnancies:
  - LHON disease-causing mutation identified in a previous child or in the mother, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy

#### **LHON targeted mutation analysis (G3460A, G11778A and T14484C )**

- Genetic Counseling:
  - Pre and post-test counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Testing:
  - No previous genetic testing for LHON, and
  - No known LHON mutation in the family, AND
- Diagnostic Testing for Symptomatic Individuals:
  - Ophthalmology examination is suggestive, but not confirmatory, of a diagnosis of LHON, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy

#### **Whole mtDNA sequencing**

- Genetic Counseling:
  - Pre and post-test counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Criteria for LHON targeted mutation analysis is met, AND
- No mutations identified in the targeted mutation analysis, AND
- Paternal transmission has been ruled out

LHON



## References

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# Legius Syndrome Genetic Testing

MOL.TS.302.A  
v1.0.2020

## Introduction

Legius syndrome testing is addressed by this guideline.

## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedure addressed by this guideline   | Procedure code |
|---|----------------|
| SPRED1 Known Familial Mutation Analysis | 81403          |
| SPRED1 Sequencing                       | 81405          |
| SPRED1 Deletion/Duplication Analysis    | 81479          |

## What is Legius Syndrome

### Definition

Legius syndrome is an autosomal dominant condition characterized by multiple café-au-lait macules and axillary or inguinal freckling, without neurofibromas or other tumor symptoms of Neurofibromatosis type 1 (NF1).<sup>1</sup>

### Incidence or Prevalence

The exact incidence of Legius syndrome is unknown. Studies have shown that approximately 2% of individuals meeting the diagnostic criteria for NF1 have Legius syndrome.<sup>1</sup>

### Symptoms/Diagnosis

Individuals with Legius syndrome have multiple café-au-lait macules and may have axillary or inguinal freckling. Other clinical features reported in some patients with Legius syndrome include macrocephaly, Noonan-like facial features, pectus excavatum or carinatum, developmental concerns, attention deficit hyperactivity disorder (ADHD), and learning difficulties.<sup>2</sup>

Genetic testing may be indicated in a patient with café-au-lait macules to confirm a diagnosis and direct long term management and surveillance. Approximately 3%-25% of individuals evaluated for NF1 who do not have an identifiable mutation in the NF1 gene are noted to have a SPRED1 pathogenic variant.<sup>3</sup> Individuals with NF1 require

long-term surveillance due to an increased risk of tumor development and other complications. Thus, the diagnosis of Legius syndrome may include molecular testing of the SPRED1 gene, and in some cases the NF1 gene.

### **Cause**

Legius syndrome is caused by mutations in the SPRED1 gene. The protein product of this gene interacts with neurofibromin, the protein product of the NF1 gene.<sup>2</sup>

### **Inheritance**

Legius syndrome is inherited in an autosomal dominant fashion. When a parent has a SPRED1 mutation, each offspring has a 50% risk of inheriting the mutation.<sup>3</sup>

### **Treatment**

Management of a child with Legius syndrome includes therapies for developmental delays, learning disorders, and ADHD.<sup>3</sup>

### **Survival**

Lifespan does not appear to be affected by Legius syndrome. Current knowledge is based on the clinical history of less than 200 individuals with confirmed diagnosis of Legius syndrome.<sup>4</sup>

## **Test Information**

### **Introduction**

Testing for Legius syndrome may be performed by SPRED1 sequencing or SPRED1 deletion/duplication analysis. Known familial mutation analysis is also available.

### **SPRED1 sequencing analysis**

SPRED1 sequencing variants, such as missense, nonsense, and splice site variants, account for up to 88% of mutations seen in Legius syndrome.<sup>3</sup>

### **SPRED1 deletion/duplication analysis**

About 10% of the disease-causing variants in Legius syndrome are multi-exon and whole gene deletions.<sup>4,5</sup>

### **Known familial mutation analysis**

Analysis for known familial mutations is typically performed by Sanger sequencing, but if available, a targeted mutation panel that includes the familial mutation may be performed.

Known familial mutations analysis is performed when a causative mutation has been identified in a close relative of the individual requesting testing.

## Guidelines and evidence

### Introduction

The following section includes relevant guidelines and evidence pertaining to Legius syndrome testing.

### Expert Authored Review

"There are different opinions on the appropriate approach when clinical information and family history cannot distinguish between Neurofibromatosis type 1 and Legius syndrome. The pros and cons assessment of molecular testing requires the consideration each individual's unique circumstances, including (but not limited to):

- Clinical findings and family history
- Age of the individual
- Differences in recommended clinical management when the diagnosis of NF1 or Legius syndrome is established with certainty versus when the diagnosis of neither can be established with confidence
- Psychological burden of a diagnosis or lack thereof
- Cost of testing and surveillance "4

## Criteria

### Introduction

Requests for SPRED1 testing are reviewed using the following clinical criteria.

### SPRED1 Known Familial Mutation Analysis

Genetic Counseling:

- Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND

Diagnostic Testing for Symptomatic Individuals:

- No previous genetic testing of SPRED1, AND
- SPRED1 mutation identified in 1st degree biological relative

## SPRED1 Sequencing

- No previous genetic testing of SPRED1, AND
- No known, pathogenic SPRED1 mutation in the member's biologic relatives, AND
- No known, pathogenic NF1 mutation in the member or the member's biologic relatives, AND
- Member has at least one of the following pigmentary findings suggestive of Legius syndrome:
  - Six or more café-au-lait macules over 5 mm in greatest diameter in prepubertal individuals, with or without freckling in the axillary or inguinal regions, or
  - Six or more café-au-lait macules over 15 mm in greatest diameter in postpubertal individuals, with or without freckling in the axillary or inguinal regions, AND
- Member's personal and/or family history are not consistent with neurofibromatosis type 1 (e.g., neurofibromas, optic glioma, Lisch nodules, sphenoid dysplasia or tibial pseudoarthrosis are not present), AND
- The results of the test will directly impact the diagnostic and treatment options that are recommended for the patient, AND
- Rendering laboratory is a qualified provider of services per the Health Plan policy.

## SPRED1 Deletion/Duplication

- Criteria for SPRED1 sequencing are met, AND
- No previous deletion/duplication analysis of SPRED1, AND
- No mutation detected in full sequencing of SPRED1

## References

### Introduction

This guideline cites the following references.

1. Muram-Zborovski T, Stevenson D, Viskochil D, et al. SPRED1 mutations in a Neurofibromatosis clinic. *J Child Neurol.* 2011;10:1203-1209.
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5. Spencer E, Davis J, Mikhail F, et al. Identification of SPRED1 deletions using RT-PCR, multiplex ligation-dependent probe amplification and quantitative PCR. *Am J Med Genet A.* 2011;155A(6):1352–9.

# Li-Fraumeni Syndrome

MOL.TS.193.A  
v1.0.2020

## Introduction

Li-Fraumeni syndrome testing is addressed by this guideline.

## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedures addressed by this guideline | Procedure codes |
|--|-----------------|
| TP53 Sequencing                        | 81405           |
| TP53 Deletion/Duplication Analysis     | 81479           |
| TP53 Known Familial Mutation Analysis  | 81403           |

## What is Li-Fraumeni syndrome

### Definition

Li-Fraumeni syndrome (LFS) is a hereditary cancer-predisposition syndrome typically associated with soft tissue sarcoma, osteosarcoma, premenopausal breast cancer, brain tumor, and adrenocortical carcinomas. People with LFS also have an increased risk of a variety of other cancers.<sup>1-3</sup>

### Cause

Historically, there are two forms of LFS: Classic LFS, and Li-Fraumeni-like syndrome (LFL).<sup>1</sup> LFL shares some of the features for LFS, but has less strict clinical diagnostic criteria.<sup>1</sup> LFS/LFL are caused by mutations in the TP53 gene.

### Prevalence

Prevalence of inherited p53 mutations is estimated to be 1 in 20,000.<sup>1</sup> The likelihood of detecting a TP53 mutation is about 70% in classic LFS cases and 40-50% in LFL cases.<sup>1</sup>

### Inheritance

This condition is inherited in an autosomal dominant manner.<sup>1</sup> Children of an affected person have a 1 in 2 (50%) chance to be affected. Most TP53 mutations are inherited

from an affected parent.<sup>1</sup> The frequency of de novo mutations is not well defined but may be as high as 20%.<sup>1</sup>

## Prognosis

About 50% of individuals with LFS/LFL will have cancer by 30 years of age, and 90% of individuals with LFS/LFL will have cancer by 60 years of age.<sup>1</sup>

## Test information

### Introduction

Testing for Li-Fraumeni may include sequence analysis, deletion/duplication analysis, or known familial mutation analysis.

### Sequence analysis

Complete TP53 gene sequencing will detect approximately 95% of known mutations.<sup>1</sup>

Limited sequencing of only certain regions of the TP53 gene is also available. The detection rate of the limited sequencing tests varies between 70-90% depending on which portions of the gene are screened.<sup>1</sup>

### Deletion/duplication testing

Deletion/duplication testing may be considered as a reflex test if a mutation is not found by sequencing. This method will identify gene rearrangements in an additional 1% of cases.

### Known familial mutation analysis

Once a mutation has been identified in the family, known familial mutation testing can be done for at-risk family members.<sup>1,2</sup>

## Guidelines and evidence

### Introduction

This section includes relevant guidelines and evidence pertaining to Li-Fraumeni testing.

### National Comprehensive Cancer Network

The National Comprehensive Cancer Network (2019) guidelines outline the following Li-Fraumeni syndrome testing criteria (quoted directly). These are considered a category 2A recommendation “lower level evidence with uniform NCCN consensus”:<sup>2</sup>



- Individuals from a family with a known TP53 mutation, OR
- Classic Li-Fraumeni syndrome when ALL of the following are present:
  - Combination of an individual diagnosed less than age 45 years of age with a sarcoma; AND
  - First-degree relative diagnosed less than 45 years of age with cancer; AND
  - An additional first- or second-degree relative in the same lineage with cancer diagnosed less than 45 years of age, or a sarcoma at any age OR
- Chompret Criteria (2015 version)<sup>4</sup>, when ANY of the following are present:
  - Individual with a tumor from LFS tumor spectrum (for example, soft tissue sarcoma, osteosarcoma, CNS tumor, breast cancer, adrenocortical carcinoma), before 46 years of age, and at least one first- or second-degree relative with any of the aforementioned cancer (other than breast cancer if the proband has breast cancer) before the age of 56 years, or with multiple primaries at any age; OR
  - Individual with multiple tumors (except multiple breast tumors), two of which belong to LFS tumor spectrum with the initial cancer occurring before the age of 46 years; OR
  - Individual with adrenocortical carcinoma or choroid plexus carcinoma or rhabdomyosarcoma of embryonal anaplastic subtype, at any age of onset, regardless of the family history
- Early onset breast cancer
  - Individual with breast cancer diagnosed before 31 years. TP53 testing can be ordered alone, concurrently with BRCA1/2 testing and/or other gene testing or as a follow up test after negative BRCA1/2 testing.

## Criteria

### Introduction

Requests for Li-Fraumeni testing are reviewed using these criteria.

### TP53 Known Familial Mutation Analysis

- Genetic Counseling:
  - Pre- and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Testing:

- No previous genetic testing of TP53, AND
- Diagnostic and Predisposition Testing for Presymptomatic/Asymptomatic Individuals\*\*:
  - Known family mutation in TP53, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

\*\* Includes prenatal testing for at-risk pregnancies.

### TP53 Sequencing

- Genetic Counseling:
  - Pre- and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy). AND
- Previous Testing:
  - No previous sequencing of TP53, and
  - No previous duplication/deletion analysis, AND
- Diagnostic Testing for Symptomatic Individuals:
  - Classic Li-Fraumeni syndrome when **ALL** of the following are present:
    - Combination of an individual diagnosed less than age 45 years of age with a sarcoma; and
    - First-degree relative diagnosed less than 45 years of age with cancer; and
    - An additional first- or second-degree relative in the same lineage with cancer diagnosed less than 45 years of age, or a sarcoma at any age, OR
  - Chompret Criteria (2015) are met when **ANY** of the following are present:
    - Individual with a tumor from LFS tumor spectrum (eg, sarcoma, CNS tumor, breast cancer, osteosarcoma, adrenocortical carcinoma, leukemia, or lung bronchoalveolar cancer) before age 46 years, and at least one first- or second-degree relative with any of the aforementioned cancers (other than breast cancer if the proband has breast cancer) under the age of 56 years or with multiple primaries at any age; or
    - Individual with multiple tumors (except multiple breast tumors), two of which are LFS tumor spectrum (eg, sarcoma, CNS tumor, breast cancer, osteosarcoma, adrenocortical carcinoma, leukemia, or lung bronchoalveolar cancer) with the initial cancer occurring before the age of 46 years, regardless of the family history; or

- Individual with adrenocortical carcinoma or choroid plexus carcinoma or rhabdomyosarcoma of embryonal anaplastic subtype, at any age of onset, regardless of the family history, OR
- Early onset breast cancer
  - Individual with breast cancer diagnosed before 31 years of age, OR
- Individual with a tumor from LFS tumor spectrum and one or more biologic relatives (1st, 2nd, or 3rd degree) with a clinical diagnosis of LFS/LFL (relative meets classic Li-Fraumeni syndrome criteria or Chompret criteria, as listed above) and no known family mutation or no testing to date, OR
- Predisposition Testing for Presymptomatic/Asymptomatic Individuals:
  - One or more biologic relatives (1st, 2nd, or 3rd degree) with a clinical diagnosis of LFS/LFL (relative meets classic Li-Fraumeni syndrome criteria or Chompret criteria as listed above) and no known family mutation or no testing to date, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

### **TP53 Deletion/Duplication Analysis**

- Genetic Counseling:
  - Pre- and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Testing:
  - No previous deletion analyses of TP53, and
  - No mutation detected on full sequencing of TP53, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

## **References**

### **Introduction**

These references are cited in this guideline.

1. Schneider K, Garber J. (Updated April 2013). Li-Fraumeni Syndrome. In: GeneReviews at GeneTests: Medical Genetics Information Resource (database online). Copyright, University of Washington, Seattle. 1997-2016. Available at: <http://www.ncbi.nlm.nih.gov/books/NBK1311/>.
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[http://www.nccn.org/professionals/physician\\_gls/pdf/genetics\\_screening.pdf](http://www.nccn.org/professionals/physician_gls/pdf/genetics_screening.pdf).

3. Hampel H et al. A practice guideline from the American College of Medical Genetics and Genomics and the National Society of Genetic Counselors: referral indications for cancer predisposition assessment. *Genet Med*. 2015;17(1):70-87. Available at: <https://www.acmg.net/docs/gim2014147a.pdf>
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# Genetic Testing for Limb-Girdle Muscular Dystrophy

MOL.TS.288.A

v1.0.2020

## Introduction

Limb-girdle muscular dystrophy (LGMD) testing is addressed by this guideline.

## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedure addressed by this guideline                           | Procedure code |
|---|----------------|
| Limb-girdle muscular dystrophy Known Familial Mutation Analysis | 81403          |
| Miscellaneous LGMD Gene Analysis                                | 81400-81408    |
| Miscellaneous LGMD Gene Analysis                                | 81479          |

## What is limb-girdle muscular dystrophy

### Definition

Limb-girdle muscular dystrophy (LGMD) is a rare, inherited, heterogeneous group of over 30 myopathies with predominant involvement of the proximal musculature.<sup>1</sup> They are typically progressive myopathies characterized by weakness and atrophy of muscle without primary involvement of the nervous system or neurogenic atrophy. The LGMDs are classified into two groups, based on inheritance pattern: LGMD1 (autosomal dominant) and LGMD2 (autosomal recessive).

### Incidence or Prevalence

Autosomal recessive LGMD is more common, with an overall prevalence of about 1/15,000.<sup>2</sup> Dominant forms are comparatively rare, representing 10% of LGMD cases.<sup>2</sup> The prevalence of specific LGMD subtypes may differ in certain populations:<sup>1</sup>

- LGMD2C is more common in Roma and Tunisian populations,
- LGMD2A is more common in Southern European, Eastern European, and British populations, and
- LGMD2I is more common in Northern European populations.

## Symptoms

Signs and symptoms typically begin anytime between childhood and adulthood depending on the subtype but are generally not congenital. Symptoms can include the following:

- Upper and lower limb weakness, proximal greater than distal weakness
- Gait weakness
- Foot drop
- Cramps
- Exercise intolerance

LGMDs are most often non-syndromic and usually limited to skeletal muscle, but not always. For example, certain subtypes involve cardiac and respiratory muscles. The clinical course can range from mild, with relatively normal activity and life span, to severe with rapid onset and progression of disease.<sup>2</sup>

The muscle atrophy in LGMD is greatest at the shoulder girdle (scapulohumeral) and pelvic girdle (pelvifemoral), although it may progress distally. Bulbar muscles (including facial muscles and oropharyngeal muscles innervated by cranial nerves VII-XII) are relatively spared depending on the subtype of LGMD. This general pattern of girdle muscle weakness as well as onset, progression, and distribution help classify LGMD and its genetic subtypes.

## Cause

There are more than 30 genes implicated in LGMD subtypes, which manifest in overlapping and variable clinical presentations.<sup>2</sup> The genes identified so far encode muscle proteins within the sarcomere- sarcolemma- sarcoplasm-extracellular-matrix network.<sup>3</sup>

## Inheritance

LGMD inheritance is typically autosomal with LGMD subtype nomenclature reflecting autosomal dominant inheritance (LGMD1 with subtypes designated by letter), and autosomal recessive inheritance (LGMD2 with subtypes designated by letter). This autosomal inheritance pattern helps distinguish LGMD from the more common X-linked dystrophies (Duchenne, Becker and Emery-Dreifuss).<sup>4</sup>

## Diagnosis

Diagnosis of muscular dystrophies is typically based on clinical phenotype and inheritance pattern.<sup>3</sup> Although classification schema are becoming more reliant on molecular test results, the 2014 American Academy of Neurology guidelines for LGMD still recommend genetic testing that is directed by clinical assessment.<sup>1</sup>

- The phenotype must be more consistent with LGMD than other myopathies

- Muscle weakness in the proximal limbs and limb girdle (i.e., scapular winging)
- Myopathic and not neuropathic symptoms
- Sparing of extra-ocular muscles (although eye anomalies are seen in some severe allelic disorders)<sup>2</sup>
- Onset is not congenital
- Course is progressive
- Biochemical/histological investigation should suggest muscle damage (although findings can be non-specific)<sup>5</sup>
  - Creatine kinase can be elevated or normal
  - EMG typically shows myopathic rather than neuropathic changes
  - Muscle biopsy shows “dystrophic” changes” (degeneration / regeneration of fibers), and immunohistochemical staining may reveal aberrant or absent muscle specific proteins.
- Dystrophinopathy and inflammatory myopathy should be excluded
- Identification of pathogenic variants in an LGMD-associated gene can confirm a clinical diagnosis of LGMD

Given the expanding number of loci involved in LGMD subtypes, a negative molecular test result does not rule out LGMD. There are more than 50 loci implicated in LGMD subtypes.

### **Treatment**

There is no cure for LGMD. Treatment is symptom driven and includes weight control, physical therapy, surgery, use of respiratory aids, and cardiology monitoring.<sup>1</sup>

### **Survival**

LGMDs have a broad range of severity. Many are life shortening and debilitating.<sup>2</sup>

### **Test information**

#### **Introduction**

Testing for LGMD disease may include targeted mutation analysis, gene by gene sequence analysis, or panel testing. Known familial mutation analysis is also available.

## Sequence analysis

Until recently, most sequencing tests used the Sanger sequencing methodology that was originally developed in the 1970s. Sanger sequencing is labor intensive and did not lend itself to high-throughput applications.

Next generation sequencing (NGS), which is also sometimes called massively parallel sequencing, was developed in 2005 to allow larger scale and more efficient gene sequencing. NGS relies on sequencing many copies of small pieces of DNA simultaneously and using bioinformatics to assemble the sequence. NGS may not perform as well as Sanger sequencing in some applications.

NGS tests vary in technical specifications (e.g., depth of coverage, extent of intron/exon boundary analysis, methodology of large deletion/duplication analysis).

Sequence analysis detects single nucleotide substitutions and small (several nucleotide) deletions and insertions. Regions analyzed typically include the coding sequence and intron/exon boundaries. Promoter regions and intronic sequences may also be sequenced if disease-causing mutations are known to occur in these regions of a gene.

The efficiency of NGS has led to an increasing number of large, multi-gene testing panels. NGS panels that test several genes at once are particularly well-suited to conditions caused by more than one gene or where there is considerable clinical overlap between conditions.

Results may be obtained that cannot be adequately interpreted based on the current knowledge base. When a sequence variation is identified that has not been previously characterized or shown to cause the disorder in question, it is called a variant of uncertain significance (VUS). VUSs are relatively common findings when sequencing large amounts of DNA with NGS.

Under certain circumstances, technologies used in multi-gene testing may fail to identify mutations that might be identifiable through single-gene testing. If high clinical suspicion exists for a particular syndrome testing for that syndrome should be performed instead of a broad multi-gene panel.

Since genes can be easily added or removed from multi-gene tests over time by a given lab, medical records must document which genes were included in the specific multi-gene test used and in which labs they were performed.

Additionally, tests should be chosen to

- maximize the likelihood of identifying mutations in the genes of interest
- contribute to alterations in patient management
- minimize the chance of finding variants of uncertain clinical significance



## **LGMD sequence analysis**

When a specific LGMD subtype is clinically favored over another, genetic testing specific to that subgroup is supported over large panels. However, given the number of loci, and phenotypic overlap among the limb girdle muscular dystrophies, panel testing grouped by inheritance pattern is acceptable.

## **Deletion/duplication analysis**

Analysis for deletions and duplications can be performed using a variety of technical platforms including exon array, MLPA, and NGS data analysis.

These assays detect gains and losses too large to be identified through sequencing technology, often single or multiple exons or whole genes.

## **LGMD deletion/duplication analysis**

Large deletions in autosomal LGMD related genes are infrequently reported. Therefore, deletion/duplication analysis is done as second tier testing or first tier in some cases to help rule out X linked dystrophies if they are a part of the differential.

## **Known familial mutation analysis**

Analysis for known familial mutations is typically performed by Sanger sequencing, but if available, a targeted mutation panel that includes the familial mutation may be performed.

Known familial mutations analysis is performed when a causative mutation has been identified in a close relative of the individual requesting testing.

## **Guidelines and evidence**

### **Introduction**

The following section includes relevant guidelines and evidence pertaining to Limb Girdle Muscular Dystrophy testing.

### **American Academy of Neurology and American Association of Neuromuscular and Electrodiagnostic Medicine**

The Guideline Development Subcommittee of the American Academy of Neurology and the Practice Issues Review Panel of the American Association of Neuromuscular and Electrodiagnostic Medicine (2014) issued recommendations for the approach to genetic testing in LGMD:<sup>1</sup>

- Clinically directed genetic testing is recommended (See Table e-2 for reference of clinical features suggestive of LGMD subtypes).

- Clinicians should use a clinical phenotype, inheritance pattern, and associated manifestations to guide genetic diagnosis (Level B)
- "In patients with suspected muscular dystrophy in whom initial clinically directed genetic testing does not provide a diagnosis, clinicians may obtain genetic consultation or perform parallel sequencing of targeted exomes, whole-exome sequencing, whole-genome sequencing, or next-generation sequencing to identify the genetic abnormality (Level C)."

## Literature Review

Studies evaluating diagnostic yield from small and large panels found both number and composition of genes sequenced have a sizeable impact. A 3-fold greater diagnostic pickup rate was seen when the LGMD panel was increased from 11 genes to a more comprehensive panel containing 41 genes (15 - 46%).<sup>6</sup>

Sequencing of 18 LGMD related genes in 35 patients suspected of having a muscular dystrophy (unknown genetic diagnosis, high CK values and dystrophic changes on muscle biopsy, DMD ruled out prior to study inclusion) was reported.<sup>6</sup> Pathogenic variants confirmed a LGMD-related molecular etiology in 20 patients (57.1%). The study population was ascertained through the neurology clinic at the University of Seoul, Korea. Information regarding consanguinity was not stated in the report and may not have been specifically queried in the study.<sup>7</sup>

While some panels are getting so large as to overlap with WES, a comprehensive panel approach has been suggested to be similar or superior to WES.<sup>6,8,9</sup> One study analyzed 50 families with an LGMD type distribution of muscle weakness.<sup>8</sup> They showed that after large LGMD panel testing as a first line diagnostic, follow-up WES did not yield further diagnosis. On the other hand, smaller panels would have missed several LGMD related genes.<sup>8</sup> Weaknesses of this study includes the specialized population investigated and the small sample size, albeit somewhat large for this rare disease. The population was suspected to be highly consanguineous (in Saudi Arabia) which authors suggest led in part to their 76% diagnostic yield. The authors also analyzed cost, and, despite the large panel size (759 OMIM genes), the actual cost of sequencing with batching was around \$150.00 per sample. This study did not include deletion/duplication analysis. Follow-up analysis after negative large panel testing was carried out with only a small cohort of nine people. Also, the size of the large sequencing panel used approximates the size of the interpretive gene set that a bioinformatician would look at when analyzing results from WES with a myopathic proband.<sup>8</sup> A large gene panel may also increase the risk of incidental findings or variant of uncertain clinical significance.

A US study of 4656 patients with clinically suspected LGMD (no prior molecular testing) underwent genetic testing via a 35-gene NGS panel (included LGMD or LGMD-like genes).<sup>10</sup> A molecular diagnosis was established in 27% (N=1259). There was a high prevalence of patients with pathogenic variants in more than one LGMD gene (N=31), raising the question of possible synergistic heterozygosity/digenic/multigenic contribution to disease presentation/progression.

A group in Australia performed exome sequencing (ES) on 60 families with LGMDs and achieved a diagnostic success rate of 45%.<sup>11</sup> All patients had normal dystrophin immunohistochemistry results. In 14 of the 60 families, pathogenic variants were identified in genes typically associated with other forms of inherited myopathy, highlighting the diagnostic challenge with overlapping clinical presentation among patients with features of LGMD.

A US study of 55 families affected by LGMD demonstrated pathogenic variants in 22 families using exome sequencing.<sup>3</sup> Most of the probands had clinical muscle biopsies, and none of the muscle biopsies led to a genetic diagnosis prior to enrollment. "Among the pathogenic mutations identified in our cohort, six were found in loci not traditionally classified as being associated with LGMD (e.g., DMD, GAA, SMCHD1, VCP, FLNC, and the D4Z4 region of 4q35)", suggesting that gene panels include a broad array of muscle disease genes, beyond just LGMD, particularly given the decreasing use of muscle biopsy in clinical settings.<sup>3</sup>

Given the degree of phenotypic overlap among LGMD subtypes, atypical presentations of non-LGMD myopathies, and variable expressivity of LGMD, panel testing may be superior to a candidate gene approach when multiple LGMD subtypes are being considered.

## Criteria

### Introduction

Requests for LGMD testing are reviewed using the following clinical criteria.

### Known Familial Mutation Analysis

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - No previous genetic testing of requested LGMD gene, AND
- Diagnostic Testing for Symptomatic Individuals:
  - Known family mutation(s) in LGMD subtype related gene in 1st or 2nd degree biologic relative, OR
- Presymptomatic Testing for Asymptomatic Individuals:
  - Age 18 years or older, and
  - At increased risk of developing an LGMD phenotype, and
  - Known family mutation(s) in LGMD subtype related gene in 1st or 2nd degree biologic relative, AND

- Rendering laboratory is a qualified provider of services per the Health Plan policy.

### **LGMD Single Gene Analysis**

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - No redundant previous LGMD related gene sequencing, and
  - No known LGMD related gene mutation in family, AND
- Diagnostic Testing for Symptomatic Individuals:
  - Member displays clinical features of LGMD by the following
    - Muscle weakness and atrophy not secondary to a neurogenic cause in a Limb-girdle distribution, and
    - Member does not have a congenital myopathy, and
    - EMG does not show evidence of a nerve etiology as the primary cause, OR
  - Member has had a muscle biopsy and results are consistent with the LGMD subtype for which testing is being requested, AND
- Inheritance pattern is consistent with the LGMD subtype for which testing is being requested, AND
- The results of the test will directly impact the diagnostic and treatment options that are recommended for the patient, AND
- Rendering laboratory is a qualified provider of services per the Health Plan policy.

### **LGMD Multi-Gene Diagnostic Panels**

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - No known molecular cause of LGMD (single disease causing mutation in dominant forms or biallelic disease-causing mutations in recessive forms) in family, and
  - No mutations or one mutation associated with recessive form of LGMD detected by single gene analysis or different mutation panel than being requested , AND

**LGMD**

- Diagnostic Testing for Symptomatic Individuals:
  - Muscle weakness and atrophy not secondary to a neurogenic cause in a limb-girdle distribution, and
  - Member does not have a congenital myopathy, and
  - EMG does not show evidence of a nerve etiology as the primary cause, and
  - Muscle biopsy, if available, shows dystrophic changes (degeneration / regeneration of fibers), and immunohistochemical staining may reveal aberrant or absent muscle specific proteins, AND
- Inheritance pattern not suggestive of Duchenne muscular dystrophy or other X-linked muscular dystrophies, AND
- The results of the test will directly impact the diagnostic and treatment options that are recommended for the patient, AND
- Rendering laboratory is a qualified provider of services per the Health Plan policy

### **Billing and Reimbursement Considerations:**

For a panel to be considered for reimbursement, it must be limited to LGMD-associated genes. Broad neuromuscular panels are not reimbursable.

If the inheritance pattern in the family is evident based on pedigree analysis, panels specific to the inheritance pattern will be reimbursable; however, panels of all LGMD genes will not.

If a muscle biopsy has been performed with IHC staining, only genes associated with findings will be reimbursable.

When multiple CPT codes are billed for components of a panel and there is a more appropriate CPT code representing the panel, the laboratory will be redirected to the appropriate panel code(s).

## **References**

### **Introduction**

This guideline cites the following references.

1. Narayanaswami P, Weiss M, Selcen D, et al. Evidence-based guideline summary: diagnosis and treatment of limb-girdle and distal dystrophies: report of the guideline development subcommittee of the American Academy of Neurology and the practice issues review panel of the American Association of Neuromuscular & Electrodiagnostic Medicine. *Neurology*. 2014;83:1453-1463.
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7. Seong MW, Cho A, Park HW, et al. Clinical applications of next-generation sequencing-based gene panel in patients with muscular dystrophy: Korean experience. *Clin Genet.* 2015;89(4): 484-488.
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# Liquid Biopsy Testing – Solid Tumors

MOL.TS.194.A

v1.0.2020

## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedures addressed by this guideline                                | Procedure codes |
|---|-----------------|
| APC Sequencing  | 81201           |
| BRAF V600 Targeted Mutation Analysis                                  | 81210           |
| BRCA1/2 Sequencing  | 81163           |
| BRCA1 Sequencing  | 81165           |
| BRCA2 Sequencing  | 81216           |
| EGFR Targeted Mutation Analysis                                       | 81235           |
| JAK2 V617F Targeted Mutation Analysis                                 | 81270           |
| KIT Targeted Sequence Analysis  | 81272           |
| KIT D816 Targeted Mutation Analysis                                   | 81273           |
| KRAS Exon 2 Targeted Mutation Analysis                                | 81275           |
| KRAS Targeted Mutation Analysis, Additional Variants                  | 81276           |
| MGMT Promoter Methylation Analysis                                    | 81287           |
| MLH1 Sequencing   | 81292           |
| MSH2 Sequencing   | 81295           |
| MSH6 Sequencing   | 81298           |
| NPM1 Exon 12 Targeted Mutation Analysis                               | 81310           |
| NRAS Exon 2 and Exon 3 Analysis                                       | 81311           |
| PDGFRA Targeted Sequence Analysis                                     | 81314           |
| PMS2 Sequencing   | 81317           |
| PTEN Sequencing   | 81321           |
| Solid Organ Neoplasm Molecular Profiling                              | 81445           |
| Solid Organ or Hematolymphoid Neoplasm Molecular Profiling - Expanded | 81455           |

| Procedures addressed by this guideline    | Procedure codes |
|---|-----------------|
| TERT Targeted Sequence Analysis           | 81345           |
| NeoLAB Prostate                           | 0011M           |
| Miscellaneous Molecular Tumor Marker Test | 81400           |
| Miscellaneous Molecular Tumor Marker Test | 81401           |
| Miscellaneous Molecular Tumor Marker Test | 81402           |
| Miscellaneous Molecular Tumor Marker Test | 81403           |
| Miscellaneous Molecular Tumor Marker Test | 81404           |
| Miscellaneous Molecular Tumor Marker Test | 81405           |
| Miscellaneous Molecular Tumor Marker Test | 81406           |
| Miscellaneous Molecular Tumor Marker Test | 81407           |
| Miscellaneous Molecular Tumor Marker Test | 81408           |
| Miscellaneous Molecular Tumor Marker Test | 81479           |
| Miscellaneous Molecular Tumor Marker Test | 88271           |

## What is liquid biopsy testing

### Definition

The use of cell-free circulating tumor DNA (ctDNA) to identify genetic mutations present in a tumor is also referred to as a liquid biopsy.

- The National Cancer Institute defines a liquid biopsy as “a test done on a sample of blood to look for cancer cells from a tumor that are circulating in the blood or for pieces of DNA from tumor cells that are in the blood. A liquid biopsy may be used to help find cancer at an early stage. It may also be used to help plan treatment or to find out how well treatment is working or if cancer has come back. Being able to



take multiple samples of blood over time may also help doctors understand what kind of molecular changes are taking place in a tumor.”<sup>2</sup>

- Cell-free circulating tumor DNA (ctDNA) is released into circulation by tumors.<sup>1</sup> It can be found in various substances, including blood, urine, saliva, etc.
- Analysis of ctDNA is performed to help identify indicators of disease recurrence or disease progression. It can also help to determine if a specific treatment is indicated.
- Liquid biopsies can be used to more easily obtain serial sampling of a tumor. This is particularly useful since somatic mutations that are used in treatment decisions can change as the tumor progresses.<sup>1</sup> ctDNA is also thought to be a more representative sample of the entire tumor genome as well as any metastases that may be present.<sup>1</sup>
- Traditional methods of performing biopsies on tumor tissue pose the following problems:<sup>1,3</sup>
  - Biopsies are invasive, involve risks, are typically costly, and are typically difficult to obtain.
  - Treatment decisions often rely on one single biopsy, while tumors are usually heterogeneous in nature, tumor characteristics can evolve, and information regarding metastases may not be known.<sup>1</sup>
- The use of liquid biopsies can help overcome some of the above problems with traditional biopsies since they can be completed non-invasively.
- This policy will only address the use of ctDNA as a liquid biopsy in solid tumors. Although circulating tumor cells (CTCs) can be used to help obtain information about a person’s cancer prognosis and treatment options, this policy also does not address CTCs. For information on coverage for CTC assays, please see the policy titled **CellSearch Circulating Tumor Cell Count for Breast Cancer Prognosis**. This policy also does not address the use of ctDNA as a liquid biopsy in hematological malignancies.

## Test information

- Testing methodology relies on the presence of ctDNA in circulation.
- Various laboratories have recently developed liquid biopsy tests (e.g. Guardant Health, Trovagene, Biocept, Transgenomic).
- ctDNA is typically analyzed by one of the following methods:
  - Standard testing methodologies, such as PCR or sequencing, are used to identify targeted mutations commonly present in tumors of a specific type.
  - Methodologies such as NGS-based sequencing or array-CGH are used to identify both novel and recurrent mutations. These include whole genome

sequencing or whole exome sequencing. These approaches analyze single genes, panels of genes, exomes, or genomes. Use of these approaches allows testing with no prior knowledge of genetic mutations that are present in the patient's tumor.

## Guidelines and evidence

- The National Comprehensive Cancer Network (NCCN, 2019) states the following in regards to liquid biopsies for testing in patients with non-small cell lung cancer:<sup>4</sup>
  - “Cell-free/circulating tumor DNA testing should not be used in lieu of a tissue diagnosis.”
  - “The use of cell-free/circulating tumor DNA testing can be considered in specific clinical circumstances, most notably:”
    - “If a patient is medically unfit for invasive tissue sampling”
    - “In the initial diagnostic setting, if following pathologic confirmation of a NSCLC diagnosis there is insufficient material for molecular analysis, cell-free/circulating tumor DNA should be used only if follow-up tissue-based analysis is planned for all patients in which an oncogenic driver is not identified”
- Many laboratories are developing liquid biopsy assays. For many of these assays, analytical validity studies have been performed; however, data regarding the clinical validity and clinical utility of these tests is still emerging.<sup>3,5-14</sup>
- The TRACERx study (Tracking Non-small cell lung cancer evolution through therapy (Rx)) is a large, prospective clinical trial being conducted to evaluate “the relationship between intra-tumor heterogeneity and clinical outcome following surgery and adjuvant therapy.”<sup>15</sup> Researchers plan to analyze patient’s tumors before surgery and multiple times after surgery during their treatment regimen. Tumor tissue and ctDNA in patient’s blood will be examined in approximately 840 patients with NSCLC. This trial is expected to continue until 2023.<sup>15</sup>
- Limited evidence suggests that liquid biopsy with Guardant360, in patients with advanced NSCLC, may be a reasonable non-invasive alternative to tumor biopsy, particularly in patients unable to undergo standard tissue biopsy or in cases where tumor tissues are lacking or insufficient for proper mutation analysis.<sup>16-26</sup>

## Criteria

### Guardant360 testing for non-small cell lung cancer (NSCLC)

- When Guardant360 is being requested, the panel will be considered medically necessary when the following criteria are met:

- The member has a diagnosis of metastatic or recurrent NSCLC, AND
- NSCLC diagnosis has been confirmed based on a histopathologic assessment of tumor tissue, AND
- No previous multi-gene panel testing has been performed for NSCLC, AND
- Insufficient tumor tissue is available for broad molecular profiling and member is unable to undergo an additional standard tissue biopsy due to documented medical reasons (i.e., invasive tissue sampling is contraindicated due to the member's clinical condition)

### EGFR targeted mutations

Liquid biopsy testing for EGFR targeted mutations is addressed in the guideline *EGFR Testing for Non-Small Cell Lung Cancer TKI Response*.

### Other considerations

- All other liquid biopsy multi-gene panels are considered investigational and/or experimental and therefore, not eligible for reimbursement.
- Liquid biopsy for all other indications is considered investigational and/or experimental and therefore, not eligible for reimbursement.
- The Guardant360 multi-gene panel will only be considered for reimbursement when billed with an appropriate panel CPT code. When multiple CPT codes are billed for components of the panel, eviCore will redirect to the appropriate panel code.

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# Long QT Syndrome Testing

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## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedures addressed by this guideline            | Procedure codes |
|---|-----------------|
| Long QT Syndrome Sequencing Multigene Panel       | 81413           |
| Long QT Syndrome Deletion/Duplication Panel       | 81414           |
| Long QT Syndrome Known Familial Mutation Analysis | 81403           |
| ANK2 Sequencing                                   | 81479           |
| CASQ2 Sequencing                                  | 81405           |
| CAV3 Sequencing                                   | 81404           |
| KCNE1 Sequencing                                  | 81479           |
| KCNE2 Sequencing                                  | 81479           |
| KCNH2 Sequencing                                  | 81406           |
| KCNJ2 Sequencing                                  | 81403           |
| KCNQ1 Sequencing                                  | 81406           |
| RYR2 Sequencing                                   | 81408           |
| SCN5A Sequencing                                  | 81407           |
| SCN4B Sequencing                                  | 81479           |
| AKAP9 Sequencing                                  | 81479           |
| SNTA1 Sequencing                                  | 81479           |
| KCNJ5 Sequencing                                  | 81479           |
| CALM1 Sequencing                                  | 81479           |
| CALM2 Sequencing                                  | 81479           |
| CACNA1C Sequencing                                | 81479           |

## What is Long QT syndrome

### Definition

Long QT Syndrome (LQTS) is caused by mutations in a number of genes, most of which are related to the functioning of sodium or potassium ion channels in the heart.<sup>1</sup> Testing may offer prognostic information in some cases, as specific genes and even specific mutations within those genes may have some correlation to risk for sudden death, effectiveness of beta-blocker therapy, and preventive strategies.<sup>1-4</sup>

- Signs and symptoms of long QT syndrome (LQTS) are variable, but may include a prolonged QT interval on an electrocardiogram, torsades de pointes, syncope, seizures, cardiac arrest, and sudden cardiac death.<sup>1,2</sup> Many patients with LQTS can be largely asymptomatic, with cardiac arrest or sudden cardiac death as the first and only symptom.
- Symptoms typically occur in young individuals who are otherwise healthy.<sup>1</sup> Certain events — such as exercise, emotional stress, a startle, or sleep — can trigger arrhythmia in individuals with LQTS.<sup>1</sup> Patients are recommended to avoid these activities when possible.<sup>1</sup>
- Screening for LQTS is by electrocardiography (ECG or EKG), and sometimes includes an ambulatory ECG (Holter monitor), and/or an exercise- or medication-induced stress test.<sup>1,3</sup> In many cases, the diagnosis of LQTS can be made based on personal and family history and clinical findings.<sup>1</sup> However, approximately 10-40% of LQTS patients will not have diagnostic ECG changes.<sup>4</sup>
- Several forms of LQTS exist. The autosomal dominant Romano-Ward syndrome is the most common form, with a prevalence of 1 in 3000 to 1 in 5000.<sup>1,2</sup> It affects all ethnic groups.<sup>1</sup> All forms of LQTS are estimated to affect at least 1 in 2500 people.<sup>4</sup>
- Genetic LQTS must be differentiated from acquired LQTS, which can be caused by exposure to certain medications, certain heart conditions, bradycardia, electrolyte imbalances, dietary deficiencies, or intracranial disease.<sup>1</sup>

### Test information

- Genetic testing for LQTS is typically performed with a **sequencing panel**. Commercially available genetic testing exists for the AKAP9, ANK2, CACNA1C, CAV3, CALM1, CALM2, CALM3, KCNE1, KCNE2, KCNH2 (hERG), KCNJ2, KCNJ5, KCNQ1, SCN4B, SCN5A, SNTA1, and TRDN genes associated with LQTS.<sup>1</sup> Mutations in three genes (KCNQ1, KCNH2, and SCN5A) account for the majority of cases.<sup>1,2</sup> Testing will find a mutation in approximately 75% of patients with a clinical diagnosis of LQTS.<sup>4</sup> Composition of test panels varies by laboratory.
- **Deletion/duplication testing** for the AKAP9, ANK2, CACNA1C, CAV3, KCNE1, KCNE2, KCNH2, KCNJ2, KCNQ1, SCN4B, SCN5A, SNTA1 genes is also available.

- Once the causative mutation has been identified in a family member, other at-risk relatives only need to be tested for that mutation — not a panel of genes. Testing by **known familial mutation analysis** is greater than 99% accurate.<sup>1</sup>

## Guidelines and evidence

A 2013 expert consensus statement from the Heart Rhythm Society (HRS), the European Heart Rhythm Association (EHRA), and the Asia Pacific Heart Rhythm Society incorporates genetic test results into the recommended diagnostic criteria:<sup>5</sup>

- LQTS is diagnosed:
  - In the presence of an LQTS risk score  $\geq 3.5$  in the absence of a secondary cause for QT prolongation and/or
  - In the presence of an unequivocally pathogenic mutation in one of the LQTS genes or
  - In the presence of a corrected QT interval for heart rate using Bazett's formula (QTc)  $\geq 500$  ms in repeated 12-lead electrocardiogram (ECG) and in the absence of a secondary cause for QT prolongation.
- LQTS can be diagnosed in the presence of a QTc between 480 and 499 ms in repeated 12-lead ECGs in a patient with unexplained syncope in the absence of a secondary cause for QT prolongation and in the absence of a pathogenic mutation.

A 2011 expert consensus statement from the Heart Rhythm Society (HRS) and the European Heart Rhythm Association (EHRA) makes the following recommendations regarding genetic testing:<sup>4</sup>

- “Comprehensive or LQT1-3 (KCNQ1, KCNH2, and SCN5A) targeted LQTS genetic testing is recommended for any patient in whom a cardiologist has established a strong clinical index of suspicion for LQTS based on examination of the patient's clinical history, family history, and expressed electrocardiographic (resting 12-lead ECGs and/or provocative stress testing with exercise or catecholamine infusion) phenotype.” [Class I, “is recommended”]<sup>4</sup>
- “Comprehensive or LQT1-3 (KCNQ1, KCNH2, and SCN5A) targeted LQTS genetic testing is recommended for any asymptomatic patient with QT prolongation in the absence of other clinical conditions that might prolong the QT interval (such as electrolyte abnormalities, hypertrophy, bundle branch block, etc., i.e., otherwise idiopathic) on serial 12-lead ECGs defined as QTc $>480$ ms (prepuberty) or  $>500$ ms (adults).” [Class I, “is recommended”]<sup>4</sup>
- “Comprehensive or LQT1-3 (KCNQ1, KCNH2, and SCN5A) targeted LQTS genetic testing may be considered for any asymptomatic patient with otherwise idiopathic QTc values $>460$ ms (prepuberty) or  $>480$ ms (adults) on serial 12-lead ECGs.” [Class IIb “may be considered”]<sup>4</sup>



- “Mutation specific genetic testing is recommended for family members and other appropriate relatives subsequently following the identification of the LQTS-causative mutation in an index case.” [Class I, “is recommended”]<sup>4</sup>
- Older American College of Cardiology/American Heart Association/European Society of Cardiology (2006) guidelines on the management of ventricular arrhythmias made no specific evidence-based recommendations about genetic testing for LQTS, but do state:
  - “[Genetic testing is] useful for risk stratification and for making therapeutic decisions,” and they highlight the benefit for identifying family members for counseling and preventative management. They conclude: “Although genetic analysis is not yet widely available, it is advisable to try to make it accessible to LQTS patients.”<sup>3</sup>

The 2015 European Society of Cardiology Guidelines for the management of patients with ventricular arrhythmias and the prevention of sudden cardiac death state:<sup>6</sup>

- “LQTS is diagnosed in the presence of a confirmed pathogenic LQTS mutation, irrespective of the QT duration.” [Class I, Level C recommendation]

## Criteria

### Long QT Syndrome Known Familial Mutation Analysis

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - No previous genetic testing for Long QT Syndrome inclusive of known family mutation, AND
- Diagnostic and Predisposition Testing:
  - Long QT Syndrome family mutation identified in 1st degree relative(s). (Note: 2nd or 3rd degree relatives may be considered when 1st degree relatives are unavailable or unwilling to be tested), AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy

### Long QT Syndrome Sequencing or Multigene Panel

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND

- Previous Genetic Testing:
  - No previous genetic testing for Long QT Syndrome, AND
- Diagnostic Testing for Symptomatic Individuals:
  - Clinical signs indicating moderate to high pre-test probability of Long QT syndrome, but diagnosis cannot be made with certainty by other methods (i.e. Schwartz criteria of 2-3), or
  - Confirmation of prolonged QTc or T-wave abnormalities [ $>460$ ms (prepuberty) or  $>480$ ms (adults) on serial 12-lead ECGs] on exercise or ambulatory ECG, or during pharmacologic provocation testing and acquired cause has been ruled out, or
  - A prolonged or borderline prolonged QT interval on ECG or Holter monitor and acquired cause has been ruled out, or
  - Profound congenital bilateral sensorineural hearing loss and prolonged QTc, OR
- Predisposition Testing for Presymptomatic/Asymptomatic Individuals:
  - Biologic relative(s) (1st degree) diagnosed with LQTS clinically whose genetic diagnosis is unknown, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

### **Long QT Syndrome Deletion/Duplication Analysis**

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - No mutation identified with long QT full gene sequence analysis, or
  - Neither or only one mutation in KCNQ1 or KCNE1 identified in an individual with profound congenital bilateral sensorineural hearing loss and prolonged QTc, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy

### **Billing and Reimbursement Considerations**

When multiple CPT codes are billed for components of a panel and there is a more appropriate CPT code representing the panel, eviCore will redirect to the panel code(s).

If the laboratory will not accept redirection to a panel code, the medical necessity of each billed component procedure will be assessed independently.

- In general, only a limited number of panel components that are most likely to explain the member's presentation will be reimbursable. The remaining panel components will not be reimbursable.
- When the test is billed with multiple stacked codes, only the following genes may be considered for reimbursement:
  - KCNQ1
  - KCNH2
  - SCN5A

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# Lynch Syndrome Genetic Testing

**MOL.TS.197.A**  
v1.0.2020

## Introduction

Lynch syndrome genetic testing is addressed by this guideline.

## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedures addressed by this guideline         | Procedure codes |
|--|-----------------|
| MLH1 Known Familial Mutation Analysis          | 81293           |
| MSH2 Known Familial Mutation Analysis          | 81296           |
| MSH6 Known Familial Mutation Analysis          | 81299           |
| PMS2 Known Familial Mutation Analysis          | 81318           |
| Known Familial Variant Not Otherwise Specified | 81403           |
| MLH1 Sequencing                                | 81292           |
| MLH1 Deletion/Duplication Analysis             | 81294           |
| MSH2 Sequencing                                | 81295           |
| MSH2 Deletion/Duplication Analysis             | 81297           |
| MSH6 Sequencing                                | 81298           |
| MSH6 Deletion/Duplication Analysis             | 81300           |
| PMS2 Sequencing                                | 81317           |
| PMS2 Deletion/Duplication Analysis             | 81319           |
| EPCAM Deletion/Duplication Analysis            | 81403           |

## What is Lynch syndrome

### Definition

Lynch syndrome, also called hereditary, non-polyposis colorectal cancer (HNPCC), is a hereditary cancer syndrome that is the most common cause of colon and endometrial cancer.

## Prevalence

Lynch syndrome affects approximately 1 in 35 colorectal and endometrial cancer patients and around 1 in 370 individuals in the general population. Lynch syndrome accounts for 2-4% of all colorectal cancer cases.<sup>1-3</sup>

### Cancer risks

Lynch syndrome is associated with an 82% lifetime risk for colorectal cancer and a 15-60% risk of endometrial cancer.<sup>4,5</sup> The risk also increases for development of the following cancer types:

- small bowel
- stomach
- ovarian
- pancreatic
- ureteral and renal pelvis
- biliary tract
- brain
- sebaceous adenoma, and
- keratoacanthoma tumors.<sup>1, 5</sup>

### Onset

The average ages of diagnosis for colorectal, endometrial, and gastric cancers are 44-61, 48-62, and 56 years, respectively.<sup>4</sup> Ovarian cancer diagnoses are typically earlier, with an average age of diagnosis of 42.5 years, roughly one-third of cases being diagnosed before the age of 40.<sup>4</sup>

### Diagnosis

Lynch syndrome should be suspected when the personal and family cancer history meets the *Revised Bethesda Guidelines* or the *Amsterdam II Criteria* (see below).<sup>6,7</sup>

### Cause

Lynch syndrome is caused by mutations in any one of at least the following five genes: MLH1, MSH2, MSH6, PMS2, and EPCAM.<sup>4,8</sup>

### Inheritance

Lynch syndrome is an autosomal dominant syndrome that is associated with a germline mutation in one of at least five genes: MLH1, MSH2, MSH6, PMS2, and EPCAM. Children of an affected individual have a 50% risk to inherit a mutation.<sup>4</sup>

Lynch syndrome mutations inherited in an autosomal recessive manner cause constitutional MMR deficiency syndrome (CMMR-D). Testing for CMMR-D is not addressed in this summary.<sup>4,5</sup>

### Associated syndromes

Lynch syndrome includes the variants Muir-Torre syndrome (one or more Lynch syndrome-associated cancers and sebaceous neoplasms of the skin) and Turcot syndrome (Lynch syndrome with glioblastoma).<sup>4</sup>

## Test information

### Introduction

Testing for Lynch syndrome may include tumor testing, gene sequencing, deletion/duplication analysis, known familial mutation testing, or multigene panel testing.

### Testing approaches

Testing those with a suspected Lynch syndrome-related cancer should begin with microsatellite instability or immunohistochemistry testing on tumor tissue. The following table lists and describes the various testing scenarios.

| When ...   | Then ...  |
|--|---|
| tumor tests suggest Lynch syndrome   | that individual should be offered genetic testing to look for a mutation that causes Lynch syndrome. <sup>1, 8-10</sup>                                   |
| immunohistochemistry studies are abnormal  | those results may suggest which mismatch repair genes is likely to harbor a mutation.   |
| tumor tests are normal, and there is a young age of diagnosis or a strong family history of Lynch syndrome-associated cancers is present | genetic testing may still be warranted, or tumor testing in another family member with the most suspicious cancer history may be considered. <sup>8</sup> |
| tumor screening is not possible, and the individual meets the guideline criteria   | direct genetic testing may be reasonable.   |

### Genetic testing

Genetic testing usually starts either with sequencing and deletion/duplication analysis of the gene identified from tumor IHC results, or with a comprehensive gene panel. The National Comprehensive Cancer Network has outlined a comprehensive strategy for

molecular testing of Lynch Syndrome.<sup>1</sup> The first person tested should be the relative most likely to have Lynch Syndrome in the family.

When the family Lynch syndrome mutation is known, at-risk relatives should be tested for that specific mutation only. This is often called single site mutation analysis. Detection rates approach 100%.

## Guidelines and evidence

### Introduction

This section includes relevant guidelines and evidence pertaining to Lynch syndrome genetic testing.

### Multiple society recommendations

The US Multi-Society Task Force (2014), the National Society of Genetic Counselors and the Collaborative Group of the Americas on Inherited Colorectal Cancer (NSGC/CGA-ICC, jointly published, 2012), the National Comprehensive Cancer Network (NCCN, 2018), and the American College of Gastroenterology (ACG); (2015) have practice guidelines that address Lynch syndrome genetic testing. Generally, these recommendations agree.<sup>1,8,9,11</sup>

- Test colorectal or endometrial tumors by microsatellite instability and/or immunohistochemistry first when tissue is available.
- Individuals with abnormal microsatellite instability and/or immunohistochemistry results (and no demonstrated BRAF mutation or hypermethylation of MLH1) should be offered genetic testing to identify a Lynch syndrome disease-causing mutation. Results from tumor testing should guide the genetic testing cascade. When tumor testing is not possible or results are inconclusive, genetic testing for an inherited mutation is indicated if a patient with a suspected Lynch syndrome-related cancer meets one of the first three Bethesda Guidelines or the family meets the Amsterdam Criteria (see tables below). If no affected family member is available for testing, at-risk relatives can consider genetic testing if the family meets the Amsterdam Criteria. However, only a mutation positive result can be clearly interpreted. Mutation negative results must be interpreted with caution; the chance of inconclusive results is high because the family mutation may not be detectable. Once a Lynch syndrome disease-causing mutation has been identified, at-risk relatives should be offered genetic testing for that specific mutation.

“The Multi-Society Task Force is composed of gastroenterology specialists with a special interest in CRC, representing the following major gastroenterology professional organizations: American College of Gastroenterology, American Gastroenterological Association Institute, and the American Society for Gastrointestinal Endoscopy. Also, experts on LS from academia and private practice were invited authors of this guideline. Representatives of the Collaborative Group of the Americas on Inherited Colorectal Cancer and the American Society of Colon and Rectal Surgeons also



reviewed this manuscript. In addition to the Task Force and invited experts, the practice committees and Governing Boards of the American Gastroenterological Association Institute, American College of Gastroenterology, American Society for Gastrointestinal Endoscopy reviewed and approved this document.”

### **Society of Gynecologic Oncology**

The Society of Gynecologic Oncology recommends “all women who are diagnosed with endometrial cancer should undergo systematic clinical screening for Lynch syndrome (review of personal and family history) and/or molecular screening. Molecular screening of endometrial cancer for Lynch syndrome is the preferred strategy when resources are available.” Universal molecular tumor testing for either all endometrial cancer or cancers diagnosed at age less than 60, regardless of personal or family cancer history, is a sensitive strategy for identifying women with Lynch syndrome.<sup>12</sup>

### **Revised Bethesda Guidelines**

According to the *Revised Bethesda Guidelines*<sup>6</sup>, consider Lynch syndrome tumor screening when any one of the following criteria are met:

- colorectal cancer is diagnosed before the age of 50
- presence of synchronous or metachronous colorectal cancer, or other Lynch syndrome-associated tumor<sup>\*\*\*</sup>, regardless of age
- microsatellite unstable (MSI-H) tumor pathology before the age of 60, examples include
  - tumor-infiltrating lymphocytes
  - Crohn’s-like lymphocytic reaction
  - mucinous or signet-ring differentiation
  - medullary growth pattern, or
  - other reported features
- at least one first-degree relative, including parent, sibling, or child with a Lynch syndrome-related tumor<sup>\*\*\*</sup>, one of whom was diagnosed before the age of 50, or
- at least two first- or second-degree relatives with Lynch syndrome-related tumors<sup>\*\*\*</sup> at any age.

### **Amsterdam II Criteria**

According to *Amsterdam II Criteria*<sup>7</sup>, Lynch syndrome is likely when all of the following criteria are met:

- there are at least three relatives with Lynch syndrome associated tumors<sup>\*\*\*</sup>

- one affected relative is a first-degree relative (parent, sibling, child) of the other two
- affected relatives are in two or more successive generations
- at least one Lynch syndrome-related tumor was diagnosed before age 50, and
- FAP has been excluded on the basis of no polyposis.

Tumors must be verified by pathology.

\*\*\*Lynch syndrome-associated tumors include

- colorectal
- endometrial
- small bowel
- stomach
- ovarian
- pancreatic
- ureteral and renal pelvis
- biliary tract
- brain tumors, usually glioblastomas associated with Turcot syndrome variant
- sebaceous adenomas, and
- keratoacanthomas, associated with a Muir-Torre syndrome variant.

## Criteria

### Known Familial Mutation Analysis

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Testing:
  - No previous testing for inherited Lynch syndrome mutations, AND
- Family History:
  - Known MLH1, MSH2, MSH6, PMS2, or EPCAM mutation in a close blood relative (1st, 2nd, or 3rd degree), AND
- Age- 18 years and older, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

**Gene Sequencing and/or Deletion/Duplication Analysis of MLH1, MSH2, MSH6, PMS2, or EPCAM**

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Testing:
  - Gene requested has not been tested previously by the same methodology (i.e., sequencing or deletion/duplication analysis), AND
- Age- 18 years or older, AND
- Familial adenomatous polyposis (FAP) has been ruled out, AND
- Diagnostic Testing for Symptomatic Individuals<sup>1,13</sup>
  - Personal history of colorectal cancer (or other Lynch syndrome-related tumor<sup>\*\*\*</sup>), and
  - If colorectal cancer (see figure A):
    - MSI testing of tumor tissue shows MSI-high, or
    - IHC testing of tumor tissue detects absence of MLH1, MSH2, MSH6, and/or PMS2 encoded protein products, and
    - BRAF mutation analysis and/or MLH1 hypermethylation analysis performed if indicated (according to figure A) and not consistent with sporadic CRC (sporadic CRC is likely when the tumor has MLH1 promoter hypermethylation and/or the BRAF V600E mutation.), or
  - If other Lynch syndrome-associated tumor:
    - Endometrial cancer diagnosed before age 50, or
    - Endometrial cancer diagnosed at any age with abnormal tumor testing indicative of a mutation in a mismatch repair gene, or
    - Presence of synchronous or metachronous Lynch syndrome-associated tumors, regardless of age, or
    - Amsterdam II criteria are met:
      - $\geq 3$  close blood relatives (1st, 2nd, or 3rd degree) with Lynch syndrome-associated tumor (symptomatic member can be one of the three), and
      - One should be a first-degree relative of the other two, and
      - $\geq 2$  successive generations affected, and
      - $\geq 1$  diagnosed before age 50, or

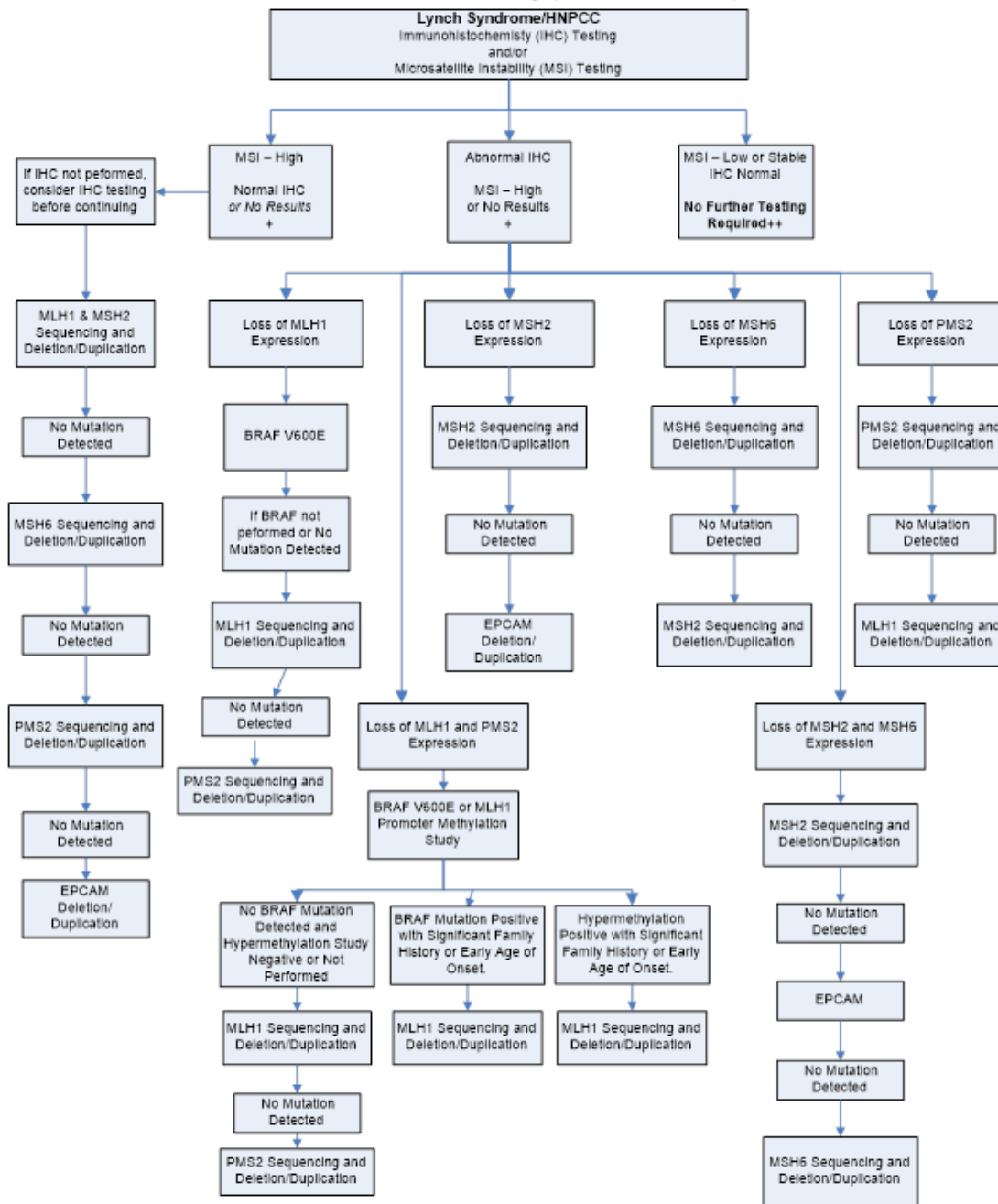
- 5% or greater risk of Lynch syndrome based on one of the following mutations prediction models (MMRPro or MMRPredict)<sup>1,10,11</sup>, or
- 2.5% or greater risk of Lynch syndrome based on PREMM[5],<sup>14</sup> OR
- Predisposition Testing for Presymptomatic/Asymptomatic Individuals:<sup>1</sup>
  - ≥ 3 close blood relatives (1st, 2nd, or 3rd degree) with Lynch syndrome-associated tumor, where Amsterdam II criteria are met:
    - One should be a first degree relative of the other two, and
    - ≥ 2 successive generations affected, and
    - ≥ 1 diagnosed before age 50, and
  - IHC and/or Lynch syndrome genetic testing results from affected family member are unavailable, OR
  - 5% or greater risk of Lynch syndrome based on one of the following mutations prediction models (MMRPro or MMRPredict)<sup>1, 10, 11</sup>, OR
  - 2.5% or greater risk of Lynch syndrome based on PREMM[5]<sup>14</sup>, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy

\*\*\*Lynch syndrome-associated tumors include colorectal, endometrial, small bowel, stomach, ovarian, pancreatic, ureteral and renal pelvis, biliary tract, brain/CNS tumors (usually glioblastomas associated with Turcot syndrome variant), sebaceous adenomas, and keratoacanthomas (associated with Muir-Torre syndrome variant).

### **Billing and reimbursement considerations**

- For individuals that have had previous tumor testing (MSI and/or IHC), the testing algorithm as outlined in Figure A must be followed for payment of claim.
- Lynch syndrome genetic testing for those with colorectal cancer is generally not indicated in the absence of abnormal MSI and/or IHC results on the colorectal tumor. MSI and/or IHC became part of the standard NCCN recommended evaluation for all people with colorectal cancer under the age of 70 (at a minimum) in May 2013. As a result, most people affected with colorectal cancer who are appropriate candidates for Lynch syndrome testing should have access to MSI and/or IHC. Lynch syndrome genetic testing without MSI and/or IHC results will only be considered necessary in extenuating circumstances and will require medical necessity review.

Figure A 3.11  
 Diagnostic Testing for Individuals with  
 Previous Tumor Testing (MSI and/or IHC)



+ “Individuals with abnormal MSI and/or IHC tumor results and no germline mutation detected in the corresponding gene(s) may still have undetected Lynch syndrome. At this time, no consensus has been reached as to whether these patients should be managed as Lynch syndrome or managed based on personal/family history. Growing evidence suggests that the majority of these individuals with abnormal tumor results and no germline mutation found have double somatic mutations/changes in the MMR

Lynch Syndrome Genetic Testing

genes. Although the efficacy has not yet been proven, genetic testing of the corresponding gene(s) could be performed on tumor DNA to assess for somatic mutations. Individuals found to have double somatic mutations/changes in the MMR genes likely do not have Lynch syndrome and management should be based on personal/family history.”<sup>1</sup>

++“If strong family history (i.e. Amsterdam criteria) or additional features of hereditary cancer syndromes (multiple colon polyps) are present, additional testing may be warranted in the proband, or consider tumor testing in another affected family member due to the possibility of a phenocopy.”<sup>11</sup>

+++ Per NCCN guidelines, only MLH1 promoter mutation analysis is recommended for endometrial tumors when IHC testing has indicated a loss of MLH1 protein.<sup>1</sup>

## References

### Introduction

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# Lynch Syndrome Tumor Screening - First-Tier

MOL.TS.198.A

v1.0.2020

## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedures addressed by this guideline                               | Procedure codes |
|--|-----------------|
| Microsatellite Instability   | 81301           |
| Immunohistochemistry Tumor Screening (MLH1, MSH2, MSH6, and/or PMS2) | 88341           |
| Immunohistochemistry Tumor Screening (MLH1, MSH2, MSH6, and/or PMS2) | 88342           |

## What is Lynch syndrome tumor screening

### Definition

Lynch syndrome, also called hereditary non-polyposis colorectal cancer (HNPCC), is the most common known hereditary cause of colon and endometrial cancer. It affects approximately 1 in 35 colorectal and endometrial cancer patients and around 1 in 370 individuals in the general population. Lynch syndrome accounts for 2-4% of all colorectal cancer cases.<sup>1-3</sup>

- Lynch syndrome is associated with a high lifetime risk for colorectal cancer (up to 82%) and endometrial cancer (15-60%), diagnosed at an earlier than usual age. The risk is also increased for small bowel, stomach, ovarian, pancreatic, ureteral and renal pelvis, biliary tract, brain, sebaceous adenoma, and keratoacanthoma tumors.<sup>1,4,5</sup>
- Lynch syndrome is caused by mutations in the following mismatch repair genes: MLH1, MSH2, MSH6, and PMS2.<sup>4</sup> An additional gene called EPCAM (or TACSTD1) has been found to account for about 1-3% of Lynch syndrome cases.<sup>4</sup>
- Lynch syndrome gene mutations are inherited in an autosomal dominant manner (children of an affected individual have a 50% risk to inherit a mutation), but family history alone is unreliable for identifying Lynch syndrome cases.<sup>1,4</sup> Lynch syndrome mutations inherited in an autosomal recessive manner cause Constitutional MMR-Deficiency syndrome (CMMR-D).<sup>4,5</sup>



- Individuals with colorectal or endometrial cancer due to Lynch syndrome often have abnormal immunohistochemistry (IHC) and/or microsatellite instability (MSI) results on their tumors. These tests have good sensitivity and can identify individuals at sufficient risk for Lynch syndrome to warrant follow-up genetic testing.<sup>1</sup>
- Tumor screening is generally offered to those with colorectal or endometrial cancer (see guidelines below).<sup>1,6,7,8</sup>
- Identifying at-risk individuals is necessary for appropriate surveillance and risk reduction.<sup>1</sup>

## Test information

- Both immunohistochemistry and microsatellite instability evaluate formalin-fixed, paraffin-embedded tumor tissue for evidence of mismatch repair defects. Lynch syndrome is caused by mutations in mismatch repair genes.
  - **Immunohistochemistry (IHC)** detects the presence or absence of MLH1, MSH2, MSH6, ± PMS2 mismatch repair proteins.<sup>1,5</sup> Most Lynch syndrome-causing mutations result in protein truncation or absent protein expression<sup>7</sup>, which leads to abnormal IHC staining. As a result, IHC will detect an estimated 83%-94% of underlying Lynch syndrome mutations in colorectal tumors.<sup>2,9</sup> IHC has the distinct benefit of identifying the gene most likely to have a mutation.<sup>4,9</sup> DNA testing can then be targeted to that specific gene.
  - **Microsatellite Instability (MSI)** compares normal and tumor tissue to detect microsatellite (stretches of repetitive DNA) size changes. Lynch syndrome mutations often cause the size of microsatellites to be unstable.<sup>3</sup> When tumor tissue shows high microsatellite instability (MSI-H), it is indirect evidence of an underlying Lynch syndrome gene mutation. Depending on the panel of MSI markers, 80-91% of MLH1 and MSH2 mutations and 55-77% of MSH6 and PMS2 mutations will be detected by MSI testing.<sup>2</sup>
- No specific tumor screening strategy has been recommended, but studies suggest that both MSI and IHC are cost-effective.<sup>1,2</sup>
- MSI and IHC together have better sensitivity for Lynch syndrome than either test alone<sup>4</sup>, and may be used simultaneously or sequentially.

## Guidelines and evidence

- The National Comprehensive Cancer Network (NCCN, 2018) has published practice guidelines that address MSI and IHC tumor screening for Lynch syndrome:<sup>1</sup>
  - Routine tumor testing for Lynch syndrome is supported either for all CRC patients or CRC patients diagnosed at < 70 years and also those ≥70 years who meet the Bethesda guidelines.

- “IHC and/or MSI screening of all colorectal and endometrial cancers (usually from surgical resection but may be performed on biopsies) regardless of age at diagnosis or family history, has been implemented at some centers to identify individuals at risk for Lynch syndrome. This approach was recently endorsed for colorectal cancer by the Evaluation of Genomic Applications in Practice and Prevention Working Group from the CDC and shown to be cost effective.”
- “An alternative approach is to test all patients with CRC diagnosed prior to age 70 years plus patients diagnosed at older ages who meet the Bethesda guidelines.”
 

“This approach gave a sensitivity of 95.1% (95%CI, 89.8-99.0%) and a specificity of 95.5% (95%CI, 94.7-96.1%). This level of sensitivity was better than that of both the revised Bethesda and Jerusalem (testing all patients diagnosed with CRC at age <70) recommendations. While this new selective strategy failed to identify 4.9% of Lynch syndrome cases, it resulted in approximately 35% fewer tumors undergoing MMR testing.”
- “Endometrial cancer <50 y is not included in the revised Bethesda guidelines; however, recent evidence suggests that these individuals should be evaluated for Lynch syndrome.”
- Consider Lynch syndrome tumor screening if any one of the following are met:<sup>10</sup>
  - Colorectal cancer diagnosed before age 50
  - Presence of synchronous or metachronous colorectal cancer, or colorectal cancer with other Lynch syndrome-associated tumors,\*\* regardless of age
  - Microsatellite unstable (MSI-H) tumor pathology before age 60 (e.g., tumor-infiltrating lymphocytes, Crohn’s-like lymphocytic reaction, mucinous/signet-ring differentiation, medullary growth pattern, or other reported features)
  - Colorectal cancer diagnosed in a patient with at least one first-degree relative (parent, sibling, child) with a Lynch syndrome-related tumor\*, one of whom was diagnosed before age 50
  - Colorectal cancer diagnosed in a patient with at least two first- or second-degree relatives with Lynch syndrome-related tumors \* at any age

\*\*Lynch syndrome-associated tumors include colorectal, endometrial, small bowel, stomach, ovarian, pancreatic, ureteral and renal pelvis, biliary tract, brain tumors (usually glioblastomas associated with Turcot syndrome variant), sebaceous adenomas, and keratoacanthomas (associated with Muir-Torre syndrome variant).

- An evidence-based recommendation from the Centers for Disease Control and Prevention sponsored Evaluation of Genomic Applications in Practice and Prevention Working Group (EGAPP, 2009) found sufficient evidence to recommend Lynch syndrome tumor screening to all individuals with newly diagnosed colorectal cancer since morbidity and mortality can be significantly improved for the patient and at-risk relatives through management changes once Lynch syndrome is

diagnosed.<sup>2</sup> Although not yet standard of care, some centers have instituted screening for all newly diagnosed colorectal and endometrial cancer.<sup>1</sup>

- A National Society of Genetic Counselors and the Collaborative Group of the Americas on Inherited Colorectal Cancer (2012) Joint Practice Guideline makes the following recommendations:<sup>11</sup>
  - “Microsatellite instability (MSI) and immunohistochemistry (IHC) tumor analyses should be performed on CRC or endometrial cancers as the first-line testing strategy for any patient being evaluated for Lynch syndrome (this includes individuals with CRC or endometrial cancer who meet Amsterdam I or II criteria or Bethesda guidelines).”
  - “MSI testing should include, at a minimum, the five markers included in the NCI panel.”
  - “MSI and IHC should be performed on pretreated specimens.”
  - “MSI and IHC can be technically challenging assays and should be performed in laboratories that have experience with these tests to minimize the possibility of false positive or false negative results.”
  - “MSI and IHC should be performed, when possible, on an affected relative’s tumor when an unaffected patient is being evaluated for Lynch syndrome.”
  - “Direct germline genetic testing (refers to both DNA sequencing and a technology that detects large rearrangements, insertions, deletions and duplications) may be considered on an affected or unaffected patient being evaluated for Lynch syndrome when MSI and IHC testing are not feasible.”
  - This guideline also notes that “Approximately 25% of individuals with Lynch syndrome are not going to meet Amsterdam or Bethesda criteria so limiting MSI and IHC to individuals who meet these criteria only is inadequate and will miss a large number of individuals with Lynch syndrome.”
- The Multi-Society Task Force (2014)<sup>7</sup> recently published a consensus statement on genetic evaluation for Lynch syndrome and recommended that “Testing for MMR deficiency of newly diagnosed CRC should be performed. This can be done for all CRCs, or CRC diagnosed at age 70 years or younger, and in individuals older than 70 years who have a family history concerning for LS. Analysis can be done by IHC testing for the MLH1 / MSH2 / MSH6 / PMS2 proteins and / or testing for MSI. Tumors that demonstrate loss of MLH1 should undergo BRAF testing or analysis of MLH1 promoter hypermethylation.” The Multi-Society Task Force on Colorectal Cancer additionally endorsed utilizing The Colorectal Cancer Risk Assessment Tool to aid in identifying individuals with possible Lynch syndrome.<sup>7,12</sup>
  - The Multi-Society Task Force is composed of gastroenterology specialists with a special interest in CRC, representing the following major gastroenterology professional organizations: American College of Gastroenterology, American Gastroenterological Association Institute, and the American Society for Gastrointestinal Endoscopy. Also, experts on LS from academia and private

practice were invited authors of this guideline. Representatives of the Collaborative Group of the Americas on Inherited Colorectal Cancer and the American Society of Colon and Rectal Surgeons also reviewed this manuscript. In addition to the Task Force and invited experts, the practice committees and Governing Boards of the American Gastroenterological Association Institute, American College of Gastroenterology, American Society for Gastrointestinal Endoscopy reviewed and approved this document.

- The American Gastroenterology Association (AGA; 2015) recommends “testing the tumors of all patients with colorectal cancer with either immunohistochemistry (IHC) or for microsatellite instability (MSI) to identify potential cases of Lynch syndrome versus doing no testing for Lynch syndrome.”<sup>6</sup>
- The American College of Gastroenterology (ACG; 2015) states that “All newly diagnosed colorectal cancers (CRCs) should be evaluated for mismatch repair deficiency. Analysis may be done by immunohistochemical testing for the MLH1/MSH2/MSH6/PMS2 proteins and/or testing for microsatellite instability (MSI). Tumors that demonstrate loss of MLH1 should undergo BRAF testing or analysis for MLH1 promoter hypermethylation.”<sup>13</sup>
- The Society of Gynecologic Oncology recommends “all women who are diagnosed with endometrial cancer should undergo systematic clinical screening for Lynch syndrome (review of personal and family history) and/or molecular screening. Molecular screening of endometrial cancer for Lynch syndrome is the preferred strategy when resources are available.” Universal molecular tumor testing for either all endometrial cancer or cancers diagnosed at age less than 60, regardless of personal or family cancer history, is a sensitive strategy for identifying women with Lynch syndrome.<sup>14</sup>
- The US Food and Drug Administration (FDA) has approved “Keytruda for the treatment of adult and pediatric patients with unresectable or metastatic solid tumors that have high microsatellite instability (MSI-H) or mismatch repair deficiency (dMMR). This indication covers patients with solid tumors that have progressed following prior treatment and who have no satisfactory alternative treatment options and patients with colorectal cancer that has progressed following treatment with certain chemotherapy drugs.”<sup>15</sup>

## Criteria

- Testing may be considered for individuals who meet ANY of the following criteria:
  - All colorectal cancers regardless of age, OR
  - All endometrial cancers regardless of age, OR
  - Treatment with Keytruda is being considered, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy

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# Lynch Syndrome Tumor Screening - Second-Tier

**MOL.TS.199.A**  
v1.0.2020

## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedures addressed by this guideline | Procedure codes |
|--|-----------------|
| BRAF V600 Targeted Mutation Analysis   | 81210           |
| MLH1 Promoter Methylation Analysis     | 81288           |

## What are BRAF mutation and MLH1 promoter methylation testing for Lynch Syndrome

### Introduction

Lynch syndrome, also called hereditary non-polyposis colorectal cancer (HNPCC), is the most common known hereditary cause of colon and endometrial cancer. It affects approximately 1 in 35 colorectal and endometrial cancer patients and around 1 in 370 individuals in the general population. Lynch syndrome accounts for 2-4% of all colorectal cancer cases.<sup>1-4</sup>

- Lynch Syndrome is associated with a high lifetime risk for colorectal cancer (up to 82%) and endometrial cancer (15-60%), diagnosed at an earlier than usual age. The risk is also increased for small bowel, stomach, ovarian, pancreatic, ureteral and renal pelvis, biliary tract, brain, sebaceous adenoma, and keratoacanthoma tumors.<sup>1,5,6</sup> Lynch syndrome is an autosomal dominant syndrome that is associated with a germline mutation in one of at least five genes: MLH1, MSH2, MSH6, PMS2, and EPCAM. Children of an affected individual have a 50% risk to inherit a mutation.<sup>5</sup>
- People suspected to have colorectal or endometrial cancer caused by Lynch syndrome generally have tumor screening studies first.<sup>1,7,8</sup> Tumors caused by Lynch syndrome often show microsatellite instability (MSI) and absent protein from one or more mismatch repair genes (MLH1, MSH2, MSH6, +/- PMS2) by immunohistochemistry (IHC).<sup>1,5</sup>
- If MSI or IHC shows signs of Lynch syndrome, the next step is usually Lynch syndrome genetic testing.<sup>1,2,5</sup>

- However, another step may be useful before genetic testing when IHC indicates absent MLH1 protein. Absent MLH1 may be caused by Lynch syndrome, but is also frequently a sporadic finding in colorectal and endometrial cancers. Additional testing can help determine whether MLH1-negative colorectal and endometrial tumors (not other Lynch syndrome-associated tumors) are sporadic or are associated with Lynch syndrome.<sup>1,2,5</sup>
- The most common cause of absent MLH1 protein is sporadic methylation of the MLH1 gene, which causes the gene to make no protein.<sup>3</sup>
- This MLH1 methylation is often associated with a sporadic mutation in the BRAF gene (in colorectal tumors only; not endometrial).
- BRAF is part of a cell signaling pathway that helps control cell growth. About 6-8% of colorectal cancer tumors have a BRAF mutation.<sup>9</sup> A single mutation, called V600E (previously called V599E), accounts for about 90% of these BRAF mutations.<sup>3</sup>
- When MLH1 protein is absent and a BRAF mutation is present, the colorectal cancer is rarely caused by Lynch syndrome (i.e., the cancer is usually sporadic).<sup>3</sup>
- When MLH1 protein is absent, the tumor is negative for a BRAF V600 codon mutation, and MLH1 promoter methylation is present, the cancer is still generally sporadic. However, other types of mutations (e.g., MLH1 epimutations that cause widespread hypermethylation or MLH1 promoter variants) may cause this result.<sup>1,2</sup>
- BRAF gene mutations that are inherited or occur in tumors are relevant to several other diagnoses, including:
  - Colorectal Cancer Anti-EGFR Therapy Response
  - Thyroid Cancer Prognosis
  - Noonan Syndrome

## Test information

- For Lynch syndrome-related testing, BRAF mutation analysis +/- MLH1 promoter methylation studies are done on colorectal tumor tissue. MLH1 promoter methylation studies (not BRAF) are done on endometrial tumor tissue. Sporadic BRAF mutations do not appear to be responsible for MLH1 methylation in endometrial tumors.<sup>2</sup>
- When BRAF is being tested because MLH1 protein was absent on colorectal tumor IHC, most laboratories test only for the BRAF V600 codon mutation. However, some laboratories sequence all or part of the BRAF gene (sometimes for reasons other than Lynch syndrome screening). Targeted mutation analysis is generally less expensive than gene sequencing. Because the V600 codon mutation accounts for most BRAF colorectal cancer mutations, targeted mutation analysis for this one



mutation is sufficient. Results of testing for this single mutation are expected to be reliable.<sup>3</sup>

- BRAF mutation analysis and MLH1 promoter methylation studies may be offered as panels or in reflex options. For instance, BRAF mutation analysis may be a reflex test when MLH1 IHC results are abnormal. MLH1 promoter methylation studies may be done as reflex test if BRAF mutation analysis is negative.

## Guidelines and evidence

The following organizations address when BRAF and/or MLH1 promoter methylation studies should be employed in evaluating the likelihood a tumor is caused by Lynch syndrome. This section does not address who should have MSI and/or IHC tumor screening for Lynch syndrome at the time of cancer diagnosis.

- The National Comprehensive Cancer Network (NCCN, 2018) includes BRAF V600 codon mutation and MLH1 promoter methylation status in their table that outlines “tumor testing results and additional testing strategies.”<sup>1</sup>
- For colorectal tumors that show no MLH1 protein by IHC (+/- PMS2 negative), they state “consider BRAF/methylation studies.”
- They recommend the following based on the BRAF results:

| BRAF V600E Mutation | MLH1 Promoter Methylation | Lynch Syndrome Genetic Testing?  |
|---------------------|---------------------------|--|
| Positive            | Not necessary             | No   |
| Negative            | Positive                  | Most likely a sporadic cancer; genetic testing only if young age of onset or the family history is compelling. |
| Negative            | Negative                  | Pursue MLH1 and/or PMS2 genetic testing**.   |

**Note** \*\* If genetic testing is negative, consider somatic MMR genetic testing.<sup>1</sup>

- If one somatic mutation only or LOH of one allele only is identified in the tumor, this could mean that the patient has Lynch syndrome due to an unidentifiable germline mutation and these represent the “second hit” in the tumor.
- The National Society of Genetic Counselors and the Collaborative Group of the Americas on Inherited Colorectal Cancer (NSGC/CGA-ICC, jointly published, 2012) guidelines state:<sup>2</sup>
  - “Both somatic hypermethylation of the MLH1 gene (an epigenetic change) and somatic mutations of the BRAF gene have been described in sporadic CRCs

- exhibiting MSI and/or loss of expression of MLH1. These somatic events are rarely seen in LS CRCs and therefore may be useful in determining whether a MSI-high CRC is more likely to be sporadic.”
- “MLH1 promoter methylation and BRAF V600E mutation testing may help to reduce the number of germline genetic tests needed when IHC reveals absence of MLH1 and PMS2. However, NSGC and the CGAICC did not find enough data to recommend one test over the other or both concomitantly.”
  - The likelihood of identifying a germline MLH1 with both DNA sequencing and deletion/duplication analysis is approximately 33% when MLH1 +/- PMS2 are absent on IHC and MLH1 promoter hypermethylation is not present.
- The American Gastroenterology Association (AGA; 2015) suggests “that in patients with colorectal cancer with IHC absent for MLH1, second-stage tumor testing for a BRAF mutation or for hypermethylation of the MLH1 promoter should be performed rather than proceeding directly to germline genetic testing.” <sup>7</sup>

## Criteria

### **BRAF V600 Codon Mutation Analysis or MLH1 Promoter Methylation Status**

- Previous Testing:
  - IHC testing has been performed and indicates a loss of MLH1 protein, AND
- Diagnostic Testing for Symptomatic Individuals:
  - Personal history of colorectal or endometrial\*\*\*\* cancer, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy

\*\*\*\* MLH1 methylation only

## References

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# Macula Risk

MOL.TS.300.A  
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## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedures addressed by this guideline   | Procedure code |
|--|----------------|
| CHF/ARMS2 common variants  | 81401          |
| Unlisted molecular pathology procedure (e.g. ABCA1, ApoE, C2, C3, CETP, CFB, CFI, COL8A1, LIPC, TIMP3) | 81479          |

## What is age-related macular degeneration

### Definition

Age-related macular degeneration (AMD) is the leading cause of blindness and irreversible vision loss among older adults (>65 years).

The etiology of AMD is believed to be multifactorial, and includes modifiable and non-modifiable genetic risk factors that affect the progression of AMD to more advanced stages. The Age-Related Eye Disease Study (AREDS) evaluated the effects of supplements with antioxidants (vitamin E, C, and beta-carotene) and zinc. Results showed that patients taking these supplements experienced a 25% reduced risk of disease progression to advanced AMD in at least one eye over a period of 5 years. More recent data from the AREDS2 study found that omega-3 acids or lutein and zeaxanthin added to the original AREDS formulation had no additional treatment effect on AMD progression to advanced disease. However, some clinical study results of genetic subgroup analyses have shown a differential treatment effect of supplementation on progression based on genotype.<sup>1</sup> For example, some results suggest that complement factor H gene (CFH) and age-related maculopathy susceptibility 2 gene (ARMS2) genetic polymorphisms have different effects on the progression risk of AMD in different treatment groups of AREDS, while other studies fail to report any differential effect. As a result, there is ongoing controversy regarding the impact of nutritional supplementation on disease progression to advanced AMP for those patients with specific genotypes.<sup>2,3</sup>

## Test information

### Introduction

According to the manufacturer (ArcticDx, Inc.), Macula Risk PGx AMD testing is intended to assist in the selection of eye supplement formulations for patients diagnosed with intermediate dry age-related macular degeneration (AMD).

The Macula Risk PGx is a combined pharmacogenetic and prognostic DNA test that assesses a patient's risk of progression to advanced AMD based on their individual risk profile and is designed to aid in the selection of eye supplement formulations.<sup>4</sup>

## Guidelines and evidence

### Introduction

The following section includes relevant guidelines and evidence pertaining to Macula Risk testing.

#### American Society of Retina Specialists

In a 2017 Genetics Task Force Special Report, the American Society of Retina Specialists states:<sup>5</sup>

- “At present, there is no clinical evidence that altering the management of genetically higher risk progression patients, for example, with more frequent office visits and/or improved lifestyle changes, results in better visual outcomes for these patients compared with individuals of lower genetic susceptibility. As such, prospective studies are needed before patient care is modified.”
- “Although genetic testing to determine the optimal nutritional supplementation may in the future prove useful, at present there is insufficient data to support the use of genetic testing in patients with AMD prior to recommendation of current Age-Related Eye Disease Study (AREDS) nutritional supplement use.”

#### American Academy of Ophthalmology

In a 2015 update to their Preferred Practice Pattern document, the American Academy of Ophthalmology states that routine genetic testing is not supported by the literature and is not currently recommended, citing the need for prospectively designed clinical trials to demonstrate clinical value.<sup>6</sup>

### Literature review

Several retrospective post-hoc subgroup analyses have evaluated the clinical usefulness of identifying specific genotypes to guide optimal nutritional supplementation among patients with ARMD.<sup>1-3,7-11</sup>

Most, if not all, available studies are association studies conducting retrospective post-hoc analyses of the same population sample of the previous RCT evaluating the efficacy of the AREDS formulation on AMD progression. These studies conducted several repeat analysis using differing methodologies of various subsets of the patient population enrolled in the AREDS Study. Results of these studies are conflicting and inconsistent. One study that conducted a re-analysis of the AREDS data failed to detect an association between genetics and nutritional supplements in AMD prophylaxis.<sup>8</sup> Another study showed a treatment benefit of zinc to reduce progression to advanced AMD among patients without risk alleles for CFH and 1 or 2 risk alleles for ARMS2.<sup>5</sup> Another analysis by the same author found that among patients treated with zinc, the risk increased for those with a CFH allele, while the risk lessened for patients with ARMS2 allele.<sup>2</sup>

More recently, three studies have found that CFH and ARMS gene variants either do or do not influence progression of disease to advanced AMD, further demonstrating inconsistent study results.<sup>3,9,10</sup> Thus, there is considerable uncertainty regarding the clinical usefulness of genotyping to guide use of nutritional supplements.

There is also a lack of direct evidence regarding the clinical utility of genetic testing for AMD progression. Well-designed research that consistently replicates findings of significant associations between genotype and disease progression following AREDS supplementation is needed before the patient-specific genotype testing is used to guide decisions regarding nutritional supplementation in clinical practice.

## Criteria

### Introduction

Requests for Macula Risk are reviewed using the following criteria.

This test is considered investigational and/or experimental.

- Investigational and experimental (I&E) molecular and genomic (MolGen) tests refer to assays involving chromosomes, DNA, RNA, or gene products that have insufficient data to determine the net health impact, which typically means there is insufficient data to support that a test accurately assesses the outcome of interest (analytical and clinical validity), significantly improves health outcomes (clinical utility), and/or performs better than an existing standard of care medical management option. Such tests are also not generally accepted as standard of care in the evaluation or management of a particular condition.
- In the case of MolGen testing, FDA clearance is not a reliable standard given the number of laboratory developed tests that currently fall outside of FDA oversight and FDA clearance often does not assess clinical utility.

## References

### Introduction

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# MammaPrint 70-Gene Breast Cancer Recurrence Assay

MOL.TS.200.A  
v1.0.2020

## Procedure addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedure addressed by this guideline | Procedure code |
|---------------------------------------|----------------|
| MammaPrint 70 Gene Signature          | 81521          |

## What is MammaPrint

### Definition

MammaPrint® is a 70-gene expression test designed to predict the chance of later-in-life recurrence of breast cancer in women with newly diagnosed, early stage breast cancer.<sup>1</sup> It is FDA cleared for use along with other standard prognostic methods, such as disease staging, grading and other tumor marker analyses.<sup>2</sup>

- MammaPrint is intended to assist patients and providers considering treatment with adjuvant chemotherapy. Patients assigned a “low risk” may choose hormone therapy (tamoxifen) alone and forego chemotherapy. Patients assigned a “high risk” may benefit from more aggressive treatment and choose to do chemotherapy.<sup>1</sup>
- MammaPrint is designed for women with breast cancer who have:<sup>1,2</sup>
  - Stage I or II invasive carcinoma
  - Tumor size <5.0 cm
  - Node-negative (no metastasis to lymph nodes)
  - Estrogen receptor-positive (ER+) or -negative (ER-) disease

### Test information

- MammaPrint uses a microarray platform to analyze the expression level of 70 genes in the tumor. These 70 genes are thought to be critical in the cellular pathways to cancer metastasis.<sup>1</sup>
- Based on the test results, patients are assigned either a low risk or a high risk for a distant recurrence. Low risk corresponds to a 10% risk of recurrence by 10 years



without any additional adjuvant treatment. In contrast, those in the high risk group have a 29% risk of recurrence by 10 years without any additional adjuvant treatment.<sup>1</sup>

## Guidelines and evidence

### National Comprehensive Cancer Network

The National Comprehensive Cancer Network (NCCN) 2018 Clinical Practice Guidelines for Breast Cancer state that:<sup>3</sup>

- MammaPrint is considered evidence and consensus category 1 for prognostic assessment in node-negative and 1-3 node positive breast cancer.
- Use of the test for predictive purposes has not been determined.

### American Society of Clinical Oncology

Evidence-based clinical guidelines from the American Society of Clinical Oncology (ASCO, updated 2017) state the following:<sup>4</sup>

- “If a patient has ER/PgR–positive, HER2-negative, node-negative, breast cancer, the MammaPrint assay may be used in those with high clinical risk per MINDACT categorization to inform decisions on withholding adjuvant systemic chemotherapy due to its ability to identify a good prognosis population with potentially limited chemotherapy benefit (Type: evidence based; Evidence quality: high; Strength of recommendation: strong).”
- “If a patient has ER/PgR–positive, HER2-negative, node-negative, breast cancer, the MammaPrint assay should not be used in those with low clinical risk per MINDACT categorization to inform decisions on withholding adjuvant systemic chemotherapy, because women in the low clinical risk category had excellent outcomes and did not appear to benefit from chemotherapy even with a genomic high-risk cancer (Type: evidence based; Evidence quality: high; Strength of recommendation: strong).”
- “If a patient has ER/PgR–positive, HER2-negative, node-positive, breast cancer, the MammaPrint assay may be used in patients with one to three positive nodes and at high clinical risk per MINDACT categorization to inform decisions on withholding adjuvant systemic chemotherapy due to its ability to identify a good prognosis population with potentially limited chemotherapy benefit. However, such patients should be informed that a benefit of chemotherapy cannot be excluded, particularly in patients with greater than one involved lymph node (Type: evidence based; Evidence quality: high; Strength of recommendation: moderate).”
- “If a patient has ER/PgR–positive, HER2-negative, node-positive, breast cancer, the MammaPrint assay should not be used in patients with one to three positive nodes and at low clinical risk per MINDACT categorization to inform decisions on

withholding adjuvant systemic chemotherapy. There are insufficient data on the clinical utility of MammaPrint in this specific patient population (Type: informal consensus; Evidence quality: low; Strength of recommendation: moderate).”

- “If a patient has HER2-positive breast cancer, the clinician should not use the 70-gene assay (MammaPrint) to guide decisions on adjuvant systemic therapy. (Type: informal consensus. Evidence quality: low. Strength of recommendation: moderate).”
- “If a patient has TN breast cancer, the clinician should not use the 70-gene assay (MammaPrint) to guide decisions on adjuvant systemic therapy. (Type: informal consensus. Evidence quality: insufficient. Strength of recommendation: strong).”

### **St. Gallen International Expert Consensus**

St. Gallen International Expert Consensus (updated 2017):<sup>5</sup>

- “The panel agreed that there was no role in clinical low risk cases [such as pT1a/b, grade 1 (G1), ER high, N0] and similar settings where chemotherapy would not be indicated under any circumstances.”
- “The panel agreed that a number of gene expression signatures served as prognostic markers in the setting of adjuvant endocrine therapy in node-negative breast cancers, including the 21 gene recurrence score, the 70 gene signature, the PAM50 ROR score<sup>V R</sup>, the EpClin score<sup>V R</sup>, and the Breast Cancer Index <sup>V R</sup>. The Panel endorsed all of these assays for guiding the decision on adjuvant chemotherapy in node-negative tumors as they all identify node-negative cases at low risk, with an excellent prognosis that would not warrant chemotherapy.”
- “The panel agreed that gene expression signatures offered information that can refine the prognosis for node-positive breast cancers. However, the Panel did not uniformly endorse the use of gene expression signatures for making treatment decisions regarding adjuvant chemotherapy in node positive cases.”
- “The panel did not recommend the use of gene expression signatures for choosing whether to recommend extended adjuvant endocrine treatment, as no prospective data exist and the retrospective data were not considered sufficient to justify the routine use of genomic assays in this setting.”
- “In patients who are not candidates for adjuvant chemotherapy owing to comorbid health conditions or tumor stage/risk, or in patients who ‘obviously’ need adjuvant chemotherapy, typically including stage III breast cancer, there is no routine need for genomic tests.”
- “In general the zone ‘in between’ is where genomic assays may be most valuable. These would often be patients with tumors between 1 and 3 cm, with zero to two or three positive lymph nodes, and intermediate proliferative fraction. Multigene assay should not be the only factor considered in making a decision to proceed or to avoid chemotherapy.”

## European Society of Medical Oncology

European Society of Medical Oncology (ESMO) 2015:<sup>6</sup>

- “Gene expression profiles, such as MammaPrint (Agendia, Amsterdam, the Netherlands), Oncotype DX Recurrence Score (Genomic Health, Redwood City, CA), Prosigna (Nanostring Technologies, Seattle, WA) and EndoPredict (Myriad Genetics), may be used to gain additional prognostic and/or predictive information to complement pathology assessment and to predict the benefit of adjuvant chemotherapy. The three latter tests are designed for patients with ER-positive early breast cancer only.”
- “In cases of uncertainty regarding indications for adjuvant chemotherapy (after consideration of other tests), gene expression assays, such as MammaPrint, Oncotype DX, Prosigna and Endopredict, may be used, where available.”
- “In cases when decisions might be challenging, such as luminal B HER2-negative and node-negative breast cancer, commercially available molecular signatures for ER-positive breast cancer, such as Oncotype DX, EndoPredict, Prosigna, and for all types of breast cancer (pN0–1), such as MammaPrint and Genomic Grade Index, may be used in conjunction with all clinicopathological factors, to help in treatment decision making.”

## Evaluation of Genomic Applications in Practice and Prevention

The Evaluation of Genomic Applications in Practice and Prevention (EGAPP, 2009) Working Group reviewed the evidence for MammaPrint and concludes:<sup>7</sup>

- “It is unclear what population of patients would derive benefit from use of the test, and what the magnitude of that benefit would be. Prospective data from trials like MINDACT will be extremely valuable.”
- “Overall, published evidence supports MammaPrint as a better predictor of the risk of distant recurrence than traditionally used tumor characteristics or algorithms, but its performance in therapeutically homogeneous populations is not yet known with precision, and it is unclear for how many women the lowest predicted risks are low enough to forgo chemotherapy.”
- “No evidence is available to permit conclusions regarding the clinical utility of MammaPrint to select women who will benefit from chemotherapy.”
- “To conclude, the literature on the 70-gene signature includes numerous studies that focused more on its biological underpinning and less on the clinical implications of this gene expression profile, although it has now received FDA approval for clinical use.”

## US Food and Drug Administration

The US Food and Drug Administration (FDA) cleared MammaPrint for clinical use on fresh tissue samples in 2007.<sup>2</sup> The FDA cleared MammaPrint for clinical use on FFPE samples in 2015.<sup>1</sup>

## Literature Review

While the clinical validity of the test has been established, data regarding the clinical utility of MammaPrint is still emerging.<sup>8-24</sup>

- The current evidence base, consisting of a single open-label RCT and a number of small, retrospective studies, is limited and of poor to moderate quality, to conclude that foregoing chemotherapy is a safe and will not lead to increased risk of recurrence and death. It remains unclear if decisions to forego adjuvant chemotherapy based on MammaPrint results lead to significantly improved patient health outcomes, including long-term overall survival, distant-free survival, and QOL.
- There is a lack of direct evidence regarding clinical utility. Future well-designed clinical studies with long-term follow-up data are necessary to capture late distant recurrence occurring beyond 5 years. Study designs should include comparisons of survival outcomes following treatment guided by MammaPrint and clinical assessment to adequately assess clinical utility including quality of life measures in well-designed clinical trials are also necessary to help understand the complete value of MammaPrint and to help weigh the benefits and harms of foregoing chemotherapy in clinical practice.

## Criteria

- Previous Testing:
  - No repeat MammaPrint testing on the same sample when a result was successfully obtained, and
  - No previous gene expression assay (e.g. Prosigna) performed on the same sample when a result was successfully obtained, AND
- Testing Multiple Samples:
  - When more than one breast cancer primary is diagnosed:
    - There should be reasonable evidence that the tumors are distinct (e.g., bilateral, different quadrants, different histopathologic features, etc.), and
    - There should be no evidence from either tumor that chemotherapy is indicated with or without knowledge of the MammaPrint test result (e.g., histopathologic features or previous MammaPrint result of one tumor suggest chemotherapy is indicated), and

- If both tumors are to be tested, both tumors must independently meet the required clinical characteristics outlined below.
- Required Clinical Characteristics:
  - Invasive breast cancer meeting all of the following criteria:
    - Tumor size >0.5cm (5mm) in greatest dimension (T1b-T3), and
    - Estrogen receptor positive (ER+), and
    - HER2 negative, and
  - Patient has no regional lymph node metastasis (pN0) or only micrometastases (pN1mi, malignant cells in regional lymph node(s) not greater than 2.0mm), and
  - Chemotherapy is a treatment option for the patient; results from this MammaPrint test will be used in making chemotherapy treatment decisions, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

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# Mammostrat Breast Cancer Recurrence Assay

MOL.TS.201.A  
v1.0.2020

## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedure addressed by this guideline | Procedure codes |
|---------------------------------------|-----------------|
| Mammostrat Breast Cancer Assay        | 84999<br>S3854  |

## What is the Mammostrat Breast Cancer Recurrence Assay

### Definition

The Mammostrat<sup>®</sup> Breast Cancer Recurrence Assay is an immunohistochemical (IHC) assay that measures levels of five proteins in tumor tissue associated with risk of breast cancer recurrence.<sup>1</sup>

- It is used in people with newly diagnosed, early stage breast cancer.
- The assay looks at five proteins and determines their expression levels in the tumor. The expression levels of these five markers are thought to influence whether the tumor will metastasize, increasing the patient's chance of recurrence. These levels are then translated into a risk index, given as a percent chance of recurrence over 10 years.
- Physicians and patients may use the risk index as one factor in determining the course of treatment. Patients in the high risk category may benefit more from aggressive treatment, whereas patients in the low risk category may elect to forgo the aggressive chemotherapy.<sup>2</sup>

### Test information

- The Mammostrat assay measures the expression level of five proteins by immunohistochemistry. These markers are believed to be associated with breast cancer recurrence:<sup>3</sup>
  - **p53** plays a role in cell cycle regulation. Mutations in the p53 gene are associated with tumor growth.



- **HTF9C** is implicated in DNA replication and cell cycle control.
  - **CEACAM5** is normally expressed in embryonic tissue, but is also found in some tumors.
  - **NDRG1** may have a role in helping tumors survive aggressive treatment.
  - **SLC7A5** can, when overexpressed, help sustain the high growth rate of cancer.
- These levels are then translated into a quantitative “risk index” via a proprietary algorithm, which divides patients into groups with low, moderate, or high risk of recurrence:<sup>1</sup>

| Risk index | Risk of breast cancer recurrence over 10 years |
|------------|--|
| Low        | 7.6%   |
| Moderate   | 16.3%  |
| High       | 20.9%  |

## Guidelines and evidence

- The NCCN does not specifically mention the use of Mammostrat in its most recent guidelines.
- The American Society of Clinical Oncology (ASCO, 2016)<sup>4</sup> published a clinical practice guideline on the use of biomarkers to guide decision-making in women with early-stage invasive breast cancer. They recommend:<sup>4</sup>
  - “If a patient has ER/PgR-positive, HER2-negative (node-positive or node-negative) breast cancer, the clinician should not use the five-protein assay (Mammostrat; Clariant, a GE Healthcare company, Aliso Viejo, CA) to guide decisions on adjuvant systemic therapy. Type: evidence based. Evidence quality: intermediate. Strength of recommendation: moderate.”
  - “If a patient has HER2-positive breast cancer or TN breast cancer, the clinician should not use the five-protein assay (Mammostrat) to guide decisions on adjuvant systemic therapy. Type: informal consensus. Evidence quality: insufficient. Strength of recommendation: strong.”
- A 2010 clinical study tested the assay’s ability to accurately predict risk of breast cancer recurrence in a cohort of 1,812 women with early stage breast cancer:<sup>1</sup>
  - “The Mammostrat markers are biologically independent of one another and measure aspects of physiology distinct from proliferation, HER2 status, and hormone receptor status already assessed by IHC assays that are standard of care. Collectively these data add support to a potential role for Mammostrat in management of early-stage breast cancer.”

## Criteria

- This test is considered investigational and/or experimental.
  - Investigational and experimental (I&E) molecular and genomic (MolGen) tests refer to assays involving chromosomes, DNA, RNA, or gene products that have insufficient data to determine the net health impact, which typically means there is insufficient data to support that a test accurately assesses the outcome of interest (analytical and clinical validity), significantly improves health outcomes (clinical utility), and/or performs better than an existing standard of care medical management option. Such tests are also not generally accepted as standard of care in the evaluation or management of a particular condition.
  - In the case of MolGen testing, FDA clearance is not a reliable standard given the number of laboratory developed tests that currently fall outside of FDA oversight and FDA clearance often does not assess clinical utility.

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# Marfan Syndrome Genetic Testing

MOL.TS.202.A

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## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedures addressed by this guideline  | Procedure codes |
|---|-----------------|
| FBN1 Known Familial Mutation Analysis   | 81403           |
| TGFBR1 Known Familial Mutation Analysis | 81403           |
| TGFBR2 Known Familial Mutation Analysis | 81403           |
| FBN1 Sequencing                         | 81408           |
| FBN1 Deletion/Duplication Analysis      | 81479           |
| TGFBR1 Sequencing                       | 81405           |
| TGFBR2 Sequencing                       | 81405           |

## What is Marfan syndrome

### Definition

Marfan syndrome is an autosomal dominant disorder that affects connective tissue in many parts of the body. It affects about 1 in 5000 to 1 in 10000 individuals.<sup>1</sup>

- Symptoms can present in males or females at any age and typically worsen over time. Infants who present with symptoms typically have the most severe disease course.<sup>1</sup>
- Signs and symptoms of Marfan syndrome usually include (some combination of the following):<sup>1</sup>
  - Cardiovascular system — dilatation of the aorta, predisposition for aortic tear or rupture, mitral valve prolapse (with or without congestive heart failure), tricuspid valve prolapse, and enlargement of the proximal pulmonary artery.<sup>1</sup>
  - Skeletal system — long bone overgrowth and joint laxity, long arms and legs, scoliosis, sternum deformity (pectus excavatum or carinatum), pes planus, long thin fingers and toes, micrognathia, retrognathia, high-arched palate, deep set eyes, malar hypoplasia, down- slanting palpebral fissures, and long thin face.<sup>1</sup>

- Ocular system — severe myopia, dislocated lens of eye (ectopia lentis), elongation of the globe with or without flattened cornea, detached retina, glaucoma, early cataracts.<sup>1</sup>
- Other symptoms – dural ectasia (stretching of the dural sac), hernias, stretch marks on the skin, and lung bullae.<sup>1</sup>
- Clinical diagnosis-Ghent Criteria<sup>1-3</sup>
  - With no known family history, a Marfan syndrome diagnosis is confirmed if any ONE of the following is met:<sup>1-3</sup>
    - Significant aortic dilation (Z-score  $\geq 2$ )/dissection + ectopia lentis\*\*
    - Significant aortic dilation (Z-score  $\geq 2$ )/dissection + FBN1 mutation
    - Aortic dilation/dissection + sufficient points from other system findings\*\*
    - Ectopia lentis + FBN1 mutation known to be associated with aortic disease
  - With a known family history, the presence of any ONE of the following is diagnostic:<sup>1-3</sup>
    - Ectopia lentis
    - Significant aortic root enlargement (Z-score  $\geq 2$  in those >20 years of age or  $\geq 3$  in those <20 years of age)\*\*
    - Sufficient points ( $\geq 7$ ) from other system findings\*\*
- \*\* Marfan syndrome can be clinically diagnosed in these cases, provided there are not other findings that more strongly suggest Sphrintzen-Goldberg syndrome, Loeys-Dietz syndrome, or vascular Ehlers-Danlos syndrome, which have clinical overlap. Or, these conditions are unlikely based on genetic or collagen testing.
- Systemic scoring system<sup>1-3</sup>
  - Wrist and Thumb Sign - 3 points
  - Wrist or Thumb Sign - 1 point
  - Pectus Carinatum deformity - 2 points
  - Pectus Excavatum or chest asymmetry -1 point
  - Hindfoot deformity - 2 points
  - Plan pes planus -1 point
  - Pneumothorax - 2 points
  - Dural Ectasia - 2 points
  - Protrusio Acetabulae - 2 points

- Reduced upper seg/lower seg and inc. arm span and height ratio - 1 point
  - Scoliosis or thoracolumbar kyphosis - 1 point
  - Reduced elbow extension - 1 point
  - 3 of 5 facial features: Dolichocephaly, enophthalmos, downslanting palpebral fissures, malar hypoplasia, retrognathia - 1 point
  - Skin striae - 1 point
  - Myopia - 1 point
  - Mitral Valve Prolapse - 1 point
- According to the Ghent criteria, many of the manifestations of Marfan syndrome can emerge with age. Therefore, it is not advisable to establish definitive alternative diagnosis in individuals younger than age 20 years who have some physical manifestations of Marfan syndrome but not enough for a clinical diagnosis. In this circumstance, the following is suggested:<sup>1,2</sup>
    - “If the systemic score is <7 and/or borderline aortic root measurements (Z-score <3) are present (without an FBN1 pathogenic variant), use of the term ‘nonspecific connective tissue disorder’ is suggested until follow-up echocardiographic evaluation shows aortic root dilation (Z-score  $\geq 3$ ).”<sup>1,2</sup>
    - “If an FBN1 pathogenic variant is identified in simplex or familial cases but aortic root Z-score is below 3.0, the term ‘potential Marfan syndrome’ should be used until the aorta reaches this threshold.”<sup>1,2</sup>
- Diagnostic evaluations recommended:
    - Ophthalmologist evaluation with someone familiar with Marfan<sup>1</sup>
    - Evaluation for skeletal manifestations by an orthopedist<sup>1</sup>
    - Cardiovascular evaluations<sup>1</sup>
    - Medical genetics evaluation<sup>1</sup>

## Genetics

- Marfan syndrome is caused by mutations in the FBN1 gene, located on chromosome 15.<sup>1,4</sup>
- Marfan syndrome is inherited in an autosomal dominant fashion. Everyone has 2 copies of the FBN1 gene. If one of these genes has a mutation, it is enough to cause Marfan syndrome. It affects males and females equally.<sup>1</sup>
- A person who is found to have a FBN1 mutation has a 50% chance to pass the mutation to his/her children. Prenatal testing is available when the FBN1 mutation in the family is known.

- Genetic testing for Marfan syndrome typically starts with sequencing of the FBN1 gene. If negative, deletion/duplication of FBN1 should be considered.<sup>1</sup>
- Mutations in the TGFBR1 or TGFBR2 gene have been found in some individuals with a clinical suspicion of MFS and no identifiable FBN1 mutation.<sup>1</sup> Mutations in TGFBR1/2 are associated with Loeys-Dietz syndrome (LDS). Some features of MFS and LDS overlap. However, people with LDS typically have a greater risk of frequent aortic dissection and rupture at smaller dimensions and in early childhood.<sup>1</sup>
- The presence of a mutation in the FBN1 gene alone does not diagnose Marfan syndrome. FBN1 mutations may cause conditions other than Marfan syndrome. Conversely, some people who meet the clinical diagnostic criteria for Marfan syndrome do not have an identifiable FBN1 mutation.<sup>1</sup>
- Approximately 25% of cases of Marfan syndrome are the result of a new genetic change (de novo mutation) in the affected person and are not inherited from a carrier parent.<sup>1</sup>

## Test information

- ***FBN1 Sequencing*** identifies an FBN1 gene mutation in approximately 70-93% of people with a clinical diagnosis of Marfan syndrome.<sup>1</sup>
- ***FBN1 Deletion/Duplication Analysis*** can be performed to look for other types of gene mutations when sequencing is negative. The percentage of people with a clinical diagnosis of Marfan syndrome and a deletion/duplication mutation is unknown.<sup>1</sup>
- ***FBN1 Known Familial Mutation***. If a FBN1 mutation is found in an affected person, other family members may be offered testing.<sup>1,5</sup>
- ***Additional Testing Information***
  - ***TGFBR1/2 Testing***. If a mutation is not found in FBN1 and there is a strong clinical suspicion of Marfan syndrome, TGFBR1/2 genetic testing may be indicated. Given the increased risk of aortic dissection and rupture at smaller dimensions and in early childhood in LDS,<sup>1</sup> it is important to confirm whether there is a mutation in one of these two genes.
  - ***Panel Testing***. There are other conditions which can cause familial aortic aneurysm and dissections and/or have overlapping features with Marfan syndrome. Many laboratories offer panel testing for FBN1 as well as other genes that cause these conditions.<sup>1</sup> Detection rates of expanded panels vary by laboratory and depend on the genes included and the methods used for testing.<sup>1</sup> A thorough clinical evaluation along with appropriate imaging studies will point to a specific diagnosis in many cases.<sup>1</sup> Testing for conditions that are clinically indicated is most appropriate.<sup>1</sup> Testing multiple genes, without supporting clinical features, has the potential to yield results that are difficult to interpret.<sup>1</sup> The chance that a variant of uncertain significance will be found increases as more

genes are tested. According to the American College of Medical Genetics and Genomics, “There is no case of classic, bona fide MFS due to mutations in a gene other than FBN1.”<sup>6</sup> Therefore, when there is a strong clinical suspicion for Marfan syndrome, genetic testing for genes other than FBN1 is typically not needed, with the exception of TGFBR1/2 testing.

## Guidelines and evidence

- The European Society of Cardiology (ESC, 2014) stated the following:<sup>7</sup>
  - “Once a familial form of TAAD is highly suspected, it is recommended to refer the patient to a geneticist for family investigation and molecular testing.” (Class I, Level C)
- The Canadian Cardiovascular Society (2014) stated the following:<sup>8</sup>
  - “We recommend clinical and genetic screening for suspected Marfan syndrome to clarify the nature of the disease and provide a basis for individual counseling” (Strong recommendation, High quality evidence)
  - “We recommend that genetic counseling and testing be offered to first degree relatives of patients in whom the causal mutation of a TAD-associated gene is identified. We recommend that aortic imaging be offered only to mutation carriers.” (Strong recommendation, low quality evidence)
- Joint evidence-based guidelines from ACCF/AHA/AATS/ACR/ASA/SCA/SCAI/SIR/STS/SVM (2010) for the diagnosis and management of thoracic aortic disease include Marfan syndrome. Genetic testing for Marfan syndrome is addressed in the following guidelines statements:
  - “If the mutant gene (FBN1, TGFBR1, TGFBR2, COL3A1, ACTA2, MYH11) associated with aortic aneurysm and/or dissection is identified in a patient, first-degree relatives should undergo counseling and testing. Then, only the relatives with the genetic mutation should undergo aortic imaging.”<sup>5</sup> [Class 1, Level of Evidence C. Recommendation that procedure or treatment is useful/effective. It is based on very limited populations evaluated and only expert opinion, case studies or standard of care.]<sup>5</sup>
  - “The criteria for Marfan syndrome is based primarily on clinical findings in the various organ systems affected in the Marfan syndrome, along with family history and FBN1 mutations status.”<sup>5</sup>
  - Recommend echo at baseline, repeat at 6 months to look for progression then yearly if stable (Class 1).
  - Determining genetic etiology guides prophylactic aortic surgery.
- An international group of Marfan syndrome experts initially proposed clinical diagnostic criteria for Marfan syndrome in 1996, called the Ghent nosology that gained wide acceptance.<sup>9</sup>

- The Ghent criteria were updated in 2010 and now address the role of FBN1 genetic testing in the diagnosis of Marfan syndrome.<sup>2</sup> They do not include guidelines about when to test for a familial mutation, but do indicate that finding a familial mutation is not sufficient evidence alone to make a definitive diagnosis, stating: “If an FBN1 mutation is identified in sporadic or familial cases but aortic root measurements are still below Z=3, we propose to use the term 'potential MFS' [Marfan syndrome] until the aorta reaches threshold.”
- According to the American College of Medical Genetics and Genomics, “There is no case of classic, bona fide MFS due to mutations in a gene other than FBN1. However, current clinical molecular testing of FBN1 successfully detects mutations in such unequivocal patients in only about 90-95% of cases. For all of these reasons, searching for mutations in FBN1 continues to have a circumscribed role in the diagnosis of equivocal cases. Said differently, MFS remains, by and large, a clinical diagnosis.”<sup>6</sup>
- Cardiac Society of Australia and New Zealand (CSANZ) Cardiovascular Genetic Diseases Council (2017):<sup>10</sup>
  - “A definitive molecular genetic diagnosis can clarify an equivocal clinical picture or result in a diagnosis in an apparently phenotypically normal individual. It is unknown at this stage what proportion of patients with these different genetic mutations will develop aortic dilatation or dissection. Identification of a causal mutation allows for the provision of accurate genetic counseling, the screening of at-risk family members and offers the possibility of accurate prenatal or preimplantation genetic diagnosis.”
  - “Molecular confirmation of a suspected clinical diagnosis is increasingly important for guiding patient management. As an example, an individual who looks marfanoid will have more extensive arterial imaging screening if identified to have a SMAD3 mutation as opposed to an FBN1 mutation.”

## Criteria

### FBN1 Known Familial Mutation Analysis

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - No previous genetic testing of FBN1, and
  - FBN1 mutation identified in 1<sup>st</sup> degree biological relative, OR
- Prenatal Testing for At-Risk Pregnancies:



- FBN1 mutation identified in a previous child or either parent, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

### **FBN1 Sequencing**

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Testing:
  - No previous FBN1 sequencing, and
  - No known FBN1 mutation in the family, AND
- Diagnostic Testing for Symptomatic Individuals:
  - Genetic testing is necessary because there is uncertainty in the clinical diagnosis, and
    - Aortic root enlargement (Z-score greater than or equal to 2.0) and a systemic score less than 7, without ectopia lentis, or
    - Ectopia lentis, or
  - An individual has a clinical diagnosis of Marfan syndrome based on the revised Ghent Criteria, and
    - Genetic testing is needed in order to offer testing to family members, or
    - Genetic testing is needed for prenatal diagnosis purposes, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

### **FBN1 Deletion/Duplication Analysis**

- Criteria for FBN1 Sequencing are met, AND
- No previous deletion/duplication analysis of FBN1, AND
- No mutations detected in full sequencing of FBN1, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

### **TGFBR1/2 Known Familial Mutation Analysis**

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND

- Previous Genetic Testing:
  - No previous genetic testing of TGFBR1/2, and
  - TGFBR1/2 mutation identified in 1<sup>st</sup> degree biological relative, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

### **TGFBR2 Sequencing**

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - No previous TGFBR2 testing performed, and
  - No mutations detected in full sequencing of FBN1, and
  - No mutations detected in deletion/duplication analysis of FBN1, AND
- Diagnostic Testing for Symptomatic Individuals:
  - There is a strong clinical suspicion of MFS based on the Ghent criteria (Member met testing guidelines for FBN1 sequencing), AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

### **TGFBR1 Sequencing**

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - No previous TGFBR1 testing performed, and
  - No mutations detected in full sequencing or deletion/duplication analysis of FBN1, and
  - No mutations detected in full sequencing of TGFBR2, AND
- Diagnostic Testing for Symptomatic Individuals:
  - There is a strong clinical suspicion of MFS based on the Ghent criteria (Member met testing guidelines for FBN1 sequencing), AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

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# Maturity-Onset Diabetes of the Young (MODY) Testing

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v1.0.2020

## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedures addressed by this guideline | Procedure codes |
|--|-----------------|
| ABCC8 Sequencing                       | 81407           |
| BLK Sequencing                         | 81479           |
| CEL Sequencing                         | 81479           |
| CEL Exon 11 Sequencing                 | 81403           |
| GCK Sequencing                         | 81406           |
| GCK Deletion/Duplication               | 81479           |
| HNF1A Sequencing                       | 81405           |
| HNF1A Deletion/Duplication             | 81479           |
| HNF1B Sequencing                       | 81405           |
| HNF1B Deletion/Duplication             | 81404           |
| HNF4A Sequencing                       | 81406           |
| HNF4A Deletion/Duplication             | 81479           |
| INS Sequencing                         | 81404           |
| KCNJ11 Sequencing                      | 81403           |
| KLF11 Sequencing                       | 81479           |
| NEUROD1 Sequencing                     | 81479           |
| PAX4 Sequencing                        | 81479           |
| PDX1 Sequencing                        | 81404           |

## What is MODY

### Definition

Maturity-onset diabetes of the young (MODY) is a type of monogenic diabetes

characterized by non-insulin-dependent diabetes and early onset (usually before age 35).<sup>1-4</sup>

## Incidence and Prevalence

Diabetes affects 29.1 million people in the United States, or 9.3% of the population.<sup>5</sup> The most common types of diabetes are type 1 and type 2. The genetic basis of these types of diabetes is largely unknown. The disease is thought to be the result of a combination of multiple genetic and environmental risk factors.<sup>5</sup> Monogenic forms of diabetes are rare, accounting for approximately 2% of all diabetes cases.<sup>1-3</sup>

## Symptoms

Diabetes is a disorder that results in elevated blood glucose. Over time, the disorder can cause various health problems, including diseases of the heart, kidneys, eyes, and nervous system.

## Cause

Monogenic forms of diabetes are caused by a mutation in a single gene. There are 14 known MODY genes, and three account for the majority of cases.<sup>1-3</sup>

- **MODY3:** Mutations in the hepatocyte nuclear factor-1 alpha (HNF1A) gene are the most common cause of MODY, accounting for about half of cases. This type is characterized by a progressive insulin secretory defect due to beta-cell failure. Laboratory evaluations are negative for pancreatic islet cell antibodies (ruling out type 1) and glycosuria is detectable even at low blood glucose levels (<10 mmol/l). Treatment of choice for people with this type of MODY is sulfonylureas, and a majority of patients can be transferred from insulin to oral agents.
- **MODY2:** Mutations in the glucokinase gene (GCK) are the next most common cause of MODY, accounting for about 20-25% of cases. GCK encodes the glucokinase enzyme, which acts as the pancreatic glucose sensor. Mutations result in lifelong, stable, mild fasting hyperglycemia. HbA1C values are usually just above the high normal range. People with GCK mutations rarely require treatment. This type of MODY may be detected during pregnancy, when glucose tolerance testing is routinely performed.
- **MODY1:** Mutations in the hepatocyte nuclear factor-4 alpha (HNF4A) gene cause a clinical presentation similar to HNF1A. However, mutations in this gene are much less common (less than 10% of MODY). Age of onset may be later, and there is not a low renal threshold. HNF4A mutations can also cause high birth weight in newborns and transient neonatal hypoglycemia. These patients are also more sensitive to sulfonylurea treatment.

The remaining genes are rare causes of MODY, each accounting for less than 1% of cases.<sup>1-3</sup>

- MODY5: Caused by heterozygous mutations in HNF1B. The vast majority of HNF1B mutations cause Renal Cysts and Diabetes Syndrome, which is associated with diabetes, renal cysts, genitourinary malformations, pancreatic atrophy, hyperuricemia, and abnormal liver function tests.
- MODY8: Caused by heterozygous mutations in CEL. Affected individuals also have pancreatic exocrine dysfunction (diabetes-pancreatic-exocrine dysfunction syndrome).
- Others include: MODY4 (PDX1/IPF-1), MODY6 (NEUROD1), MODY7 (KLF11), MODY9 (PAX4), MODY10 (INS), MODY11 (BLK), MODY12 (ABCC8) and MODY13 (KCNJ11), APPL1 (MODY14).

Other monogenic causes of pediatric diabetes include the following (not meant to be an all-inclusive list):<sup>2,7-8</sup>

- Permanent neonatal diabetes mellitus (PNDM), defined as persistent hyperglycemia in the first 6 months of life. It is most commonly caused by mutations in the ABCC8, KCNJ11, and INS genes. Biallelic mutations in GCK and PDX1 are less common causes.
- Transient neonatal diabetes mellitus (TNDM), which accounts for ~50% of all neonatal diabetes. Affected individuals are at risk for recurrence later in life. 70% of TNDM cases are due to 6q24 methylation defects, while ABCC8 and KCNJ11 combined account for an additional 26% of cases.
- Cystic fibrosis, caused by biallelic CFTR mutations (for more information, see test-specific guideline, *Cystic Fibrosis Testing*)
- Immune dysregulation, polyendocrinopathy, and enteropathy, X-linked (IPEX syndrome), due to mutations in FOXP3
- Maternally inherited diabetes and deafness (MIDD), caused by mutations in mitochondrial genes: MT-TL1, MT-TK, or MT-TE
- Wolcott-Rallison syndrome, due to mutations in EIF2AK3
- Wolfram syndrome, caused by mutations in WFS1 and less often CISD2
- Other genes associated with PNDM and extra-pancreatic features include GATA6, GLIS3, IER3IP1, NEUROG3, PTF1A, and RFX6.

## Inheritance

MODY is typically inherited in an autosomal dominant manner. When a parent has a MODY mutation, each of her/his offspring have a 50% risk of inheriting the mutation.<sup>1-4</sup> Mutations that occur de novo in an affected individual, reduced penetrance, and variable expressivity have been reported.<sup>4</sup> Thus, the absence of a family history does not, by itself, rule out a diagnosis of MODY.

## Diagnosis

Diabetes evaluations may include assessment of pancreatic autoantibodies, plasma glucose levels, hemoglobin A1C assessment (HbA1C), and oral glucose tolerance testing (OGTT). For young individuals in whom a diagnosis of type 1 or type 2 diabetes is considered unlikely, genetic testing for monogenic diabetes may be considered, especially in the presence of a strong family history.<sup>5</sup>

## Treatment

Like other forms of diabetes, monogenic diabetes is treated with diet, oral antidiabetic agents, and/or insulin, as required for blood sugar regulation.<sup>4</sup> Most patients with MODY are not insulin-dependent. Knowledge of the specific genetic cause of MODY may help guide management.

## Survival

Survival of affected individuals was reduced when compared with unaffected relatives, specifically with regard to cardiovascular-related causes of death.<sup>6</sup>

## Test information

### Introduction

Testing for MODY may include single gene sequence analysis, single gene deletion/duplication analysis, or multi-gene panels of various sizes.

### Sequence analysis

Until recently, most sequencing tests used the Sanger sequencing methodology that was originally developed in the 1970s. Sanger sequencing is labor intensive and did not lend itself to high-throughput applications.

Next generation sequencing (NGS), which is also sometimes called massively parallel sequencing, was developed in 2005 to allow larger scale and more efficient gene sequencing. NGS relies on sequencing many copies of small pieces of DNA simultaneously and using bioinformatics to assemble the sequence. NGS may not perform as well as Sanger sequencing in some applications.

NGS tests vary in technical specifications (e.g., depth of coverage, extent of intron/exon boundary analysis, methodology of large deletion/duplication analysis).

Sequence analysis detects single nucleotide substitutions and small (several nucleotide) deletions and insertions. Regions analyzed typically include the coding sequence and intron/exon boundaries. Promoter regions and intronic sequences may also be sequenced if disease-causing mutations are known to occur in these regions of a gene.

The efficiency of NGS has led to an increasing number of large, multi-gene testing panels. NGS panels that test several genes at once are particularly well-suited to conditions caused by more than one gene or where there is considerable clinical overlap between conditions.

Results may be obtained that cannot be adequately interpreted based on the current knowledge base. When a sequence variation is identified that has not been previously characterized or shown to cause the disorder in question, it is called a variant of uncertain significance (VUS). VUSs are relatively common findings when sequencing large amounts of DNA with NGS.

Under certain circumstances, technologies used in multi-gene testing may fail to identify mutations that might be identifiable through single-gene testing. If high clinical suspicion exists for a particular syndrome testing for that syndrome should be performed instead of a broad multi-gene panel.

Since genes can be easily added or removed from multi-gene tests over time by a given lab, medical records must document which genes were included in the specific multi-gene test used and in which labs they were performed.

Additionally, tests should be chosen to

- maximize the likelihood of identifying mutations in the genes of interest
- contribute to alterations in patient management
- minimize the chance of finding variants of uncertain clinical significance

### **MODY gene sequence analysis**

MODY multi-gene panels include a wide variety of genes associated with MODY and monogenic diabetes in general. Some panels may also include genes associated with other types of monogenic diabetes and glycemic disorders, such as neonatal diabetes, syndromic diabetes, and familial hyperinsulinism.

### **Deletion/duplication analysis**

Analysis for deletions and duplications can be performed using a variety of technical platforms including exon array, MLPA, and NGS data analysis.

These assays detect gains and losses too large to be identified through sequencing technology, often single or multiple exons or whole genes.

## **Guidelines and evidence**

### **American Diabetes Association**

The American Diabetes Association (2017) states: “Children and adults, diagnosed in early adulthood, who have diabetes not characteristic of type 1 or type 2 diabetes that occurs in successive generations (suggestive of an autosomal dominant pattern of



inheritance) should have genetic testing for maturity-onset diabetes of the young”(Grade A recommendation).<sup>9</sup>

### **National Academy of Clinical Biochemistry**

The National Academy of Clinical Biochemistry (2011) states (direct quotes):<sup>10</sup>

- Routine measurement of genetic markers is not of value at this time for the diagnosis or management of patients with type 1 diabetes. For selected diabetic syndromes, including neonatal diabetes, valuable information can be obtained with definition of diabetes-associated mutations. A (moderate)
- There is no role for routine genetic testing in patients with type 2 diabetes. These studies should be confined to the research setting and evaluation of specific syndromes. A (moderate)

### **International Society for Pediatric and Adolescent Diabetes**

The International Society for Pediatric and Adolescent Diabetes (2014) makes the following recommendations:<sup>2</sup>

- “The diagnosis of maturity-onset diabetes of the young (MODY) should be suspected in cases with”
  - “A family history of diabetes in one parent and first degree relatives of that affected parent in patients who lack the characteristics of type 1 diabetes [no islet autoantibodies, low or no insulin requirements 5 yr after diagnosis (stimulated C-peptide >200 pmol/L)] and lack the characteristics type 2 diabetes (marked obesity, acanthosis nigricans).”
  - “Mild stable fasting hyperglycemia which does not progress. Such cases should be tested for glucokinase (GCK) gene mutations, which is the commonest cause of persistent, incidental hyperglycemia in the pediatric population (B).”
- “Specific features can suggest subtypes of MODY, such as renal developmental disease or renal cysts (HNF1B-MODY) and macrosomia and/or neonatal hypoglycemia (HNF4A-MODY) (C).”
- “In familial autosomal dominant symptomatic diabetes, mutations in the hepatocyte nuclear factor 1 $\alpha$  (HNF1A) gene (HNF1A-MODY) should be considered as the first diagnostic possibility, while mutations in the GCK gene are the most common cause in the absence of symptoms or marked hyperglycemia (B).”
- “Three genes are responsible for the majority of MODY cases (GCK, HNF1A, and HNF4A) ... However, up to 13 different genes have been reported to cause autosomal dominant non-insulin dependent diabetes but these are so unusual they do not need to be tested for in children with diabetes except in a research setting or when there are additional phenotypes such as pancreatic exocrine dysfunction.”

## European Molecular Genetics Quality Network

The European Molecular Genetics Quality Network (2008) makes the following recommendations for testing (paraphrased due to their length):<sup>3</sup>

- Testing for GCK mutations (presentation outside of pregnancy):
  - Persistent, stable elevation of fasting blood glucose (5.5-8 mmol/l)
  - HbA1c just above the upper limit of normal (rarely exceeds 7.5%)
  - Oral glucose tolerance testing demonstrates a small increment (4.6 mmol/l is often used to prioritize testing)
  - May have a family history consistent with autosomal dominant inheritance
- Testing for GCK mutations (for evaluation of gestational diabetes):
  - Persistent elevation of fasting blood glucose (5.5-8 mmol/l) before, during and after pregnancy
  - At least one oral glucose tolerance test with an increment of <4.6 mmol/l (either during or after pregnancy)
- Testing for HNF1A mutations:
  - Young-onset diabetes (<25 years old)
  - Non-insulin-dependent diabetes
  - Family history of diabetes (at least two generations)
  - Absence of pancreatic islet autoantibodies
  - Glycosuria at blood glucose levels <10 mmol/l
  - Marked sensitivity to sulfonylureas
  - Features suggestive of monogenic diabetes (lack of obesity or evidence of insulin resistance, absence of acanthosis nigricans, etc)
- Testing for HNF4A mutations:
  - Should be considered when HNF1A analysis is normal but the clinical features are strongly suggestive of HNF1A
  - “When diabetic family members have marked macrosomia (>4.4 kg at term) or if diazoxide-responsive neonatal hyperinsulinism has been diagnosed in the context of familial diabetes.”
  - “Macrosomic babies with diazoxide-responsive hyperinsulinism and a strong family history of diabetes should be considered for HNF4A mutation screening.”

- Syndromic forms of diabetes, including HNF1B and CEL mutations, “are not included in these guidelines since testing is guided by the non-endocrine pancreatic or extra-pancreatic clinical features.”

## Literature Review

An expert-authored review (2018) suggests that MODY has an onset in adolescence or young adulthood, typically less than 35 years.<sup>4</sup>

- “Molecular genetic testing approaches to determine the associated MODY gene can include a combination of gene-targeted testing (serial single-gene or multigene panel) and comprehensive genomic testing (chromosomal microarray analysis or exome sequencing), depending on the phenotype.”
- “Serial single-gene testing. Sequence analysis of the most likely genes is performed first. If no pathogenic variant is found, gene-targeted deletion/duplication analysis to detect exon-sized deletions could be considered, especially for those genes (CEL, GCK, HNF1A, HNF1B, and HNF4A) in which whole-gene or multiexon deletions have been identified.”
- “A MODY multigene panel that includes the 14 known MODY-related genes and other genes of interest is most likely to identify the genetic cause of MODY at the most reasonable cost while limiting identification of variants of uncertain significance and pathogenic variants in genes that do not explain the underlying phenotype [Ellard et al 2013, Alkorta-Aranburu et al 2016].”
  - a) “The genes included in the panel and the diagnostic sensitivity of the testing used for each gene vary by laboratory and are likely to change over time.”
  - b) “Some custom laboratory-designed multigene panels may include genes not associated with MODY but possibly associated with other types of monogenic diabetes; other custom laboratory-designed panels may not include the genes that rarely cause MODY.”
  - c) “In some laboratories, panel options may include a custom laboratory-designed panel and/or custom phenotype-focused exome analysis that include genes specified by the clinician.”

## Criteria

This guideline applies to all MODY testing, including single genes as well as multi-gene panels, which are defined as assays that simultaneously test for more than one MODY gene. Medical necessity determination generally relies on criteria established for testing individual genes.

Medical necessity criteria differ based on the type of testing being performed (i.e., individual MODY genes separately chosen versus pre-defined panels of MODY genes) and how that testing will be billed (one or more individual MODY gene procedure codes, specific panel procedure codes, or unlisted procedure codes).

These guidelines are for gene testing in the context of MODY evaluation only. For gene testing in non-MODY contexts (e.g., neonatal diabetes, familial hyperinsulinism, etc.), refer to the general policies, *Genetic Testing to Diagnose Non-Cancer Conditions* and *Genetic Testing by Multigene Panels*, as appropriate.

### **HNF1A Sequencing and Deletion/Duplication Analysis**

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - No previous HNF1A gene sequencing or deletion/duplication analysis, and
  - No known MODY mutation in biologic relative, AND
- Diagnostic Testing for Symptomatic Individuals:
  - Member has a diagnosis of diabetes prior to 35 years of age, and
  - Member has a biological parent with diabetes, and
  - Member does NOT have symptoms consistent with a specific condition or specific gene mutation, and
  - Member does NOT have any of the following features:
    - Extra-pancreatic manifestations (e.g., congenital malformations and other signs of syndromic diabetes), or
    - Pancreatic autoantibodies suggestive of type 1 diabetes, or
    - Body mass index (BMI) greater than or equal to 35 kg/m<sup>2</sup>, or
    - Acanthosis nigricans, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

### **HNF4A Sequencing and Deletion/Duplication Analysis**

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - No previous HNF4A gene sequencing or deletion/duplication analysis, and
  - No known MODY mutation in biologic relative, and
  - Member has previous HNF1A testing with no deleterious mutation found, AND

- Rendering laboratory is a qualified provider of service per the Health Plan policy.

### **GCK Sequencing and Deletion/Duplication Analysis**

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - No previous GCK gene sequencing or deletion/duplication analysis, and
  - No known MODY mutation in biologic relative, AND
- Diagnostic Testing for Symptomatic Individuals:
  - Member has previous HNF1A testing with no deleterious mutation found, or
  - Member has a personal history of the following features presenting outside of pregnancy:
    - Persistent, stable elevation of fasting blood glucose (5.5-8 mmol/L), and
    - HbA1C that is no more than mildly elevated (less than or equal to 7.5%), and
    - At least one oral glucose tolerance test demonstrates a small increment (less than 4.6 mmol/L), or
  - Member has a personal history of the following features in the context of gestational diabetes:
    - Persistent elevation of fasting blood glucose (5.5-8 mmol/L) before, during, and after pregnancy, and
    - At least one oral glucose tolerance test demonstrates a small increment (less than 4.6 mmol/L) either during or after pregnancy, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

### **Sequencing and Deletion/Duplication Analysis of ABCC8, BLK, CEL, HNF1B, INS, KCNJ11, KLF11, NEUROD1, PAX4, and PDX1**

Sequencing and deletion/duplication analysis of these genes in the context of MODY testing is not a covered benefit.

- The clinical utility of these tests for the evaluation of MODY has not been well established. Mutations in HNF1A, GCK, and HNF4A are responsible for the majority of cases of MODY, making them the most common known genetic causes of the disorder. There are other genes associated with MODY, but mutations in each gene account for greater than 1% of cases of MODY, therefore incremental mutation yield

of individual gene testing is expected to be very low. In addition, medical management guidelines have not been established for most of these forms of MODY.

- Gene testing is not covered strictly for the indication of MODY testing. Testing in other contexts may meet medical necessity criteria (e.g., HNF1B testing for individuals with symptoms of Renal Cysts and Diabetes Syndrome, CEL testing for individuals with diabetes and pancreatic exocrine dysfunction, or certain gene tests for individuals with neonatal diabetes or familial hyperinsulinism). For gene testing in non-MODY contexts, refer to *Genetic Testing for Non-Cancer Conditions*.

### **MODY Multi-Gene Panels**

When separate procedure codes will be billed for individual MODY genes (e.g., Tier 2 MoPath codes 81400-81408), each individually billed test will be evaluated separately. The below criteria for single gene testing will be applied.

If the member meets the following criteria, the entire panel will be approved. However, the laboratory will be redirected to use a panel CPT code for billing purposes (e.g. 81479):

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - No previous MODY genetic testing, and
  - No known MODY mutation in biologic relative, AND
- Diagnostic Testing for Symptomatic Individuals:
  - Member has a diagnosis of diabetes prior to 35 years of age, and
  - Member has a family history of diabetes consistent with autosomal dominant inheritance, and
  - Member does NOT have symptoms consistent with a specific condition or specific gene mutation, and
  - Member does NOT have any of the following features:
    - Extra-pancreatic manifestations (e.g., congenital malformations and other signs of syndromic diabetes), or
    - Pancreatic autoantibodies suggestive of type 1 diabetes, or
    - Body mass index (BMI) greater than or equal to 35 kg/m<sup>2</sup>, or
    - Acanthosis nigricans, AND

- Rendering laboratory is a qualified provider of service per the Health Plan policy.

When a multi-gene panel is being requested and will be billed with a single panel CPT code (e.g. 81479), the panel will be considered medically necessary when the following criteria are met:

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - No previous MODY genetic testing, and
  - No known MODY mutation in biologic relative, AND
- Diagnostic Testing for Symptomatic Individuals:
  - Member has a diagnosis of diabetes prior to 35years of age, and
  - Member has a family history of diabetes consistent with autosomal dominant inheritance, and
  - Member does NOT have symptoms consistent with a specific condition or specific gene mutation, and
  - Member does NOT have of the following features:
    - Extra-pancreatic manifestations (e.g., congenital malformations and other signs of syndromic diabetes), or
    - Pancreatic autoantibodies suggestive of type 1 diabetes, or
    - Body mass index (BMI) greater than or equal to 35 kg/m<sup>2</sup>, or
    - Acanthosis nigricans, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

### **Billing and reimbursement considerations**

- When multiple CPT codes are billed for components of a panel and there is a more appropriate CPT code representing the panel, eviCore will redirect to the panel code(s).
- If the laboratory will not accept redirection to a panel code, the medical necessity of each billed component procedure will be assessed independently.
  - In general, only a limited number of panel components that are most likely to explain the member's presentation will be reimbursable. The remaining panel components will not be reimbursable.

- When a MODY multi-gene panel is billed with multiple stacked codes, only the following genes may be considered for reimbursement:
  - HNF1A
  - GCK
  - HNF4A

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# Multiple Endocrine Neoplasia Type 2 (MEN2)

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## Introduction

Multiple Endocrine Neoplasia Type 2 (MEN2) is addressed by this guideline.

## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedures addressed by this guideline | Procedure codes |
|--|-----------------|
| RET Known Familial Mutation Analysis   | 81403           |
| RET Targeted Mutation Analysis         | 81404           |
| RET Targeted Sequencing                | 81405           |
| RET Full Gene Sequencing               | 81406           |

## What is Multiple Endocrine Neoplasia Type 2

### Definition

Multiple Endocrine Neoplasia Type 2 (MEN2) is a group of autosomal dominant hereditary cancer predisposition syndromes caused by mutations in the RET proto-oncogene. There are two different clinical subtypes of MEN2: MEN2A, which includes the familial medullary thyroid cancer subtype (91%; 35% with isolated FMTC), and MEN2B (9%)<sup>1</sup>.

### Incidence or Prevalence

The prevalence of all subtypes of MEN2 worldwide is estimated to be 1/35,000 to 1/40,000.<sup>1,2</sup>

### Symptoms

#### MEN 2A

MEN2A is further subclassified:

- Classic MEN2A

- MEN2A with cutaneous lichen amyloidosis (CLA)
- MEN2A with Hirschsprung's disease (HD)
- Familial medullary thyroid cancer (FMTC) was once considered to be a separate subtype from MEN2A, and is now widely considered to be a variant of MEN2A with decreased penetrance of pheochromocytoma and primary hyperparathyroidism (PHPT).<sup>1</sup>

MEN 2A should be suspected in individuals with one or more specific endocrine tumors- medullary thyroid cancer (and/or its precursor, C-cell hyperplasia), pheochromocytoma, or parathyroid adenoma/hyperplasia.

- Approximately 95% of individuals will have medullary thyroid cancer (MTC), typically at a younger age of onset than sporadic MTC, as a presenting symptom. The MTC is more often associated with C-cell hyperplasia and tends to be multifocal or bilateral.
- Approximately 50% of individuals with MEN2A will develop pheochromocytoma (PCC). PCC has the tendency to be adrenal and bilateral.<sup>3-5</sup> PCC is the first sign in approximately 13-27% of individuals with MEN2A.<sup>6,7</sup>
- Approximately 20-30% of individuals with MEN2A will develop primary hyperparathyroidism. <sup>1</sup>

## **MEN2B**

MEN2B should be suspected in individuals with distinctive facies (including lip mucosal neuromas resulting in thick vermilion of the upper and lower lip), mucosal neuromas of the lips and tongue, medullated corneal nerve fibers, marfanoid habitus, and MTC.

- MEN2B is characterized by early development of an aggressive form of MTC in all affected individuals.<sup>2</sup>
- PCCs occur in 50% of individuals with MEN2B, where approximately half are multiple and often bilateral.
- Clinically significant parathyroid disease is absent in MEN2B.
- MEN2B may be identified in infancy or early childhood by the presence of mucosal neuromas on the anterior dorsal surface of the tongue, palate, or pharynx, and a distinct facial appearance. Approximately 40% of affected individuals have diffuse ganglioneuromatosis of the gastrointestinal tract. Approximately 75% of affected individuals have a marfanoid habitus, often with kyphoscoliosis or lordosis, joint laxity, and decreased subcutaneous fat.

## **Cause**

Over 95% cases of MEN 2 are due to mutations in RET, a proto-oncogene and tyrosine kinase. Gain of function mutations allow activation without dimerization of the protein or

dimerization of the protein in the absence of ligand (constitutive activation).<sup>2</sup> Pathogenic variants have been reported in exons 5, 8, 10, 11, 13, 14, 15, and 16 (with mutations in exons 10 and 11 comprising 95% of individuals with MEN2A). Genotype-phenotype correlations are known for RET mutations.<sup>1</sup>

## Inheritance

MEN2 is inherited in an autosomal dominant pattern, meaning that an affected individual has inherited one RET mutation from an affected parent. MEN2 can also result from a new (de novo) RET mutation in the affected individual.

Individuals with MEN2 have a 50% chance of passing the mutation to their children. Additionally, parents and siblings of known carriers have a 50% chance of being carriers of the same mutation.

Approximately 5-9% of MEN2A and 50% of MEN2B are caused by de novo RET mutations not inherited from an affected parent.<sup>1,2</sup> Siblings would still need to be tested to rule out germline mutations.

MEN2 is associated with high penetrance and variable expressivity.

## Diagnosis

The diagnosis of MEN2 is established based on clinical presentation, family history, and genetic testing. Identification of a pathogenic RET variant establishes the diagnosis if clinical features are inconclusive.<sup>2</sup> Genetic testing to identify germline RET mutations is indicated in all individuals with primary C-cell hyperplasia or medullary thyroid cancer or a clinical diagnosis of MEN2, regardless of whether there is a family history.

### MEN2A

- The occurrence of two or more specific endocrine tumors (medullary thyroid cancer, pheochromocytoma, and/or parathyroid adenoma/hyperplasia) in the patient or in close relatives
- Familial medullary thyroid carcinoma (FMTC) is suspected in families with four or more cases of MTC in the absence of pheochromocytoma or parathyroid adenoma/hyperplasia.<sup>2</sup> However, distinguishing this subtype from classical MEN2A can be challenging for some small families.

### MEN2B

- The presence of early-onset medullary thyroid cancer, mucosal neuromas of the lips and tongue, medullated corneal nerve fibers, distinctive facies with enlarged lips, and a marfanoid body habitus.<sup>2</sup>

MEN2

## Treatment

Management and prevention strategies for those with or at-risk for MEN2 include prophylactic thyroidectomy, biochemical screening for functioning pheochromocytoma, and ongoing monitoring for residual MTC, hypoparathyroidism, and pheochromocytoma.

## Survival

Survival in MEN2 can be reduced and is largely dependent on clinical presentation and stage of cancer at the time of diagnosis.<sup>1</sup>

## Test information

### Introduction

Testing for MEN2 may include targeted mutation analysis, sequence analysis, or known familial mutation testing.

### Targeted mutation analysis

Targeted mutation analysis use hybridization, single nucleotide extension, select exon sequencing, or similar methodologies to assess a set of disease-causing mutations.

This analysis identifies common and/or recurring mutations.

Targeted mutation panels or select exon sequencing may have differing clinical sensitivities dependent upon patient ethnicity, phenotypic presentation, or other case-specific characteristics.

RET targeted sequencing may evaluate exons 5, 8, 10, 11, and 13-16, where most disease-causing mutations have been reported. Such testing will detect 98% of mutations associated with MEN2A and 95% of mutations associated with FMTC.<sup>8-11</sup>

Targeting 2 RET mutations (p.Met918Thr and p.Ala883Phe) will detect 98% of RET mutations associated with MEN2B.<sup>12,13</sup> As the phenotype is distinct from MEN2A, targeting these two mutations may be more efficient than select exon sequencing for MEN2B.

### Full Gene Sequence analysis

Sequence analysis detects single nucleotide substitutions and small (several nucleotide) deletions and insertions. Regions analyzed typically include the coding sequence and intron/exon boundaries. Promoter regions and intronic sequences may also be sequenced if disease-causing mutations are known to occur in these regions of a gene.

Results may be obtained that cannot be adequately interpreted based on the current knowledgebase. When a sequence variation is identified that has not been previously

characterized or shown to cause the disorder in question, it is called a variant of uncertain significance (VUS). VUSs are relatively common findings when sequencing large amounts of DNA with NGS.

Additionally, tests should be chosen to

- maximize the likelihood of identifying mutations in the genes of interest
- contribute to alterations in patient management
- minimize the chance of finding variants of uncertain clinical significance.

### **Deletion/duplication analysis**

Analysis for deletions and duplications can be performed using a variety of technical platforms including exon array, MLPA, and NGS data analysis.

Deletion/duplication panels may be billed separately from sequencing panels.

These assays detect gains and losses too large to be identified through sequencing technology, often single or multiple exons or whole genes.

Deletion/duplication analysis for MEN2 is typically not a consideration as the mutational mechanism is gain of function caused by missense variants and small in frame deletions and duplications.

### **Known familial mutation analysis**

Analysis for known familial mutations is typically performed by Sanger sequencing, but if available, a targeted mutation panel that includes the familial mutation may be performed.

Known familial mutations analysis is performed when a causative mutation has been identified in a close relative of the individual requesting testing.

## **Guidelines and evidence**

### **Introduction**

The following section includes relevant guidelines and evidence pertaining to MEN2 testing.

### **National Comprehensive Cancer Network**

Evidence-based guidelines from the National Comprehensive Cancer Network (NCCN, 2019) support genetic counseling and RET genetic testing for the following:<sup>14</sup>

- An individual with a diagnosis of medullary thyroid cancer, a clinical diagnosis of MEN2, or primary C-cell hyperplasia
- An at-risk relative of an individual with a known germline RET mutation

## American Thyroid Association

Revised Guidelines from the American Thyroid Association for the Management of Medullary Thyroid Carcinoma (2015) recommend the following as Grade B Recommendations (based on fair evidence of health outcomes improvement):<sup>8</sup>

- MEN2A (Recommendations 3 and 4): initial testing of “either a single or multi-tiered analysis to detect RET mutations in exon 10 (codons 609, 611, 618, and 620), exon 11 (codons 630 and 634), and exons 8, 13, 14, 15, and 16. Sequencing of the entire coding region should be reserved for situations in which no RET mutation is identified or there is a discrepancy between the MEN2 phenotype and the expected phenotype.”
- MEN2B (Recommendation 5): “Patients with the MEN2B phenotype should be tested for the RET codon M918T mutation (exon 16), and if negative, the RET codon A883F mutation (exon 15). If there are no mutations identified in these two exons, the entire RET coding region should be sequenced.”
- MTC (Recommendation 6): “Patients with presumed sporadic MTC should have genetic testing to detect a RET germline mutation.”
- Other groups who should be tested (Recommendation 7): “Genetic counseling and genetic testing for RET germline mutations should be offered to:
  - First-degree relatives of patients with proven hereditary MTC,
  - Parents whose infants or young children have the classic phenotype of MEN2B,
  - Patients with CLA
  - Infants or young children with Hirschsprung’s Disease<sup>2,15</sup> and exon 10 RET germline mutations and adults with MEN2A and exon 10 mutations who have symptoms suggestive of Hirschsprung’s Disease”

## Criteria

### Introduction

Requests for MEN2 testing are reviewed using the following criteria.

### RET Known Familial Mutation Analysis

- Genetic Counseling:
  - Pre- and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Testing:
  - No previous genetic testing of RET, AND

- Diagnostic and Predisposition Testing:
  - Known deleterious family mutation in RET identified in 1st, 2nd, or 3rd degree biological relative(s), AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy

### **RET Targeted Mutation Analysis**

- Genetic Counseling:
  - Pre- and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Testing:
  - No previous genetic testing of RET, AND
- Diagnostic Testing for Symptomatic Individuals:
  - Personal history of medullary thyroid cancer, or
  - Personal history of primary C-cell hyperplasia, or
  - Personal history of a clinical diagnosis of MEN2A: occurrence of two or more specific endocrine tumors (medullary thyroid cancer, pheochromocytoma, and/or parathyroid adenoma/hyperplasia), or
  - Personal history of a clinical diagnosis of FMTC: families with four or more cases of medullary thyroid cancer in the absence of pheochromocytoma or parathyroid adenoma/hyperplasia, or
  - Personal history of a clinical diagnosis of MEN2B: the presence of early-onset medullary thyroid cancer, mucosal neuromas of the lips and tongue, medullated corneal nerve fibers, distinctive facies with enlarged lips, and a marfanoid body habitus, OR
- Predisposition Testing for Presymptomatic/Asymptomatic Individuals:
  - First-degree relative of an individual with a clinical diagnosis of MEN2A, MEN2B, or FMTC (Note: whenever possible, an affected family member should be tested first), AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

### **RET Full Gene Sequencing**

- Genetic Counseling:
  - Pre- and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Testing:



- No previous RET full gene sequencing, and
  - Previous RET targeted analysis performed and no mutations found, and
  - No known familial mutation, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy

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# MGMT Testing for Malignant Glioma Alkylating Agent Response

MOL.TS.203.A

v1.0.2020

## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedure addressed by this guideline | Procedure code |
|---------------------------------------|----------------|
| MGMT Promoter Methylation Analysis    | 81287          |

## What is MGMT

### Definition

MGMT is the O6-methylguanine- DNA methyltransferase gene, which encodes an essential DNA repair enzyme. MGMT expression in tumors causes resistance to DNA-alkylating drugs. MGMT repairs the damage produced by these DNA cross linking agents.<sup>1</sup>

- Gene methylation is a control mechanism that regulates gene expression. If the MGMT gene is hypermethylated, its expression is absent (“turned off”) or reduced (“turned down”). With less MGMT DNA repair protein present, the tumor is typically more responsive to alkylating drugs.<sup>2</sup>
- Glioblastoma is a common and aggressive brain tumor that is often treated with alkylating drugs.<sup>2</sup> Temozolomide is a standard systemic chemotherapy shown to be effective for malignant gliomas.<sup>2</sup>
- About 40-50% of glioblastoma tumors exhibit MGMT hypermethylation, leading to increased chemosensitivity.<sup>3,4</sup>
- Treatment of gliomas often includes resection, radiation, and chemotherapy. For frail or elderly patients, combined treatment may not be tolerated; therefore, treatment with a single agent (radiation therapy or chemotherapy) or chemotherapy with deferred radiation therapy may be considered.<sup>1</sup>

## Test information

- MGMT promoter methylation testing is performed on paraffin embedded tumor tissue. Quantitative methylation-sensitive PCR or pyrosequencing is used to determine MGMT gene promoter methylation levels.

## Guidelines and evidence

- The National Comprehensive Cancer Network (NCCN, 2018) states:
  - “MGMT promoter methylation is an essential part of molecular diagnostics for all high grade gliomas (grade III and IV).”
  - “MGMT promoter methylation is particularly useful in treatment decisions for elderly patients with high grade gliomas (grades III-IV).”
  - “Patients with glioblastoma that are not MGMT promoter methylated derive less benefit from treatment with temozolomide compared to those whose tumors are methylated.”
- In September 2012, Alberta Health Services published a *Clinical Practice Guideline on Glioblastoma*. It concluded:<sup>5</sup>
  - “Determination of MGMT promoter methylation status may assist in determination of prognosis.”
  - “...whenever possible, determination of MGMT promoter methylation status should be conducted, as it may assist in determination of prognosis.”
- An analysis of epigenetic promoter methylation of the MGMT gene in 206 patients with glioblastoma demonstrated:<sup>6</sup>
  - Significantly improved median survival for those with a methylated MGMT promoter—21.7 months for those treated with temozolomide compared to 15.3 months for those treated with radiotherapy alone ( $p=0.007$ ).
  - Marginally improved median survival for those without a methylated MGMT promoter—12.7 months for those treated with temozolomide versus 11.8 months for those treated with radiotherapy alone ( $p=0.06$ ).
  - MGMT promoter methylation was an independent prognostic factor for favorable response to any glioblastoma treatment (HR=0.45, 95% CI 0.32 – 0.61;  $p<0.001$ ).

## Criteria

- Testing criteria:
  - Diagnosis of glioblastoma (or gliosarcoma)<sup>1</sup>, and
  - Adjuvant temozolomide chemotherapy is being considered<sup>1</sup>, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

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# Mitochondrial DNA Deletion Syndromes

MOL.TS.244.A

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## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedure addressed by this guideline | Procedure code |
|---------------------------------------|----------------|
| mtDNA Deletion Analysis               | 81465          |

## What are mtDNA deletion syndromes

### Definition

Mitochondrial DNA deletion syndromes include three overlapping phenotypes: Kearns-Sayre syndrome (KSS), Pearson syndrome, and progressive external ophthalmoplegia (PEO).<sup>1,2</sup>

- The three phenotypes may be observed in different members of the same family or may evolve in a given individual over time.<sup>1</sup>
  - **KSS** is a multisystem disorder defined by three key signs and symptoms: onset before age 20 years (typically in childhood), pigmentary retinopathy, and PEO. Affected individuals must also have at least one of the following: cardiac conduction block, cerebrospinal fluid protein concentration >100 mg/dL, or cerebellar ataxia. Other findings may include short stature, hearing loss, dementia, limb weakness, diabetes mellitus, hypoparathyroidism, and growth hormone deficiency.<sup>1,2</sup>
  - **Pearson syndrome** includes the findings of sideroblastic anemia and exocrine pancreas dysfunction. It is usually fatal in infancy. Those surviving into childhood develop features of KSS.<sup>1,3</sup>
  - Symptoms may first occur between the first and fifth decade of life and may not appear in any particular order.<sup>1</sup>
  - **PEO** is a mitochondrial myopathy characterized by findings including drooping of the eyelids (ptosis), paralysis of the extraocular muscles (ophthalmoplegia), and variably severe proximal limb weakness.<sup>1</sup>
  - Rarely Leigh syndrome can manifest due to a mtDNA deletion which is characterized by basal ganglia and brain stem lesions.<sup>1</sup>
- These conditions are caused by pathogenic variants in mitochondrial DNA (mtDNA). Pathogenic variants can be sporadic (not inherited) or maternally

inherited. A female who carries the mtDNA mutation at high mutation load will typically pass it on to all of her children. A male who carries the mtDNA mutation cannot pass it on to his children.<sup>1-3</sup>

- The wide variability in clinical presentation depends on how much mutant mtDNA is present in a tissue (heteroplasmy), which organs and tissues have mutant mtDNA, and how vulnerable those tissues are to impaired mitochondrial function (threshold effect).<sup>1</sup>
- Management is usually symptomatic and supportive.<sup>1</sup> Consensus based recommendations have been published by the Mitochondrial Medicine Society for the routine care and management of individuals with mitochondrial disease, including those with mtDNA deletions.<sup>4</sup>
- An epidemiologic study of an adult population in the North East of England estimated the prevalence of large-scale mtDNA deletions at 1.2:100,000.<sup>5</sup>

## Test information

- Diagnosis of mtDNA deletion syndromes is based on a combination of clinical findings and genetic testing.<sup>1,2</sup>
- Findings in KSS and PEO may include elevated lactate and pyruvate levels in blood and cerebrospinal fluid while at rest, with excessive increases in blood after moderate activity. MRI can demonstrate leukoencephalopathy, often associated with cerebral or cerebellar atrophy or basal ganglia lesions.<sup>1</sup> Biochemical studies may also be performed, though: “It is important to note that biochemical abnormalities may not be present during periods when the mitochondrial disease is quiescent/dormant.”<sup>6</sup>
- Detection rate for cases of KSS and PEO by deletion/duplication analysis is 90% and 50% respectively.<sup>1</sup>
  - In cases of KSS and PEO, the disease-causing rearrangements can be detected on a muscle specimen but typically are undetectable in blood (especially in PEO), therefore mutational analysis is best obtained through skeletal muscle biopsy by NGS.<sup>1</sup> The same would apply to the rare cases of Leigh syndrome.<sup>1</sup>
  - For Pearson syndrome, the rearrangements can best be detected in blood by whole mitochondrial genome amplification followed by massively parallel sequencing detecting about 90% of those affected.<sup>1,2</sup>
- Any molecular genetic test for a mtDNA mutation should ideally be directed by the clinical phenotype and results of other clinical, laboratory, and radiological investigations.<sup>2</sup>
- Genetic test results alone cannot predict the exact course or phenotype of the disease. Therefore, testing is not appropriate for asymptomatic at-risk individuals.<sup>1,2</sup>

## Guidelines and evidence

- No specific evidence-based U.S. testing guidelines were identified.
- Case reports and a limited number of case series are the primary evidence base available for the diagnosis of mitochondrial disease. There are few prospective studies. The Mitochondrial Medicine Society developed consensus recommendations using the Delphi method and published them in 2015.<sup>7</sup>
  - Recommendations for DNA testing
    - “Massively parallel sequencing/NGS of the mtDNA genome is the preferred methodology when testing mtDNA and should be performed in cases of suspected mitochondrial disease instead of testing for a limited number of pathogenic point mutations.”
    - “Patients with a strong likelihood of mitochondrial disease because of a mtDNA mutation and negative testing in blood, should have mtDNA assessed in another tissue to avoid the possibility of missing tissue-specific mutations or low levels of heteroplasmy in blood; tissue-based testing also helps assess the risk of other organ involvement and heterogeneity in family members and to guide genetic counseling.”
    - “Heteroplasmy analysis in urine can selectively be more informative and accurate than testing in blood alone, especially in cases of MELAS due to the common m.3243 A>G mutation.”
    - “When considering nuclear gene testing in patients with likely primary mitochondrial disease, NGS methodologies providing complete coverage of known mitochondrial disease gene is preferred. Single-gene testing should usually be avoided because mutations in different genes can produce the same phenotype. If no mutation is identified via known NGS panels, then whole exome sequencing should be considered.”
- A workshop of the National Institute of Neurological Disorders and Stroke (2008) summarizes:<sup>6</sup>
  - “The diagnosis of mitochondrial diseases is complicated by their heterogeneous presentations and by the lack of screening procedures or diagnostic biomarkers that are both sensitive and specific. The workshop panelists explained that diagnosis is often a lengthy process beginning with a general clinical evaluation followed by metabolic screening and imaging and finally by genetic tests and more invasive biochemical and histological analyses. The identification of known mitochondrial mutations in tissue has greatly aided diagnosis. However, even when clinical features and family history strongly suggest mitochondrial disease, the underlying genetic mutation can elude detection, and there is no current screening procedure that would be practical for all cases of suspected mitochondrial disease.”
- The European Federation of Neurological Sciences (2009)<sup>8</sup> provided molecular diagnostic evidence-based guidelines for these conditions:



- “If the phenotype suggests syndromic MID [mitochondrial disorders] due to mtDNA deletion (mtPEO, KSS, Pearson’s syndrome), mtDNA analysis starts with RFLP or Southern-blot from appropriate tissues. mtDNA deletions with low heteroplasmy rate may be detected only by long-range PCR. If neither a single deletion nor multiple deletions are found, mtDNA sequencing is recommended.”

## Criteria

### Known Familial Mutation Testing

- Genetic Counseling
  - Pre and post-test counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing
  - No previous genetic testing in the individual for mtDNA deletion syndromes, and
  - A maternal deletion identified in the mother, AND
- Diagnostic Testing for Symptomatic Individual:
  - Clinical exam and/or biochemical testing suggestive, but not confirmatory, of a diagnosis of a mtDNA deletion syndrome, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy

### mtDNA Deletion Testing

- Genetic Counseling
  - Pre and post-test counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Testing:
  - No previous genetic testing for mtDNA deletions,\*\* and
  - No known mitochondrial pathogenic variants or deletions in the family, AND
- Diagnostic Testing for Symptomatic Individuals:
  - Clinical exam and/or biochemical testing suggestive, but not confirmatory, of a diagnosis of a mtDNA deletion syndrome, and
  - Genetic testing is needed to confirm the diagnosis, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy

\*\* Exceptions may be considered if technical advances in testing demonstrate significant advantages that would support a medical need to retest.

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# Mitochondrial Encephalomyopathy, Lactic Acidosis, and Stroke-like Episodes (MELAS) Genetic Testing

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## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedures addressed by this guideline | Procedure codes |
|--|-----------------|
| MELAS Known Familial Mutation Analysis | 81403           |
| MT-TL1 Targeted Mutation Analysis      | 81401           |
| MT-ND5 Targeted Mutation Analysis      | 81401           |
| Whole Mitochondrial Genome             | 81460           |

## What is MELAS

### Definition

Mitochondrial Encephalomyopathy, Lactic Acidosis, and Stroke-like Episodes (MELAS) is a progressive, multisystem genetic disease.<sup>1</sup>

- The estimated prevalence of MELAS is about 16-18/100,000 individuals.<sup>2,3</sup>
- MELAS symptoms can present at any age. Most cases present in childhood, with 65%-76% developing symptoms before age 20. Few cases present before age 2 (5%-8%) and after age 40 (1%-6%).<sup>1</sup>
- Individuals with MELAS typically experience disease progression that results in death. Median survival time from point of diagnosis is about 16.9 years, with a subgroup of 20.8% who are more severely affected and die within 7.3 years of diagnosis.<sup>1</sup> Overall, children and young adults diagnosed with MELAS who have classical symptoms have a shorter lifespan than older adults with milder symptoms.
- Typical initial clinical presentation includes stroke-like episodes or cortical blindness often occurring with generalized tonic-clonic seizures, and these episodes may be recurrent and associated with altered consciousness. Almost all individuals with MELAS (94%) have lactic acidemia. Individuals may also have recurrent headaches, anorexia, recurrent vomiting, possibly exercise intolerance or proximal limb weakness, Wolff-Parkinson-White syndrome, and diabetes mellitus. Short

stature in children and sensorineural hearing loss in both children and adults are also common.<sup>1</sup>

- The natural history of MELAS involves gradual impairment of motor abilities, vision, and cognitive ability by adolescence or young adulthood due to recurring stroke-like episodes.<sup>1</sup>
- There is no cure for MELAS. Several types of treatment, however, have demonstrated benefit in affected individuals. The use of oral and intravenous (IV) L-arginine and citrulline has shown reduction of frequency and/or severity of stroke-like episodes.<sup>4-9</sup> Both endurance and resistance exercise have been studied and shown to increase mitochondrial metabolism.<sup>6</sup> Vitamin and cofactor supplementation including CoQ10, alpha lipoic acid, and riboflavin should be offered, and addition of folinic acid and L-carnitine should be considered, especially if there is documented deficiency.<sup>4</sup>
- At-risk individuals may benefit from assessment to initiate baseline evaluations (neurology, cardiology, ophthalmology, and audiology) and potential intervention prior to exhibiting clinical manifestations.<sup>5</sup> Screening for diabetes mellitus by fasting serum glucose concentration and glucose tolerance test is recommended.<sup>1</sup>
- Diagnosis of MELAS is based on a combination of clinical and laboratory findings and genetic testing.<sup>1,11</sup>
- MELAS is caused by mutations in the mitochondrial DNA (mtDNA) that are always maternally inherited. This means that a female who carries the mtDNA mutation will pass it on to all of her children. A male who carries the mtDNA mutation will not pass it on to his children.<sup>1,11</sup>
- Mutations in the mtDNA gene, MT-TL1, cause MELAS. A majority of affected individuals with classic symptoms, about 80%, have a specific mutation, A3243G.<sup>1,10,11</sup> Other rare mtDNA mutations in the MT-TL1 gene, T3271C and A3252G, and in 9 other mtDNA genes are also associated with MELAS.<sup>1,11</sup>
- Genetic test results alone cannot predict the exact course or phenotype of the disease.<sup>1,11</sup> For all mtDNA mutations, clinical expressivity depends on the three following factors:<sup>1</sup>
  - The relative abundance of mutant mtDNA, mutational load (heteroplasmy)
  - The organs and tissues in which the mutant mtDNA is found (tissue distribution), and
  - The vulnerability of each tissue to impaired oxidative metabolism (threshold effect).
- There is suggested clinical utility with the use of genetic testing for MELAS at the present time. Each patient and family is unique; therefore, it is necessary to consider the specific case to determine the clinical utility in regards to impactful management.<sup>11</sup> This may include changes to stroke treatment, treatment during illness, the use of anesthesia, the use of exercise as treatment, and the use of vitamin and xenobiotics.<sup>6</sup>

## Test information

- The investigation and diagnosis of patients with mitochondrial respiratory chain disease often necessitates a combination of techniques including muscle histochemistry, biochemical assessment and molecular genetic studies along with clinical assessment. Any molecular genetic test for a mtDNA mutation should ideally be directed by the clinical phenotype and results of these other investigations.<sup>11</sup>
- Targeted mutation testing for MELAS is available at many laboratories. The specific mutations included in these targeted tests can vary by laboratory; however, they typically include the most common pathogenic variant found in MELAS, m.3243 A>G.
- The common MELAS mutations are also included on a number of more general mitochondrial targeted mutation panels (in conjunction with genes for LHON, MERRF and Leigh syndrome).
- Full sequencing of the entire mitochondrial genome can be done to identify the remaining rare mtDNA mutation in individuals affected with MELAS. Since the mitochondrial genome is highly polymorphic, this is not routinely offered unless clinical suspicion is very high and paternal transmission has been ruled out.<sup>1</sup> If the status of heteroplasmy is of concern, next generation testing with high read depth may be preferable.
- A number of large panels sequence the mitochondrial genome in conjunction with nuclear-encoded mitochondrial genes for a broad approach to testing.
- DNA testing can be performed on a blood specimen. Muscle biopsy is generally not necessary, but some labs accept blood, saliva and muscle samples.
- A muscle biopsy or heteroplasmy analysis in urine may be recommended for testing of A3243G variant in cases with a clinical presentation of classic MELAS and where the variant is not detected on blood or urine specimens.<sup>1</sup> If the status of heteroplasmy is of concern, next generation testing with high read depth may be preferable, however certain targeted mutation analysis can detect low level heteroplasmy.

## Guidelines and evidence

- No specific evidence-based U.S. testing guidelines for MELAS were identified.
- The Mitochondrial Medicine Society (2015)<sup>4</sup> developed consensus recommendations for the diagnosis and management of mitochondrial disease. Testing strategies, including strategies for genetic testing, were discussed.
  - Recommendations for DNA testing
    - “Massively parallel sequencing/NGS of the mtDNA genome is the preferred methodology when testing mtDNA and should be performed in cases of

- suspected mitochondrial disease instead of testing for a limited number of pathogenic point mutations.”
- “Patients with a strong likelihood of mitochondrial disease because of a mtDNA mutation and negative testing in blood, should have mtDNA assessed in another tissue to avoid the possibility of missing tissue-specific mutations or low levels of heteroplasmy in blood; tissue-based testing also helps assess the risk of other organ involvement and heterogeneity in family members and to guide genetic counseling. Heteroplasmy analysis in urine can selectively be more informative and accurate than testing in blood alone, especially in cases of MELAS due to the common m.3243A>G mutation.”<sup>2</sup>
  - Recommendations for pathology testing
    - “Muscle (and/or liver) biopsies should be performed in the routine analysis for mitochondrial disease when the diagnosis cannot be confirmed with DNA testing.”
  - The European Federation of Neurological Sciences (EFNS, 2009) provided molecular diagnostic consensus-based guidelines based on literature reviews:<sup>1,2</sup>
    - “If the phenotype suggests syndromic mitochondrial disease due to mtDNA point mutations (MELAS, MERRF, NARP, LHON) DNA-microarrays using allele-specific oligonucleotide hybridization, real-time-PCR or single-gene sequencing are indicated.”<sup>5</sup>
  - The clinical utility of genetic testing for MELAS was described by a workshop of the National Institute of Neurological Disorders and Stroke (2008):<sup>1,3</sup>
    - “The diagnosis of mitochondrial diseases is complicated by their heterogeneous presentations and by the lack of screening procedures or diagnostic biomarkers that are both sensitive and specific. The workshop panelists explained that diagnosis is often a lengthy process beginning with a general clinical evaluation followed by metabolic screening and imaging and finally by genetic tests and more invasive biochemical and histological analyses. The identification of known mitochondrial mutations in tissue has greatly aided diagnosis. However, even when clinical features and family history strongly suggest mitochondrial disease, the underlying genetic mutation can elude detection, and there is no current screening procedure that would be practical for all cases of suspected mitochondrial disease.”
    - Initial screening includes testing for blood lactate, urine amino acids, acyl-carnitine profile, and MRI. “It is important to note that biochemical abnormalities may not be present during periods when the mitochondrial disease is quiescent/dormant.”
  - The Clinical Molecular Genetics Society (CMGS) of UK (2008) provided practice-based guidelines for the molecular diagnosis of mitochondrial disease:<sup>11</sup>

- In cases with strong clinical evidence, testing should begin with checking for the common A3243G mutation. Testing for the rare mutations including T3271C and A3252G is not routinely indicated unless there is strong clinical diagnosis of MELAS testing.

## Criteria

### MELAS Known Familial Mutation Testing

- Genetic Counseling
  - Pre and post-test counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing
  - No previous genetic testing in the individual for MELAS, and
  - MELAS pathogenic variant identified in 1<sup>st</sup> degree biological maternal relative, AND
- Predictive Testing for Asymptomatic Individual:
  - 18 years of age or older, or
  - Under the age of 18 years, and
    - Presymptomatic screening for diabetes mellitus is being considered, OR
- Diagnostic Testing for Symptomatic Individual:
  - Clinical exam and biochemical testing suggestive, but not confirmatory, of a diagnosis of MELAS, OR
- Prenatal Testing for At-Risk Pregnancies:
  - MELAS causing pathogenic variant in a previous child or in the mother, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy

### MELAS Targeted Mutation Analysis (A3243G)

- Genetic Counseling
  - Pre and post-test counseling by an appropriate provider(as deemed by the Health Plan policy), AND
- Previous Testing:
  - No previous genetic testing for MELAS, and
  - No known MELAS pathogenic variants in the family, AND

- Diagnostic Testing for Symptomatic Individuals:
  - Clinical exam and biochemical testing suggestive, but not confirmatory, of a diagnosis of MELAS by one or more of the following:
    - Lactic acidosis both in blood and in the CSF,<sup>1</sup> and/or
    - Muscle biopsy showing ragged red fibers,<sup>1</sup> and/or
    - Respiratory chain enzyme studies that are consistent with a diagnosis of MELAS,<sup>1</sup> and/or
    - Stroke-like episodes before the age of 40 years,<sup>1</sup> and/or
    - Encephalopathy with seizures and/or dementia,<sup>1</sup> and
  - Genetic testing is needed to confirm the diagnosis, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy

### **MELAS Targeted Mutation Analysis (G13513A, T3271C, and A3252G)**

- Genetic Counseling
  - Pre and post-test counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Criteria for MELAS targeted mutation analysis (A3243G) is met, AND
- No pathogenic variants identified in the targeted mutation analysis (A3243G)

### **Whole mtDNA Sequencing**

- Genetic Counseling
  - Pre and post-test counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Criteria for MELAS targeted mutation analysis is met, AND
- No pathogenic variants identified in the targeted mutation analysis (A3243G, G13513A, T3271C, and A3252G), AND
- Paternal transmission has been ruled out

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# Mitochondrial Genetic Testing

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## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedures addressed by this guideline                   | Procedure codes |
|--|-----------------|
| Whole Mitochondrial Genome Sequencing                    | 81460           |
| Whole Mitochondrial Genome Deletion/Duplication Analysis | 81465           |
| Nuclear Encoded Mitochondrial Gene Sequencing Panel      | 81440           |

## What are mitochondrial disorders

### Definition

Mitochondrial disorders arise from mutations in both nuclear and mitochondrial (mtDNA) components of the respiratory chain. They comprise a clinically diverse group of diseases that may present at any age and affect a single organ or present as a multi-system condition in which neurologic and myopathic features predominate. Extensive clinical variability and phenotypic overlap exists among the many discrete mitochondrial disorders.

- Mitochondrial disease is suspected in patients with a combination of clinical features in:
  - Muscle: proximal myopathy or cardiomyopathy
  - Nervous system: encephalopathy, seizures, dementia, stroke-like episodes, ataxia and spasticity and migraine
  - Eye: ptosis, ophthalmoparesis, ophthalmoplegia, optic atrophy, pigmentary retinopathy
  - Sensorineural hearing loss
  - Diabetes mellitus
  - Mid or late pregnancy loss
- Mitochondrial disease is not curable. However, in some cases, specific treatment recommendations can be made based on a person's definitive diagnosis.

Consensus based recommendations have been published by the Mitochondrial Medicine Society for the routine care and management of individuals with mitochondrial disease.<sup>1</sup> Individuals at-risk for mitochondrial conditions may also benefit from clinical assessment to initiate baseline evaluations (neurology, cardiology, ophthalmology, and audiology) and potential intervention prior to exhibiting clinical manifestations.<sup>2,3</sup>

- Mitochondrial conditions caused by nuclear DNA variants can be maternally or paternally inherited and may follow autosomal dominant, autosomal recessive, and X-linked inheritance.
- Mitochondrial conditions caused by mtDNA are always maternally inherited. Pathogenic variants in the mtDNA may be de novo or maternally inherited. This means that a female who carries a mtDNA mutation at high mutation load will typically pass it on to all of her children. However, due to the meiotic bottleneck, the heteroplasmy level may vary significantly between generations. A male who carries the mtDNA mutation will not pass it on to his children.<sup>2,4</sup> mtDNA deletions are rarely transmitted (less than 1% empiric risk).<sup>5</sup> If the mother is symptomatic, then the recurrence risk is approximately 4%.<sup>6</sup>
- For all mtDNA mutations, clinical expressivity depends on the three following factors:<sup>2</sup>
  - The ratio of mutant mtDNA, mutational load (heteroplasmy)
  - The organs and tissues in which the mutant mtDNA is found (tissue distribution), and
  - The vulnerability of each tissue to impaired oxidative metabolism (threshold effect).
- Analysis of an individual's family history may provide information regarding most likely inheritance patterns for a suspected mitochondrial condition. This may guide decisions to perform mtDNA sequencing, mtDNA deletion/duplication testing, nuclear encoded DNA sequencing, and/or nuclear encoded DNA deletion/duplication testing.
- While genetic test results alone cannot predict the exact course or phenotype of the disease, severity does correlate with mutation load for mitochondrial DNA mutations.<sup>4,7</sup>
- Identification of a pathogenic variant in a proband can allow for informative testing of relatives at risk for diabetes, seizures, hearing loss, optic atrophy, and other findings.

## Test information

- The investigation and diagnosis of patients with mitochondrial disease often necessitates a combination of techniques including muscle histocytochemistry, biochemical assessment and molecular genetic studies along with clinical

assessment.<sup>4</sup> Any molecular genetic test for a mtDNA mutation should ideally be directed by the clinical phenotype and results of these other investigations.<sup>4</sup>

- While biochemical analyses of an affected tissue may be informative, they are not sensitive or specific enough to definitively diagnose most mitochondrial conditions.
- Due to overlap of clinical findings of mitochondrial conditions and non-mitochondrial conditions, affected individuals are more likely to have multiple tests performed before a molecular genetic cause is identified. If an individual's clinical findings clearly correlate with a specific mitochondrial condition, then testing can be focused on the most appropriate approach for that condition. However, if the clinical picture strongly suggests a mitochondrial condition but there is uncertainty about which subset of conditions, then larger mtDNA or nuclear DNA testing panels may be appropriate.
- “Approaches to molecular genetic testing of a proband to consider are serial testing of single genes, multi-gene panel testing (simultaneous testing of multiple genes), and/or genomic testing (e.g., sequencing of the entire mitochondrial genome, genome sequencing, or exome sequencing to identify a pathogenic variant in a nuclear gene). In many individuals in whom molecular genetic testing does not yield or confirm a diagnosis, further investigation of suspected mitochondrial disease can involve a range of different clinical tests, including muscle biopsy for respiratory chain function.”<sup>5</sup>
- The efficiency of next generation sequencing (NGS) has led to an increasing number of large, multi-gene testing panels. NGS panels that test several genes at once are particularly well-suited to conditions caused by more than one gene or where there is considerable clinical overlap between conditions making it difficult to reliably narrow down likely causes. As a result, several laboratories have begun to combine genes involved in certain conditions which often have both of those characteristics.
- Mitochondrial Genome Sequencing Panels and Mitochondrial Genome Deletion/Duplication Panels:
  - Whole Mitochondrial Genome Sequencing: NGS testing is capable of simultaneously detecting point mutations, deletions, and point mutation heteroplasmies. Typically, Sanger sequence analysis will miss heteroplasmy below 20%. With suitable depth of coverage, NGS can detect heteroplasmy down to ~1%.<sup>8-9</sup>
  - Mitochondrial Genome Deletion/Duplication Panels: Testing for deletions and duplications of the entire mitochondrial genome.
- For some, but not all, mtDNA conditions, such as MERFF, if mtDNA genetic testing is negative in a blood sample in a person with symptoms of the mtDNA condition, testing can be done on other specimens. Typically this is done when the phenotype is highly suggestive of the presence of a mutation associated with a specific gene or set of genes, or when there is a need to assess reproductive risk.

- The potential for informativeness versus the invasiveness and procedural costs are factors to consider. For instance, muscle biopsy also allows enzymatic analysis of the electron transport chain, light and ultrastructural microscopy, and mtDNA copy number analysis, which may provide highly useful information for some conditions, such as MERFF.
- Genetic testing can also be done on skin fibroblasts, urinary sediment, or buccal mucosa.<sup>2</sup> If cultured fibroblasts are used, measures such as limited passaging and uridine supplementation should be taken to reduce selection against mutant genotypes.
- Nuclear Encoded Mitochondrial Gene Sequencing Panel: A number of large panels are available that sequence numerous nuclear-encoded mitochondrial genes for a broad approach to testing. Multi-gene panel tests, even for similar clinical scenarios, vary considerably laboratory by laboratory in the genes that are included and in technical specifications (e.g. depth of coverage, extent of intron/exon boundary analysis, methodology of large deletion/duplication analysis).

## Guidelines and evidence

- No specific evidence-based U.S. testing guidelines were identified.
- The Mitochondrial Medicine Society developed consensus recommendations using the Delphi method and published them in 2015.<sup>10</sup>
  - Recommendations for DNA testing
    - Massively parallel sequencing/NGS of the mtDNA genome is the preferred methodology when testing mtDNA and should be performed in cases of suspected mitochondrial disease instead of testing for a limited number of pathogenic point mutations.
    - Patients with a strong likelihood of mitochondrial disease because of a mtDNA mutation and negative testing in blood, should have mtDNA assessed in another tissue to avoid the possibility of missing tissue-specific mutations or low levels of heteroplasmy in blood; tissue-based testing also helps assess the risk of other organ involvement and heterogeneity in family members and guides genetic counseling.
    - When considering nuclear gene testing in patients with likely primary mitochondrial disease, NGS methodologies providing complete coverage of known mitochondrial disease genes is preferred. Single-gene testing should usually be avoided because mutations in different genes can produce the same phenotype. If no mutation is identified via known NGS panels, then whole exome sequencing should be considered.
  - Recommendations for pathology testing

- Biopsy should only be considered when the diagnosis cannot be confirmed with DNA testing of other more accessible tissues. Muscle (and/or liver) biopsies are often not necessary and should be avoided when possible due to their invasive nature, unless other types of analyses such as pathology, enzymology, or mtDNA copy number analyses are required for diagnosis.
- The American College of Medical Genetics and Genomics (ACMG, 2013) states the following regarding testing individuals with isolated autism for mitochondrial disorders:<sup>11</sup>
  - “As with metabolic disorders, testing for mitochondrial disorders in persons with ASDs is recommended only if supporting symptoms or laboratory abnormalities are present.”
- The European Federation of Neurological Sciences (2009)<sup>12</sup> provided molecular diagnostic consensus-based guidelines based on literature reviews: “If the phenotype suggests syndromic mitochondrial disease due to mtDNA point mutations (MELAS, MERRF, NARP, LHON) DNA-microarrays using allele-specific oligonucleotide hybridisation, real-time-PCR or single-gene sequencing are indicated.”
- The Clinical Molecular Genetics Society (CMGS) of the United Kingdom (2008)<sup>13</sup> practice-based guidelines for the molecular diagnosis of mitochondrial disease state that: “ In cases with strong clinical evidence, testing should begin with checking for the common mutation, m.8344A>G. Subsequent testing for other mutations, such as m.8356T>C, may be indicated in cases with a strong clinical indication of MERRF”. “For routine referrals for NARP, presence of T8993G and T8993C mutations should be investigated.”

## Criteria

### Whole mtDNA Sequencing

- Pre and post-test counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Member has not had previous whole mtDNA sequencing performed, AND
- Biochemical testing appropriate for the suspected disorder has been performed and is not confirmatory of a diagnosis of a specific mitochondrial condition, AND
- Member has multiple organ system involvement defined as altered function in two or more organ systems, AND
- Member has one or more of the following clinical features: proximal myopathy, cardiomyopathy, encephalopathy, seizures, dementia, stroke-like episodes, ataxia, spasticity, ptosis, ophthalmoparesis, ophthalmoplegia, optic atrophy, pigmentary retinopathy, sensorineural hearing loss, diabetes mellitus, mid- or late pregnancy

- loss, MRI and/or MRS imaging results consistent with a mitochondrial process, and/or pathology results consistent with a mitochondrial process, AND
- Member's clinical presentation does not fit a well-described syndrome for which single-gene or targeted panel testing is available (e.g. LHON), AND
  - Alternate etiologies have been considered and ruled out when possible (e.g., environmental exposure, injury, infection), AND
  - Family history strongly suggests mitochondrial inheritance (e.g. paternal transmission has been ruled out)

### **Whole mtDNA Deletion/Duplication Analysis**

- Pre and post-test counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Member has not had previous whole mtDNA deletion/duplication analysis performed, AND
- Biochemical testing appropriate for the suspected disorder has been performed and is not confirmatory of a diagnosis of a specific mitochondrial condition, AND
- Member has multiple organ system involvement defined as altered function in two or more organ systems, AND
- Member has one or more of the following clinical features: proximal myopathy, cardiomyopathy, encephalopathy, seizures, dementia, stroke-like episodes, ataxia, spasticity, ptosis, ophthalmoparesis, ophthalmoplegia, optic atrophy, pigmentary retinopathy, sensorineural hearing loss, diabetes mellitus, mid- or late pregnancy loss, MRI and/or MRS imaging results consistent with a mitochondrial process, and/or pathology results consistent with a mitochondrial process, AND
- Member's clinical presentation does not fit a well-described syndrome for which single-gene or targeted panel testing is available (e.g. LHON), AND
- Alternate etiologies have been considered and ruled out when possible (e.g., environmental exposure, injury, infection), AND
- Family history strongly suggests mitochondrial inheritance (e.g. paternal transmission has been ruled out)

### **Nuclear Encoded Mitochondrial Gene Sequencing Panel**

- Pre and post-test counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Member has not had a previous nuclear encoded mitochondrial gene sequencing panel testing performed, AND
- Biochemical testing appropriate for the suspected disorder has been performed and is not confirmatory of a diagnosis of a specific mitochondrial condition, AND

- Member has multiple organ system involvement defined as altered function in two or more organ systems, AND
- Member has one or more of the following clinical features: proximal myopathy, cardiomyopathy, encephalopathy, seizures, dementia, stroke-like episodes, ataxia, spasticity, ptosis, ophthalmoparesis, ophthalmoplegia, optic atrophy, pigmentary retinopathy, sensorineural hearing loss, diabetes mellitus, mid- or late pregnancy loss, MRI and/or MRS imaging results consistent with a mitochondrial process, and/or pathology results consistent with a mitochondrial process, AND
- Member's clinical presentation does not fit a well-described syndrome for which single-gene or targeted panel testing is available (e.g. LHON), AND
- Alternate etiologies have been considered and ruled out when possible (e.g., environmental exposure, injury, infection), AND
- Family history DOES NOT strongly suggest mitochondrial inheritance (e.g. paternal transmission is observed, autosomal inheritance is likely)

### Exclusions

- Testing addressed in this guideline applies to patients in whom a mitochondrial disorder is suspected based on a constellation of findings commonly seen in these conditions, while not fitting clearly into one of the discrete mitochondrial syndromes. This guideline is not applicable in the following cases:
  - The patient's findings fit into a discrete mitochondrial syndrome for which more specific testing is appropriate. Please see one of the following guidelines for information on specific mitochondrial conditions (MELAS, LHON, MNGIE, MERRF, NARP, etc); or
  - The patient's findings could be explained nonspecifically by a mitochondrial disorder or other neurological or myopathic condition not related to mitochondrion for which a different genetic test may be considered ; or
  - Individuals who have no increased risk above the general population risk to have inherited a mitochondrial disease and have just one of the following findings in isolation: fatigue; muscle weakness; developmental delay ; autism; migraines; abnormal biochemical test results (e.g., elevated lactate); psychiatric symptoms.

### Billing and reimbursement considerations

- Whole mtDNA Sequencing will only be considered for coverage when billed under the appropriate panel CPT code: 81460
- Whole mtDNA Deletion/Duplication will only be considered for coverage when billed under the appropriate panel CPT code: 81465



- Nuclear Encoded Mitochondrial Gene Sequencing Panels will only be considered for coverage when billed under the appropriate panel CPT code: 81440
- If the panel will be billed with separate procedure codes for each gene analyzed and the member meets criteria for Whole mtDNA Sequencing, Whole mtDNA Deletion/Duplication, or Nuclear Encoded Mitochondrial Gene Sequencing Panel, the testing will be approved but the laboratory will be redirected to the appropriate CPT code for billing purposes.
- If the panel cannot be redirected to 81460, 81465, or 81440 for any reason, the medical necessity of each billed procedure will be assessed independently.
- If more than one test or procedure code is requested at one time, the member meets criteria for all tests requested, and each test is equally likely based on personal history, clinical findings, and family history, the testing will be tiered in the following order: 81460, 81465, 81440.

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# Mitochondrial Neurogastrointestinal Encephalopathy (MNGIE)

MOL.TS.243.A

v1.0.2020

## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedures addressed by this guideline | Procedure codes |
|--|-----------------|
| TYMP Known Familial Mutation           | 81403           |
| TYMP Sequencing                        | 81405           |
| TYMP Deletion/Duplication              | 81479           |

## What is MNGIE

### Definition

Mitochondrial Neurogastrointestinal Encephalopathy (MNGIE) is a multisystem mitochondrial disease.<sup>1</sup> MNGIE is typically characterized by progressive gastrointestinal dysmotility, which may present with nausea, dysphagia, reflux, early satiety, vomiting after a meal, episodic abdominal pain, bloating, and/or diarrhea. Additionally individuals may present with cachexia (a wasting syndrome), ptosis/ophthalmoplegia (drooping/weakness of the eyelid), leukoencephalopathy on brain MRI, or peripheral neuropathy (tingling, numbness, and/or pain in the extremities).<sup>1</sup> Symptoms may first occur between the first and fifth decade of life and may not appear in any particular order.<sup>1</sup>

- MNGIE is caused by biallelic mutations in the nuclear TYMP gene on chromosome 22 and is inherited in an autosomal recessive pattern, meaning parents of an affected individual must be obligate carriers. The chance of having another child with MNGIE to the same parents is 25%.
- Prevalence of MNGIE is largely unknown but the condition appears to be rare. Approximately 120 cases have been reported. No ethnic predilection for MNGIE disease has been observed. Parental consanguinity is common.<sup>1</sup>
- Management can be supportive, and may include assistance with swallowing difficulties, medication for nausea and vomiting, gastrostomy and parenteral nutrition for nutritional support, pain medications for neuropathy, and physical therapy and occupational therapy.<sup>1</sup>

- In individuals with advanced illness, liver transplant or allogeneic hematopoietic stem cell transplant, have been suggested as possible curative treatment options, although risks and benefits of these procedures must be properly weighed.<sup>2,3</sup>
- Peritoneal dialysis has also been suggested as a method of reduction of the thymidine concentration and should be considered as an additional or alternative form of treatment.<sup>4</sup>

## Test information

- “The TYMP gene encodes thymidine phosphorylase, a cytosolic enzyme that catalyzes the phosphorylation of thymidine or deoxyuridine to thymine or uracil, and is thus essential for the nucleotide salvage pathway.”<sup>5</sup>
- Mutations that disrupt the function of TYMP will therefore disrupt the enzyme activity causing it to decrease and levels of thymidine or deoxyuridine to increase.
- Reduced thymidine phosphorylase enzyme activity or elevated thymidine and deoxyuridine levels are consistent with a diagnosis of MNGIE.<sup>1</sup>
- Genetic testing of the TYMP gene can help to diagnosis a person with MNGIE.
  - The overwhelming majority (nearly 100%) of TYMP mutations are detected by gene sequencing. TYMP deletions and duplications are less common (prevalence unknown).
    - Complete sequencing of TYMP for pathogenic mutations is necessary to diagnosis MNGIE.
    - If only one TYMP mutation is identified or variant of uncertain significance results are returned, pursue gene TYMP deletion/duplication analysis.<sup>1</sup>

## Guidelines and evidence

- No specific evidence-based U.S. testing guidelines were identified.
- Although not specific to genetic testing for MNGIE, the Mitochondrial Medicine Society (2015)<sup>6</sup> developed consensus recommendations for the diagnosis and management of mitochondrial disease. Testing strategies, including strategies for genetic testing, were discussed.
  - Recommendations for DNA testing include the following:
    - “Massively parallel sequencing/NGS of the mtDNA genome is the preferred methodology when testing mtDNA and should be performed in cases of suspected mitochondrial disease instead of testing for a limited number of pathogenic point mutations.”

- “Patients with a strong likelihood of mitochondrial disease because of a mtDNA mutation and negative testing in blood, should have mtDNA assessed in another tissue to avoid the possibility of missing tissue-specific mutations or low levels of heteroplasmy in blood; tissue-based testing also helps assess the risk of other organ involvement and heterogeneity in family members and to guide genetic counseling.”
  - “Heteroplasmy analysis in urine can selectively be more informative and accurate than testing in blood alone, especially in cases of MELAS due to the common m.3243 A>G mutation.”
  - “When considering nuclear gene testing in patients with likely primary mitochondrial disease, NGS methodologies providing complete coverage of known mitochondrial disease gene is preferred. Single-gene testing should usually be avoided because mutations in different genes can produce the same phenotype. If no mutation is identified via known NGS panels, then whole exome sequencing should be considered.”
- The European Federation of Neurological Sciences (2009) provided molecular diagnostic consensus-based guidelines based on literature reviews: “Sequencing of TYMP should be performed only if serum thymidine is elevated.”<sup>7</sup>
  - Evidence from peer reviewed journals provide symptoms, clinical findings, imaging, and family history suggestive of MNGIE.<sup>8,9</sup>
    - Severe gastrointestinal dysmotility, cachexia, ptosis, external ophthalmoparesis/ophthalmoplegia, and sensorimotor neuropathy.
    - Brain MRI that demonstrates abnormal brain white matter (increased FLAIR or T2-weighted signal) consistent with asymptomatic leukoencephalopathy. In the absence of leukoencephalopathy, MNGIE disease is very unlikely.<sup>1</sup>
    - Family history consistent with autosomal recessive inheritance.

## Criteria

### TYMP Known Familial Mutation Testing

- Genetic Counseling
  - Pre and post-test counseling by an appropriate provider(as deemed by the Health Plan policy), AND
- Previous Genetic Testing
  - No previous genetic testing in the individual for MNGIE, and
  - TYMP pathogenic variant(s) identified in parents and/or sibling(s), AND
- Predictive Testing for Asymptomatic Individual:

- 18 years of age or older, OR
- Diagnostic Testing for Symptomatic Individual:
  - Clinical exam and/or biochemical testing suggestive, but not confirmatory, of a diagnosis of MNGIE, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy

### **TYMP Sequencing**

- Genetic Counseling
  - Pre and post-test counseling by an appropriate provider(as deemed by the Health Plan policy), AND
- Previous Testing:
  - No previous genetic testing for MNGIE, and
  - No known TYMP pathogenic variants in the family, AND
- Diagnostic Testing for Symptomatic Individuals:
  - Clinical exam and/or biochemical testing suggestive, but not confirmatory, of a diagnosis of MNGIE, and
  - Genetic testing is needed to confirm the diagnosis, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy

### **TYMP Deletion/Duplication**

- Genetic Counseling
  - Pre and post-test counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Criteria for TYMP sequencing is met, AND
- No pathogenic variants or only one pathogenic variant identified in TYMP Sequencing.

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# Molecular Gastrointestinal Pathogen Panel (GIPP) Testing

MOL.CS.277.A

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## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedures addressed by this guideline  | Procedure codes |
|---|-----------------|
| BioFire FilmArray Gastrointestinal (GI) Panel, BioFire Diagnostics  | 0097U           |
| Gastrointestinal Pathogen (eg, Clostridium difficile, E. coli, Salmonella, Shigella, Norovirus, Giardia), Includes Multiple Reverse Transcription, When Performed, And Multiplex Amplified Probe Technique, Multiple Types Or Subtypes, 3-5 Targets   | 87505           |
| Gastrointestinal Pathogen (eg, Clostridium difficile, E. coli, Salmonella, Shigella, Norovirus, Giardia), Includes Multiple Reverse Transcription, When Performed, And Multiplex Amplified Probe Technique, Multiple Types Or Subtypes, 6-11 Targets  | 87506           |
| Gastrointestinal Pathogen (eg, Clostridium difficile, E. coli, Salmonella, Shigella, Norovirus, Giardia), Includes Multiple Reverse Transcription, When Performed, And Multiplex Amplified Probe Technique, Multiple Types Or Subtypes, 12-25 Targets | 87507           |

## What are nucleic acid amplified probe techniques (NAAT) for the identification of microorganisms via GIPP

### Definition

Tests performed by NAAT uses a microorganism's DNA or RNA to directly identify specific bacteria, viruses, and/or protozoa rather than standard microorganism detection techniques such as bacterial culture, microscopy with and without stains,



direct fluorescent antibody testing, rapid antigen testing, qualitative and quantitative immunoassay for identification of antigens or toxins from stool and single-plex PCR assays. Multiplex NAAT tests are included in the larger grouping of culture-independent diagnostic tests (CIDT). CIDT includes but is not limited to simplex direct probe and amplified probe techniques.

- This technology offers results in a matter of hours, rather than 2-3 days of time-consuming and labor intensive bacterial cultures and immunoassays for processing stool specimens. CIDT are touted as providing a more comprehensive assessment of disease etiology by increasing the diagnostic yield compared with conventional diagnostic tests permitting earlier initiation of appropriate therapeutic agents targeted to the detected pathogen(s), if any, rather than empirical therapy until culture results are available.
- However, above and beyond microorganism detection, this type of testing does not provide the culture isolates that are needed for antimicrobial susceptibility testing, serotyping, subtyping and whole genome sequencing that are critical for monitoring trends, detecting clusters of illness and investigating outbreaks.<sup>1</sup>

## Test information

- In addition to CLIA-regulated laboratory developed tests (LDTs) by specialty (e.g. academic) laboratories, several commercial GIPP assays are currently available. For example, Binnicker has evaluated three FDA-cleared GIPP assays,<sup>2</sup> and all are closed system tests that do not allow random access for physician's to select likely etiologic agents of diarrhea.
- Please note that the NAAT results of GIPP assays can be inconclusive and non-specific, such as the inability to always distinguish pathogenic from non-pathogenic organisms.<sup>3</sup>

## Guidelines and evidence

- American College of Gastroenterology 2016 Clinical Guidelines<sup>4</sup> include two relevant sections pertaining to GIPP:
  - “Traditional methods of diagnosis (bacterial culture, microscopy with and without special stains and immunofluorescence, and antigen testing) fail to reveal the etiology of the majority of cases of acute diarrheal infection. If available, the use of Food and Drug Administration-approved culture-independent methods of diagnosis can be recommended at least as an adjunct to traditional methods. (Strong recommendation, low level of evidence).”
  - “The new diagnostics’ best applicability is for the clinician in practice, seeing one patient at a time rather than in the public health setting, e.g., in outbreak investigations. One potential drawback of molecular technologies is the need to predefine the particular microbes being sought. In addition the significance of an

identified organism may not be clear as these molecular technologies, which involve nucleic acid amplification, are limited to our existing knowledge of a microbes' genome and do not discriminate between viable and non-viable organisms. As a result they can detect microbes at non-pathogenic levels. Given the high rates of asymptomatic carriage of enteropathogens, this can be a considerable problem. To confound matters, further multiplex techniques are more commonly associated with increased detection of mixed infections and the relative importance of each pathogen may be unclear.”

- Infectious Diseases Society of America 2017 Clinical Practice Guidelines for the Diagnosis and Management of Infectious Diarrhea<sup>5</sup> make the following recommendations:
  - “A broad differential diagnosis is recommended in immunocompromised people with diarrhea, especially those with moderate and severe primary or secondary immune deficiencies... Some experts have proposed that these assays may be particularly well suited for making an organism-specific diagnosis in immunocompromised patients.”
  - “Culture-independent, including panel-based multiplex molecular diagnostics from stool and blood specimens, and, when indicated, culture-dependent diagnostic testing should be performed when there is a clinical suspicion of enteric fever or diarrhea with bacteremia (strong, moderate).”
  - “Multipathogen nucleic acid amplification tests can simultaneously detect viral, parasitic, and bacterial agents, including some pathogens that previously could not be easily detected in the clinical setting such as norovirus, and enterotoxigenic E. coli (ETEC), enteropathogenic E. coli (EPEC), and enteroaggregative E. coli (EAEC) in less time than traditional methods. The short time to results could reduce inappropriate use of antimicrobial agents to treat infections that do not require antimicrobial therapy and could shorten the time to targeted management and isolation measures for certain infections such as STEC O157. With these assays, it is common to detect the presence of >1 pathogen that may differ with regard to clinical management.”
  - “Even a positive result for 1 pathogen should be interpreted in the context of the patient’s clinical presentation, because less is known about the clinical significance of tests that detect nucleic acid as compared with traditional assays that generally detect viable organisms. The importance of detection of multiple pathogens in the same specimen is often unclear; it is unknown if all pathogens detected in the specimen are clinically relevant or if one is more strongly associated with the illness.”
- Acute diarrhea, often called gastroenteritis, can be defined as the passage of a greater number of stools of decreased form from the normal lasting < 14 days. Acute diarrhea is generally associated with clinical features of nausea, vomiting, abdominal pain and cramps, bloating, flatulence, fever, passage of bloody stools, tenesmus and fecal urgency. It is the leading cause of outpatient visits,

hospitalizations, and lost quality of life occurring domestically and those traveling abroad.

- Many episodes of acute diarrhea are self-limited and require fluid replacement and supportive care. Oral rehydration is indicated for patients who are mildly to moderately dehydrated. IV fluids may be required for more severe dehydration. Routine use of antidiarrheal agents is not recommended because many of these agents have potentially serious adverse effects, particularly in infants and young children. Antimicrobial therapy is typically warranted for adult and pediatric patients with immune systems which are severely weakened from medications, age and other primary/secondary immunocompromising illnesses/conditions.<sup>6-7</sup>
- Laboratory testing algorithms for infectious causes of diarrhea generally agree that testing is NOT warranted for community-acquired diarrhea of <7 days duration without signs or symptoms of severe (fever, bloody diarrhea, dysentery, severe abdominal pain, dehydration, hospitalization and immunocompromised state) disease. In general, when community-acquired diarrhea persists for ≥7 days, or the diarrhea is travel-related, or there are signs/symptoms of severe disease, GIPP testing may be warranted. Additional directed testing may be indicated if the GIPP results are negative and diarrhea persists. No additional testing is indicated for GIPP-positive result unless the clinical picture changes. Clostridium difficile molecular testing is warranted on health-care associated diarrhea with onset after the 3rd inpatient day or after recent antibiotic use.
- Whereas a majority of microorganisms can be identified with up to 5 targets, typically including Salmonella, Campylobacter, Shigella, Cryptosporidium, and Shiga toxin producing E.coli, additional agents may be in the working differential diagnosis, such as (but not limited to) Clostridium difficile, additional E. coli variants, Yersinia enterocolitica, Vibrio parahaemolyticus, Giardia, Cryptosporidium, and viruses including norovirus, rotavirus, and enteric adenoviruses.
- Salient illustrations<sup>8-10</sup> of the literature have annotated a diverse set of offending infectious agents (bacterial, parasitic and viral) in patients presenting with acute diarrhea. However, it must be emphasized that such original study recruitment criteria were not designed to stratify probability/incidence distributions of causative organisms, according to more carefully specified patient presentation categories. Furthermore, the molecular predilection for mixed infectious agent identification is a confounding factor when clinicians are trying to pinpoint the precise etiology of acute diarrhea, given the dilemma between pathogenicity and non-pathogenicity, which was briefly cited above.
- As a result, when the patient history, clinical presentation and symptoms, etc. suggest a specific microbial etiology and/or therapy, a broad GIPP consisting of >5 infectious targets is not indicated. However, broader GIPP molecular panels (e.g. 6-25 targets) might occasionally be indicated when a patient presents with a clinical scenario and overlapping symptoms consistent with multiple possible microbiological etiologies, where both diagnosis and treatment are particularly challenging (e.g., as noted above for immunocompromised patients).

## Criteria

- The following clinical indications can support the use of molecular GIPP testing.
  - a) Individuals with acute diarrhea with moderate-to-severe symptoms (such as fever, dysentery, severe dehydration).
  - b) Individuals with community-acquired diarrhea that persists for more than seven days, or individuals with travel-associated diarrhea of uncertain etiology.
  - c) Immunocompromised individuals with acute diarrhea. Immunocompromise may support the use of a relatively large number of testing targets, in concert with other supporting clinical documentation in the medical record.
- The following are contraindications to GIPP testing, with any number of targets:
  - a) Immunocompetent individuals with mild diarrhea, particularly of  $\leq 7$  days' duration.
  - b) Individuals in whom the clinical presentation of acute diarrhea suggests a specific infectious etiology, unless first-line laboratory testing should fail to detect the suspected organism, and there is still a high clinical suspicion of infectious etiology.
- Molecular GIPP testing is limited to the minimum number of targets needed for therapeutic decision making. When ordering any configuration of infectious disease targets, whether using GIPP or conventional culture, the medical record should clearly indicate the differential diagnosis of possible microorganisms based upon patient history and presenting signs/symptoms.

## Billing and reimbursement

If any molecular test panels are ordered, which have variable numbers and configurations of infectious agent targets, then the following guidelines apply to the reimbursement of CPT codes 87505, 87506, and 87507, respectively:

- 87505 (3-5 targets) or 87506 (6-11 targets) is supported by ICD-10-CM R19.7 (Diarrhea, unspecified), A09 (Infectious gastroenteritis and colitis, unspecified), A04.9 (Bacteria intestinal infection, unspecified), or K52.9 (Noninfective gastroenteritis and colitis, unspecified).
- 87507 (12-25 targets) is supported by ICD-10-CM R19.7 (Diarrhea, unspecified), A09 (Infectious gastroenteritis and colitis, unspecified), A04.9 (Bacterial intestinal infection, unspecified), or K52.9 (Noninfective gastroenteritis and colitis, unspecified) plus at least one of the immunodeficiency-related codes in the table: *ICD10 Codes Indicating Immunocompromise*
- If the laboratory's testing platform consists solely of a multiplexed panel of 12 or more targets, yet only a subset of the organisms are considered medically necessary based on the above criteria, the lab may request reimbursement for that subset of organisms using a procedure code that does not represent all organisms included on the panel.

## Exclusions and other considerations

Although outbreak investigations may sometimes require use of GIPP testing, the public health evaluations of such outbreaks are beyond the scope and domain of this guideline.

## ICD10 codes

ICD10 codes in this section may be used to support medical necessity as described in the above criteria.

### ***ICD10 Codes Indicating Immunocompromise***

| ICD10 code or range | Description  |
|---------------------|--|
| B20                 | Human immunodeficiency virus [HIV] disease                                     |
| D80.X               | Immunodeficiency with predominantly antibody defects                           |
| D81.0               | Combined immunodeficiencies  |
| D82.X               | Immunodeficiency associated with other major defects                           |
| D83.X               | Common variable immunodeficiency   |
| D84.X               | Other immunodeficiencies   |
| D89.X               | Other disorders involving the immune mechanism, not elsewhere classified       |
| Y92.238             | Other place in hospital as the place of occurrence of the external cause       |
| Y92.239             | Unspecified place in hospital as the place of occurrence of the external cause |

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# MTHFR Variant Analysis for Hyperhomocysteinemia

MOL.TS.205.A

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## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedure addressed by this guideline | Procedure code |
|---------------------------------------|----------------|
| MTHFR Genotyping                      | 81291          |

## What is hyperhomocysteinemia

### Definition

Hyperhomocysteinemia generally refers to mild to moderate elevations of plasma homocysteine levels, which may be defined as 15 to 40  $\mu\text{mol/L}$ .<sup>1</sup>

- Hyperhomocysteinemia may be caused by nutritional deficiencies, various medical conditions, certain drugs, smoking, and inherited factors — such as MTHFR gene variants.<sup>1</sup>
- The MTHFR gene encodes the 5, 10-methylenetetrahydrofolate reductase (MTHFR) enzyme. MTHFR is involved in folate metabolism. The major circulating form of folate is key to converting homocysteine into methionine. Therefore, MTHFR gene variants that reduce MTHFR enzyme function may predispose one to impaired folate metabolism and ultimately mild to moderate hyperhomocysteinemia. However, homocysteine levels are usually normal if folate intake is sufficient.<sup>1</sup>
- Both hyperhomocysteinemia in general, and MTHFR variants specifically, have been reported in association with cardiovascular disease, venous thromboembolism, pregnancy complications, and certain birth defects, such as neural tube defects.<sup>1,2</sup> However, data is inconsistent and associated risks generally small.

### Test information

- MTHFR genetic testing looks for two very common gene variants: C677T and A1298C.<sup>2</sup>
- Individuals who have two variants, including at least one C677T, may have an increased risk for hyperhomocysteinemia. However, the connection between these

MTHFR variants, hyperhomocysteinemia itself, and ultimate disease risk remains unclear.<sup>3,4</sup>

- Many experts suggest that measuring homocysteine levels directly is more informative than MTHFR variant testing.<sup>5</sup>
- Note that pathogenic variants in the MTHFR gene (not the common benign variants discussed here) are rarely associated with a genetic disorder called homocystinuria.<sup>2</sup> MTHFR variant testing will not find the pathogenic variants that cause homocystinuria.
- MTHFR gene testing may be a component of panels for thrombophilia, cardiovascular disease risk, psychiatric conditions, or preeclampsia. There is insufficient evidence in the peer-reviewed literature to establish clinical utility for any of these indications for testing.

## Guidelines and evidence

- As part of the Choosing Wisely campaign, the American College of Medical Genetics and Genomics (2015) released “Five Things Physicians and Patients Should Question,” which states:<sup>6</sup>
  - “Don’t order MTHFR genetic testing for the risk assessment of hereditary thrombophilia. The common MTHFR gene variants, 677C>T and 1298A>G, are prevalent in the general population. Recent meta-analyses have disproven an association between the presence of these variants and venous thromboembolism.”
- Also as part of the Choosing Wisely campaign, the Society for Maternal Fetal Medicine (2014) released “Five Things Physicians and Patients Should Question,” which states:<sup>7</sup>
  - “Don’t do an inherited thrombophilia evaluation for women with histories of pregnancy loss, intrauterine growth restriction (IUGR), preeclampsia and abruptio. Scientific data supporting a causal association between either methylenetetrahydrofolate reductase (MTHFR) polymorphisms or other common inherited thrombophilias and adverse pregnancy outcomes, such as recurrent pregnancy loss, severe preeclampsia and IUGR, are lacking.”
- The American College of Medical Genetics and Genomics (ACMG, 2013) states:<sup>8</sup>
  - “It was previously hypothesized that reduced enzyme activity of MTHFR led to mild hyperhomocysteinemia which led to an increased risk for venous thromboembolism, coronary heart disease, and recurrent pregnancy loss. Recent meta-analyses have disproven an association between hyperhomocysteinemia and risk for coronary heart disease and between MTHFR polymorphism status and risk for venous thromboembolism. There is growing evidence that MTHFR polymorphism testing has minimal clinical utility



and, therefore should not be ordered as a part of a routine evaluation for thrombophilia.”

- The American College of Obstetricians and Gynecologists (ACOG, 2013) states:<sup>9</sup>
  - “Because of the lack of association between either heterozygosity or homozygosity for the methylenetetrahydrofolate reductase (MTHFR) C677T polymorphism and any negative pregnancy outcomes, including any increased risk for venous thromboembolism, screening with either MTHFR mutation analyses or fasting homocysteine levels is not recommended.”
- The National Society of Genetic Counselors (NSGC, 2005) state that MTHFR variant testing is specifically not justified in the case of recurrent pregnancy loss based on available studies.<sup>10</sup>

## Criteria

This test is considered investigational and/or experimental.

- Investigational and experimental (I&E) molecular and genomic (MolGen) tests refer to assays involving chromosomes, DNA, RNA, or gene products that have insufficient data to determine the net health impact, which typically means there is insufficient data to support that a test accurately assesses the outcome of interest (analytical and clinical validity), significantly improves health outcomes (clinical utility), and/or performs better than an existing standard of care medical management option. Such tests are also not generally accepted as standard of care in the evaluation or management of a particular condition.
- In the case of MolGen testing, FDA clearance is not a reliable standard given the number of laboratory developed tests that currently fall outside of FDA oversight and FDA clearance often does not assess clinical utility.

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# Multiple Endocrine Neoplasia Type 1 (MEN1)

MOL.TS.285.A  
v1.0.2020

## Introduction

Multiple Endocrine Neoplasia Type 1 (MEN1) is addressed by this guideline.

## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedures addressed by this guideline | Procedure codes |
|--|-----------------|
| MEN1 Known Familial Mutation Analysis  | 81403           |
| MEN1 Deletion/Duplication Analysis     | 81404           |
| MEN1 Full Gene Sequencing              | 81405           |

## What is Multiple Endocrine Neoplasia Type 1

### Definition

Multiple Endocrine Neoplasia Type 1 (MEN1) is an autosomal dominant disorder characterized by the development of multiple endocrine and non-endocrine tumors.

### Incidence or Prevalence

MEN1 has a prevalence of 1/10,000 to 1/100,000 individuals.<sup>1</sup>

### Symptoms

The presenting symptom in approximately 90% of individuals with MEN1 is primary hyperparathyroidism (PHPT). Parathyroid tumors cause overproduction of parathyroid hormone which leads to hypercalcemia. The average age of onset is 20-25 years. Parathyroid carcinomas are rare in individuals with MEN1.<sup>2,3,4</sup>

Pituitary tumors are seen in 30-40% of individuals and are the first clinical manifestation in 10% of familial cases and 25% of simplex cases. Tumors are typically solitary and there is no increased prevalence of pituitary carcinoma in individuals with MEN1.<sup>2,5</sup>

- Prolactinomas are the most commonly seen pituitary subtype and account for 60% of pituitary adenomas. They manifest as amenorrhea, oligomenorrhea, and/or galactorrhea in females and sexual dysfunction and gynecomastia in males.
- Growth hormone (GH)-secreting adenomas account for 25% of pituitary adenomas, with acromegaly as a common manifestation.
- Growth hormone/prolactin (GH/PRL)-secreting adenomas are seen in approximately 5% of individuals with MEN1. Manifestations can include acromegaly, as well as amenorrhea, oligomenorrhea, and/or galactorrhea in females and sexual dysfunction and gynecomastia in males.
- Adrenocorticotrophic hormone (ACTH)-secreting adenomas occur in less than 5% of individuals with MEN1 and are associated with Cushing's syndrome.
- Thyroid-stimulating hormone (TSH)-secreting adenomas are rare and manifest as symptoms of hyperthyroidism.
- Non-secreting tumors occur in less than 5% of individuals with MEN1 and manifest as enlarging pituitary tumors which can compress adjacent structures.

Well-differentiated endocrine tumors of the gastro-entero-pancreatic (GEP) tract include tumors of the stomach, duodenum, pancreas, and intestinal tract.<sup>2,6,7</sup>

- Gastrinoma resulting in Zollinger-Ellison syndrome (ZES). More than 80% of MEN1-associated gastrinomas are found in the first and second portion of the duodenum. They are frequently multiple and usually malignant.
- Insulinoma resulting in hypoglycemia, which is observed in 10% of individuals with MEN1.
- Glucagonoma resulting in hyperglycemia, gastrointestinal problems, venous thrombosis, and skin rash. They are seen in less than 1% of individuals with MEN1.
- VIPoma (Vasoactive intestinal peptide-secreting tumor). These growths are typically malignant with high metastatic potential.

Other tumor types may include:

- Carcinoid tumors with bronchopulmonary, thymic, and gastric subtypes<sup>2</sup>
- Adrenocortical tumors including cortisol-secreting, aldosterone-secreting, and rarely, pheochromocytoma<sup>2</sup>
- Non-endocrine tumors (facial angiofibromas, collagenomas, lipomas, meningiomas, ependymomas, and leiomyomas)

## Cause

Almost all cases of MEN1 are due to inactivating mutations in the MEN1 gene. The MEN1 gene codes for a tumor suppressor called menin. An inherited inactivating mutation plus an acquired (somatic) change in the other gene copy causes clonal

growth that leads to tumors.<sup>1</sup> Pathogenic mutations in MEN1 are identified in 80% to 95% of familial cases and 65% to 70% of de novo cases.<sup>8</sup>

Germline MEN1 mutations have been reported in approximately 20% to 57% of individuals with familial isolated hyperparathyroidism (FIHP) and rarely in individuals with familial pituitary tumor.<sup>1</sup>

## Inheritance

MEN1 mutations are inherited in an autosomal dominant manner, meaning that a person only needs a mutation in one copy of the gene to be affected. A child of an affected person has a 50% chance to inherit the mutation. The de novo mutation rate is approximately 10%. The age-related penetrance for all clinical features surpasses 50% by age 20 years and 95% by age 40 years.<sup>2,9,10</sup>

## Diagnosis

Clinical diagnosis of MEN1 is made when two neuroendocrine tumors of the parathyroid, pituitary, or GEP tract are identified.<sup>1</sup> Diagnostic tests may include biochemical testing for hormone and calcium levels, imaging, and molecular testing of the MEN1 gene, depending on clinical presentation and family history.

## Treatment/Surveillance

Management and prevention strategies for those with or at-risk for MEN1 include treatment of specific tumor symptoms. This may include surgeries to remove the affected glands and specific medical therapies. Presymptomatic screening protocols in MEN1 carriers have been established and are based on the youngest age of disease manifestations that has been reported.<sup>8</sup> Regular monitoring of hormone levels, as well as abdominal, chest, and head CTs and/or MRIs may be recommended.

## Survival

Survival in MEN1 can be reduced and is largely dependent on clinical presentation and stage of cancer at the time of diagnosis. Thymic tumors in individuals with MEN1 are aggressive and median survival after diagnosis is less than 10 years.<sup>1</sup>

## Test information

### Introduction

Testing for MEN1 may include sequence analysis, deletion/duplication analysis, or known familial mutation testing.

## Full Gene Sequence analysis

Sequence analysis detects single nucleotide substitutions and small (several nucleotide) deletions and insertions. Regions analyzed typically include the coding sequence and intron/exon boundaries. Promoter regions and intronic sequences may also be sequenced if disease-causing mutations are known to occur in these regions of a gene.

MEN1 sequencing evaluates each DNA nucleotide to identify mutations throughout the gene and should detect a mutation in 80-95% of familial cases of MEN1 and 65-75% of *de novo* cases of MEN1.<sup>11-13</sup>

- The likelihood of detecting an MEN1 pathogenic variant is highest when an individual has more main tumors (parathyroid, pancreatic, and pituitary), especially those families with hyperparathyroidism and pancreatic islet tumors.<sup>14,15</sup>
- The likelihood of detecting an MEN1 pathogenic variant increases in *de novo* cases with the presence of pancreatic lesions or with the presence of two main manifestations of MEN1.<sup>16</sup>
- Individuals who have a single MEN1-related tumor and no family history of MEN1 syndrome rarely have germline MEN1 pathogenic variants.<sup>14</sup>

## Deletion/duplication analysis

Analysis for deletions and duplications can be performed using a variety of technical platforms including exon array, MLPA, and NGS data analysis to identify single or multiple or whole gene deletions.

Deletion/duplication panels may be billed separately from sequencing panels.

The likelihood of identifying a deletion or duplication in an individual with MEN1 and no mutation identified by gene sequencing is 1-4%.<sup>14,15,17-21</sup>

## Known familial mutation analysis

Analysis for known familial mutations is typically performed by Sanger sequencing, but if available, a targeted mutation panel that includes the familial mutation may be performed.

Known familial mutations analysis is performed when a causative mutation has been identified in a close relative of the individual requesting testing.

## Guidelines and evidence

### Introduction

The following section includes relevant guidelines and evidence pertaining to MEN1 testing.

## National Comprehensive Cancer Network

Evidence-based guidelines from the National Comprehensive Cancer Network (NCCN, 2018) support the use of MEN1 genetic testing in those with a clinical diagnosis of MEN1 or an at-risk relative of an individual with a known MEN1 germline mutation. A clinical diagnosis for MEN1 includes two or more MEN1-associated tumors:<sup>22</sup>

- multi-gland parathyroid hyperplasia;
- pancreatic NET; or
- pituitary tumors

## Literature review

An expert-authored review (2012)<sup>2</sup> of MEN1 states MEN1 germline mutation testing should be offered to probands with MEN1 and their first-degree relatives, including relatives who are either asymptomatic or have clinical manifestations of MEN1. MEN1 germline mutation testing should be offered at the earliest opportunity as MEN1 manifestations may occur by the age of 5 years. A diagnosis of MEN1 may be established by one of the three criteria:

- The occurrence of two or more primary MEN1-associated endocrine tumors (such as parathyroid adenoma, enteropancreatic tumor, and pituitary adenoma);
- The occurrence of one of the MEN1-associated tumors in a first-degree relative of a patient with a clinical diagnosis of MEN1;
- The identification of a germline MEN1 mutation in an individual who may be asymptomatic and has not yet developed serum biochemical or radiological abnormalities indicative of tumor development.

Comprehensive MEN1 testing should also be considered in any person under the age of 30 with primary hyperparathyroidism (PHPT), pancreatic precursor lesions, or pancreatic islet tumor regardless of family history.<sup>8</sup>

## Criteria

### Introduction

Requests for MEN1 testing are reviewed using the following criteria.

### MEN1 Known Familial Mutation Analysis

- Genetic Counseling:
  - Pre- and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND

- Previous Testing:
  - No previous genetic testing of MEN1, AND
- Diagnostic and Predisposition Testing:
  - Known disease-causing family mutation in MEN1 identified in 1st, 2nd, or 3rd degree biological relative(s), AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy

### **MEN1 Full Gene Sequencing**

- Genetic Counseling:
  - Pre- and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Testing:
  - No previous genetic testing of MEN1, AND
- Diagnostic Testing for Symptomatic Individuals
  - Personal history of two or more of the following:
    - Parathyroid tumor, and/or
    - Pituitary tumor, including prolactinoma, GH-secreting adenoma, GH/PRL-secreting adenoma, TSH-secreting adenoma, ACTH-secreting adenoma, non-secreting pituitary adenoma, and/or
    - Well-differentiated endocrine tumors of the gastro-entero-pancreatic (GEP) tract, including gastrinoma, insulinoma, glucagonoma, VIPoma, non-secreting adenoma, pancreatic polypeptide-secreting adenoma, and/or
    - Carcinoid tumor, and/or
    - Adrenocortical tumor, OR
  - Any person under the age of 30 with PHPT, pancreatic precursor lesions, or pancreatic islet tumor regardless of family history, OR
- Predisposition Testing for Presymptomatic/Asymptomatic Individuals:
  - First-degree relative of an individual with a clinical diagnosis of MEN1 (Note: whenever possible, an affected family member should be tested first), AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy

### **MEN1 Duplication/Deletion Analysis**

- Genetic Counseling:



- Pre- and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Testing:
  - No previous duplication/deletion testing, and
  - Previous MEN1 sequencing performed and no mutations found, and
  - No known familial mutation, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy

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# MUTYH Associated Polyposis Testing

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## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedures addressed by this guideline | Procedure codes |
|--|-----------------|
| MUTYH Known Familial Mutation Analysis | 81403           |
| MUTYH Targeted Mutation Analysis       | 81401           |
| MUTYH Sequencing                       | 81406           |
| MUTYH Deletion/Duplication Analysis    | 81479           |

## What is MUTYH-associated polyposis

### Definition

MUTYH-associated polyposis (MAP) is an inherited colorectal cancer syndrome caused by mutations in the MUTYH gene (also called MYH). MAP is estimated to account for 0.7% of all colorectal cancer.<sup>1</sup>

- MAP clinical findings overlap those of familial adenomatous polyposis (FAP) and attenuated FAP (AFAP). Affected patients most often have fewer than 100 adenomas, but cases of hundreds and occasionally over 1000 polyps have been reported.<sup>1,2</sup> Hyperplastic and sessile serrated, and traditional serrated adenomatous polyps have also been seen in individuals with MAP, although adenomas remain the most common polyp type in MAP.<sup>1,3</sup> Duodenal adenomas occur in 17-25% of individuals with MAP and gastric polyps have been reported in about 11%.<sup>1,3,4</sup> Additionally, approximately one third of individuals with MAP have been described with colorectal cancer and no polyps or only a few polyps.<sup>1</sup>
- Up to 26% of people who meet clinical diagnostic criteria for classic or attenuated FAP, but have normal FAP genetic test results, will have a MAP mutation.<sup>1</sup>
- Because MAP is not clinically distinguishable from FAP or AFAP, the identification of two MUTYH mutations is required to make a MAP diagnosis.<sup>1,5</sup>
- Adenomas and colorectal cancer tend to present later than FAP. The diagnosis of colorectal cancer is often 50 years (range of 45-59 years).<sup>1,2</sup> The lifetime risk for colorectal cancer in individuals with MAP is 43 to 100% in the absence of timely surveillance.<sup>1</sup> There is also an estimated 4-5% lifetime risk for duodenal cancer.<sup>1-3</sup>

- Unlike FAP, MAP is inherited in an autosomal recessive manner — both copies of the MUTYH gene must have a mutation to be affected. This means that siblings are the only relatives likely to be affected in the family history (i.e., you do not see inheritance from parent to child as with FAP).

## Test information

- ***MUTYH Targeted Mutation Analysis:*** Two MUTYH mutations are particularly common (Y165C and G382D) and account for over 80% of MUTYH mutations in Caucasians of Northern European descent.<sup>6</sup> It is estimated that 1%-2% of the general northern European population is a carrier for a MUTYH mutation.<sup>1</sup> Some laboratories test for only these two mutations or offer reflex options that begin with these two mutations and proceed to full gene sequencing if two mutations are not found.
- ***MUTYH Sequencing Analysis:*** MUTYH full sequencing analysis analyzes the entire gene for mutations. It is typically done in reflex to negative results from targeted mutation analysis.
- ***MUTYH Deletion/Duplication Analysis:*** If sequencing does not find two mutations, large gene deletion/duplication analysis can be performed. It remains unknown what percentage of MAP is due to large deletions/duplications/rearrangements in the gene and thus are detectable only with this technology. However, large deletions have been reported.<sup>1,7,8</sup>
- ***MUTYH Known Familial Mutation Analysis:*** Once the mutations that run in the family are known, other relatives can have testing for only those mutations. This is more accurate and cost-effective.
- ***Multi-gene Panel Test:*** A multi-gene panel that includes MUTYH and other polyposis genes may also be considered.<sup>1</sup>

## Guidelines and evidence

- Guidelines from the National Comprehensive Cancer Network (NCCN, 2018) on High-Risk Colorectal Assessment states the following:<sup>2</sup>
  - MUTYH testing criteria:
    - “Personal history of >10 adenomas”
    - “Individual meeting criteria 1 or 3 (NCCN, 2017) for Serrated Polyposis Syndrome (SPS) [formerly known as hyperplastic polyposis] with at least some adenomas.” (see below)
    - “Known deleterious MUTYH mutation(s) in the family”
  - SPS clinical diagnostic criteria:

- i. “At least 5 serrated polyps (includes hyperplastic polyps, sessile serrated adenomas/polyps, and traditional serrated adenomas) proximal to the sigmoid colon with 2 or more of these being >10mm.”
  - ii. “Any number of serrated polyps proximal to the sigmoid colon in an individual who has a first-degree relative with serrated polyposis.”
  - iii. “At least 20 serrated polyps of any size, but distributed throughout the colon.”
- Footnotes:
    - “When colonic polyposis is present in a single person with a negative family history, consider testing for a de novo APC mutation; if negative, follow with testing of MUTYH (targeted testing for the two common northern European founder mutations c.536A>G and c.1187G>A may be considered first followed by full sequencing if biallelic mutations are not found). When colonic polyposis is present only in siblings, consider recessive inheritance and test for MUTYH first. Order of testing for APC and MUTYH is at the discretion of the clinician.”
    - “MUTYH genetic testing is not indicated based on a personal history of desmoid tumor, hepatoblastoma, cribriform-morular variant of papillary thyroid cancer, or multifocal/bilateral CHRPE.”
    - “Siblings of a patient with MAP are recommended to have site-specific genetic testing for the familial biallelic mutations. Children of an affected parent with MAP are recommended to have site-specific genetic testing for the familial mutation/s. If positive for one MUTYH mutation, full sequencing of MUTYH is recommended. Full sequencing of MUTYH also may be considered in an unaffected parent when the other parent has MAP. If the unaffected parent is found to not be heterozygous for a MUTYH mutation, genetic testing in children is not necessary. If he or she is found to have a MUTYH mutation, testing for the familial mutations in the children is recommended.”
    - “It is important to note that de novo mutations can occur in APC or MUTYH. Thus, when colonic polyposis is present in an individual with a negative family history, consideration should be given to genetic testing of APC, followed by testing of MUTYH if no APC mutation is found.”
  - All recommendations are category 2A.
  - Evidence-based guidelines from the American College of Gastroenterology (ACG, 2009) state:<sup>9</sup> “Patients with classic FAP, in whom genetic testing is negative, should undergo genetic testing for bi-allelic MUTYH mutations. Patients with 10 - 100 adenomas can be considered for genetic testing for attenuated FAP and if negative, MUTYH associated polyposis” [Grade 2C: Weak recommendation, low-quality or very low-quality evidence].

## Criteria

### MUTYH Known Familial Mutation Analysis

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Testing:
  - No previous genetic testing for known MUTYH family mutation(s), AND
- Diagnostic or Predisposition Testing:<sup>1,2</sup>
  - Two known MUTYH mutations in a sibling, or
  - Both parents with one or two known MUTYH mutations, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

### MUTYH Targeted Mutation Analysis for Y179C and G396D Mutations

- Genetic Counseling
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Testing:
  - No previous MUTYH testing, and
  - No mutation detected on APC gene testing, if performed, AND
- Individual is of Northern European descent, AND
- Diagnostic Testing for Symptomatic Individuals:<sup>2,10</sup>
  - Clinical findings:
    - > 10 cumulative adenomas, or
    - At least two adenomas, AND
      - At least 5 serrated polyps proximal to the sigmoid colon (2 or more of >10mm), or
      - > 20 serrated polyps of any size, but distributed throughout the colon, AND
  - Recessive pattern of inheritance (e.g. family history positive for only an affected sibling), OR
- Testing for Presymptomatic/Asymptomatic Individuals: <sup>1,2</sup>

- Reproductive partner of a person with MAP (to determine if children at risk), AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

### **MUTYH Sequencing**

- Genetic Counseling
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Testing:
  - No previous MUTYH full sequencing, and
  - Two mutations NOT identified through MUTYH targeted mutation analysis (Y179C and G396D) if performed, and
  - No mutation detected on APC gene testing, if performed, AND
- Diagnostic Testing for Symptomatic Individuals:<sup>2,10</sup>
  - Clinical findings:
    - > 10 cumulative adenomas, or
    - At least two adenomas, AND
      - At least 5 serrated polyps proximal to the sigmoid colon (2 or more of >10mm), or
      - > 20 serrated polyps of any size, but distributed throughout the colon, AND
  - Recessive pattern of inheritance (e.g. family history positive for only an affected sibling), OR
- Testing for Presymptomatic/Asymptomatic Individuals:<sup>1,2</sup>
  - Reproductive partner of a person with MAP (to determine if children at risk), AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

### **MUTYH Deletion/Duplication Analysis**

- Genetic Counseling
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND

- Previous Testing:
  - MUTYH full sequencing performed, and
  - No mutations or only one mutation detected in MUTYH through any previous testing (founder mutation panel or full gene sequencing), and
  - No mutation detected on APC gene testing, if performed, AND
- Diagnostic Testing for Symptomatic Individuals:<sup>2,10</sup>
  - Clinical findings:
    - > 10 cumulative adenomas, or
    - At least two adenomas, AND
      - At least 5 serrated polyps proximal to the sigmoid colon (2 or more of >10mm), or
      - > 20 serrated polyps of any size, but distributed throughout the colon, AND
  - Recessive pattern of inheritance (e.g. family history positive for only an affected sibling), OR
- Testing for Presymptomatic/Asymptomatic Individuals:<sup>1,2</sup>
  - Reproductive partner of a person with MAP (to determine if children at risk), AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

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# Myeloma Prognostic Risk Signature (MyPRS)

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## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedure addressed by this guideline     | Procedure code |
|---|----------------|
| Myeloma Prognostic Risk Signature (MyPRS) | 81479          |

## What is MyPRS

### Definition

The Myeloma Prognostic Risk Signature (MyPRS®) (Signal Genetics™) has been developed to estimate the underlying activity of disease progression, in patients diagnosed with active MM. The test may be used as a potentially useful risk stratification tool to predict treatment response to chemotherapy, predict risk of survival and relapse, and tailor therapy selection. Specifically, MyPRS may identify a high-risk patient group for disease progression based on the expression levels of 70 selected genes measured at baseline. It may be helpful to stratify patients into high-, high risk-borderline, low-risk borderline, and low-risk categories to optimize individual treatment.<sup>8</sup>

- Multiple myeloma (MM) is a malignant and often incurable hematological cancer, characterized by the abnormal and uncontrolled proliferation of plasma cells in bone marrow, leading to impaired hematopoiesis and production of monoclonal immunoglobulin (Ig).<sup>1,2</sup> The disease is responsible for about 1% of all cancers worldwide and 10 to 15% of all hematological cancers. MM usually affects older adults (median age of onset is 71 and 74 years for men and women, respectively). For the period between 2009 and 2010, the relative world-wide 5-year survival rate was approximately 45%.
- Clinical features of MM include anemia (73%), bone pain (58%), fatigue (32%), and unusual weight loss (25%).<sup>3</sup> Diagnostic laboratory and clinical assessments include hypercalcemia, kidney dysfunction, anemia, and bone lesions.<sup>4</sup> In general, patients are treated with autologous stem-cell transplantation (ASCT), along with supportive measures, such as pain therapy, administration of bisphosphonates, and irradiation of skeletal/extramedullary lesions.<sup>3</sup>
- A growing body of research suggests specific genetic lesions play an important role in the tumor biology of MM. Furthermore, the high number of chromosomal

aberrations and multiple changes in gene expression of these lesions has demonstrated that the underlying genetic features of MM tumor cells are responsible for the significant degree of clinical heterogeneity typically observed in this disease. Several molecular subtypes, each with a unique path of pathogenesis and clinical presentation, have also been identified.<sup>2</sup> The inherent molecular heterogeneity of the disease is believed to translate into highly variable treatment responses and survival times (ranging from a few months to 15 years or more).<sup>5</sup> Given the considerable heterogeneity of associated outcomes, various prognostic risk factors specific to MM have been identified to predict the course of disease, define individualized treatment strategies, predict survival, and enhance overall therapeutic decision making.<sup>6</sup>

- Conventional cytogenetic methods, such as karyotyping and fluorescence in situ hybridization (FISH), are used in clinical practice to assess disease prognosis and stratify MM patients based on recurrent chromosomal changes. Risk stratification is intended to ensure patients receive proper treatment, depending on disease severity. One available risk stratification strategy is the evidence-based algorithm, the Mayo Stratification of Myeloma and Risk-Adapted Therapy (mSMART), used to inform treatment decisions for patients with newly diagnosed disease.<sup>7</sup> However, given the heterogeneity of MM, conventional prognostic methods may not accurately estimate risk.

## Test information

- According to Signal Genetics, the MyPRS test uses the Affymetrix GeneChip® 3000Dx v.2 System, a whole-genome microarray platform, and requires at least 20,000 CD138+ plasma cells in order to obtain sufficient genetic material for gene expression analysis.<sup>8</sup>
- The MyPRS gene expression profiling model consists of a continuous gene score that is a linear combination of the 70 genes along with a cutoff, such that patients with a score greater than the cutoff are categorized as high risk and otherwise low risk for disease progression.<sup>9</sup>
- The MyPRS prognostic score has the ability to predict a patient's likely event-free survival (EFS) and overall survival (OS) at the time of diagnosis or after relapse. The algorithm used to develop this prognostic score was based on mathematical models using microarray technology and multivariate analysis of independent patient cohorts over 8 years of follow-up. Results of the model indicate that on a risk score of 0 (lowest likelihood of risk; good prognosis) to 100 (highest likelihood of risk; poor prognosis), a cut-off point of 45.2 discriminates between low and high risk patients.<sup>8</sup>
- The test also provides results of a molecular subtype (7-class molecular subtype taxonomy), each associated with unique genetic lesions, altered genes, and outcome variation.

MyPRS

- Patients are provided results of virtual karyotyping to predict cytogenic abnormalities associated with MM, which is based on an 816-gene algorithm using gene expression data, and validated against multiple traditional cytogenic techniques.<sup>8</sup>
- In November 2016, Quest Diagnostics purchased MyPRS assets from Signal Genetics.<sup>10</sup>

## Guidelines and evidence

- The National Comprehensive Cancer Network (NCCN , 2017) Clinical Practice Guidelines stated the following regarding gene expression profiling (GEP):
  - “GEP is a powerful and fast tool with the potential to provide additional prognostic value to further refine risk stratification, help therapeutic decisions, and inform novel drug design and development.”<sup>11</sup>
  - “The NCCN Panel unanimously agreed that although GEP is not currently routinely used in clinical practice during diagnostic workup, GEP is a useful tool and may be helpful in selected patients to estimate the aggressiveness of the disease and individualize treatment.”
  - The NCCN Panel does not make any explicit recommendations for its use in its diagnostic and treatment pathways for cases of MM.
- There is insufficient evidence in the peer-reviewed literature to draw definitive conclusions regarding the analytical validity, clinical validity, and clinical utility of the MyPRS test to accurately provide prognostic risk stratification among patients who are newly diagnosed with MM or who have relapsed following treatment.<sup>12-15</sup>
  - The evidence base mostly consists of retrospective studies evaluating small numbers of patients that evaluated the strength of the association between the MyPRS score with various survival measures, including post relapse survival, overall survival, and progression-free survival. Although the available studies reported significant associations between MyPRS and survival measures (patients with high MyPRS scores may be at increased risk of relapse and death), with study authors concluding that MyPRS has value as a risk stratification tool, the quality of the overall evidence is low given the retrospective study designs across the evidence base, and the lack of reported accuracy measures, including sensitivity, specificity, PPV, NPV, and clinical utility values. Furthermore, there is little to no evidence regarding the comparative accuracy of MyPRS with FISH testing or MyPRS with karyotyping. It is unknown if MyPRS can be an adequate substitute for FISH testing in patients with MM as part of the routine workup of the disease.
  - Future prospective studies, allocating patients to therapies determined to be most effective based on MyPRS score, with adequate sample sizes, using gold standard diagnostic and/or prognostic measures, are necessary to elucidate its

role as an adjunct to existing risk stratification measures or as a stand-alone test. Well-designed clinical utility studies are also needed to assess whether the MyPRS test leads to improved therapeutic clinical decision-making and improved patient outcomes.

## Criteria

- This test is considered investigational and/or experimental.
  - Investigational and experimental (I&E) molecular and genomic (MolGen) tests refer to assays involving chromosomes, DNA, RNA, or gene products that have insufficient data to determine the net health impact, which typically means there is insufficient data to support that a test accurately assesses the outcome of interest (analytical and clinical validity), significantly improves health outcomes (clinical utility), and/or performs better than an existing standard of care medical management option. Such tests are also not generally accepted as standard of care in the evaluation or management of a particular condition.
  - In the case of MolGen testing, FDA clearance is not a reliable standard given the number of laboratory developed tests that currently fall outside of FDA oversight and FDA clearance often does not assess clinical utility.

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# Myoclonic Epilepsy with Ragged Red Fibers (MERRF)

MOL.TS.242.A

v1.0.2020

## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedures addressed by this guideline | Procedure codes |
|--|-----------------|
| MT-TK Targeted Mutation Analysis       | 81401           |
| Whole Mitochondrial Genome             | 81460           |

## What is MERRF

### Definition

Myoclonic Epilepsy with Ragged Red Fibers (MERRF) is a multisystem mitochondrial disease.<sup>1</sup>

- MERRF typically presents with myoclonus (brief, involuntary twitching of a muscle or a group of muscles), followed by generalized epilepsy, ataxia (lack of coordination of muscle movements), weakness, and dementia.<sup>1</sup> Ragged red fibers (RRF) are identified on muscle biopsy pathology.<sup>1</sup>
  - Other common findings include hearing loss, short stature, optic atrophy, and cardiomyopathy with Wolff-Parkinson-White syndrome (a syndrome in which there is extra electrical connection in the heart at birth causing rapid heartbeat). Occasionally pigmentary retinopathy and lipomatosis are observed.<sup>1</sup>
  - Most cases present in childhood after normal early development.<sup>1</sup>
- MERRF is caused by mutations in the mitochondrial DNA (mtDNA) and follows maternal inheritance. This means that a female who carries the mtDNA point mutation will pass it on to all of her children. A male who carries the mtDNA mutation cannot pass it on to his children.<sup>1,2</sup>
- For all mtDNA mutations, clinical expressivity depends on the three following factors:
  - The relative abundance of mutant mtDNA, or mutational load (heteroplasmy)
  - The organs and tissues in which the mutant mtDNA is found (tissue distribution), and

- The vulnerability of each tissue to impaired oxidative metabolism (threshold effect).
- The estimated prevalence of MERRF is approximately 0.25-1.5/100,000 individuals.<sup>1</sup>
- Management is usually palliative. Certain antiepileptic drugs, such as valproic acid, should be avoided as they may cause secondary carnitine deficiency or can be used with L-carnitine supplementation.<sup>1</sup>
- At-risk individuals may also benefit from clinical assessment to initiate baseline evaluations (neurology, cardiology, ophthalmology, and audiology) and potential intervention prior to exhibiting clinical manifestations.<sup>1</sup>

## Test information

- The investigation and diagnosis of patients with mitochondrial disease often necessitates a combination of techniques including muscle histocytochemistry, biochemical assessment and molecular genetic studies along with clinical assessment. Any molecular genetic test for a mtDNA mutation should ideally be directed by the clinical phenotype and results of these other investigations.<sup>3</sup>
- MERRF Mutation Panel: Heteroplasmic mutations in the mtDNA genes, MT-TK, MT-TL1, MT-TF, MT-TI, and MT-TP cause MERRF. Mutations in the mtDNA genes MT-TH, MT-TS1, MT-TS2, cause MELAS/MERRF overlap syndrome.
  - Approximately 90% of cases of MERRF are due to MT-TK mutations. 80% of MERRF cases are the result of a specific genetic change, m.8344A>G (formerly A8344G) in MT-TK.<sup>1,2,4</sup>
    - Three additional MT-TK mutations, m.8356T>C, m.8363G>A, and m.8361G>A, are present in an additional 10% of affected individuals. These three mutations can also be associated with other mitochondrial or genetic conditions.<sup>1</sup>
  - Detection rate of the four-mutation panel is about 90%.<sup>1</sup>
  - “Sequence analysis / scanning for pathogenic variants is used to detect pathogenic variants throughout mtDNA and is not specific for MERRF. The overall variant detection rate for MERRF by scanning/sequence analysis of mtDNA is 90%-95%.”<sup>1</sup>
- Due to its ability to simultaneously sequence the entire mtDNA and measure heteroplasmy at each position, next generation sequencing (NGS) is an option for assessing MERRF and overlapping syndromes. However, certain targeted mutation analyses can also estimate heteroplasmy. Typically, Sanger sequence analysis will miss heteroplasmy below 20%.
- If genetic testing is negative in a blood sample in a person with symptoms of MERRF, testing can be done on other specimens. Typically this is done when the



phenotype is highly suggestive of presence of a MERRF mutation or when there is a need to assess reproductive risk.

- Muscle may be considered as a secondary tissue since it is clinically involved as evidenced by Ragged Red Fibers. Muscle biopsy allows enzymatic analysis of the electron transport chain, light and ultra structural microscopy, and mtDNA copy number analysis—all of which may provide highly useful information. However, the invasiveness and procedural costs associated with a muscle biopsy are factors to consider.
- Genetic testing can also be done on skin fibroblasts, urinary sediment, saliva, or buccal mucosa.<sup>1</sup> If cultured fibroblasts are used, measures such as limited passaging and uridine supplementation should be taken to reduce selection against mutant genotypes that may lead to skewed heteroplasmy.

## Guidelines and evidence

- No specific evidence-based U.S. testing guidelines were identified.
- Case reports and a limited number of case series are the primary evidence base available for the diagnosis of mitochondrial disease.<sup>5-7</sup>
- The Mitochondrial Medicine Society developed consensus recommendations using the Delphi method and published them in 2015.<sup>8</sup>
  - Recommendations for DNA testing
    - “Massively parallel sequencing/NGS of the mtDNA genome is the preferred methodology when testing mtDNA and should be performed in cases of suspected mitochondrial disease instead of testing for a limited number of pathogenic point mutations.”
    - “Patients with a strong likelihood of mitochondrial disease because of a mtDNA mutation and negative testing in blood, should have mtDNA assessed in another tissue to avoid the possibility of missing tissue-specific mutations or low levels of heteroplasmy in blood; tissue-based testing also helps assess the risk of other organ involvement and heterogeneity in family members and to guide genetic counseling.”
    - “Heteroplasmy analysis in urine can selectively be more informative and accurate than testing in blood alone, especially in cases of MELAS due to the common m.3243 A>G mutation.”
    - “When considering nuclear gene testing in patients with likely primary mitochondrial disease, NGS methodologies providing complete coverage of known mitochondrial disease gene is preferred. Single-gene testing should usually be avoided because mutations in different genes can produce the same phenotype. If no mutation is identified via known NGS panels, then whole exome sequencing should be considered.”

MERRF

- The European Federation of Neurological Sciences (2009) provided molecular diagnostic consensus-based guidelines based on literature reviews: “If the phenotype suggests syndromic mitochondrial disease due to mtDNA point mutations (MELAS, MERRF, NARP, LHON) DNA-microarrays using allele-specific oligonucleotide hybridization, real-time-PCR or single-gene sequencing are indicated.”<sup>9</sup>
- The clinical utility of genetic testing for MERRF was described by a workshop of the National Institute of Neurological Disorders and Stroke (2008):<sup>10</sup>
  - “The diagnosis of mitochondrial diseases is complicated by their heterogeneous presentations and by the lack of screening procedures or diagnostic biomarkers that are both sensitive and specific. The workshop panelists explained that diagnosis is often a lengthy process beginning with a general clinical evaluation followed by metabolic screening and imaging and finally by genetic tests and more invasive biochemical and histological analyses. The identification of known mitochondrial mutations in tissue has greatly aided diagnosis. However, even when clinical features and family history strongly suggest mitochondrial disease, the underlying genetic mutation can elude detection, and there is no current screening procedure that would be practical for all cases of suspected mitochondrial disease.”<sup>10</sup>
  - Initial screening includes testing lactate and CSF protein levels, muscle biopsy, EEG, ECG, and MRI. “It is important to note that biochemical abnormalities may not be present during periods when the mitochondrial disease is quiescent/dormant.”<sup>10</sup>
- The Clinical Molecular Genetics Society of UK (2008) provided practice-based guidelines for the molecular diagnosis of mitochondrial disease: “In cases with strong clinical evidence, testing should begin with checking for the common mutation, m.8344A>G. Subsequent testing for other mutations, such as m.8356T>C, may be indicated in cases with a strong clinical indication of MERRF.”<sup>3</sup>

## Criteria

### Known MERRF Familial Mutation Testing

- Genetic Counseling
  - Pre and post-test counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing
  - No previous genetic testing in the individual for MERRF,\*\* and
  - MERRF pathogenic variant identified in matrilineal relative, AND

- Predictive Testing for Asymptomatic Individual:
  - 18 years of age or older, or
  - Under the age of 18 years, and
    - Presymptomatic screening for Wolff-Parkinson-White is being considered, OR
- Diagnostic Testing for Symptomatic Individual:
  - Clinical exam and/or biochemical testing suggestive, but not confirmatory, of a diagnosis of MERRF, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy

### **MERRF Targeted Mutation Analysis**

- Genetic Counseling
  - Pre and post-test counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Testing:
  - No previous genetic testing for MERRF,\*\* and
  - No known MERRF pathogenic variants in the family, AND
- Diagnostic Testing for Symptomatic Individuals:
  - Clinical exam and/or biochemical testing suggestive, but not confirmatory, of a diagnosis of MERRF, and
  - Genetic testing is needed to confirm the diagnosis, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy

### **Whole mtDNA Sequencing**

- Genetic Counseling
  - Pre and post-test counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Criteria for MERRF targeted mutation analysis is met, AND
- No pathogenic variants identified in the MERRF targeted mutation analysis, AND
- Paternal transmission has been ruled out

\*\* Exceptions may be considered if technical advances in testing demonstrate significant advantages that would support a medical need to retest.

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# Myotonic Dystrophy Type 1 Genetic Testing

MOL.TS.312.A  
v1.0.2020

## Introduction

Myotonic Dystrophy type 1 (DM1) genetic testing is addressed by this guideline.

## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedures addressed by this guideline   | Procedure codes |
|--|-----------------|
| DMPK Expansion Analysis Detection        | 81234           |
| DMPK Expansion Analysis Characterization | 81239           |

## What is Myotonic Dystrophy Type 1

### Definition

Myotonic dystrophy type 1 (DM1) affects multiple body systems and is characterized by myotonia (prolonged muscle contraction), muscle weakness and wasting, and cataracts.<sup>1</sup>

### Incidence and Prevalence

DM1 affects approximately 1 in 20,000 people.<sup>1</sup> The condition is considered to be nearly 100% penetrant, meaning that essentially every person with an expanded repeat mutation will show some features of DM1.<sup>1</sup>

### Symptoms

DM1 can range from mild to severe and can be grouped into three overlapping categories:<sup>1</sup>

- Mild DM1: The most mild myotonia with cataracts, but lifespan is typically normal.
- Classic DM1: More significant myotonia with physical disability in adulthood and possibly shortened lifespan. Heart conduction abnormalities are common, as well as cataracts, balding, and muscle weakness.

- Congenital DM1: The most severe form causes general weakness at birth, with respiratory insufficiency. Intellectual disability may be present and lifespan is shortened. Polyhydramnios and reduced fetal movement may be noted in pregnancy.

### Cause

DM1 is caused by expansion of a CTG trinucleotide repeat in the myotonic dystrophy protein kinase (DMPK) gene. The number of CTG repeats that an individual has is reasonably correlated with the severity of their disease:<sup>1</sup>

- 50-150 repeats: Mild DM1
- 100-1000 repeats: Classic DM1
- More than 1000 repeats: Congenital DM1

### Inheritance

DM1 is an autosomal dominant condition. A person with an affected parent has a 50% risk to also be affected with DM1<sup>1</sup>. The number of CTG repeats in the DMPK gene can expand from one generation to the next, a phenomenon called anticipation. Therefore, children and grandchildren of an affected individual have an increased risk for a more severe form of myotonic dystrophy and/or an earlier age of onset than their affected relatives.<sup>1</sup> Anticipation can occur with maternal or paternal inheritance; however, it is more commonly seen when inherited from the mother.<sup>1</sup>

### Diagnosis

DM1 should be suspected in adults who present with the following:<sup>1</sup>

- Muscle weakness (especially in leg, hands, neck, and face)
- Myotonia (for example, difficulty quickly releasing a gripped hand)
- Posterior subcapsular cataracts

DM1 should be suspected in newborns who present with the following:<sup>1</sup>

- Hypotonia (low muscle tone)
- Weakness in facial muscles
- General muscle weakness
- Positional malformations
- Respiratory problems

If DM1 is suspected, confirmation can be obtained with molecular testing to detect CTG expansions in the DMPK gene. DMPK testing has greater than a 99% detection rate for those with DM1.

Predictive testing may be considered for at-risk relatives if there is a known mutation in DMPK previously identified in the family.<sup>1,2</sup> Children at-risk for DM1 can present with conduction defects and arrhythmias at an early age, when other signs of myopathy may not be apparent. Confirming or ruling out a DM1 mutation guides cardiac screening and anticipatory management of other symptoms.<sup>2</sup>

Non-molecular testing currently is not used for diagnostic purposes, but can be used if molecular testing finds no repeat expansions in DMPK and other neuromuscular disorders are being considered. Such non-molecular testing may include:<sup>1</sup>

- Electromyography (EMG)
- Serum CK concentration
- Muscle biopsy

### **Treatment**

No cure currently exists for DM1, so treatment is focused on managing the specific symptoms with which an individual presents. Physical and/or occupational therapy can help strengthen muscles and provide appropriate assistive devices. One may consult a cardiologist as well, if the individual presents with cardiac symptoms.<sup>1</sup>

Screening and prevention strategies may include:<sup>1,2</sup>

- Annual cardiac screening for conduction abnormalities and cardiac management
- Avoidance of specific medications, such as statins, that can increase weakness
- Identify risk for malignant hyperthermia with the use of anesthesia medications (uncommon complication)

### **Survival**

Affected individuals are most likely to die from respiratory failure or cardiovascular problems. Larger CTG repeat expansions are correlated with both an earlier age of onset, and shorter expected lifespan.<sup>1</sup>

- Mild DM1: 60 years – normal lifespan
- Classic DM1: 48 – 55 years
- Congenital DM1: 45 years

### **Test Information**

#### **Introduction**

Testing for DM1 may include targeted analysis to detect repeat expansions in DMPK, characterization of repeat expansions in DMPK, or known familial mutation analysis.

## Trinucleotide repeat expansion

Characterization of repeat lengths in DMPK are as follows:<sup>1</sup>

- 5-34 CTG repeats: Normal range
- 35-49 CTG repeats: Premutation, meaning the individual is asymptomatic. However, his or her children are at an increased risk for presenting with symptoms
- 50 or more CTG repeats: Full-penetrance alleles, meaning the individual will show symptoms of this condition

## Known familial mutation analysis

Known familial mutation analysis is performed when a causative mutation has been identified in a close relative of the individual requesting testing.

Analysis for known familial mutations is typically performed by trinucleotide repeat expansion analysis.

## Guidelines and Evidence

### Introduction

This section includes relevant guidelines and evidence pertaining to genetic testing for DM1.

### Myotonic Dystrophy Foundation (MDF)

Over 65 medical experts on myotonic dystrophy from the US, Canada, the UK, and Western Europe worked on a project organized by the Myotonic Dystrophy Foundation (MDF) from 2015-2017. The goal was to develop consensus-based recommendations, which included the following:<sup>3</sup>

- "DM1 via molecular genetic testing as the first line of investigation for any patient suspected of having DM1. Muscle biopsy should no longer be performed as a diagnostic test when there is clear clinical suspicion of DM1. Patients with more than 50 CTG repeats in the 3' untranslated region of the DMPK gene on chromosome 19 are considered to have DM1."

### European Federation of Neurological Societies (EFNS)

Guidelines from the European Federation of Neurological Societies (EFNS, 2011) address the molecular diagnosis of myotonic dystrophy and other neurogenetic disorders. They state:<sup>4</sup>

- "In patients with certain distinctive phenotypes, and a suggestive family history, a molecular diagnosis can be made without additional investigations, this includes a male patient with muscular dystrophy, whose uncle had a similar phenotype, a



patient with the typical presentation of a myotonic dystrophy or of a facio-scapulohumeral dystrophy. In such cases, an analysis of the respective gene should be performed without a muscle biopsy (level B)."

### **European Molecular Genetics Quality Network (EMQN)**

Guidelines established at the European Molecular Genetics Quality Network (EMQN) Best Practice Meeting in 2008 state the following:<sup>5</sup>

- "Muscle biopsies of patients with congenital DM1 may reveal only variability in fiber size and centralization of nuclei. However, none of the characteristics found in muscle biopsies of patients with classical or adult-onset DM1 myotonic dystrophy are present. Therefore, in order to confirm a clinical suspicion of congenital DM1, the diagnosis can only be established by DNA analysis."

### **International Myotonic Dystrophy Consortium**

Eighty-three myotonic dystrophy researchers gathered at the second International Myotonic Dystrophy Consortium (1999) meeting and produced the following consensus-based guidelines:<sup>6</sup>

- "Direct analysis of the CTG repeat expansion has sensitivity and specificity, such that the combination of Southern blot and polymerase chain reaction (PCR) can detect all DM1 mutations without false positives...The gene test will increase the physician's confidence in diagnosing a patient with typical symptoms."
- "The gene test will be useful for individuals in whom DM1 is part of a wider differential diagnosis."
- "If a parent has already been diagnosed with DM1, prenatal testing can be used to assess fetal risk."

## **Criteria**

### **Introduction**

Requests for DM1 testing are reviewed using these criteria.

### **Known familial mutation analysis**

- Genetic Counseling:
  - Pre- and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - No previous DMPK gene analysis performed that would have identified the known familial mutation, AND

- Presymptomatic Testing for Asymptomatic Individuals:
  - 18 years of age or older, and
  - Known disease-causing mutation in DMPK gene identified in 1<sup>st</sup> degree relative(s), OR
- Diagnostic Testing for Symptomatic Individuals:
  - Known disease-causing mutation in DMPK gene identified in 1<sup>st</sup> degree relative(s), AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy

### **DMPK repeat analysis**

- Genetic counseling:
  - Pre- and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing
  - No previous repeat analysis of DMPK performed, AND
- Individual has a clinical suspicion of myotonic dystrophy type 1 based on the following:
  - Infant with one or more of the following without a known etiology:
    - Hypotonia
    - Weakness in facial muscles (e.g. ptosis, eyelid closure, weak smile, inverted upper lip, thin face, dull facial expression)
    - General muscle weakness
    - Positional malformations
    - Respiratory problems, or
  - Individual with one or more of the following without a known etiology:
    - Muscle weakness (especially in leg, hands, neck, and face)
    - Weakness in facial muscles (e.g. ptosis, eyelid closure, weak smile, inverted upper lip, thin face, dull facial expression)
    - Myotonia (for example, difficulty quickly releasing a gripped hand), AND
- Family history is consistent with autosomal dominant inheritance (including simplex cases), AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy

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These references are cited in this guideline.

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# NETest

MOL.TS.250.A  
v1.0.2020

## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedure addressed by this guideline | Procedure code |
|---------------------------------------|----------------|
| NETest™                               | 0007M          |

## What is NETest

### Definition

Neuroendocrine tumors (NETs) are a group of tumors that originate from epithelial cells with neuroendocrine variances; gastroenteropancreatic NETs are a subgroup of NETs that develop from the gastrointestinal tract.<sup>1</sup>

- Detection of these lesions is often delayed due to the heterogeneous cellular make-up and inconspicuous symptomology.<sup>1</sup>
- The prevalence and incidence of gastroenteropancreatic neuroendocrine tumors (NETs) have been increasing.<sup>1</sup>
- Currently, there is a lack of specific blood markers for NET detection. Measurement of the neuroendocrine secretory peptide Chromogranin A (CgA) is often used, but is characterized by flaws since it is a single value, non-specific, and assay data are highly variable.
- As a result, there is greater interest in the discovery of effective biomarkers, such as the NETest, to evaluate disease risk and new therapies targeting gastroenteropancreatic NET.<sup>2-6</sup>

### Test information

- NETest is a noninvasive blood test designed to assist in identifying activity of neuroendocrine tumor disease.
- This test examines the expression of 52 genes, which can be used to identify active disease and provide information about the biology of the tumor cell.
- As an adjunct to standard clinical assessment, the NETest provides an assessment of treatment responses in patients with NETs.<sup>2-6</sup>

- The algorithm measures the activity of RNA gene expression and calculates a risk score. Risk scores range from 0-100%. The higher the score, the higher the risk of active disease at the time of testing. The following categories have a sensitivity of 95.7%:<sup>2-6</sup>
  - Very low ( $\leq 13.4\%$ ) exhibit minimal risk for disease activity.
  - Low (13.4% - 43.4%) are classified as low active or stable disease
  - High ( $>43.4\%$ ) are classified as highly active disease.

## Guidelines and evidence

### National Comprehensive Cancer Network (NCCN)

The NCCN guidelines (2018) on Neuroendocrine and Adrenal Tumors indicate that additional research is required before potential prognostic markers and other new molecular assays are routinely used in clinical practice.<sup>1</sup> They state that “a multinational consensus meeting of experts concluded that, to date, no single currently available biomarker is sufficient as a diagnostic, prognostic, or predictive marker in patients with neuroendocrine tumors.”

### Literature Review

The overall evidence base of retrospective and prospective clinical studies assessing NETest as a diagnostic, prognostic, and as a tool for treatment monitoring is insufficient.<sup>7-19</sup> Results of individual studies suggest that NETest performs better than the conventional, single analyte, CgA, when combined with conventional prognostic indicators, and that NETest consistently shows some degree of association with measures of survival, suggesting that it may be useful in estimating the likelihood of recurrence. However, numerous limitations characterize the individual studies, which lowers the confidence in these findings (positive or negative), and hamper any definitive conclusions that can be drawn regarding the value of NETest.

It is still unclear when NETest should be used in a clinical practice setting, particularly in terms of determining the most accurate timing of blood specimen collection, as well as establishing the exact threshold metrics of the NETest to establish diagnosis, predict disease progression, and monitor treatment, such as an adjuvant therapy. There were no available studies of NETest as a companion diagnostic to accurately predict treatment responses. There were also no direct clinical utility studies that evaluated if NETest results improved health outcomes more than conventional testing or evaluated the impact of the NETest on physician treatment decisions.

Well-designed prospective studies, with consecutively enrolled, well-defined patient populations and sufficient follow-up periods are needed to evaluate the value of NETest to establish diagnosis, assess prognosis, and monitor treatment in patients with NET.

## Criteria

- This test is considered investigational and/or experimental.
  - Investigational and experimental (I&E) molecular and genomic (MolGen) tests refer to assays involving chromosomes, DNA, RNA, or gene products that have insufficient data to determine the net health impact, which typically means there is insufficient data to support that a test accurately assesses the outcome of interest (analytical and clinical validity), significantly improves health outcomes (clinical utility), and/or performs better than an existing standard of care medical management option. Such tests are also not generally accepted as standard of care in the evaluation or management of a particular condition.
  - In the case of MolGen testing, FDA clearance is not a reliable standard given the number of laboratory developed tests that currently fall outside of FDA oversight and FDA clearance often does not assess clinical utility.

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# Neurofibromatosis Type 1 Genetic Testing

MOL.TS.301.A  
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## Introduction

Neurofibromatosis Type 1 is addressed by this guideline.

## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedure addressed by this guideline | Procedure code |
|---------------------------------------|----------------|
| NF1 Known Familial Mutation Analysis  | 81403          |
| NF1 Sequencing                        | 81408          |
| NF1 Deletion/Duplication Analysis     | 81479          |

## What is Neurofibromatosis Type 1

### Definition

Neurofibromatosis Type 1 (NF1) is a neurocutaneous condition characterized by the growth of tumors along nerves in the skin, brain, eyes, and other parts of the body and changes in skin pigmentation (café-au-lait macules and freckling).<sup>1</sup>

### Incidence or Prevalence

NF1 is one of the most common dominantly inherited genetic disorders. This condition has an incidence at birth of approximately 1 in 2500 to 1 in 3000 individuals.<sup>2</sup>

### Symptoms

The signs and symptoms of NF1 develop gradually over time. Initial clinical features of NF1 are café-au-lait macules. These macules increase in size and number with age. Freckling in the axilla and inguinal area (groin) develop later in childhood. Lisch nodules are present in only 50% of affected children under the age of 5 years. However, these benign iris tumors (hamartomas) are present in almost all affected adults.

The spectrum and severity of symptoms vary greatly between individuals with NF1, even in the same family.<sup>3</sup> Skin findings and Lisch nodules may be the only clinical features in some patients with NF1. Multi-systemic manifestations of NF1 include short



stature, macrocephaly, scoliosis, distinctive osseous lesions, learning differences, seizures, and attention deficit hyperactivity disorder (ADHD). Cardiovascular complications include high blood pressure, cerebral and peripheral arterial stenosis, and stroke.<sup>4</sup>

NF1 is associated with an increased risk of benign tumors, including cutaneous and plexiform neurofibromas, optic glioma, and pheochromocytoma. There is also an increased risk of certain cancers, including malignant peripheral nerve sheath tumors, brain tumors, leukemia, and breast cancer.<sup>5</sup> Malignant peripheral nerve sheath tumors may develop by malignant transformation of neurofibromas during adolescence or adulthood.

## Diagnosis

Diagnostic criteria for NF1 were formulated by the National Institute of Health (1988).<sup>5</sup> A full description can be found in the Guidelines and Evidence section.

Individuals with NF1 typically present with one or more clinical features of the disorder. Approximately 90% of children with NF1 will exhibit clinical features from two or more diagnostic criteria by age 6, 97% by age 8 and 100% by age 20.

NF1 has overlapping clinical features with Legius syndrome, other forms of neurofibromatosis, conditions with café-au-lait and pigmented macules, and overgrowth syndromes.<sup>2</sup>

## Genotype-Phenotype Correlations

Only a few clear correlations between specific NF1 mutations and distinct clinical phenotypes have been described.

Individuals with a single amino acid deletion p.Met922del in the NF1 gene have a very mild phenotype with typical pigmentary features of NF1 without cutaneous neurofibromas or other tumors.<sup>6</sup> Missense mutations affecting p.Arg1809 are associated with a distinct presentation including pulmonic stenosis, learning disabilities, short stature, and Noonan-like features, in addition to mild NF1 phenotype.<sup>7</sup>

NF1 microdeletions are associated with early appearance of numerous cutaneous neurofibromas, severe cognitive abnormalities, somatic overgrowth, large hands and feet, and dysmorphic facial features.<sup>8</sup>

Individuals with missense mutations in codons 844-848 have a high risk of plexiform and spinal neurofibromas, optic gliomas, skeletal abnormalities, and other malignant tumors.<sup>9</sup>

## Segmental NF

Segmental NF1 is a rare subtype that results from a post-zygotic mutation in the NF1 gene leading to somatic mosaicism. Neurofibromas, café-au-lait macules, and axillary freckling are typically unilateral and localized to one area of the body, usually following the lines of Blaschko.<sup>10</sup> There is an increased risk of malignancies.

## Cause

Neurofibromatosis Type 1 is caused by mutations in the NF1 gene which produces the protein product, neurofibromin. Neurofibromin functions as a tumor suppressor. NF1 gene mutations lead to defective or missing neurofibromin resulting in uncontrolled cell proliferation and growth of tumors common in NF1.<sup>3</sup>

## Inheritance

Neurofibromatosis type 1 is inherited in an autosomal dominant fashion. Almost half of all NF1 cases are the result of a new or de novo gene mutation. The mutation rate for NF1 is among the highest known for any gene in humans.<sup>11</sup> The remainder of new NF1 cases are inherited from an affected parent. Individuals with NF1 have a 50% chance of passing the mutation to their children. Additionally, parents and siblings of known affected individuals have a 50% chance of having the same mutation. Penetrance is virtually complete after childhood; however, there is significant clinical variability.

## Treatment

There is no cure for Neurofibromatosis type 1. Long-term management includes multi-system surveillance for potential complications, treatment of bulky tumors and cancers, and therapies and medications for other systemic manifestations.<sup>4</sup> Clinical trials are underway to study new medications for the treatment of tumors common in NF1.

## Survival

The lifespan of individuals with Neurofibromatosis Type 1 is reported to be approximately 8 years less than the general population. The most important causes of early death are malignancy, especially malignant peripheral nerve sheath tumors, and vasculopathy.

## Test Information

### Introduction

Testing for Neurofibromatosis Type 1 may include NF1 gene sequencing, NF1 deletion/duplication analysis, or known familial mutation analysis.

### NF1 sequencing analysis

NF1 sequence analysis may involve a multistep protocol to increase the detection of splicing mutations. This protocol combines sequence analysis in genomic DNA and cDNA (mRNA). NF1 sequencing variants, such as missense, nonsense, and splice site variants, account for up to 95% of mutations seen in NF1.

NF1

## NF1 deletion/duplication analysis

Large deletions in NF1 are infrequently reported. Deletion/duplication analysis is done as second-tier testing after NF1 sequence analysis.

## Known familial mutation analysis

Analysis for known familial mutations is typically performed by Sanger sequencing, but if available, a targeted mutation panel that includes the familial mutation may be performed.

Known familial mutations analysis is performed when a causative mutation has been identified in a close relative of the individual requesting testing.

## Segmental NF

Testing of various sample types is available to help identify individuals with segmental NF1. “RNA-based NF1/SPRED1 testing on cultured cells from affected tissues is offered starting from biopsies of café-au-lait macules (CALM) and/or neurofibromas.”<sup>12</sup>

## Guidelines and Evidence

### Introduction

The following section includes relevant guidelines and evidence pertaining to Neurofibromatosis type 1 testing.

### American College of Medical Genetics and Genomics (ACMG)

The American College of Medical Genetics and Genomics (ACMG, 2018) stated the following in regard to genetic testing for NF1:<sup>13</sup>

- “In childhood, NF1 genetic testing can quickly establish a diagnosis and relieve anxiety, but that is less likely an issue for adults.”
- “Most adults with NF1 are clinically diagnosed in childhood, according to NIH consensus criteria. The criteria are both highly specific and sensitive in adults with NF1.”

### National Institute of Health (NIH)

The diagnostic criteria set forth by the National Institute of Health (NIH Consensus Development Conference, 1988 ) are met for NF1 in individuals who have at least two or more of following findings:<sup>5</sup>

- Six or more café-au-lait macules >5 mm in greatest diameter in prepubertal individuals and >15 mm in greatest diameter in postpubertal individuals

- Two or more neurofibromas of any type or one plexiform neurofibroma
- Freckling in the axillary and/or inguinal (groin) regions
- Optic glioma
- Two or more Lisch nodules (iris hamartomas)
- A distinctive osseous lesion such as sphenoid dysplasia or tibial pseudoarthrosis
- A first-degree relative with NF1 as defined by the above criteria

### Expert authored review

“Genetic testing is indicated for individuals in whom NF1 is suspected but who do not fulfill the NF1 diagnostic criteria. This is rarely necessary after early childhood. Testing may be useful in a young child with a serious tumor (e.g., optic glioma) in whom establishing a diagnosis on NF1 immediately would affect management. Testing of an adult with NF1 is necessary if prenatal or preimplantation genetic diagnosis in a current or future pregnancy is anticipated.”

“A multistep detection protocol that combines analysis of genomic DNA and cDNA (mRNA) and testing for whole-gene or exon copy number changes is recommended if molecular genetic testing is indicated. This approach identifies more than 95% of NF1 pathogenic variants in individuals fulfilling the NIH diagnostic criteria. Because of the variety and rarity of individual pathogenic variants found in people with NF1 and the frequency of pathogenic variants that affect splicing, methods that include cDNA sequencing have higher detection rates than methods based solely on analysis of gDNA.”<sup>14</sup>

## Criteria

### Introduction

Requests for NF1 testing are reviewed using the following clinical criteria.

### NF1 Known Familial Mutation Analysis

Genetic Counseling:

- Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND

Previous Genetic Testing:

- No previous genetic testing of NF1, AND
- NF1 mutation identified in 1st degree biological relative, OR

Prenatal Testing for At-Risk Pregnancies:

NF1

- NF1 mutation identified in a previous child or either parent

### **NF1 Sequencing**

#### Genetic Counseling:

- Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND

#### Previous Genetic Testing:

- No previous genetic testing of NF1, and
- No known pathogenic NF1 mutation in biological relatives, AND

#### Diagnostic Testing for Symptomatic Individuals:

- The member is suspected to have neurofibromatosis type 1 but the diagnosis is in question because member meets only one of the following:
  - Six or more café-au-lait macules over 5 mm in greatest diameter in prepubertal individuals, or
  - Six or more café-au-lait macules over 15 mm in greatest diameter in postpubertal individuals, or
  - Freckling in the axillary or inguinal regions, or
  - Two or more neurofibromas of any type or one plexiform neurofibroma, or
  - Optic glioma, or
  - Two or more Lisch nodules (iris hamartomas), or
  - A distinctive osseous lesion (e.g., sphenoid dysplasia or tibial pseudoarthrosis), or
  - The member displays at least two of the following findings:
    - Less than 6 café-au-lait macules of any size
    - One neurofibroma
    - One Lisch nodule, AND
- The results of the test will directly impact the diagnostic and treatment options that are recommended for the patient, AND
- Rendering laboratory is a qualified provider of services per the Health Plan policy.

### **NF1 Deletion/Duplication Analysis**

- Criteria for NF1 Sequencing are met, AND
- No previous deletion/duplication analysis of NF1, AND

- No mutation detected in full sequencing of NF1

### NF1 Testing on Tissue Samples

Requests for NF1 testing on café au lait macules or neurofibromas after negative NF1 testing on a blood sample in individuals with a clinical suspicion of segmental NF will be reviewed on a case by case basis.

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# Neurogenic Muscle Weakness, Ataxia, and Retinitis Pigmentosa (NARP)

MOL.TS.245.A

v1.0.2020

## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedures addressed by this guideline | Procedure codes |
|--|-----------------|
| MT-ATP6 Targeted Mutation Analysis     | 81401           |
| Whole Mitochondrial Genome             | 81460           |

## What is NARP

### Definition

Neurogenic muscle weakness, Ataxia, and Retinitis Pigmentosa is a multisystem mitochondrial disease.<sup>1</sup> NARP is characterized by proximal neurogenic muscle weakness with sensory neuropathy, ataxia, learning difficulties, and pigmentary retinopathy.<sup>1</sup> Most cases present in childhood with ataxia and learning difficulties. Seizures may also be present.<sup>1</sup> Additional clinical features include short stature, sensorineural hearing loss, progressive external ophthalmoplegia, and cardiac conduction defects (heart block).<sup>1</sup>

- NARP is caused by mutations in the mitochondrial DNA (mtDNA) and follows maternal inheritance. This means that a female who carries the mtDNA mutation at high mutation load will typically pass it on to all of her children. A male who carries the mtDNA mutation cannot pass it on to his children.<sup>1,2</sup>
- For all mtDNA mutations, clinical expressivity depends on the three following factors:<sup>1</sup>
  - The relative abundance of mutant mtDNA, mutational load (heteroplasmy)
  - The organs and tissues in which the mutant mtDNA is found (tissue distribution), and
  - The vulnerability of each tissue to impaired oxidative metabolism (threshold effect).
- The mutation load in given tissues can change over time, and mtDNA deletions are not usually detectable in white blood cells from adults.<sup>1</sup>



- The exact prevalence of NARP is unknown.<sup>1</sup>
- Management of NARP is generally supportive. Regular neurologic, ophthalmologic, and cardiologic screenings are recommended for affected individuals. Anti-epileptic drugs that affect the mitochondrial respiratory chain should be avoided, as they may cause secondary carnitine deficiency or can be used with L-carnitine supplementation.<sup>1</sup>

## Test information

- The investigation and diagnosis of patients with mitochondrial disease often necessitates a combination of techniques including muscle histochemistry, biochemical assessment, and molecular genetic studies along with clinical assessment. Any molecular genetic test for a mtDNA mutation should ideally be directed by the clinical phenotype and results of these other investigations.<sup>2</sup>
- NARP Targeted Mutation Analysis
  - m.8993T>G (T8993G) and m.8993T>C (T8993C) in MT-ATP6 cause ~50% of cases of NARP.<sup>1</sup>
  - If negative, whole genome sequencing of mitochondrial DNA can detect more rare mutations associated with NARP, but does not significantly increase the detection rate over testing for the common two mutations.<sup>1</sup>
- The clinical course for mitochondrial diseases is subject to the concepts of heteroplasmy, tissue distribution, and threshold effect.<sup>1,3</sup> While genetic test results alone cannot predict the exact course or phenotype of the disease, severity does correlate with mutation load.<sup>1,4</sup>
- Due to its ability to simultaneously sequence the entire mtDNA and measure heteroplasmy at each position, next generation sequencing (NGS) is an attractive option for assessing NARP and overlapping syndromes. However, certain targeted mutation analyses can estimate heteroplasmy. Typically, Sanger sequence analysis will miss heteroplasmy below 20%.
- Genetic testing can also be done on skin fibroblasts, urinary sediment, or buccal mucosa.<sup>1</sup> If cultured fibroblasts are used, measures such as limited passaging and uridine supplementation should be taken to reduce selection against mutant genotypes that may lead to skewed heteroplasmy.
- If genetic testing is negative in a blood sample in a person with symptoms of NARP, testing can be done on other specimens. Typically this is done when the phenotype is highly suggestive of presence of a NARP mutation or when there is a need to assess reproductive risk for offspring with higher mutant load and risk for developing Leigh disease.
  - Muscle may be considered as a secondary tissue. Muscle biopsy allows enzymatic analysis of the electron transport chain, light and ultrastructural

microscopy, and mtDNA copy number analysis—all of which may provide highly useful information.

- However, muscle (and/or liver) biopsies are often not necessary and should be avoided when possible due to their invasive nature. Biopsies should only be considered when the diagnosis cannot be confirmed with DNA testing of other more accessible tissues.

## Guidelines and evidence

- No specific evidence-based U.S. testing guidelines were identified.
- Case reports and a limited number of case series are the primary evidence base available for the diagnosis of mitochondrial disease.<sup>4-6</sup>
- The Mitochondrial Medicine Society developed consensus recommendations using the Delphi method and published them in 2015.<sup>7</sup>
  - Recommendations for DNA testing
    - “Massively parallel sequencing/NGS of the mtDNA genome is the preferred methodology when testing mtDNA and should be performed in cases of suspected mitochondrial disease instead of testing for a limited number of pathogenic point mutations.”
    - “Patients with a strong likelihood of mitochondrial disease because of a mtDNA mutation and negative testing in blood, should have mtDNA assessed in another tissue to avoid the possibility of missing tissue-specific mutations or low levels of heteroplasmy in blood; tissue-based testing also helps assess the risk of other organ involvement and heterogeneity in family members and to guide genetic counseling.”
    - “Heteroplasmy analysis in urine can selectively be more informative and accurate than testing in blood alone, especially in cases of MELAS due to the common m.3243 A>G mutation.”
    - “When considering nuclear gene testing in patients with likely primary mitochondrial disease, NGS methodologies providing complete coverage of known mitochondrial disease gene is preferred. Single-gene testing should usually be avoided because mutations in different genes can produce the same phenotype. If no mutation is identified via known NGS panels, then whole exome sequencing should be considered.”
- A workshop of the National Institute of Neurological Disorders and Stroke (2008)<sup>3</sup> summarizes:
  - “The diagnosis of mitochondrial diseases is complicated by their heterogeneous presentations and by the lack of screening procedures or diagnostic biomarkers that are both sensitive and specific. The workshop panelists explained that diagnosis is often a lengthy process beginning with a general clinical evaluation

followed by metabolic screening and imaging and finally by genetic tests and more invasive biochemical and histological analyses. The identification of known mitochondrial mutations in tissue has greatly aided diagnosis. However, even when clinical features and family history strongly suggest mitochondrial disease, the underlying genetic mutation can elude detection, and there is no current screening procedure that would be practical for all cases of suspected mitochondrial disease.”

- The Clinical Molecular Genetics Society (CMGS) of the United Kingdom (2008)<sup>2</sup> practice-based guidelines for the molecular diagnosis of mitochondrial disease state that: “For routine referrals for NARP, presence of T8993G and T8993C mutations should be investigated.”
- The European Federation of Neurological Sciences (2009)<sup>8</sup> evidence-based molecular diagnostic guidelines state: “If the phenotype suggests syndromic mitochondrial disease due to mtDNA point mutations (MELAS, MERRF, NARP, LHON) DNA-microarrays using allele-specific oligonucleotide hybridization, real-time-PCR or single-gene sequencing are indicated.”

## Criteria

### Known NARP Familial Mutation Testing

- Genetic Counseling
  - Pre- and post-test counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing
  - No previous genetic testing in the individual for NARP\*, and
  - NARP pathogenic variant identified in matrilineal relative, AND
- Predictive Testing for Asymptomatic Individual:
  - 18 years of age or older, or
  - Under the age of 18 years, and
    - Screening for learning disabilities, retinitis pigmentosa, and/or ataxia is being considered, OR
- Diagnostic Testing for Symptomatic Individual:
  - Clinical exam and/or biochemical testing suggestive, but not confirmatory, of a diagnosis of NARP, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy

NARP

## NARP Targeted Mutation Analysis

- Genetic Counseling
  - Pre- and post-test counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Testing:
  - No previous genetic testing for NARP\*, and
  - No known NARP pathogenic variants in the family, AND
- Diagnostic Testing for Symptomatic Individuals:
  - Clinical exam and/or biochemical testing suggestive, but not confirmatory, of a diagnosis of NARP, and
  - Genetic testing is needed to confirm the diagnosis, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy

## Whole mtDNA Sequencing

- Genetic Counseling
  - Pre- and post-test counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Criteria for NARP targeted mutation analysis is met, AND
- No pathogenic variants identified in the NARP targeted mutation analysis, AND
- Paternal transmission has been ruled out

\* Exceptions may be considered if technical advances in testing demonstrate significant advantages that would support a medical need to retest.

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# Niemann-Pick Disease Types A and B Testing

MOL.TS.207.A  
v1.0.2020

## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedures addressed by this guideline | Procedure codes |
|--|-----------------|
| Acid Sphingomyelinase Enzyme Activity  | 82657           |
| SMPD1 Known Familial Mutation          | 81403           |
| SMPD1 Targeted Mutation Analysis       | 81330           |
| SMPD1 Sequencing                       | 81479           |
| SMPD1 Deletion/Duplication Analysis    | 81479           |

## What is Niemann-Pick disease types A and B

### Definition

Niemann-Pick disease is a genetic disorder caused by an inability to process lipids (fats), which results in a toxic buildup of lipids in some organs.<sup>1-3</sup>

- Two types of Niemann-Pick disease are caused by a deficiency of the acid sphingomyelinase enzyme:
  - Type A, also called the “neurological” or “neuronopathic” type, causes symptoms beginning in infancy. These include an enlarged liver and spleen (hepatosplenomegaly), psychomotor impairment with neurologic deterioration, interstitial lung disease, and eventually a classic cherry-red spot of the retina. Affected individuals usually do not survive beyond childhood.<sup>1-3</sup>
  - Type B, also called the “non-neurological” or “non-neuronopathic” type, causes some symptoms similar to type A, but symptoms are usually milder and begin later. Additional symptoms include hyperlipidemia (high fat levels in blood) and thrombocytopenia (low platelets). Affected individuals can survive to adulthood.<sup>1,3</sup>
- The SMPD1 gene encodes the acid sphingomyelinase (ASM) enzyme. Gene mutations in the SMPD1 gene lead to reduced or absent sphingomyelinase enzyme activity, causing the symptoms of Niemann-Pick disease.<sup>1,3</sup>

- Niemann-Pick disease is suspected when a patient presents with hepatosplenomegaly, interstitial lung disease, and depending on the subtype, neurological symptoms in infancy or abnormal blood findings.<sup>3</sup> However, a diagnosis cannot be made clinically.
- When Niemann-Pick disease is suspected, acid sphingomyelinase enzyme activity testing should be performed first.<sup>3</sup> People with Niemann-Pick disease type A or B usually have less than 10% of normal ASM activity compared to healthy individuals.<sup>3</sup>

Measuring ASM enzyme activity in peripheral blood lymphocytes or cultured skin fibroblasts is a reliable way to confirm a suspected case of Niemann-Pick disease.<sup>3</sup> However, false-negative and inconclusive results are possible.<sup>3,4</sup> In such cases, genetic testing may be useful to resolve a diagnosis.

- About 1 in 250,000 people have Niemann-Pick disease.<sup>1,3</sup> Type A is more common in persons of Ashkenazi Jewish descent than in the general population. In the Ashkenazi Jewish population, the frequency of Niemann-Pick disease is 1 in 40,000.<sup>1,3</sup>
- Niemann-Pick disease is an autosomal recessive disorder. An affected individual must inherit SMPD1 gene mutations from both parents.<sup>1,3</sup>
  - Individuals who inherit only one mutation are called carriers. Carriers do not show symptoms of Niemann-Pick disease, but have a 50% chance of passing on the mutation to their children.
  - Two carriers of Niemann-Pick disease have a 25% chance of having a child with the disorder.
  - Prenatal diagnosis for at-risk pregnancies can be performed by molecular genetic testing (if the mutations in both parents are known).<sup>3</sup>
- Individuals at increased risk to have a child with Niemann-Pick disease should routinely be offered carrier screening. This includes those with:<sup>4,5</sup>
  - Ashkenazi Jewish ancestry (1 in 90 carrier risk<sup>3,5</sup>)
  - A family history of Niemann-Pick disease (regardless of ethnicity)
  - A partner who is a known carrier of Niemann-Pick disease (or affected with the milder type)

## Test information

- ***SMPD1 Mutation Analysis*** tests for four of the most common SMPD1 gene mutations.
  - Three mutations - R496L, L302P, fsP330 - account for 97% of all cases of Niemann-Pick disease type A in Ashkenazi Jewish people.<sup>5</sup>

- The fourth mutation - deltaR608 - is a common cause of Niemann-Pick disease type B in people of North African descent.<sup>3</sup>
- Carrier screening by SMPD1 mutation panel for Niemann-Pick disease is widely available as part of an “Ashkenazi Jewish Panel” that includes several other genetic disease that are more common in this population. (See Ashkenazi Jewish Carrier Screening.)
- **SMPD1 Sequencing** analyzes the entire coding region of the SMPD1 is available to detect less common mutations that cannot be detected on a common mutation analysis panel. SMPD1 sequencing detects more than 95% of all SMPD1 mutations.<sup>3</sup>
- **SMPD1 Deletion/Duplication Analysis** is available to detect large gene rearrangements that cannot be detected by sequencing. However, the frequency of such mutations is unknown.<sup>3</sup>
- **SMPD1 Known Familial Mutation Testing** can be performed for at-risk relatives when the familial mutation is known and is not one of the common mutations.<sup>3</sup>

## Guidelines and evidence

- Professional guidelines generally support Niemann-Pick disease carrier screening for those at increased risk.<sup>4,5</sup>
- Consensus guidelines from the American College of Obstetricians and Gynecologists (ACOG, 2017) address carrier screening and prenatal diagnosis for Niemann-Pick disease:
  - “Individuals with a positive family history of one of these disorders [including Niemann-Pick disease] should be offered carrier screening for the specific disorder and may benefit from genetic counseling.”
  - Carrier screening for Ashkenazi Jewish people is routinely recommended for some disorders (i.e., Tay-Sachs, Canavan, cystic fibrosis, familial dysautonomia). ACOG states: “Some experts have advocated for a more comprehensive screening panel for those of Ashkenazi descent, including tests for several diseases that are less common (carrier rates 1 in 15 to 1 in 168) [including Niemann-Pick disease]”.
  - “If it is determined that this individual [an Ashkenazi Jewish descent partner] is a carrier, the other partner should be offered screening.”
  - “When both partners are carriers of one of these disorders, they should be referred for genetic counseling and offered prenatal diagnosis.”
  - “The prevalence of these disorders [including Niemann-Pick disease] in non-Jewish populations is unknown, and the sensitivity of these carrier tests in non-Jewish populations has not been established. Because the mutations in other populations may vary, counseling on the residual risks after negative carrier



screening can be complicated in non-Jewish individuals. For couples in which one partner is a carrier and the other is of non-Jewish ancestry, genetic counseling may be useful in determining the best approach to risk estimation."

- Consensus guidelines from the American College of Medical Genetics (2008) recommend routine carrier screening for a group of disorders that includes Niemann-Pick when at least one member of the couple is Ashkenazi Jewish and that couple is pregnant or planning pregnancy.<sup>5</sup>
- No evidence-based US diagnostic testing guidelines have been identified.
- A 2015 expert-authored review recommends the following testing strategy for diagnosis of an affected person:<sup>3</sup>
  - "The diagnosis of ASM deficiency is established by detection of either biallelic pathogenic variants in SMPD1 on molecular genetic testing or residual ASM enzyme activity that is less than 10% of controls (in peripheral blood lymphocytes or cultured skin fibroblasts)."
  - Molecular testing approaches include single-gene testing and use of a multi-gene panel.
  - For individuals from populations in which common SMPD1 pathogenic variants occur (e.g., individuals of Ashkenazi Jewish background with a severe neurodegenerative form of the disease suggestive of NPD-A, individuals of North African descent with NPD-B, or individuals from Chile, Saudi Arabia, and Turkey):
    - Perform targeted analysis for pathogenic variants.
    - If targeted analysis does not identify both pathogenic variants in individuals from these populations, sequence analysis of SMPD1 is appropriate.
  - For individuals who are not in the populations discussed above:
    - Perform sequence analysis.
    - "If no or only one pathogenic variant is identified, consider gene-targeted deletion/duplication analysis."

## Criteria

### Niemann Pick Type A or B Known Familial Mutation Analysis

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Testing:

- No previous genetic testing for Niemann Pick A or B, AND
- Diagnostic and Predisposition Testing:
  - Niemann Pick A or B family mutation identified in biologic relative(s), OR
- Prenatal Testing:
  - Niemann Pick A or B mutation identified in both biologic parents, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

### **Niemann Pick A or B Targeted Mutation Analysis**

- Genetic Counseling
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - No previous genetic testing for Niemann Pick A or B
- Diagnostic Testing for Symptomatic Individuals:
  - Measurement of acid sphingomyelinase (ASM) enzyme activity in peripheral blood lymphocytes or cultured skin fibroblasts (in symptomatic individuals) with negative or equivocal result where suspicion of clinical diagnosis remains high, and
  - Hepatosplenomegaly, and/or
  - Evidence of interstitial lung disease on chest radiograph, and/or
  - Developmental Delay, and/or
  - Cherry Red Maculae, and/or
  - Hyperlipidemia, and/or
  - Thrombocytopenia, OR
- Predisposition/Carrier Testing for Presymptomatic/Asymptomatic Individuals:
  - Biologic relative(s) (1<sup>st</sup> degree) diagnosed with Niemann Pick A or B clinically, and no family mutation identified, or
  - Ashkenazi Jewish ancestry and intention to reproduce, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

### **Niemann Pick A or B Sequencing**

- Genetic Counseling:

- Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - If Ashkenazi Jewish, common mutations have been tested and resulted negative
- Diagnostic Testing for Symptomatic Individuals:
  - Measurement of acid sphingomyelinase (ASM) enzyme activity in peripheral blood lymphocytes or cultured skin fibroblasts (in symptomatic individuals) with negative or equivocal result where suspicion of clinical diagnosis remains high, and
  - Hepatosplenomegaly, and/or
  - Evidence of interstitial lung disease on chest radiograph, and/or
  - Developmental Delay, and/or
  - Cherry Red Maculae, and/or
  - Hyperlipidemia, and/or
  - Thrombocytopenia, OR
- Predisposition Testing for Presymptomatic/Asymptomatic Individuals:
  - Biologic relative(s) (1<sup>st</sup> degree) diagnosed with Niemann Pick A or B clinically, and no family mutation identified, and
  - If Ashkenazi Jewish, common mutations have been tested and resulted negative, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

#### **Niemann Pick A or B Deletion/Duplication Analysis**

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - No previous large rearrangement testing, and
  - Previous SMPD1 sequencing performed and no mutations found, and
  - No known familial mutation, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy

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# Niemann-Pick Type C Testing

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## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedures addressed by this guideline | Procedure codes |
|--|-----------------|
| NPC1 Known Familial Mutation Analysis  | 81403           |
| NPC2 Known Familial Mutation Analysis  | 81403           |
| NPC1 Sequencing                        | 81406           |
| NPC2 Sequencing                        | 81404           |
| NPC1 Deletion/Duplication Analysis     | 81479           |
| NPC2 Deletion/Duplication Analysis     | 81479           |

## What is Niemann-Pick Disease type C

### Definition

Niemann-Pick Disease, type C (NPC) is a lipid storage condition that can present at any age, though the classic presentation is in mid-to-late childhood. Symptoms fall into one of three categories: visceral, neurological and psychological.<sup>1</sup>

- The presentation of clinical symptoms at each stage is different:<sup>2,3</sup>
  - Infants typically present with hypotonia and developmental delay, with or without lung and liver disease. Liver disease can be severe, resulting in the death of an infant in a few days to a few months.
  - Children with NPC exhibit progressive ataxia, vertical supranuclear gaze palsy (VSGP) and dementia.
  - Adults who develop NPC usually have an onset of progressive cognitive impairment or other psychiatric symptoms.
- There is wide variability with disease progression and survival rate, which can range from just a few days to, in rare circumstances, 60 years. Most individuals survive between 10-25 years.<sup>4</sup>
- Two genes have been associated with NPC: NPC1 and NPC2. The proteins of these genes are thought to work together in the cellular transport of cholesterol and other molecules. Most (90-95%) individuals with NPC have at least one identifiable

gene mutation in NPC1.<sup>5,6</sup> Only 30 families have been found to have mutations in the NPC2 gene, making mutations in this gene rare (about 4% of NPC cases).<sup>1,5,7</sup>

- There have been over 200 mutations described that cause NPC.<sup>8</sup> Genotype-phenotype correlation is difficult to determine as most individuals are compound heterozygotes; however, there has been observation of some alleles being associated with mild or severe disease.<sup>8-10</sup>
- NPC is pan-ethnic with a prevalence of 1 in 100,000 live births.<sup>1</sup> There are a few populations that have a founder effect, including French Acadians of Nova Scotia, Canada originally from Normandy France<sup>7</sup>; individuals of Hispanic descent in the Upper Rio Grande valley of the United States<sup>7</sup>; and a Bedouin group in Israel.
- NPC is inherited in an autosomal recessive inheritance pattern. Because NPC is recessive, individuals usually do not have other affected family members. Males and females are equally likely to be affected. When both parents are known carriers, there is a 1/4 (25%) chance for each pregnancy to be affected. Preimplantation and prenatal genetic diagnosis are available for at-risk pregnancies when the causative mutations in the family are known.
- The NPC-suspicion index assists in the diagnosis of adult patients with NPC, with strong indicators including cognitive and psychiatric symptoms, and the combination of neurological with psychiatric signs is highly suggestive of NPC.<sup>1,11</sup>
- Once a diagnosis of NPC is suspected clinically, the diagnosis can be confirmed through a combination of biochemical and genetic studies.
- Healthcare management after diagnosis includes treatment for current symptoms. This generally includes medications to prevent the onset of seizures, although treatment of liver disease, sleeping dysfunction or other symptoms should be considered as well. There is no definitive therapy available for NPC. Bone marrow transplantation (BMT), liver transplantation or the use of cholesterol lowering drugs did not prevent the progression of neurological disease.

## Test information

- **Oxysterols (cholesterol oxidation products)** includes measurement of the oxysterols cholestane-3  $\beta$ , 5 $\alpha$ , 6 $\beta$ -triol (C-triol) and 7-ketocholesterol (7-KC) in blood. Both are sensitive markers for NPC.<sup>1,12,13</sup>
  - When this testing indicates an individual is affected, the diagnosis must be confirmed by sequence/mutation analysis and if necessary, filipin test.
    - Carrier testing is not reliable through biochemical testing.
- **Filipin biochemical testing for Niemann-Pick type C** involves demonstration of abnormal intracellular cholesterol homeostasis in cultured fibroblasts.<sup>7,14</sup> Fibroblasts are cultured in an LDL-enriched medium, and then fixed and stained with a compound called “filipin”. To perform biochemical testing, filipin interacts with

unesterified cholesterol to make specific cholesterol-filled complexes in ~80-85% of cases.

- The filipin test is no longer considered a first line test for the diagnosis of NPC. It is still an extremely useful test for cases in which molecular or biochemical results are not conclusive.<sup>1</sup>
- Carrier testing is not available through biochemical testing, as there is overlap of enzyme activity between carriers and non-carriers.
- The biochemical assay can be used for prenatal diagnosis if both mutations are not known.<sup>7</sup>
- ***NPC1 sequence analysis*** can identify ~80-90% of mutations in the NPC1 gene.<sup>15</sup>
- ***NPC2 sequence analysis*** identifies virtually 100% of mutations in the NPC2 gene.<sup>15</sup>
- ***NPC1 and NPC2 deletion/duplication analysis*** is available clinically for individuals who test negative on sequence analysis.
- ***NPC1 and NPC2 known familial mutations***: Once a disease-causing mutation has been identified, relatives of affected individuals can be tested. Because of the variability of age of onset and presenting symptoms, individuals undergoing carrier testing should be aware that they could be identified as carrying two mutant alleles, and thus affected. Preimplantation or prenatal testing can be performed through mutation analysis on CVS or amniocytes if both parental mutations are known.<sup>15</sup>

## Guidelines and evidence

- Consensus-based diagnostic recommendations are available from the International NP-C Disease Registry (2018), an international, collaborative group of disease experts:<sup>1</sup>
  - “Once NPC is suspected clinically, diagnosis can be confirmed by the combination of biochemical and molecular genetic studies.<sup>16</sup> In recent years, several plasma metabolites (cholestane-3 $\beta$ , 5 $\alpha$ , 6 $\beta$ -triol, lyso-sphingomyelin isoforms and bile acid metabolites) have emerged as sensitive and specific diagnostic biomarkers for NPC and their study, completed by genetic analyses, should now be considered as the first line laboratory testing.<sup>16,17</sup> The filipin test, although still very useful, is no longer considered as the primary tool.”
  - “Assessment of biomarkers should be considered as a first-line test to screen for NPC. Three classes of biochemical markers are either currently in use (oxysterols; lyso-SM-509 and lyso-sphingomyelin) or are in development (bile acid derivatives). They can be used alone or in combination to enhance sensitivity and specificity. The diagnosis, however, must in all cases be confirmed by mutation analysis and if necessary, filipin test.”

- “Any individual in whom the diagnosis of NPC is considered based on their clinical manifestation and/or abnormal biomarker profile should undergo genetic testing for NPC genes to confirm the diagnosis. Referral to a clinical geneticist or genetic counsellor should be considered upon the diagnosis of NPC.”
- “Filipin test is no longer considered a first line test for the diagnosis of NPC. It still remains an extremely useful diagnostic tool in uncertain cases in which biomarkers and/or molecular analysis present inconclusive results and to assess the pathogenicity of novel genetic variants.”
- Regarding genetic testing:
  - “Mutation analysis of NPC1 and NPC2 genes is mandatory to confirm the diagnosis of NPC. In addition, it is the only reliable method to diagnose NPC carriers within the family and the highly preferred strategy for prenatal diagnosis. This testing will also expedite identification of potentially pre-symptomatic affected siblings.”
  - “Although genotype/phenotype correlations are difficult to establish, some conclusions can be drawn from current evidence.”

## Criteria

### Niemann -Pick Disease Type C Known Familial Mutation Analysis

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Testing:
  - No previous genetic testing for Niemann-Pick C, AND
- Diagnostic and Predisposition Testing:
  - Niemann-Pick C family mutation identified in biologic relative(s), OR
- Carrier Testing:
  - Niemann-Pick C family mutation identified in biologic relative(s), OR
- Prenatal Testing:
  - Niemann-Pick C mutation identified in both biologic parents AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.



**Niemann -Pick C Disease Sequencing**

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - Biochemical testing performed showing abnormal biomarkers, and
  - No previous genetic testing for Niemann-Pick C, AND
- Diagnostic Testing for Symptomatic Individuals:
  - Hepatosplenomegaly and/or liver failure, or
  - Central hypotonia or low muscle tone characterized by frequent falls and clumsiness, or
  - Ocular motor abnormalities, especially saccadic eye movements (SEM) and vertical supranuclear gaze palsy, or
  - Delayed or arrested speech development with or without cognitive impairment, or
  - Cerebellar ataxia, or
  - Seizures, or
  - Dystonia, or
  - Dysphagia, OR
- Predisposition Testing for Presymptomatic/Asymptomatic Individuals:
  - Biologic relative(s) (1<sup>st</sup>, 2<sup>nd</sup>, or 3<sup>rd</sup> degree) diagnosed with NPC clinically, and no family mutation identified, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

**Niemann -Pick C Disease Deletion/Duplication Analysis**

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - Biochemical testing performed showing abnormal biomarkers, and
  - NPC1 and NPC2 sequencing performed and no mutations or only one mutation identified, AND

- Diagnostic Testing for Symptomatic Individuals:
  - Hepatosplenomegaly and/or liver failure, or
  - Central hypotonia or low muscle tone characterized by frequent falls and clumsiness, or
  - Ocular motor abnormalities, especially saccadic eye movements (SEM) and vertical supranuclear gaze palsy, or
  - Delayed or arrested speech development with or without cognitive impairment, or
  - Cerebellar ataxia, or
  - Seizures, or
  - Dystonia, or
  - Dysphagia, OR
- Predisposition Testing for Presymptomatic/Asymptomatic Individuals:
  - Biologic relative(s) (1<sup>st</sup>, 2<sup>nd</sup>, or 3<sup>rd</sup> degree) diagnosed with NPC clinically, and no family mutation identified, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

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# Non-Invasive Prenatal Screening

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## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedures addressed by this guideline                               | Procedure codes |
|--|-----------------|
| Non-Invasive Prenatal Screening for Fetal Aneuploidy                 | 81420           |
| Non-Invasive Prenatal Screening for Fetal Aneuploidy with Risk Score | 81507           |
| Non-Invasive Prenatal Screening for Fetal Aneuploidy with Risk Score | 0009M           |
| Non-Invasive Prenatal Screening for Fetal Chromosomal Microdeletions | 81422           |
| Non-Invasive Prenatal Screening for Single-Gene Mutations            | 81105-81479     |

## What is a chromosome abnormality

### Definition

A chromosome abnormality is any difference in the structure, arrangement, or amount of genetic material packaged into the chromosomes.<sup>1</sup>

- Humans usually have 23 pairs of chromosomes. Each chromosome has a characteristic appearance that should be the same in each person.
- Chromosome abnormalities can lead to a variety of developmental and reproductive disorders. Common chromosome abnormalities that affect development include Down syndrome (trisomy 21), trisomy 18, trisomy 13, Turner syndrome, and Klinefelter syndrome.
- About 1 in 200 newborns has some type of chromosome abnormality and a higher percentage of pregnancies are affected but lost during pregnancy. About 6%-11% of stillbirths or neonatal deaths are associated with a chromosome abnormality.<sup>2, 3</sup>
- The risk of having a child with an extra chromosome, notably Down syndrome, increases as a woman gets older.<sup>3</sup> However, many babies with Down syndrome are born to women under 35 and the risk of having a child with other types of

chromosome abnormalities, such as Turner syndrome or 22q11 deletion syndrome, is not related to maternal age. Therefore, prenatal screening for Down syndrome and certain other chromosome abnormalities is now routinely offered to all pregnant women. As a result, prenatal diagnosis via amniocentesis or chorionic villus sampling is now also an option for most pregnant women.

## Test information

- Non-invasive prenatal screening (NIPS) is performed on a maternal plasma sample generally collected after 9 weeks' gestation.<sup>4</sup>
- Testing methodology relies on the presence of cell-free placental DNA in maternal circulation.<sup>4</sup> Approximately 10% of DNA in maternal circulation is of placental origin.<sup>5</sup>
- Analysis of cell-free placental DNA is performed to identify pregnancies at increased risk for chromosomal aneuploidy. Detection rates for trisomies 21, 18, and 13 are greater than 98%, with false positive rates of less than 0.5%.<sup>4</sup>
- Some laboratories also test for sex chromosome aneuploidies (such as Turner syndrome or Klinefelter syndrome) as well as rare chromosome microdeletion syndromes, with variable performance.
- Each commercial or academic laboratory offering NIPS has a proprietary platform and bioinformatics pipeline.
- Chromosome analysis via CVS and amniocentesis is also routinely available for diagnosis of fetal chromosome abnormalities in pregnancy.

## Guidelines and evidence

### The American College of Obstetricians and Gynecologists

The American College of Obstetricians and Gynecologists (ACOG, 2019) issued a practice advisory on the use of cell-free DNA to screen for single-gene disorders stating the following:<sup>6</sup>

- “The continued innovation in cell-free technology combined with the desire for a maternal blood test to predict the risk for fetal genetic disorders during a pregnancy has broadened the application of cell-free DNA screening beyond aneuploidy to single-gene disorders. Examples of single-gene disorders include various skeletal dysplasias, sickle cell disease and cystic fibrosis. Although this technology is available clinically and marketed as a single-gene disorder prenatal screening option for obstetric care providers to consider in their practice, often in presence of advanced paternal age, there has not been sufficient data to provide information regarding accuracy and positive and negative predictive value in the general population. For this reason, single-gene cell-free DNA screening is not currently recommended in pregnancy.”

:

### **The National Society of Genetic Counselors**

The National Society of Genetic Counselors (NSGC, 2018) issued a position statement regarding the use of prenatal cell-free DNA screening:<sup>7</sup>

- “The National Society of Genetic Counselors supports prenatal cell-free DNA (cfDNA) screening, also known as NIPT or NIPS, as an option for pregnant patients.”
- “Diagnostic testing should be offered to patients with increased-risk results to facilitate informed decision making.”

### **American College of Medical Genetics and Genomics**

- The American College of Medical Genetics and Genomics (ACMG, 2016) published a position statement regarding Non Invasive Prenatal Screening (NIPS), recommending the following:<sup>5</sup>
  - “Informing all pregnant women that NIPS is the most sensitive screening option for traditionally screened aneuploidies (i.e., Patau, Edwards, and Down syndrome).”
  - “Informing all pregnant women of the availability of the expanded use of NIPS to screen for clinically relevant copy number variations (CNV’s) when the following conditions can also be met:”
    - “Obstetric care providers should discuss with their patients the desire for prenatal screening as opposed to diagnostic testing (i.e., CVS or amniocentesis).”
    - “Obstetric care providers should discuss with their patients the desire for maximum fetal genomic information through prenatal screening.”
    - “Obstetric care providers should inform their patients of the higher likelihood of false-positive and false-negative results for these conditions as compared to results obtained when NIPS is limited to common aneuploidy screening.”
    - “Obstetric care providers should inform their patients of the potential for results of conditions that, once confirmed, may have an uncertain prognosis.”
  - “Referring patients to a trained genetics professional when an increased risk of aneuploidy is reported after NIPS.”
  - “Offering diagnostic testing when a positive screening test result is reported after NIPS.”

- “Offering diagnostic testing for a no-call NIPS result due to low fetal fraction if maternal blood for NIPS was drawn at an appropriate gestational age. A repeat blood draw is NOT appropriate.”
- “Informing all pregnant women, as part of pretest counseling for NIPS, of the availability of the expanded use of screening for sex chromosome aneuploidies.”
- Offering aneuploidy screening other than NIPS in cases of significant obesity.
- The ACMG specifically recommended against the following:
  - “NIPS to screen for genome-wide CNVs. If this level of information is desired, then diagnostic testing (e.g., chorionic villous sampling or amniocentesis) followed by CMA is recommended.”
  - “NIPS to screen for autosomal aneuploidies other than those involving chromosomes 13, 18, and 21.”

### **The American College of Obstetricians and Gynecologists and Society for Maternal Fetal Medicine**

In 2016, The American College of Obstetricians and Gynecologists (ACOG) and the Society for Maternal Fetal Medicine (SMFM) published a joint practice bulletin stating the following:<sup>8</sup>

- “All women should be offered the option of aneuploidy screening or diagnostic testing for fetal genetic disorders, regardless of maternal age.”
- “Obstetrician-gynecologists and other obstetric care providers should become familiar with the available screening and diagnostic testing options for their patients within their practice and adopt a standard approach for counseling.”
- “Because cell-free DNA is a screening test with the potential for false-positive and false-negative results, such testing should not be used as a substitute for diagnostic testing.”
- “All women with a positive cell-free DNA test result should have a diagnostic procedure before any irreversible action, such as pregnancy termination, is taken.”
- “Women whose cell-free DNA screening test results are not reported, are indeterminate, or are uninterpretable (a no call test result) should receive further genetic counseling and be offered comprehensive ultrasound evaluation and diagnostic testing because of an increased risk of aneuploidy.”
- “Cell-free DNA screening tests for microdeletions have not been validated clinically and are not recommended at this time.”

## The International Society for Prenatal Diagnosis

The International Society for Prenatal Diagnosis (ISPD) first issued a position statement on NIPT in January 2011 and then updated its recommendations in April 2013 and again in April 2015. ISPD summarizes that:<sup>9</sup>

- “The following protocol options are currently considered appropriate:”
  - “cfDNA screening as a primary test offered to all pregnant women.”
  - “cfDNA secondary to a high risk assessment based on serum and ultrasound screening protocols.”
  - “When cfDNA screening is extended to microdeletion and microduplication syndromes or rare trisomies the testing should be limited to clinically significant disorders or well-defined severe conditions.”

## Criteria

### Cell-free DNA-based prenatal screening for fetal aneuploidy

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Prenatal Screening:
  - Cell-free DNA-based prenatal screening for fetal aneuploidy (e.g. trisomy 13, 18, and 21) is considered medically necessary when all of the following criteria are met:
    - Singleton pregnancy, AND
    - Gestational age within the window validated by the selected testing laboratory, AND
    - Rendering laboratory is a qualified provider of service per the Health Plan policy.
- Cell-free DNA screening is not considered medically necessary in the following circumstances:
  - Singleton pregnancies in which the demise of a twin has occurred.
  - Multiple gestation pregnancies, which may be defined by the presence of one of the following ICD codes: O30.X. O31.X.
  - More than one cell-free DNA screen performed per pregnancy defined as no more than one paid cell-free DNA procedure code within 10 weeks.



- When karyotyping, aneuploidy FISH, and/or cytogenomic microarray analysis (CMA) have already been performed on the pregnancy defined as any of these procedure codes paid within 10 weeks of the cell-free DNA screen.

### **Screening for chromosome microdeletions by cell-free DNA**

This test is considered investigational and/or experimental.

- Investigational and experimental (I&E) molecular and genomic (MolGen) tests refer to assays involving chromosomes, DNA, RNA, or gene products that have insufficient data to determine the net health impact, which typically means there is insufficient data to support that a test accurately assesses the outcome of interest (analytical and clinical validity), significantly improves health outcomes (clinical utility), and/or performs better than an existing standard of care medical management option. Such tests are also not generally accepted as standard of care in the evaluation or management of a particular condition.
- In the case of MolGen testing, FDA clearance is not a reliable standard given the number of laboratory developed tests that currently fall outside of FDA oversight and FDA clearance often does not assess clinical utility.

### **Screening for single-gene mutations by cell-free DNA**

This test is considered investigational and/or experimental.

- Investigational and experimental (I&E) molecular and genomic (MolGen) tests refer to assays involving chromosomes, DNA, RNA, or gene products that have insufficient data to determine the net health impact, which typically means there is insufficient data to support that a test accurately assesses the outcome of interest (analytical and clinical validity), significantly improves health outcomes (clinical utility), and/or performs better than an existing standard of care medical management option. Such tests are also not generally accepted as standard of care in the evaluation or management of a particular condition.
- In the case of MolGen testing, FDA clearance is not a reliable standard given the number of laboratory developed tests that currently fall outside of FDA oversight and FDA clearance often does not assess clinical utility.

### **Billing and reimbursement considerations**

- Non-specific procedure codes (e.g. 81479, 81599, 84999) or any procedure codes that do not accurately describe the test methodology performed (e.g. 88271) are not eligible for reimbursement.
- Screening for aneuploidy of the X and Y chromosomes and/or detection of less common trisomies, are not separately reimbursable under these coverage guidelines. Additional procedure codes billed with cell-free DNA screening for this purpose are not eligible for reimbursement.

- Prenatal diagnosis by amniocentesis or CVS following NIPS is generally only indicated when NIPS results are abnormal or additional information becomes available throughout the pregnancy that suggests additional risk factors. Amniocentesis or CVS billed after NIPS is subject to medical necessity review.

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# Oncotype DX Breast DCIS

MOL.TS.255.A

v1.0.2020

## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedure addressed by this guideline | Procedure code |
|---------------------------------------|----------------|
| Oncotype DX Breast DCIS Assay         | 0045U          |

## What is Oncotype DX for breast cancer prognosis

### Definition

Oncotype DX<sup>®</sup> is a gene expression assay designed to determine the risk of a breast cancer recurrence within 10 years of the original diagnosis.<sup>1</sup>

- It is intended for early stage, hormone receptor-positive, lymph node-negative breast cancer.<sup>1-4</sup>
- Oncotype DX should be used with other standard methods of breast cancer assessment such as disease staging, grading, and other tumor markers.<sup>1,2</sup>
- Oncotype DX results appear to correlate with chemotherapy benefit, which may help with the decision between tamoxifen only and adjuvant chemotherapy.<sup>5,6</sup> Studies have demonstrated that the addition of Oncotype DX results changed treatment recommendations and decisions in 25% to 44% of patients, with the majority of recommendations changing from chemotherapy plus tamoxifen to tamoxifen only.<sup>7-9</sup>
- OncotypeDX can be used in individuals with ductal carcinoma in situ (DCIS) in addition to individuals with invasive carcinoma.

### Test information

- Depending on the risk being calculated (local or distant metastasis), either a DCIS Breast Score<sup>®</sup> (DCIS or invasive carcinoma) or a Breast Recurrence Score<sup>®</sup> (invasive carcinoma) is calculated.<sup>10-13</sup>
- The Oncotype DX DCIS Breast Score<sup>®</sup> algorithm is intended for use in women with DCIS treated by local excision, with or without tamoxifen treatment. The score result is reported as a number between 0 and 100, with lower scores representing a low chance of recurrence and a higher score representing a high chance of recurrence within 10 years.<sup>10-13</sup>

- Oncotype DX measures the expression level of 21 genes (16 cancer and 5 reference) from paraffin-embedded breast tumor tissue.<sup>1</sup> These sixteen genes consistently correlated with distant recurrence-free survival in three studies that explored the expression of 250 genes in breast tumor samples.<sup>5</sup>
- The Oncotype DX DCIS score is calculated using a subset of 12 of the 21 gene Oncotype DX panel, including 7 cancer-related and 5 reference genes. On the patient report, average 10 year rates for any local/same breast recurrence (DCIS and invasive) as well as local invasive rate only are reported for a given DCIS Breast Score. Results of the DCIS Breast Score have the potential to change the treatment decision based on risk of local recurrence.<sup>10-13</sup>
- The results are provided as a Recurrence Score<sup>®</sup> (RS, 0-100) with higher scores reflecting higher risk of recurrence. Three risk categories help characterize prognosis:<sup>1,2</sup>
  - Low risk (RS<18), ~50% of patients tested
    - Least aggressive tumors
    - Metastasis unlikely
    - 7% recurrence by 10 yrs
  - Intermediate risk (RS 18-30), ~25% of patients tested
    - More aggressive tumors
    - Metastasis more likely
    - 14% recurrence by 10 yrs
  - High risk (RS 31 or higher), ~25% of patients tested
    - Most aggressive tumors
    - Metastasis most likely
    - 31% recurrence by 10 yrs
- Patients with high scores benefit the most from chemotherapy, showing a substantial reduction in 10 year recurrence. Patients with intermediate scores show questionable benefit from chemotherapy, whereas those with low scores benefit the least from chemotherapy.<sup>2,5,6</sup>

## Guidelines and evidence

### National Comprehensive Cancer Network

- The National Comprehensive Cancer Network (NCCN, 2019) breast cancer treatment guidelines recommend the 21-gene Oncotype DX Breast assay in their

treatment algorithm for hormone receptor-positive, HER2-negative breast cancer in both node-negative (category of evidence 1, predictive and prognostic purposes, preferred test status) and node-positive (category of evidence 1, predictive and prognostic purposes) invasive cancer.<sup>14</sup>

- Multigene assays are not included in the diagnostic or treatment algorithms for non-invasive cancer, such as DCIS.<sup>14</sup>

### **American Society of Clinical Oncology**

The evidence-based guidelines from the American Society of Clinical Oncology (ASCO) about breast cancer tumor marker use (2007, updated 2016) state:

- “In newly diagnosed patients with node-negative, estrogen-receptor positive breast cancer, the Oncotype DX assay can be used to predict the risk of recurrence in patients treated with tamoxifen. Oncotype DX may be used to identify patients who are predicted to obtain the most therapeutic benefit from adjuvant tamoxifen and may not require adjuvant chemotherapy. In addition, patients with high recurrence scores appear to achieve relatively more benefit from adjuvant chemotherapy (specifically (C)MF) than from tamoxifen. There are insufficient data at present to comment on whether these conclusions generalize to hormonal therapies other than tamoxifen, or whether this assay applies to other chemotherapy regimen.”<sup>3</sup>
- In 2016, the American Society of Clinical Oncology (ASCO), stated: “If a patient has ER/PgR-positive, HER2-negative (node-negative) breast cancer, the clinician may use the 21-gene recurrence score (RS; Oncotype DX; Genomic Health, Redwood City, CA) to guide decisions on adjuvant systemic chemotherapy. Type: evidence based. Evidence quality: high. Strength of recommendation: strong.”<sup>4</sup>

### **Evaluation of Genomic Applications in Practice and Prevention**

The Evaluation of Genomic Applications in Practice and Prevention Working Group (EGAPP, 2009 and updated in 2016) found:

- “Insufficient evidence to make a recommendation for or against the use of tumor gene expression profiles to improve outcomes in defined populations of women with breast cancer. In the updated 2016 publication, “evidence of clinical validity for Oncotype DX was confirmed as adequate. With regard to clinical utility, although there was evidence from prospective retrospective studies that the Oncotype DX test predicts benefit from chemotherapy, and there was adequate evidence that the use of Oncotype DX gene expression profiling in clinical practice changes treatment decisions regarding chemotherapy, no direct evidence was found that the use of Oncotype DX testing leads to improved clinical outcomes. Until definitive evidence for clinical utility is available, clinicians must decide on a case-by-case basis whether to offer the test to patients.”<sup>15,16</sup>

## St. Gallen International Expert Consensus

The 14th St. Gallen International Breast Cancer Conference (2015) Expert Panel confirmed previously published recommendations:

- Regarding Oncotype DX, the 2011 recommendations stated: “Several tests are available which define prognosis. These may indicate a prognosis so good that the doctor and patient decide that chemotherapy is not required. A strong majority of the Panel agreed that the 21-gene signature (Oncotype DX) may also be used where available to predict chemotherapy responsiveness in an endocrine responsive cohort where uncertainty remains after consideration of other tests...”<sup>17</sup>
- In 2015, the Panel “considered the role of multiparameter molecular marker assays for prognosis separately in years 1-5 and beyond 5 years and their value in selecting patients who require chemotherapy.” The Panel concluded that “only Oncotype DX commanded a majority in favor of its value in predicting the usefulness of chemotherapy.”<sup>18</sup>

## European Society of Medical Oncology

The European Society of Medical Oncology (ESMO) in 2015 stated:<sup>19</sup>

- “Gene expression profiles, such as MammaPrint (Agendia, Amsterdam, the Netherlands), Oncotype DX Recurrence Score (Genomic Health, Redwood City, CA), Prosigna (Nanostring Technologies, Seattle, WA) and EndoPredict (Myriad Genetics), may be used to gain additional prognostic and/or predictive information to complement pathology assessment and to predict the benefit of adjuvant chemotherapy. The three latter tests are designed for patients with ER-positive early breast cancer only.”
- “In cases of uncertainty regarding indications for adjuvant chemotherapy (after consideration of other tests), gene expression assays, such as MammaPrint, Oncotype DX, Prosigna and Endopredict, may be used, where available.”
- “In cases when decisions might be challenging, such as luminal B HER2-negative and node-negative breast cancer, commercially available molecular signatures for ER-positive breast cancer, such as Oncotype DX, EndoPredict, Prosigna, and for all types of breast cancer (pN0–1), such as MammaPrint and Genomic Grade Index, may be used in conjunction with all clinicopathological factors, to help in treatment decision making.”

## Literature Review

- The available evidence is insufficient to assess if Oncotype DX DCIS provides a reliable, accurate, and clinically meaningful risk score to estimate local recurrence, facilitate treatment decisions, and potentially reduce the effects of overtreatment with radiotherapy in women with DCIS who have undergone surgical excision. The best available data on OncotypeDx for DCIS is from two clinical validity studies

(published in three publications).<sup>20-22</sup> They reported that the Oncotype DX Score was significantly associated with the risk of recurrence in women after surgical excision. Some study results also suggested that Oncotype DX DCIS independently predicted risk of recurrence beyond clinicopathologic variables. However, depending on the scope of the recurrence being assessed in the study (ie, local recurrence; invasive carcinoma, DCIS recurrence), these differences were not statistically significant, and suggested that Oncotype DX DCIS score was not consistently predictive.

- A few studies reported on the degree of association between Oncotype DX DCIS Score and conventional prognostic measures. In general, these studies were small, lacked controls, and conducted at single institutions or centers, and did not provide substantive evidence to the current base of evidence.<sup>23-26</sup>
- Several observational studies provide surrogate measures of clinical utility, but no direct clinical utility studies were identified that evaluated the impact of the use of Oncotype DX DCIS on survival outcomes relative to conventional prognostic risk assessments.<sup>27-30</sup>

## Criteria

- This test is considered investigational and/or experimental.
  - Investigational and experimental (I&E) molecular and genomic (MolGen) tests refer to assays involving chromosomes, DNA, RNA, or gene products that have insufficient data to determine the net health impact, which typically means there is insufficient data to support that a test accurately assesses the outcome of interest (analytical and clinical validity), significantly improves health outcomes (clinical utility), and/or performs better than an existing standard of care medical management option. Such tests are also not generally accepted as standard of care in the evaluation or management of a particular condition.
  - In the case of MolGen testing, FDA clearance is not a reliable standard given the number of laboratory developed tests that currently fall outside of FDA oversight and FDA clearance often does not assess clinical utility.

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# Oncotype DX for Breast Cancer Prognosis

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v1.0.2020

## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedure addressed by this guideline | Procedure code |
|---------------------------------------|----------------|
| Oncotype DX Breast Cancer Assay       | 81519          |

## What is Oncotype DX for breast cancer prognosis

### Definition

Oncotype DX<sup>®</sup> is a gene expression assay designed to determine the risk of a breast cancer recurrence within 10 years of the original diagnosis.<sup>1</sup>

- It is intended for early stage, hormone receptor-positive, lymph node-negative breast cancer.<sup>1-4</sup>
- Oncotype DX should be used with other standard methods of breast cancer assessment such as disease staging, grading, and other tumor markers.<sup>1,2</sup>
- Oncotype DX results appear to correlate with chemotherapy benefit,<sup>5,6</sup> which may help with the decision between tamoxifen only and adjuvant chemotherapy. Studies have demonstrated that the addition of Oncotype DX results changed treatment recommendations and decisions in 25% to 44% of patients, with the majority of recommendations changing from chemotherapy plus tamoxifen to tamoxifen only.<sup>7-9</sup>

### Test information

- Oncotype DX measures the expression level of 21 genes (16 cancer and 5 reference) from paraffin-embedded breast tumor tissue.<sup>1</sup> These sixteen genes consistently correlated with distant recurrence-free survival in three studies that explored the expression of 250 genes in breast tumor samples.<sup>5</sup>
- The results are provided as a Recurrence Score<sup>®</sup> (RS, 0-100) with higher scores reflecting higher risk of recurrence. Three risk categories help characterize prognosis:<sup>1,2</sup>
  - Low risk (RS<18), ~50% of patients tested

- Least aggressive tumors
- Metastasis unlikely
- 7% recurrence by 10 yrs
- Intermediate risk (RS 18-30), ~25% of patients tested
  - More aggressive tumors
  - Metastasis more likely
  - 14% recurrence by 10 yrs
- High risk (RS 31 or higher), ~25% of patients tested
  - Most aggressive tumors
  - Metastasis most likely
  - 31% recurrence by 10 yrs
- Patients with high scores benefit the most from chemotherapy, showing a substantial reduction in 10 year recurrence. Patients with intermediate scores show questionable benefit from chemotherapy, whereas those with low scores benefit the least from chemotherapy.<sup>2,5,6</sup>

## Guidelines and evidence

- The National Comprehensive Cancer Network (NCCN, 2019) breast cancer treatment guidelines recommend the 21-gene Oncotype DX Breast assay in their treatment algorithm for hormone receptor-positive, HER2-negative breast cancer in both node-negative (category of evidence 1, predictive and prognostic purposes, preferred test status) and node-positive (category of evidence 2A, prognostic purposes only) breast cancer.<sup>10</sup>
- The National Institute for Health and Care Excellence (NICE) 2018 stated the following:<sup>11</sup>
  - “EndoPredict (EPclin score), Oncotype DX Breast Recurrence Score and Prosigna are recommended as options for guiding adjuvant chemotherapy decisions for people with oestrogen receptor (RE)-positive, human epidermal growth factor receptor 2 (HER2)-negative and lymph node (LN)-negative (including micrometastatic disease; see section 5.4) early breast cancer, only if:”
    - “they have intermediate risk of distant recurrence using a validated tool such as PREDICT or the Nottingham Prognostic index”
    - “information provided by the test would help them choose, with their clinician, whether or not to have adjuvant chemotherapy taking into account their preference”

- The Evaluation of Genomic Applications in Practice and Prevention Working Group (EGAPP, 2009 and updated in 2016) found:
  - “Insufficient evidence to make a recommendation for or against the use of tumor gene expression profiles to improve outcomes in defined populations of women with breast cancer. In the updated 2016 publication, “evidence of clinical validity for Oncotype DX was confirmed as adequate. With regard to clinical utility, although there was evidence from prospective retrospective studies that the Oncotype DX test predicts benefit from chemotherapy, and there was adequate evidence that the use of Oncotype DX gene expression profiling in clinical practice changes treatment decisions regarding chemotherapy, no direct evidence was found that the use of Oncotype DX testing leads to improved clinical outcomes. Until definitive evidence for clinical utility is available, clinicians must decide on a case-by-case basis whether to offer the test to patients.”<sup>12,13</sup>
- The 14th St Gallen International Breast Cancer Conference (2015) Expert Panel confirmed previously published recommendations:
  - Regarding Oncotype DX, the 2011 recommendations stated: “Several tests are available which define prognosis. These may indicate a prognosis so good that the doctor and patient decide that chemotherapy is not required. A strong majority of the Panel agreed that the 21-gene signature (Oncotype DX) may also be used where available to predict chemotherapy responsiveness in an endocrine responsive cohort where uncertainty remains after consideration of other tests...”<sup>14</sup>
  - In 2015, the Panel “considered the role of multiparameter molecular marker assays for prognosis separately in years 1-5 and beyond 5 years and their value in selecting patients who require chemotherapy.” The Panel concluded that “only Oncotype DX commanded a majority in favor of its value in predicting the usefulness of chemotherapy.”<sup>15</sup>
- The 2007 evidence-based guidelines from the American Society of Clinical Oncology (ASCO) about breast cancer tumor marker use state:
  - “In newly diagnosed patients with node-negative, estrogen-receptor positive breast cancer, the Oncotype DX assay can be used to predict the risk of recurrence in patients treated with tamoxifen. Oncotype DX may be used to identify patients who are predicted to obtain the most therapeutic benefit from adjuvant tamoxifen and may not require adjuvant chemotherapy. In addition, patients with high recurrence scores appear to achieve relatively more benefit from adjuvant chemotherapy (specifically (C)MF) than from tamoxifen. There are insufficient data at present to comment on whether these conclusions generalize to hormonal therapies other than tamoxifen, or whether this assay applies to other chemotherapy regimens.”<sup>3</sup>
  - In 2016, the American Society of Clinical Oncology (ASCO) stated “If a patient has ER/PgR-positive, HER2-negative (node-negative) breast cancer, the

clinician may use the 21-gene recurrence score (RS; Oncotype DX; Genomic Health, Redwood City, CA) to guide decisions on adjuvant systemic chemotherapy. Type: evidence based. Evidence quality: high. Strength of recommendation: Strong.”<sup>4</sup>

- Additional clinical application issues:
  - **Male gender** — No studies specific to the application of Oncotype DX in men with breast cancer have been identified. In general, the NCCN breast cancer treatment guidelines do not differentiate treatment on the basis of gender<sup>10</sup>, which suggests Oncotype DX would not be excluded for males who meet NCCN clinical criteria for considering such testing.
  - **Multiple primary breast tumors** — No studies specific to the application of Oncotype DX in those with multiple breast primary cancers have been identified. Guidelines do not address this issue. A single poster summarized data in a study that used the Oncotype DX test to help assess if synchronous breast cancers were independent neoplastic events or spread of a single tumor. Of 11 patients who met criteria, 5 had different risk scores by Oncotype DX testing (with 3 of these patients having tumors assigned to different risk categories). Of these 5 with significantly different scores, 4 involved bilateral tumors and the other involved tumors in different quadrants. Comparing tumors by histology, 4 of 5 had clearly different histology and 1 had equivocal histology. Of the 6 with similar risk scores, 3 had the same histology, 2 equivocal, and in only 1 case was histology clearly different between the two tumors. This very limited data suggests Oncotype DX may be useful in multiple primaries when tumors independently meet criteria. A study published in 2016 noted that “Among women with synchronous bilateral ER-positive HER2-negative breast cancer, Oncotype DX recurrence scores were concordant in 67% of cases. These data suggest that testing of both tumors should be considered in patients who are candidates for adjuvant chemotherapy.”<sup>16</sup>
  - **Positive lymph nodes** — There is currently insufficient evidence in the peer-reviewed literature regarding the use of Oncotype DX in women with early stage (ER+/HER2-) node-positive breast cancer who are considering adjuvant chemotherapy.
    - Several prospective and retrospective-prospective studies were identified evaluating the use of Oncotype DX in early stage, node-positive breast cancer, and results suggest that use of Oncotype DX allows for prognostic risk stratification.<sup>17-29</sup> However, without chemotherapy, the risk of recurrence for patients with positive nodes appears to be notably higher than patients with negative nodes, and as such, it is not clear if patients with positive nodes can safely avoid chemotherapy treatment regimens based on Oncotype DX test results.
    - There is at least one clinical trial underway, RxPonder, to evaluate the utility of the Oncotype DX Breast Cancer assay for women with 1-3 positive lymph nodes (ER/PR-positive, HER2-negative).<sup>30</sup> This trial aims to support chance

findings from a retrospective subset analysis of the SWOG-8814 trial data that suggested Oncotype DX high and low risk scores were able to predict chemotherapy benefit regardless of node status. An abstract presented at the European Breast Cancer Conference in 2016 presented the 5-year outcome data from a prospective trial with the conclusion of: WSG PlanB for the first time shows excellent 5-year disease free survival of 94% in a population of high risk node-negative and node-positive (pN1) (41.1% had node-positive disease) early BC patients (HR+ HER2-) who omitted adjuvant CT based on RS  $\leq 11$ . These 5-year outcome data from a prospective trial incorporating the RS support the incorporation of the assay in combination with nodal status, grade and tumor size for adjuvant treatment decisions in early HR+ HER2- breast cancer.<sup>31</sup>

- Currently, evidence to support use in node-positive disease remains limited.
- **Ductal Carcinoma In Situ** — There is currently insufficient evidence in the peer-reviewed literature regarding the use of Oncotype DX in women with ductal carcinoma in situ (DCIS) who are considering radiation therapy.
  - Rakovitch et al. (2015) conducted a population cohort study (n=3320 women with DCIS) with a median follow-up period of 9.6 years.<sup>32</sup> Study authors demonstrated that the DCIS Score independently predicted the risk of local recurrence in women with DCIS treated with breast conserving surgery (HR, 2.15; 95% CI, 1.43-3.22). Patients considered low risk via the DCIS Score (62%) had 10-year local recurrence of 13%; intermediate risk (17%) patients had 10-year local recurrence of 33%; and high risk (21%) patients had 10-year local recurrence of 28%. The DCIS Score is intended to provide a quantified risk score for local recurrence to help clinicians guide treatment decisions and potentially reduced the effects of overtreatment with radiotherapy. Study results of this trial and others indicate that despite the ability of Oncotype DX to reclassify patients into different risk groups, it is not clear if the risk estimation is accurate enough to induce changes in treatment strategies or disease management, or if the 10-year local recurrence of approximately 13% is still low enough for patients to successfully avoid radiation therapy and the risk of its associated complications.<sup>33</sup>

## Criteria

- Previous Testing:
  - No repeat Oncotype DX® testing on the same sample when a result was successfully obtained, and
  - No previous gene expression assay (e.g. Prosigna) performed on the same sample when a result was successfully obtained, AND
- Testing Multiple Samples:

- When more than one breast cancer primary is diagnosed:
  - There should be reasonable evidence that the tumors are distinct (e.g., bilateral, different quadrants, different histopathologic features, etc.), and
  - There should be no evidence from either tumor that chemotherapy is indicated with or without knowledge of the Oncotype DX test result (e.g., histopathologic features or previous Oncotype DX result of one tumor suggest chemotherapy is indicated), and
  - If both tumors are to be tested, both tumors must independently meet the required clinical characteristics outlined below.
- Required Clinical Characteristics:
  - Invasive breast cancer meeting all of the following criteria:
    - Tumor size >0.5cm (5mm) in greatest dimension (T1b-T3), and
    - Estrogen receptor positive (ER+), and
    - HER2 negative, and
  - Patient has no regional lymph node metastasis (pN0) or only micrometastases (pN1mi, malignant cells in regional lymph node(s) not greater than 2.0mm), and
  - Chemotherapy is a treatment option for the patient; results from this Oncotype DX test will be used in making chemotherapy treatment decisions, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

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# Oncotype DX for Colorectal Cancer Recurrence Risk

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## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedure addressed by this guideline | Procedure code |
|---------------------------------------|----------------|
| Oncotype DX Colon Cancer Assay        | 81525          |

## What is the Oncotype DX Colon Cancer Assay

### Definition

The Oncotype DX<sup>®</sup> Colon Cancer Assay measures the expression of a panel of genes in stage II colon cancer tumors to predict the risk of future recurrence.<sup>1</sup>

- Stage II colon cancer is defined by a primary tumor that has grown into or through the outermost layers of the colon, but has not spread to nearby lymph nodes or more distant metastasis.<sup>2</sup> At least 12 to 13 lymph nodes should be evaluated.<sup>3,4</sup>
- Stage II colon cancer is often treated with surgery alone with good prognosis.<sup>3,4</sup> Adjuvant chemotherapy is not routinely recommended because it does not appear to improve 5-year survival rates by more than 5% among all people with stage II disease.<sup>3,4</sup>
- However, up to 25% of people with stage II disease will have a recurrence within 5 years.<sup>3</sup> The decision about adjuvant chemotherapy is currently influenced by factors that help predict a higher recurrence risk, including:<sup>3,4</sup>
  - Inadequately sampled lymph nodes
  - Tumor characteristics such as T4 lesion (tumor penetrates to visceral peritoneum or adheres/invaded other organs<sup>2</sup>), perforation, poorly differentiated histology
  - Microsatellite instability and/or mismatch repair expression test results (particularly if considering 5-FU therapy only)
- These prognostic markers are imperfect and the need for additional validated prognostic markers is recognized.<sup>3</sup>
- The Oncotype DX Colon Cancer Assay proposes an additional method for stratifying recurrence risk to assist in the adjuvant chemotherapy decision. Genomic

Health, who markets the assay, suggests the optimal use may be for people with “standard risk” stage II colon cancer (T3 tumor, mismatch repair proficient/microsatellite stable) following surgery, where other accepted prognostic factors do not make the chemotherapy decision clearer.<sup>1</sup>

## Test information

- The Oncotype DX Colon Cancer Assay quantifies the expression of 12 genes from paraffin-embedded primary colon cancer tissue samples.<sup>1</sup>
  - Seven cancer genes associated with recurrence-free interval: Ki-67, C-MYC, MYBL2, FAP, BGN, INHBA, GADD45B
  - Five reference genes (to normalize expression levels): ATP5E, PGK1, GPX1, UBB, VDAC2
- The results are provided as a Recurrence Score, which translates into a percent recurrence risk at three years. Further risk information is provided based on such characteristics as T3/T4 tumor grade and mismatch repair results.<sup>1</sup>

## Guidelines and evidence

### National Comprehensive Cancer Network

- The National Comprehensive Cancer Network (NCCN, 2018) colon cancer guidelines state the following:<sup>4</sup>
  - “Several multigene assays have been developed in hopes of providing prognostic and predictive information to aid in decisions regarding adjuvant therapy in patients with stage II or III colon cancer.”
  - “In summary, the information from these tests can further inform the risk of recurrence over other risk factors, but the panel questions the value added. Furthermore, there is no evidence of predictive value in terms of the potential benefit of chemotherapy to any of the available multigene assays. The panel believes that there are insufficient data to recommend the use of multigene assays to determine adjuvant chemotherapy.”

### Literature Review

There is insufficient evidence of clinical validity and clinical utility for the use of Oncotype DX for colon cancer as a prognostic or predictive assay among stage II and stage III A/B colon cancer patients.<sup>5-14</sup> Several decision impact studies suggest that use of Oncotype DX leads to changes in treatment management, but study authors do not evaluate if such changes lead to improved survival or other health outcomes. No studies directly assessed clinical utility.

Overall, it is still unclear if use of this assay will accurately identify a subset of patients with stage II/III A/B colon cancer who can safely avoid the complications of unnecessary treatments, or if use of the assay will accurately identify a subset of patients who would most benefit from a particular chemotherapy regimen.

## Criteria

- This test is considered investigational and/or experimental.
  - Investigational and experimental (I&E) molecular and genomic (MolGen) tests refer to assays involving chromosomes, DNA, RNA, or gene products that have insufficient data to determine the net health impact, which typically means there is insufficient data to support that a test accurately assesses the outcome of interest (analytical and clinical validity), significantly improves health outcomes (clinical utility), and/or performs better than an existing standard of care medical management option. Such tests are also not generally accepted as standard of care in the evaluation or management of a particular condition.
  - In the case of MolGen testing, FDA clearance is not a reliable standard given the number of laboratory developed tests that currently fall outside of FDA oversight and FDA clearance often does not assess clinical utility.

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# Oncotype DX for Prostate Cancer

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## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedures addressed by this guideline | Procedure codes |
|--|-----------------|
| OncotypeDX Genomic Prostate Score      | 0047U           |

## What are gene expression profiling tests for prostate cancer

### Definition

Prostate cancer (PC) is the most common cancer and a leading cause of cancer-related deaths worldwide. It is considered a heterogeneous disease with highly variable prognosis.<sup>1</sup>

- High-risk prostate cancer (PC) patients treated with radical prostatectomy (RP) undergo risk assessment to assess future disease prognosis and determine optimal treatment strategies. Post-RP pathology findings, such as disease stage, baseline Gleason score, time of biochemical recurrence (BCR) after RP, and PSA doubling-time, are considered strong predictors of disease-associated metastasis and mortality. Following RP, up to 50% of patients have pathology or clinical features that are considered at high risk of recurrence and these patients usually undergo post-RP treatments, including adjuvant or salvage therapy or radiation therapy, which can have serious risks and complications. According to clinical practice guideline recommendations, high risk patients should undergo 6 to 8 weeks of radiation therapy (RT) following RP. However, approximately 90% of high-risk patients do not develop metastases or die of prostate cancer, and instead may be appropriate candidates for alternative treatment approaches, including active surveillance (AS). As such, many patients may be subjected to unnecessary follow-up procedures and their associated complications, highlighting the need for improved methods of prognostic risk assessment.<sup>2,3</sup>
- Several genomic biomarkers have been commercially developed to augment the prognostic ability of currently available routine clinical and pathological tests and identify those patients most and least likely to benefit from a specific treatment strategy. Prognostic genomic tests, including gene expression profiling tests, may help to avoid overtreatment by reclassifying those men originally identified as high risk, but who are unlikely to develop metastatic disease. Genomic biomarkers may

also play a role in assisting clinicians to tailor personalized and more appropriate treatments for subgroups of PC patients, and improve overall health outcomes.<sup>2,3</sup>

## Test information

- Gene expression profiles (GEPs) evaluate the expression of several genes using one sample. Gene expression is determined through RNA analysis, using either reverse transcriptase (RT) polymerase chain reaction (PCR) or DNA microarrays.<sup>4</sup>
- Oncotype DX<sup>®</sup> Genomic Prostate Score (GPS) (Genomic Health)<sup>5</sup>
  - According to the manufacturer, Oncotype DX prostate cancer assay is a multi-gene expression profiling assay that produces a genomic prostate score (GPS), ranging from 0-100, representing tumor aggressiveness. The Oncotype DX GPS provides risk stratification to properly classify patients. This test is designed to help patients with newly diagnosed, early-stage PC make informed treatment decisions, including active surveillance.
  - Oncotype DX GPS uses quantitative RT-PCR for 12 prostate cancer-related genes and 5 control genes (total of 17 genes). It was developed for use with fixed paraffin-embedded (FPE) diagnostic prostate needle biopsies (≥1 mm prostate tumor).

## Guidelines and evidence

### National Comprehensive Cancer Network

- The National Comprehensive Cancer Network (NCCN) 2019 Clinical Practice Guidelines on Prostate Cancer state the following regarding molecular assays:<sup>6</sup>
  - “Men with low or favorable intermediate-risk disease may consider the use of the following tumor-based molecular assays: Decipher, Oncotype DX Prostate, Prolaris, ProMark during initial risk stratification.”
  - “Retrospective studies have shown that molecular assays performed on prostate biopsy or radical prostatectomy specimens provide prognostic information independent of NCCN or CAPRA risk groups. These include, but are not limited to, likelihood of death with conservative management, likelihood of biochemical progression after radical prostatectomy or external beam therapy, and likelihood of developing metastasis after radical prostatectomy or salvage radiotherapy.”
  - “Although full assessment of their clinical utility requires prospective randomized clinical trials, which are unlikely to be done, the panel believes that men with with low or favorable intermediate disease may consider the use of Decipher, Oncotype DX Prostate, Prolaris, or ProMark during initial risk stratification.”



- According to NCCN, the Molecular Diagnostic Services Program (MoIDX) recommendations stated the following:<sup>6</sup>
  - Oncotype DX Prostate: “Cover post-biopsy for NCCN very-low, low-risk, and favorable intermediate-risk prostate cancer in patients with at least 10 years life expectancy who have not received treatment for prostate cancer and are candidates for active surveillance or definitive therapy.”

### **American Association of Clinical Urologists**

The American Association of Clinical Urologists has issued a position statement on genomic testing in prostate cancer that states the following:<sup>7</sup>

- “The AACU supports the use of tissue-based molecular testing as a component of risk stratification in prostate cancer treatment decision making.”

### **American Urological Association, ASTRO, and the Society of Urologic Oncology**

The AUA/ASTRO/SUO guideline for clinically localized prostate cancer states the following:<sup>8</sup>

- “Among most low-risk localized prostate cancer patients, tissue based genomic biomarkers have not shown a clear role in the selection of candidates for active surveillance.”

### **OncotypeDX Prostate**

OncotypeDX Prostate Literature Review<sup>9-31</sup>

- The proposed use of Oncotype DX GPS varied across available studies. Two older clinical validity studies primarily included patients with NCCN-very low to favorable-intermediate risk disease to demonstrate the use of Oncotype DX GPS as an adjunct to confirm decisions regarding active surveillance. In contrast, recently published studies sought to evaluate the clinical validity of Oncotype DX GPS in NCCN-intermediate-risk patients to identify a subset of these patients who harbor aggressive tumors undetected by biopsy and who may benefit from treatment intervention instead of active surveillance.
- Several studies reported that Oncotype DX improved prediction of adverse pathology beyond currently used clinical parameters and nomograms in patients with very low, low-, and intermediate risk disease; however, these studies did not consistently report precision estimates, and when reported, wide confidence intervals suggested inadequate precision. One study conducted at a single institution evaluated long term survival outcomes of distant metastasis and disease-related mortality (~10 years). Analyses assessing the ability of Oncotype DX GPS to detect tumor aggressiveness in NCCN intermediate risk groups versus other risk groups mostly reported results with very wide confidence intervals (imprecise estimates) or P values showing no statistical significance between risk groups. In addition, across the majority of studies, the use of adverse pathology as a surrogate

for survival outcomes (disease-related mortality and metastasis at 10 years) is an inadequate indicator of the performance of Oncotype DX GPS to detect aggressive tumors that necessitate treatment. In addition, it is not clear if the available study results of Oncotype DX in patient populations who underwent RP would reliably translate to newly diagnosed, untreated patients in clinical practice, with very low-, low-, or intermediate risk of disease per NCCN risk classification.

- Direct evidence of clinical utility of Oncotype DX is lacking. Indirect clinical utility studies suggest that Oncotype DX GPS has an impact on physician and patient decision making; however, there is no evidence whether these changes lead to relevant improvements in overall health. As such, clinical utility studies in real-world urologic clinical practice are needed to evaluate if treatment practices change with test use, and if these changes result in improved patient-important outcomes, including overall survival and disease-specific survival. Evidence is also lacking regarding how to conduct ongoing monitoring of men who are determined to be low risk with Oncotype DX testing, but high risk with clinical assessment.

## Clinical Trials

### Engaging Newly Diagnosed Men About Cancer Treatment Options (ENACT)<sup>32</sup>

- “This research is being done to better understand how a new lab test called the Oncotype DX Prostate Cancer Assay may impact what treatment men decide to get and how they feel and think about their choice of treatment.”
- NCT02668276
- Recruiting

## Criteria

- This test is considered investigational and/or experimental.
  - Investigational and experimental (I&E) molecular and genomic (MolGen) tests refer to assays involving chromosomes, DNA, RNA, or gene products that have insufficient data to determine the net health impact, which typically means there is insufficient data to support that a test accurately assesses the outcome of interest (analytical and clinical validity), significantly improves health outcomes (clinical utility), and/or performs better than an existing standard of care medical management option. Such tests are also not generally accepted as standard of care in the evaluation or management of a particular condition.
  - In the case of MolGen testing, FDA clearance is not a reliable standard given the number of laboratory developed tests that currently fall outside of FDA oversight and FDA clearance often does not assess clinical utility.

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# OVA1

MOL.TS.260.A  
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## Procedure addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedure covered by this guideline | Procedure code |
|-------------------------------------|----------------|
| OVA1                                | 81503          |

## What is ovarian cancer

### Definition

With an estimated 22,440 new cases a year, ovarian cancer is one of the most common gynecological cancers in women.<sup>1</sup> In 2017, there were 14,080 deaths from ovarian cancer making it the 5<sup>th</sup> most common cancer mortality.<sup>1,2</sup>

- Signs and symptoms of ovarian cancer include the following:<sup>1</sup>
  - “Pain, swelling, or a feeling of pressure in the abdomen or pelvis.”<sup>1</sup>
  - “Vaginal bleeding that is heavy or irregular, especially after menopause.”<sup>1</sup>
  - “Vaginal discharge that is clear, white, or colored with blood.”<sup>1</sup>
  - “A lump in the pelvic area.”<sup>1</sup>
  - “Gastrointestinal problems such as gas, bloating, or constipation.”<sup>1</sup>
- Current screening methods include gynecological assessment, vaginal ultrasound, and CA-125 assay.<sup>1</sup> However, these screening methods have low predictive value and many times cancer is widespread by the time it is detected.<sup>1</sup>
- As a result, there is greater interest in the discovery of better screening methods in order to identify ovarian cancer at early stages.
- One finding that may raise concern for ovarian cancer is a pelvic mass. Approximately 20% of women will have a pelvic mass during their lifetime.<sup>3</sup> However, not all pelvic masses are cancerous.
- OVA1™ was designed by Vermillion to identify individuals with a pelvic mass who are more likely to have ovarian cancer and who should seek consultation with a gynecological surgeon.

## Test information

- The OVA1 test is indicated for the pre-surgical evaluation of women with an ovarian tumor or mass, suspected of having an ovarian neoplasm, when the clinical and radiological evaluations do not suggest the presence of malignancy.<sup>3</sup>
- This test examines the following 5 markers to assess risk:<sup>2</sup>
  - Transthyretin, Apolipoprotein A1, Transferrin, Beta-2 microglobulin, CA-125
- OVA1 test scores range from 0-10.
  - For premenopausal women, an elevated risk is considered 5 or greater.
  - For postmenopausal women, an elevated risk score is 4.4 or greater.

## Guidelines and evidence

- The National Comprehensive Cancer Network (NCCN, 2019) stated the following regarding OVA1:<sup>2</sup>
  - “The Society of Gynecologic Oncology (SGO), the FDA, and the Mayo Clinic have stated that the OVA1 test should not be used as a screening tool to detect ovarian cancer. The OVA1 test uses 5 markers (including transthyretin, apolipoprotein A1, transferrin, beta-2 microglobulin, and CA-125) to assess who should undergo surgery by an experienced gynecologic oncologist and who can have surgery in the community.”
  - “Based on data documenting an increased survival, NCCN Guidelines Panel Members recommend that all patients should undergo surgery by an experienced gynecologic oncologist (category 1).”
- The American College of Obstetrics and Gynecologists (ACOG, 2016) stated the following regarding OVA1:<sup>4</sup>
  - “Serum biomarker panels may be used as an alternative to CA 125 level alone in determining the need for referral to or consultation with a gynecologic oncologist when an adnexal mass requires surgery. These biomarker panels are not recommended for use in the initial evaluation of an adnexal mass, but may be helpful in assessing which women would benefit from referral to a gynecologic oncologist.”
  - “The multivariate index assay has demonstrated higher sensitivity and negative predictive value for ovarian malignancy when compared with clinical impression and CA 125 alone.”
- Several clinical studies in the peer-reviewed publication literature have evaluated the use of OVA1.<sup>5-15</sup>

- OVA1 has the potential to improve some aspects of diagnostic accuracy, particularly sensitivity and negative predictive value, beyond the current disease management strategies for ovarian tumors. When used alongside a clinician's assessment, some studies have shown that OVA1 has the ability to increase accurate detection of ovarian malignancies, although specificity and positive predictive values suffer. Compared with clinical assessment alone or ACOG guidelines, OVA1 improves diagnostic assessment, and OVA1 appears to demonstrate improvement over its predecessor test for CA-125.

## Criteria

Coverage for OVA1 will be granted when the following criteria are met:

- The member has surgery planned for an ovarian adnexal mass that is neither clearly benign nor clearly malignant based on clinical or ultrasound evaluation, AND
- No previous successful OVA1 testing for the current ovarian adnexal mass, AND
- The member is over 18 years of age, AND
- The member has not yet been referred to a gynecologic oncologist, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

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# PALB2 Genetic Testing for Breast Cancer Risk

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## Procedure addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedure(s) addressed by this guideline | Procedure code(s) |
|--|-------------------|
| PALB2 Known Familial Mutation Analysis   | 81403             |
| PALB2 Sequencing                         | 81406             |
| PALB2 Deletion/Duplication Analysis      | 81479             |

## What is PALB2 genetic testing

### Definition

Breast cancer is the most frequently diagnosed malignancy and the leading cause of cancer mortality in women around the world. Hereditary breast cancer accounts for 5% to 10% of all breast cancer cases.

- Screening with breast magnetic resonance imaging (MRI) is recommended for women with a greater than 20% lifetime risk for disease based on estimates of risk models that are largely dependent on family history. A large body of evidence indicates that an increased lifetime risk of >20% can also be established through genetic testing. In particular, two cancer susceptibility genes, BRCA1 and BRCA2, are implicated in about 20% of all hereditary breast cancer cases. Other genes have also been identified in the literature as being associated with inherited breast cancer risk, including ATM, CDH1, CHEK2, NBM, NF1, PALB2, PTEN, STK11, and TP53.<sup>1,2</sup>
- In particular, PALB2 is a gene that encodes a protein that may be involved in tumor suppression, and is considered a partner and localizer of BRCA2. Specifically, ~50 truncating mutations in PALB2 have been detected among breast cancer families worldwide. Kluska et al. (2017) estimates that a relative risk (RR) of 2.3 (95% CI, 1.4 to 3.9) is conferred by PALB2 mutations, indicating an approximate two-fold increased risk of developing hereditary breast cancer.<sup>3</sup> A meta-analysis of three studies estimated a relative risk of 5.3 (90% CI, 3.0-9.4).<sup>4</sup>
- The availability of multiple gene panel testing of variant genes implicated in hereditary breast cancer has led to increased interest in hereditary risk assessment

in clinical practice. Clinical decisions based on risk assessment measures include screening with breast magnetic resonance imaging (MRI) and risk-reduction surgery, which have been shown to reduce the morbidity and mortality associated with breast cancer. However, results of peer-reviewed published clinical studies evaluating the clinical validity and clinical utility of multiple gene panels, particularly of unknown clinical significance, or of low-to-moderate penetrance, are still unclear. Broad application of such testing has yet to be fully adopted.<sup>5</sup>

- Genetic testing allows patients with an increased risk of cancer to receive appropriate medical management that may reduce risk for themselves and their family members. Early identification of at-risk women allows for increased clinical surveillance and may prompt more aggressive prevention strategies, such as prophylactic surgery or chemoprevention. The National Comprehensive Cancer Network (NCCN) guidelines have been expanded to incorporate genes known to be associated with an increased risk of breast cancer into medical management recommendations.<sup>6,7</sup>

## Test information

- **Full sequence analysis** of the PALB2 gene looks at all of the coding regions of the PALB2 gene.
- **Deletion/duplication analysis** looks for large rearrangements, duplications, and deletions in the PALB2 gene.
- **Known familial mutation testing** looks for a specific mutation in the PALB2 gene previously identified in a family member.

## Guidelines and evidence

- The National Comprehensive Cancer Network (NCCN, 2019) includes breast cancer risk and management recommendations for individuals with PALB2 in a table located in their Genetic/Familial High-Risk Assessment: Breast and Ovarian guideline. However, it is noted that, “The inclusion of a gene on this table below does not imply endorsement either for or against multi-gene testing for moderate-penetrance genes.” Recommendations are as follows:<sup>7</sup>
  - “Screening: Annual mammogram with consideration of tomosynthesis and consider breast MRI with contrast at 30y.” This may be modified based on family history. Typically begin screening 5-10 years earlier than the youngest diagnosis in the family but not later than 30y.
  - “RRM: Evidence insufficient, manage based on family history.”
- The American Society of Breast Surgeons (2019) published a consensus guideline on genetic testing for hereditary breast cancer. They state the following:<sup>8</sup>

- "Breast surgeons, genetic counselors, and other medical professionals knowledgeable in genetic testing can provide patient education and counseling and make recommendations to their patients regarding genetic testing and arrange testing. When the patient's history and/or test results are complex, referral to a certified genetic counselor or genetics professional may be useful. Genetic testing is increasingly provided through multi-gene panels. There are a wide variety of panels available, with different genes on different panels. There is a lack of consensus among experts regarding which genes should be tested in different clinical scenarios. There is also variation in the degree of consensus regarding the understanding of risk and appropriate clinical management of mutations in some genes."
- "Genetic testing should be made available to all patients with a personal history of breast cancer. Recent data support that genetic testing should be offered to each patient with breast cancer (newly diagnosed or with a personal history). If genetic testing is performed, such testing should include BRCA1/BRCA2 and PALB2, with other genes as appropriate for the clinical scenario and family history. For patients with newly diagnosed breast cancer, identification of a mutation may impact local treatment recommendations (surgery and potentially radiation) and systemic therapy. Additionally, family members may subsequently be offered testing and tailored risk reduction strategies."
- "Genetic testing should be made available to all patients with a personal history of breast cancer. Every patient being seen by a breast surgeon, who had genetic testing in the past and no pathogenic variant was identified, should be re-evaluated and updated testing considered. In particular, a patient who had negative germline BRCA1 and 2 testing, who is from a family with no pathogenic variants, should be considered for additional testing. Genetic testing performed prior to 2014 most likely would not have had PALB2 or other potentially relevant genes included and may not have included testing for large genomic rearrangements in BRCA1 or BRCA2."
- The European Society for Medical Oncology (ESMO, 2016) states the following prevention and screening strategies for individuals with a PALB2 mutation:<sup>9</sup>
  - "Clinical breast examination every 6-12 months starting from age 20-25"
  - "Annual breast MRI from age 20-29"
  - "Annual breast MRI and/or mammogram at age 30-75."
  - "Consider risk-reducing mastectomy."
- ESMO (2016) also states the following regarding PALB2 testing, "The following genes might have moderate- to high-penetrance germline mutations for breast or ovarian cancer: p53, PTEN, CDH1, PALB2, CHEK2, ATM, RAD51C, STK11, RAD51D, BRIP1, MLH1, MSH, MSH6, and PMS2. Prevention and screening strategies for these mutations are summarized in Table 1 – due to limited research in individuals harboring these mutations, the level of evidence for these

recommendations is mostly expert opinion, and a full discussion is beyond the scope of these guidelines.”<sup>9</sup>

- The Third International Consensus Conference for Breast Cancer in Young Women (BCY3, 2017) led to publication of consensus recommendations. The following is stated regarding PALB2 genetic testing:<sup>10</sup>
  - “Although BRCA1/2 are the most frequently mutated genes, other additional moderate-to high-penetrance genes may be considered if deemed appropriate by the geneticist/genetic counselor. When a hereditary cancer syndrome is suspected and a mutation in BRCA1/2 has not been identified, multi-gene panel testing may be considered. Practice should be guided by high quality national/international guidelines. As commercially available multi-gene panels include different genes, the choice of the specific panel and quality-controlled laboratory is crucial, and should at least include high penetrance genes (BRCA1/2, p53, PTEN) and moderate-high penetrance genes (e.g., CDH1, CHEK2, PALB2, RAD51C, BRIP1, ATM).”
- A review of the available PALB2 literature revealed the following:<sup>11-22</sup>
  - Direct evidence from a number of case control studies reporting relative risk and odds ratio values suggest that PALB2 testing accurately identifies PALB2 mutations, which are associated with an increased risk of developing breast cancer. Indirect evidence suggests that the clinical utility of PALB2 testing may alter clinical decision making enough to lead to improved patient health outcomes.
  - Direct and indirect evidence regarding clinical validity and clinical utility suggest that expanded panel testing may be used to identify more women who can benefit from appropriate breast cancer risk reduction strategies. However, gaps in knowledge persist regarding the precise cancer risk estimates for PALB2 (e.g., wide confidence intervals) and the predictive value in individuals and relatives who test negative for pathogenic variants yet have a strong family history of disease.
  - Before clinical utility of PALB2 testing can be adequately established, well-designed studies are crucial to directly evaluate the clinical utility of PALB2 to alter treatment and overall disease management strategies and improve morbidity and mortality outcomes in women who develop hereditary breast cancer.

## Criteria

### Known Familial Mutation Analysis

- Genetic Counseling:

- Pre- and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - No previous full sequence testing or deletion/duplication analysis, and
  - Known family mutation in PALB2 identified in 1st, 2nd, or 3rd degree relative(s), AND
- Age 18 years or older, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

### Full Sequence Analysis

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - Member has had BRCA1/2 analysis and no mutations were found, and
  - Member had not had previous PALB2 sequencing, AND
- Diagnostic Testing in Symptomatic Individuals and Presymptomatic Testing in Asymptomatic individuals:
  - Member has met criteria for BRCA1/2 analysis,\*\* AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

\*\*Please see the guideline **BRCA Analysis** for criteria

### Deletion/Duplication Analysis

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - Member meets above criteria for PALB2 full sequence analysis, and
  - Member has had PALB2 full sequence analysis and no mutations were found, and
  - Member had not had previous PALB2 deletion/duplication analysis, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

PALB2

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# PancraGEN

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## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedure addressed by this guideline | Procedure code |
|---------------------------------------|----------------|
| PancraGEN                             | 81479          |

## What are pancreatic cysts

### Definition

Four of the most common types of pancreatic cysts are serous cystadenomas (SCA), solid-pseudopapillary neoplasm (SPN), mucinous cystic neoplasm (MCN), and intraductal papillary mucinous neoplasms (IPMN).<sup>1</sup>

- Pancreatic cysts are reported as incidental findings in 3 to 13% of individuals undergoing imaging procedures. Given that pancreatic cancer is a rare, but lethal disease, proper assessment of pancreatic cysts is crucial for the definitive diagnosis and optimal treatment of individuals with malignant disease.
- Clinicians typically rely on imaging, cytology, and fluid chemistry to assess the malignancy risk of pancreatic cysts. Despite first-line assessments, individuals often undergo invasive surgery to treat suspicious pancreatic cysts.
- In cases where an individual's diagnosis based on conventional pathologic and imaging approaches is inconclusive, PancraGEN has been proposed as an adjunctive risk stratification tool to provide additional clarifying information to inconclusive results of standard diagnostic tools, including imaging, carcinoembryonic antigen (CEA), cytology, and clinical risk factors.

### Test information

- PancraGEN represents a form of topographic genotyping, a process that combines conventional imaging and pathologic analyses with molecular analyses.
- According to the test manufacturer, PancraGEN provides molecular results for DNA quantity and quality, oncogene point mutations (KRAS and GNAS), and tumor suppressor gene mutations to stratify patients according to their risk of progression to malignancy.<sup>2</sup>

- The test requires specimens of pancreatobiliary fluid, pancreatic masses, or pancreatic tissue usually obtained by endoscopic ultrasound (EUS) guided fine needle aspiration (FNA).<sup>2,3</sup>
- The PancraGEN report categorizes patients into one of four groups: low risk category that supports surveillance (a. benign; b. statistically indolent) or high risk category that supports treatment intervention decisions (c. statistically higher risk; d. aggressive).
- This test is intended to determine a patient's risk of cancer progression and assess the best course of treatment. Based on test results, low-risk patients with benign cysts may benefit from early disease surveillance and avoidance of invasive surgical resection, while higher risk patients with aggressive cysts can receive proper surgical treatment for malignant lesions.<sup>2</sup>

## Guidelines and evidence

- A small base of evidence comprised of a few clinical studies have evaluated the correlation between genetic testing using the PancraGen test and histology, cytology and pathology of surgical or biopsy specimens of pancreatic tissue.<sup>4-8</sup> Two of the most relevant studies, both published by the manufacturer and evaluating the same patient population, reported results of a retrospective analysis of the National Pancreatic Cyst Registry study (n=492).
  - In the study by Al-Haddad et al. (2015), patients underwent testing with PathFinderTG (now PancraGEN) and were followed to evaluate disease progression to malignancy.<sup>4</sup> Diagnostic performance of PathFinder TG testing were compared with a set of international consensus guidelines, published in 2012, used for disease management in clinical practice.<sup>5</sup> After a median follow-up of 35 months, negative predictive values and sensitivity values for PathFinderTG and consensus guidelines were comparable, although positive predictive value and positive likelihood ratios were significantly improved for PathFinder TG. Study authors concluded that the PathFinder TG test may improve disease management by supporting a surveillance decision established by the Sendai guideline criteria.
  - In the same study population from the National Pancreatic Cyst Registry described in by Al-Haddad et al. (n=491), Loren et al. (2016)<sup>6</sup> compared the association between diagnoses made with PancraGEN and those made with the consensus guidelines by Sendai and Fukouka (2012), and also reported on the subsequent clinical decisions made in the real world regarding choices made for either surveillance or surgical intervention. Study results suggest that testing with PancraGEN testing is significantly associated with real-world decisions, although it is not known if physician influence or patient preferences could have also impacted these decisions. Study results suggest that PancraGEN testing might properly reclassify some patients misclassified by consensus guidelines.

- Farrell and colleagues assessed the incremental value of DNA markers when applied against a clinically stratified patient population, rather than using the clinical information in aggregate as part of Integrated Molecular Pathology scoring.<sup>9</sup> The absence of DNA abnormalities allowed a reduction in malignancy risk in patients with worrisome clinical findings (incremental relative risk of malignancy 0.4 (0.1-1.1 95% CI) to that of patients with no worrisome features or high risk stigmata.
- A retrospective assessment of the clinical utility of DNA biomarkers was performed by Arner and colleagues.<sup>10</sup> Results of DNA marker testing changed management decisions (as made by each of 2 experts in a retrospective case review) in approximately 27% of cases.
- The performance DNA markers in assessing the malignant potential of intraductal papillary mucinous neoplasm, both independently and as part of the Integrated Molecular Pathology malignancy risk score was evaluated by two studies.<sup>11,12</sup> The same study population, identified through retrospective chart-review, was used for both.
- Limitations of the evidence include retrospective study designs, limited follow-up times to adequately observe malignant progression, and a very small number of cases where results of PancreGEN and consensus guidelines do not agree.
- Given that the evidence base consists primarily of retrospective study designs, it is not clear if PancreGEN would perform well in a broad, general population of patients with pancreatic cysts. Small sample sizes may lead to imprecise estimates of test accuracy.

## Criteria

- This test is considered investigational and/or experimental.
  - Investigational and experimental (I&E) molecular and genomic (MolGen) tests refer to assays involving chromosomes, DNA, RNA, or gene products that have insufficient data to determine the net health impact, which typically means there is insufficient data to support that a test accurately assesses the outcome of interest (analytical and clinical validity), significantly improves health outcomes (clinical utility), and/or performs better than an existing standard of care medical management option. Such tests are also not generally accepted as standard of care in the evaluation or management of a particular condition.
  - In the case of MolGen testing, FDA clearance is not a reliable standard given the number of laboratory developed tests that currently fall outside of FDA oversight and FDA clearance often does not assess clinical utility.

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# PCA3 Testing for Prostate Cancer

MOL.TS.215.A

v1.0.2020

## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedure addressed by this guideline | Procedure code |
|---------------------------------------|----------------|
| PCA3 Score                            | 81313          |

## What is prostate cancer antigen 3 (PCA3)

### Definition

Prostate cancer gene 3 (PCA3) is a non-protein-coding messenger RNA (mRNA) that is highly overexpressed in >95% prostate cancer tissue compared with normal prostate tissue or benign prostatic hyperplasia.<sup>1</sup>

- The strong association between PCA3 mRNA levels and prostate cancer led to the development of a urinary assay to measure this analyte to aid in cancer detection.<sup>1</sup>

### Test information

- Following a digital rectal examination, urine is collected and the mRNAs for the PCA3 gene and the PSA gene are quantified. A PCA3 score is calculated from the ratio of PCA3 RNA to PSA RNA.
- A high (>25) PCA3 Score indicates an increased likelihood of a positive biopsy. A low (<25) PCA3 Score is associated with a decreased likelihood of a positive biopsy.<sup>2</sup>
- A multi-center study which included a total of 466 men found that at a score cutoff of 25 for men with at least one previous negative biopsy, PCA3 demonstrated 77.5% sensitivity, 57.1% specificity, and negative and positive predictive values of 90% and 33.6%, respectively. Men with a PCA3 score of <25 were 4.56 times more likely to have a negative repeat biopsy than men with a score of >25.<sup>3</sup>

### Guidelines and evidence

- Data from many peer-reviewed publications suggest that PCA3 gene testing, when used with other patient information, may help address some of the well-known

challenges urologists face, such as identifying prostate cancers while reducing unnecessary repeat biopsies.<sup>4-6</sup>

- The U.S Food and Drug Administration (2012) approved the ProgenSA PCA3 assay with the following intended use:<sup>7</sup>
  - “The PROGENSA® PCA3 Assay is indicated for use in conjunction with other patient information to aid in the decision for repeat biopsy in men 50 years of age or older who have had one or more previous negative prostate biopsies and for whom a repeat biopsy would be recommended by a urologist based on current standard of care, before consideration of PROGENSA PCA3 Assay results.”
  - “The Clinical Study only included men who were recommended by urologists for repeat biopsy. Therefore, the performance of the PROGENSA PCA3 Assay has not been established in men for whom a repeat biopsy was not already recommended.”
  - “Black Box Warning: The PROGENSA PCA3 Assay should not be used for men with atypical small acinar proliferation (ASAP) on their most recent biopsy. Men with ASAP on their most recent biopsy should be treated in accordance with current medical guidelines.”
- The National Comprehensive Cancer Network (NCCN, 2018) guidelines for prostate cancer early detection recognize the FDA-approved use of PCA3 testing and state:<sup>8</sup>
  - “Results were reported from an NCI Early Detection Research Network (EDRN) validation study of the PCA3 urinary assay in 859 men scheduled for a diagnostic prostate biopsy in 11 centers. The primary outcomes were reported at a PPV of 80% (95% CI, 72%–86%) in the initial biopsy setting and an NPV of 88% (95% CI, 81%–93%) in the repeat biopsy setting. Based on the data, use of PCA3 in the repeat biopsy setting would reduce the number of biopsies by almost half, and 3% of men with a low PCA3 score would have high-grade prostate cancer that would be missed. In contrast, the risk of high-grade disease in men without prior biopsy with a low PCA3 is 13%. Thus, the panel believes that this test is not appropriate to use in the initial biopsy setting.”
  - “The FDA has approved the PCA3 assay to help decide, along with other factors, whether a repeat biopsy in men aged 50 years or older with one or more previous negative prostate biopsies is necessary. This assay is recommended for men with previous negative biopsy in order to avoid repeat biopsy by the Molecular Diagnostic Services Program (MoIDX) and is therefore covered by CMS (Centers for Medicare & Medicaid Services) in this setting.”
- The American Urological Association (AUA 2013, confirmed 2015) guideline on the early detection of prostate cancer concluded:<sup>9</sup>
  - “At this point, the use of DRE, PSA derivatives (PSA density and age specific reference ranges) and PSA kinetics (velocity and doubling time), PSA molecular

forms (percent free PSA and proPSA), novel urinary markers (PCA3), and prostate imaging should be considered secondary tests (not primary screening tests) with potential utility for determining the need for a prostate biopsy, but with unproven benefit as primary screening tests.”

- “The Panel recognizes that these tests can be used as adjuncts for informing decisions about the need for a prostate biopsy –or repeat biopsy- after PSA screening, but emphasizes the lack of evidence that these tests will increase the ratio of benefit to harm.”

## Criteria

Prostate cancer antigen testing (PCA3) may be indicated in males with ALL of the following:

- Age >50 years, and
- One or more previous negative prostate biopsies, and
- Continued clinical suspicion of prostate cancer based on digital rectal exam (DRE) or elevation of prostate specific antigen (PSA) of >3 ng/mL, and for whom a repeat biopsy would be recommended by a urologist based on current standard of care, and
- Atypical small acinar proliferation (ASAP) was NOT identified on the most recent biopsy.

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# Peutz-Jeghers Syndrome Testing

MOL.TS.216.A

v1.0.2020

## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedures addressed by this guideline | Procedure codes |
|--|-----------------|
| STK11 Sequencing                       | 81405           |
| STK11 Deletion/Duplication Analysis    | 81404           |
| STK11 Known Familial Mutation Analysis | 81403           |

## What is Peutz-Jeghers syndrome

### Definition

Peutz-Jeghers syndrome (PJS) is a genetic disorder characterized by the development of polyps (hamartomas) in the gastrointestinal (GI) tract, most commonly the small intestine. Polyps also occur in the stomach and colon and on occasion in the renal pelvis, urinary bladder, ureters, lungs, nares, and gallbladder.<sup>1</sup> About a third of affected individuals present with polyps by age 10, and by age 20, about half have clinical signs and symptoms.<sup>2</sup>

- Affected people also typically have mucocutaneous pigmented lesions — lip freckling is classic, but pigmentation may also develop in the mouth, gums, nose, perianal area, and on the fingers and toes.<sup>1,2</sup>
- In addition to gastrointestinal polyps and cancer, people with PJS have an increased risk for other cancers, including those of the pancreas, lung, breast, uterus, cervix, ovaries, and testes.<sup>1,2</sup>
- PJS is caused by mutations in the STK11 gene. STK11 is a tumor suppressor gene. Its normal role is to control growth and development of cells in the GI tract. Mutations in STK11 cause cells to grow and divide uncontrollably, leading to the development of polyps and an increased risk for cancer.<sup>1</sup>
- PJS is inherited in an autosomal dominant pattern. Children of an affected person have a 1 in 2 (50%) chance to be affected. “In large series, 60-78% of individuals with PJS had affected relatives and 17-40% of individuals represented isolated cases within their families”<sup>1</sup> The proportion of a new (de novo) mutation is unclear due to variable expressivity and the frequency of subtle signs in parents is unknown.<sup>1</sup>

- Because of the potential early onset of polyp growth, surveillance is complex and involves monitoring at-risk individuals for related cancers, starting with baseline colonoscopy and upper GI endoscopy at age 8.<sup>1-4</sup>

## Test information

- Over 200 distinct STK11 gene mutations or deletions have been identified in people with PJS.

Molecular genetic testing is performed in parallel by two methods:<sup>1</sup>

- **STK11 Sequence Analysis is used** to identify smaller mutations in STK11. Approximately 81% of individuals with PJS will have a mutation detected by this method.
- **STK11 Deletion/Duplication Analysis** is used to identify larger deletions. Approximately 15% of individuals with PJS will have a mutation detected by this method.
- Ninety-four to 96% of individuals with PJS will have an STK11 pathogenic variant.<sup>5,6</sup> The detection rate in familial versus sporadic cases is 87% and 97.8%, respectively.<sup>6</sup>
- **STK11 Known Familial Mutation Analysis:** Once an STK11 mutation is identified in an affected person, predictive testing is available for at-risk family members, as is prenatal or preimplantation genetic diagnosis<sup>1</sup> Family members should be tested using the method that can accurately identify the familial mutation.
- A multi-gene panel can also be used to test individuals suspected of having PJS.

## Guidelines and evidence

- Evidence-based guidelines for the diagnosis and management of PJS were published in 2010.<sup>2</sup> These guidelines outline clinical diagnostic criteria for PJS and surveillance recommendations, but do not specifically address the utility of genetic testing.
  - A clinical diagnosis of PJS may be made in an affected person when any ONE of the following is present (directly quoted):
    - Two or more histologically confirmed PJS polyps
    - Any number of PJS polyps detected in one individual who has a family history of PJS in close relative(s)
    - Characteristic mucocutaneous pigmentation in an individual who has a family history of PJS in close relative(s)

- Any number of PJS polyps in an individual who also has characteristic mucocutaneous pigmentation
- “No clear genotype-phenotype correlation has been demonstrated in PJS, and no clear differences found between cases with STK11 mutation and in those in whom no mutation has been detected.”
- The National Comprehensive Cancer Network (2018)<sup>3</sup> guidelines outline similar clinical diagnostic criteria and provide some guidance on surveillance, but do not address the use of genetic testing.
  - “A clinical diagnosis of PJS can be made when an individual has two or more of the following features:”
    - “Two or more Peutz-Jeghers-type hamartomatous polyps of the small intestine”
    - “Mucocutaneous hyperpigmentation of the mouth, lips, nose, eyes, genitalia, or fingers”
    - “Family history of PJS”
  - “The majority of cases occur due to mutations in the STK11 (LKB1) gene and clinical genetic testing is available.”
  - Screening procedures and intervals are outlined for breast, colon, stomach, pancreatic, small intestine, cervical, ovarian, uterine, and testicular cancers.
- Clinical diagnostic criteria have been validated by genetic testing in one series of 71 patients.<sup>7</sup> Of 56 patients who met clinical criteria for PJS, 94% had an STK11 mutation found by a combination of sequencing and deletion/duplication analysis. Twelve patients had only a “presumptive diagnosis” of PJS based on the presence of hyperpigmentation or isolated PJS polyps, with no known family history. No STK11 mutations were found in those 12 patients.
- A 2016 expert-authored review states:<sup>1</sup>
  - “Testing of at-risk asymptomatic adults for Peutz-Jeghers syndrome is available after the disease-causing STK11 mutation has been identified in an affected family member.”
  - “Testing for the disease-causing mutation in the absence of definite symptoms of the disease is predictive testing. At-risk asymptomatic adult family members may seek molecular genetic testing in order to make personal decisions regarding medical surveillance, reproduction, financial matters, and career planning.”
  - “Because early detection of at-risk individuals who have an STK11 mutation affects medical management, particularly surveillance, testing of at-risk individuals during childhood is beneficial.”

- The American Society of Clinical Oncologists (ASCO) position statement on genetic testing (originally published 1996<sup>8</sup>; revised/affirmed in 2003<sup>9</sup>, 2010<sup>10</sup>, and 2015<sup>11</sup>) outlines general recommendations for genetic testing for hereditary cancer syndromes and specifically addresses issues around genetic testing in at-risk children:
  - “Indications for Genetic Testing: ASCO recommends that genetic testing be offered when 1) the individual has personal or family history features suggestive of a genetic cancer susceptibility condition, 2) the test can be adequately interpreted, and 3) the results will aid in diagnosis or influence the medical or surgical management of the patient or family members at hereditary risk of cancer.”
  - “Special Issues in Testing Children for Cancer Susceptibility: ASCO recommends that the decision to offer testing to potentially affected children should take into account the availability of evidence-based risk-reduction strategies and the probability of developing a malignancy during childhood. Where risk-reduction strategies are available or cancer predominantly develops in childhood, ASCO believes that the scope of parental authority encompasses the right to decide for or against testing.”
  - “Tests for high-penetrance mutations in appropriate populations have clinical utility, meaning that they inform clinical decision making and facilitate the prevention or amelioration of adverse health outcomes.”

## Criteria

STK11 (LKB1) gene testing may be considered for individuals with a suspected or known clinical diagnosis of Peutz-Jeghers syndrome, or a known family history of a STK11 (LKB1) mutation.

### PJS Known Familial Mutation Analysis

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Testing:
  - No previous STK11 gene testing that would have detected the family mutation, AND
- Diagnostic and Predisposition Testing:
  - Known family mutation in the STK11 gene identified in 1st degree relative(s). (Note: 2nd or 3rd degree relatives may be considered when 1st degree relatives are unavailable or unwilling to be tested), AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

### STK11 Sequencing:

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Testing:
  - No previous STK11 gene sequencing, and
  - No known familial STK11 mutation, AND
- Diagnostic Testing for Symptomatic Individuals:
  - A clinical diagnosis of PJS based on at least two of the following features:
    - At least two PJS-type hamartomatous polyps of the gastrointestinal tract, or
    - Mucocutaneous hyperpigmentation of the mouth, lips, nose, eyes, genitalia, or fingers, or
    - A family history of PJS, AND
- Predisposition Testing for Presymptomatic/Asymptomatic Individuals:
  - Member is a 1st degree relative of someone with a clinical diagnosis of PJS who has had no previous genetic testing (Note that testing in the setting of a more distant affected relative will only be considered if the 1st degree relative is unavailable or unwilling to be tested), AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

### STK11 Deletion/duplication testing

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Testing:
  - No previous STK11 deletion/duplication analysis has been performed, and
  - Above criteria for STK11 full gene sequencing are met, and
  - STK11 sequencing was previously performed and no mutations were found, and
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

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# Pharmacogenomic Testing Panels for Major Depressive Disorder

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v1.0.2020

## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedures addressed by this guideline                | Procedure codes |
|---|-----------------|
| CYP2C19 gene analysis, common variants                | 81225           |
| CYP2D6 gene analysis, common variants                 | 81226           |
| CYP2C9 gene analysis, common variants                 | 81227           |
| CYP3A4 gene analysis, common variants                 | 81230           |
| CYP3A5 gene analysis, common variants                 | 81231           |
| CYP1A2 gene analysis, common variants                 | 81479           |
| CYP2B6 gene analysis, common variants                 | 81479           |
| HLA Class I Typing, one allele or allele group        | 81381           |
| HTR2A gene analysis, common variants                  | 81479           |
| SLC6A4 gene analysis, common variants                 | 81479           |
| UGT2B15 gene analysis, common variants                | 81479           |
| UGT1A4 gene analysis, common variants                 | 81479           |
| Unlisted multianalyte assay with algorithmic analysis | 81599           |
| Unlisted molecular pathology procedure                | 81479           |
| Unlisted chemistry procedure                          | 84999           |

## What is major depressive disorder

### Definition

Major depressive disorder (MDD) is a serious mental illness and one of the most

common mental disorders in the United States, carrying the heaviest burden of disability among all mental and behavioral disorders. In 2016, roughly 16 million adults in the United States experienced at least one major depression episode in the previous year; this number represented 6.7% of all adults in the United States.<sup>1</sup> A major depressive episode can include a number of symptoms, including depressed mood, insomnia or hypersomnia, change in appetite or weight, low energy, poor concentration, and recurrent thoughts of death or suicide, among other symptoms.

- Although mental health disorders are common in the United States, the burden of illness is concentrated among individuals with serious mental illness. In 2016, there were approximately 10.4 million adults in the United States with serious mental illness, representing 4% of all Americans. Serious mental illness (SMI) is defined as a mental, behavioral, or emotional disorder resulting in serious functional impairment, which substantially interferes with or limits one or more major life activities.<sup>2</sup> Serious mental illness can affect activities of daily living and may be accompanied by fatigue, insomnia, sudden weight loss, depressed mood, among other symptoms.
- Individuals with MDD experience high levels of recurrence; after recovery from one episode, the estimated risk of recurrence over a two year period is 40%. With each successive recurrence, the risk of a subsequent recurrence increases by 16%.<sup>3</sup>
- Treatment for MDD generally consists of a combination of psychotherapy (ie, cognitive behavioral therapy [CBT]) and pharmacotherapy (ie, antidepressants). The goal of treatment for MDD is primarily enabling remission of symptoms and restoring functioning.<sup>4</sup>
- To find the optimal treatment approach, many clinicians try different antidepressants to maximize treatment response and reduce risk of relapse. However, this “trial and error” approach is not always effective since the rates of remission are relatively low and vary considerably across individuals. Consequences of treatment failure include the continuation of disabling symptoms that adversely affect work productivity, social functioning, and increase the risk of suicide.<sup>5</sup>
- It is estimated that common genetic variants account for approximately 42.0% of individual differences in antidepressant response. The phenotype of antidepressant response is likely to be polygenic and involve a large number of SNPs with small effect sizes.<sup>6</sup>
- Pharmacogenomic testing has been developed to assist clinicians to predict those medications that could yield the most optimal treatment response and/or predict the lowest risk of side effects for an individual with mental health disorders, including MDD.
- Although this guideline will focus on the use of the GeneSight Psychotropic for management of major depressive disorder, it will apply broadly to pharmacogenomic testing for mental and behavioral health disorders. The focus of the guideline is guided by the preponderance of evidence (consisting of randomized or nonrandomized studies with control groups) in the peer-review literature available for the GeneSight test for the MDD disease indication.



## Test information

- Researchers in the field of psychiatric pharmacogenomics have identified single nucleotide polymorphisms (SNPs) within genes that affect an individual's metabolism and response to anti-depressant medications.
- These SNPs have been combined into a medication decision support tool, GeneSight Psychotropic.<sup>7</sup> Based on the composite phenotype measured for each patient, the GeneSight test has been proposed to assist clinicians in selecting psychotropic medication.<sup>8</sup> Pharmacogenomic testing may be most useful in psychiatric patients who have treatment resistance, intolerable adverse effects, or the potential for experiencing adverse events or contraindications.<sup>9</sup>
- GeneSight Psychotropic is a genetic panel that provides clinicians additional information about specific genetic variants to assist with decisions about drug selection regarding "psychotropic medications commonly prescribed to treat depression, anxiety, bipolar disorder, posttraumatic stress disorder (PTSD), obsessive compulsive disorder, schizophrenia and other behavioral health conditions." GeneSight tests for genetic variants in multiple pharmacokinetic and pharmacodynamic genes, which may impact drug tolerance and/or drug response. Specifically, the test currently analyzes 12 genes that may affect an individual's response to ~56 antidepressant and antipsychotic (psychotropic) medications (including 4 pharmacodynamic genes and 8 pharmacokinetic genes).
- Per a 2018 publication, "The combinatorial pharmacogenomic test (GeneSight Psychotropic, Assurex Health, OH, USA) included 65 alleles and variants across 12 genes: CYP1A2 (15 alleles), CYP2B6 (4 alleles), CYP2C9 (6 alleles), CYP2C19 (9 alleles), CYP2D6 (17 alleles and duplication), CYP3A4 (4 alleles), UGT1A4 (2 alleles), UGT2B15 (2 alleles), HTR2A (2 alleles), the long and short 5HTTLPR variants of the SLC6A4 serotonin transporter gene (2 alleles), HLA-A (\*3101 associated SNP rs1061235) and HLA-B (1 allele)."<sup>10</sup>
- Results of the GeneSight Psychotropic are detailed in a report provided to the clinician, describing the most common medications for the patient's diagnosed condition categorized by cautionary level. Each medication is placed into one of three color-coded categories: "Use as Directed" in green, "Moderate Gene-Drug Interaction" in yellow, or "Significant Gene-Drug Interaction" in red.<sup>7</sup>
- Additional pharmacogenomic panels or individual tests address treatment of mental health disorders, and are marketed by different labs or manufacturers. A few specific tests included in each panel are listed below:
  - Genecept™ Assay (Genomind)
  - SureGene Test for Antipsychotic and Antidepressant Response (STA2R)
  - Proove Opioid Risk panel (Proove Biosciences)
  - Mental Health DNA Insight™ panel (Pathway Genomics)
  - IDgenetix-branded tests
  - Empowering Personalized Medicine (EPM) Panel

- GeneSight ADHD, GeneSight Analgesic+
- Informed PGx ADHD, Informed PGx Depression, Informed PGx Psychotropic
- YouScript Personalized Prescribing System
- ABCB1 Genotyping
- CNSDose

## Guidelines and evidence

- The best available published evidence does not currently support the use of pharmacogenomic testing using the GeneSight Psychotropic test to aid in the treatment of the psychiatric disorders, specifically MDD.<sup>11-21</sup>
- In a large (n=1799), blinded, multicenter randomized controlled trial (RCT), the Genomics Used to Improve Depression Decisions (GUIDED) trial evaluated the effect of the GeneSight Psychotropic test compared with usual care on treatment selection in patients with major depressive disorder (MDD), who had failed at least one adequate medication trial. Patients were randomized to either treatment as usual (TAU) or GeneSight guided groups.<sup>22</sup>
  - For the primary endpoint, there were no statistically significant differences between GeneSight and TAU for the change in depression symptoms at 8 weeks. Also, there were no statistically significant differences in the mean number of side effects between the two groups at 8 weeks.
  - For the secondary endpoints of response and remission, the study results favored GeneSight-guided therapy over TAU. Response and remission significantly improved in the GeneSight-guided therapy arm versus TAU.
  - The lack of significant differences observed between groups for the primary endpoint indicate that a meaningful benefit of GeneSight to guide treatment and improve symptoms of MDD relative to usual care was not demonstrated.
  - Although significant improvements in the secondary endpoints were observed, additional well-designed clinical trials, powered on the primary endpoints of remission and/or response, are needed to confirm these findings.
  - Results of a post-hoc analyses suggest that GeneSight may have clinical utility to guide changes in treatment from less to more optimal drug therapies. However, these findings need to be replicated in a well-designed trial with a pre-specified subgroup analysis before the clinical utility of the GeneSight test can be established with certainty.
  - The study has a few notable limitations:
    - There appears to be considerable attrition. It is not clear if the sample size estimation included a specified dropout rate in the statistical plan.

- The primary endpoint was evaluated in the per-protocol (PP) population, rather than the intent-to-treat (ITT) population, which may have introduced selection bias. The PP approach did not appear to account for loss to follow-up. The ITT population should include all patients who were randomized into study groups.
  - Clinicians in the guided GeneSight arm were not required to adhere to the test result, and the number and basis of treatment decisions made in this arm were not reported.
  - The results of post-hoc analyses should be interpreted cautiously since hypotheses are typically generated after the analysis has been completed and results are subject to bias.
- No specific evidence-based U.S. testing guidelines were identified. However, the American Psychiatric Association (APA) Task Force for Novel Biomarkers and Treatments, a component of the APA Council on Research, stated that there is insufficient data to support the widespread use of pharmacogenomic tests in clinical practice to guide antidepressant treatment.<sup>23</sup>

## Criteria

- These tests are considered investigational and/or experimental.
  - Investigational and experimental (I&E) molecular and genomic (MolGen) tests refer to assays involving chromosomes, DNA, RNA, or gene products that have insufficient data to determine the net health impact, which typically means there is insufficient data to support that a test accurately assesses the outcome of interest (analytical and clinical validity), significantly improves health outcomes (clinical utility), and/or performs better than an existing standard of care medical management option. Such tests are also not generally accepted as standard of care in the evaluation or management of a particular condition.
  - In the case of MolGen testing, FDA clearance is not a reliable standard given the number of laboratory developed tests that currently fall outside of FDA oversight and FDA clearance often does not assess clinical utility.

## Billing and reimbursement considerations

- Due to these tests typically being performed as gene panels and reported out as associated risks using proprietary algorithms, individual CPT codes will also not be reimbursed under this guideline.
- If single gene testing is being requested and performed to determine an individual's response to a specific medication (e.g. CYP2D6, CYP2C19, etc), please see either the pharmacogenomic testing clinical use guideline or a test-specific guideline to determine criteria for coverage.

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# Polymerase Gamma (POLG) Related Disorders Genetic Testing

MOL.TS.276.A

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## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedures addressed by this guideline | Procedure codes |
|--|-----------------|
| POLG Known Familial Mutation Analysis  | 81403           |
| POLG Full Gene Sequencing              | 81406           |
| POLG Deletion/Duplication Analysis     | 81479           |

## What are POLG-related disorders

### Definition

“POLG-related disorders” is a term used to describe medical conditions caused by mutation in the POLG gene. This is a wide spectrum of conditions that may involve multiple organ systems and have variable severity and age at onset.<sup>1,2</sup>

### Incidence and Prevalence

Although Alpers-Huttenlocher syndrome (AHS) is clinically reported to occur in 1/51,000 individuals, disease frequency calculated based on prevalence of the most common POLG mutations may be as high as 1/10,000.<sup>1</sup>

### Symptoms

There are 6 main phenotypes attributed to POLG mutations. Most affected individuals have some features ascribed to each phenotype, but rarely have all.

- Alpers- Huttenlocher syndrome (AHS):<sup>3,4</sup>
  - Most common symptoms
    - refractory seizures
    - psychomotor regression
    - liver disease

- Other possible symptoms
  - migraine with visual auras
  - cortical blindness
  - hypotonia
  - ataxia
  - extrapyramidal movements
  - peripheral neuropathy
  - progressive spastic paraparesis
  - renal tubular acidosis
  - hearing loss
  - cyclic vomiting
  - pancreatitis
- Development is often normal until disease onset, which is typically before 4 years of age. However, congenital static encephalopathy and later juvenile-onset have also been described.<sup>2</sup> When seizure etiology is unknown, valproic acid must be used with extreme caution, as it can precipitate liver dysfunction and/or failure in AHS.<sup>5,6</sup>
- Childhood myocerebrohepatopathy spectrum (MCHS):<sup>7</sup>
  - Most common / presenting symptoms
    - failure to thrive
    - lactic acidosis
    - developmental delay
    - encephalopathy
    - dementia
    - myopathy
    - hypotonia
  - Other possible symptoms
    - liver failure
    - renal tubular acidosis
    - pancreatitis
    - cyclic vomiting

- hearing loss
- MCHS is a rapidly progressive disease with a fatal outcome that usually presents between the first few months of life and 3 years. MCHS has a similar presentation to AHS, however severe myopathy, specific liver pathology, and nonspecific brain MRI brain findings (diffuse atrophy) help differentiate MCHS from AHS. In addition, seizures are less prominent and more easily controlled in MCHS compared to AHS.
- Myoclonic epilepsy myopathy sensory ataxia (MEMSA):<sup>8</sup>
  - Common symptoms
    - epilepsy
    - myopathy
    - ataxia without ophthalmoplegia
  - MEMSA has also been known as spinocerebellar ataxia with epilepsy (SCAE). Disease onset typically occurs in adolescence and presents with cerebellar and sensory ataxia. Epilepsy usually follows, with refractory seizures leading to a progressive encephalopathy.
- Ataxia neuropathy spectrum (ANS):<sup>9</sup>
  - Common symptoms
    - migraine headaches
    - ataxia
    - neuropathy (sensory, motor, or mixed)
    - encephalopathy with seizures
    - psychiatric disturbance
  - Other possible symptoms
    - myoclonus
    - blindness
    - hearing loss
    - liver failure (varying severity)
  - Disease onset ranges between adolescence and adulthood. Migraine headaches may be the first presenting symptom and precede the other symptoms by many years. Clinical myopathy is very rare. The encephalopathy is often milder than AHS and more slowly progressive. ANS was previously referred to as mitochondrial recessive ataxia syndrome (MIRAS) and sensory ataxia neuropathy dysarthria and ophthalmoplegia (SANDO).



- Autosomal recessive progressive external ophthalmoplegia (arPEO):<sup>10</sup>
  - Common symptom
    - Progressive weakness of the extraocular eye muscles resulting in ptosis and ophthalmoparesis without associated systemic involvement.
  - Onset is typically in adulthood.
- Autosomal dominant progressive external ophthalmoplegia (adPEO):<sup>1,9</sup>
  - Common symptoms
    - progressive weakness of the extraocular eye muscles resulting in ptosis and ophthalmoparesis
    - generalized myopathy
    - sensorineural hearing loss
    - axonal neuropathy
    - ataxia
    - depression
    - Parkinsonism
    - hypogonadism
    - cataracts
  - Previously, adPEO was called Chronic Progressive External Ophthalmoplegia plus (CPEO+).
- Onset of the POLG-related disorders can range from infancy to late adulthood. Younger patients typically present with seizures and lactic acidosis.<sup>11</sup> Later in life, the most common presenting symptoms are myopathy, chronic progressive external ophthalmoplegia (CPEO), and sensory ataxia.<sup>11</sup> Liver failure may also occur, particularly with exposure to the antiepileptic drug, valproic acid.<sup>1</sup>

## Cause

POLG-related disorders are caused by mutations in the POLG gene. POLG codes for a subunit of DNA polymerase protein that replicates and repairs mitochondrial DNA (mtDNA). Disease-causing mutations can affect polymerase activity, processing, DNA binding, or subunit association.<sup>1</sup>

## Inheritance

Inheritance patterns of the 6 main POLG-related disorders varies.

- AHS, MCHS, MEMSA, ANS, and arPEO are inherited in an autosomal recessive inheritance pattern. Males and female are equally likely to be affected. If both parents are carriers of one of these conditions, the risk for a pregnancy to be affected is 1 in 4 (25%).
- adPEO is inherited in an autosomal dominant pattern. When a parent has this condition, each of her/his offspring have a 50% risk of inheriting the mutation.

## Diagnosis

As no clinical diagnostic criteria exist, genetic testing of POLG is required to confirm clinical suspicion of a disorder in this spectrum.

## Treatment

Treatment is supportive and based on presenting symptoms and typically involves referral for speech therapy, physical therapy, and occupational therapy. Respiratory and nutritional support are provided as needed.

Any medications metabolized by hepatic enzymes should be carefully dosed to avoid liver toxicity. Certain antiepileptic drugs should be avoided due to the risk for precipitating or accelerating liver disease.<sup>1</sup>

Occurrence of dehydration, fever, anorexia and infection can create physical stress and hasten medical deterioration. These events should be avoided as much as possible.

## Survival

The range of survival is broad and is largely dependent on the presenting phenotype, age at onset, and the occurrence of secondary complications.

## Test information

- Given that clinical diagnostic criteria do not exist, genetic testing of POLG is required in order to confirm the diagnosis of a POLG-related disorder.<sup>1</sup>
  - For individuals with suspected adPEO, identification of one POLG mutation is required to confirm the diagnosis.
  - For individuals presenting with clinical features consistent with one of the five other phenotypes, identification of two (biallelic) mutations is required to confirm the diagnosis.
- ***POLG Full Gene Sequencing*** can be performed to identify the remaining mutations in individuals with POLG-Related Disorders. Full sequencing is typically needed given that POLG-related disorders are mainly autosomal recessive conditions and the identification of two mutations in necessary for the diagnosis.

- **POLG Deletion/Duplication Analysis** can be performed if no mutations or only one mutation is found on targeted mutation analysis and/or full gene sequencing.
- **Multi-Gene Panels** - A number of large panels are available that sequence numerous nuclear-encoded mitochondrial genes for a broad approach to testing. Multi-gene panel tests, even for similar clinical scenarios, vary considerably laboratory by laboratory in the genes that are included and in technical specifications (e.g. depth of coverage, extent of intron/exon boundary analysis, methodology of large deletion/duplication analysis).
- While **biochemical analyses** of an affected tissue may be informative, they are not sensitive or specific enough to definitively diagnose a POLG-related disorder. Muscle biopsy can be completely normal in children and adults with a POLG-related disorder and in clinically unaffected tissue.<sup>12</sup>

## Guidelines and evidence

- The Food and Drug Administration (FDA, 2017) states that Depakene (valproate) and Depakote ER (divalproex sodium) are contraindicated for patients known to have mitochondrial disorders caused by POLG mutations and children under two years of age who are clinically suspected of having a mitochondrial disorder:<sup>13</sup>
  - “Valproate-induced acute liver failure and liver-related deaths have been reported in patients with hereditary neurometabolic syndromes caused by mutations in the gene for mitochondrial DNA polymerase  $\gamma$  (POLG) (e.g., Alpers-Huttenlocher Syndrome) at a higher rate than those without these syndromes. Most of the reported cases of liver failure in patients with these syndromes have been identified in children and adolescents.”
  - “POLG-related disorders should be suspected in patients with a family history or suggestive symptoms of a POLG-related disorder, including but not limited to unexplained encephalopathy, refractory epilepsy (focal, myoclonic), status epilepticus at presentation, developmental delays, psychomotor regression, axonal sensorimotor neuropathy, myopathy, cerebellar ataxia, ophthalmoplegia, or complicated migraine with occipital aura. POLG mutation testing should be performed in accordance with current clinical practice for the diagnostic evaluation of such disorders. The A467T and W748S mutations are present in approximately 2/3 of patients with autosomal recessive POLG-related disorders.”
- Although not specific to genetic testing for POLG, the Mitochondrial Medicine Society (2015)<sup>14</sup> developed consensus recommendations for the diagnosis and management of mitochondrial disease. Testing strategies, including strategies for genetic testing, were discussed.
  - Recommendations for DNA testing include the following:

POLG

- “When considering nuclear gene testing in patients with likely primary mitochondrial disease, NGS methodologies providing complete coverage of known mitochondrial disease gene is preferred. Single-gene testing should usually be avoided because mutations in different genes can produce the same phenotype. If no mutation is identified via known NGS panels, then whole exome sequencing should be considered.”
- The European Federation of Neurological Sciences/European Neurological Society (EFNS/ENS) 2014 consensus guidelines on the diagnosis and management of chronic ataxias in adulthood recommend POLG testing in the following evaluation of individuals with autosomal recessive cerebellar ataxia:<sup>15</sup>
  - “Step 1: mutation analysis of the FRDA gene for Friedreich’s ataxia (although one can refrain from this in the case of severe cerebellar atrophy), and biochemical testing that includes cholestanol, vitamin E, cholesterol, albumin, creatine kinase (CK) and a-fetoprotein. Also consider doing nerve conduction studies/EMG (presence versus absence of peripheral neuropathy, axonal versus demyelinating) and referral to an ophthalmologist (retinitis pigmentosa, cataract, cherry red spot etc.) (Table S2) (good practice point).”
  - “Step 2: mutation analysis of the SACS, POLG, Aprataxin (APTX) and SPG7 genes (taking into account specific phenotypes, as given in Table S2), and biochemical testing for white cell enzymes, phytanic acid and long chain fatty acids (good practice point).”
  - “Step 3: referral to a specialized centre, e.g. for skin or muscle biopsy targeted at diagnoses such as Niemann - Pick type C, recessive ataxia with coenzyme Q deficiency [aarF domain containing kinase 3 (ADCK3)/autosomal recessive spinocerebellar ataxia 9 (SCAR9)] and mitochondrial disorders, or for extended genetic screening using gene panel diagnostics (good practice point).”
- A 2014 expert-authored review suggests the following testing strategy for those with a known or suspected diagnosis of a POLG related disorder:<sup>1</sup>
  - “Standard clinical investigations can identify findings that, in the context of an appropriate family history, can suggest one of the POLG-related phenotypes.”
  - “Confirmation of the diagnosis of a POLG-related disorder requires identification of POLG pathogenic variants by molecular genetic testing.”
  - “One of the following two approaches can be used:”
    - “Direct sequencing of POLG”
    - “Two tiered analysis: targeted analysis for the common POLG pathogenic variants p.Ala467Thr, p.Trp748Ser, and p.Gly848Ser, followed by sequence analysis of the entire coding region if no pathogenic variants or only one pathogenic variant is found.”
  - “In persons meeting the diagnostic criteria of an autosomal recessive POLG-related disorder but in whom sequence analysis identifies only one disease-

causing 'POLG' allele, further testing may be considered to search for a second pathogenic variant in regulatory regions (e.g., the POLG promoter) or in related mitochondrial DNA replication genes such as C10orf2 (formerly PEO1; (encodes the twinkle helicase) and POLG2 to investigate the possibility of digenic inheritance. ”

- “Digenic inheritance has been reported in arPEO in a simplex case with pathogenic variants in POLG and C10orf2.”
- “Oligonucleotide array should be strongly considered as microdeletions involving intragenic regions of POLG are reported and therefore relevant in a symptomatic individual with a single heterozygous pathogenic variant.”
- “An alternative genetic testing strategy is use of a multi-gene panel that includes POLG and other genes of interest.”

## Criteria

### Known POLG Family Mutation Testing

- Genetic Counseling:
  - Pre and post-test counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Diagnostic Testing for Symptomatic Individuals
  - No previous genetic testing of POLG, and
  - If adPEO is suspected:
    - Clinical examination is consistent with a diagnosis of adPEO, and
    - POLG mutation identified in 1<sup>st</sup> degree biological relative, OR
  - If AHS, MCHS, MEMSA, ANS, or arPEO is suspected:
    - Clinical examination is consistent with a diagnosis of AHS, MCHS, MEMSA, ANS, or arPEO, and
    - Two POLG mutations identified in a sibling, or
    - One POLG mutation identified in both parents

### POLG Full Gene Sequencing

- Genetic Counseling:
  - Pre and post-test counseling by an appropriate provider (as deemed by the Health Plan policy), AND

- Previous Testing:
  - No previous genetic testing for POLG, and
  - No known POLG mutation in the family, AND
- Diagnostic Testing for Symptomatic Individuals:
  - If adPEO is suspected:
    - Clinical examination is consistent with a diagnosis of adPEO, and
    - Genetic testing is needed to confirm the diagnosis, OR
  - If AHS, MCHS, MEMSA, ANS, or arPEO is suspected:
    - Clinical examination is consistent with a diagnosis of AHS, MCHS, MEMSA, ANS, or arPEO, and
    - Genetic testing is needed to confirm the diagnosis, OR
  - If evaluating the risk for valproate-induced hepatic toxicity:
    - The member has epilepsy, and
    - There is suspicion for a POLG-related disorder based on the presence of at least one of the following:
      - unexplained encephalopathy, or
      - refractory epilepsy, or
      - status epilepticus at presentation, or
      - developmental delays, or
      - psychomotor regression, or
      - axonal sensorimotor neuropathy, or
      - myopathy and/or hypotonia, or
      - progressive spastic paraparesis, or
      - renal tubular acidosis, or
      - sensorineural hearing loss, or
      - cyclic vomiting, or
      - pancreatitis, or
      - cerebellar ataxia, or
      - ophthalmoplegia and/or ptosis, or
      - complicated migraine with occipital aura, and

- The member is currently on Depakene (valproate) or Depakote ER (divalproex sodium) therapy, or the use of one of these medications is being proposed.

### **POLG Deletion/Duplication Analysis**

- Genetic Counseling:
  - Pre and post-test counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Criteria for POLG Full Gene Sequencing is met, AND
- If adPEO is suspected:
  - No mutations found on POLG Full Gene Sequencing, OR
- If AHS, MCHS, MEMSA, ANS, or arPEO is suspected:
  - No mutations or only one mutation found on POLG Full Gene Sequencing, OR
- If evaluating the risk for valproate-induced hepatic toxicity:
  - No mutations or only one mutation found on POLG Full Gene Sequencing

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# Prader-Willi Syndrome Testing

MOL.TS.217.A

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## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedures addressed by this guideline             | Procedure codes |
|--|-----------------|
| Chromosome 15 Uniparental Disomy                   | 81402           |
| Chromosomal Microarray [BAC], Constitutional       | 81228           |
| Chromosomal Microarray [SNP], Constitutional       | 81229           |
| Chromosomal Microarray [CGH], Constitutional       | S3870           |
| FISH Probe for 15q11-q13 Deletion                  | 88271           |
| SNRPN/UBE3A Methylation Analysis                   | 81331           |
| Imprinting Center Defect Analysis                  | 81479           |
| Imprinting Center Known Familial Mutation Analysis | 81403           |

## What is Prader-Willi syndrome

### Definition

Features of Prader-Willi syndrome are caused when the Prader-Willi critical region (PWCR) on chromosome 15 is only inherited from the mother and there is no copy from the father. Prader-Willi syndrome can be caused by a chromosome deletion, uniparental disomy (two copies of the maternal chromosome), or imprinting defect. There are several genetic tests available that can help diagnose Prader-Willi syndrome.<sup>1,2</sup>

- Prader-Willi syndrome (PWS) is characterized by:<sup>1</sup>
  - Decreased muscle tone (hypotonia) and feeding difficulties in early infancy
  - Insatiable appetite in childhood that often results in obesity
  - Developmental delay

- Short stature
- Behavior problems
- Small hands and feet
- Underdeveloped genitalia and infertility

## Test information

- ***SNRPN Methylation Analysis:*** This test is typically the first test in the evaluation of both Angelman syndrome and Prader-Willi syndrome. It will detect about 80% of patients with Angelman syndrome and >99% of patients with Prader-Willi syndrome. However, DNA methylation analysis does not identify the underlying cause, which is important for determining the risk to future siblings. This risk ranges from less than 1% to up to 50%, depending on the genetic mechanism. Follow-up testing for these causes may be appropriate.
- ***Chromosomal Microarray or FISH Analysis for 15q11-q13 Deletion:*** If DNA methylation analysis for Angelman (AS) or Prader-Willi syndrome (PWS) is abnormal, deletion analysis is typically the next step. Approximately 70% of cases of both AS and PWS have a deletion in one copy of chromosome 15 involving the 15q11.2-q13 region. When looking specifically for this deletion, FISH (fluorescence in situ hybridization) analysis is most commonly performed. However, chromosomal microarray can also detect such deletions. If chromosomal microarray (CMA, array CGH) has already been done, FISH is not likely to be necessary.
- ***Chromosome 15 Uniparental Disomy (UPD):*** If DNA methylation analysis is abnormal but deletion analysis is normal, UPD analysis next may be appropriate for evaluation of both Angelman (AS) and Prader-Willi syndrome (PWS). About 28% of PWS cases are due maternal UPD (both chromosome 15s are inherited from the mother). Both parents must be tested to diagnose UPD.
- ***Imprinting Center Defect Analysis:*** This test may be considered in the evaluation of Angelman syndrome (AS) and Prader-Willi syndrome (PWS) when methylation is abnormal, but FISH (or array CGH) and UPD studies are normal. Individuals with such results are presumed to have an imprinting defect. An abnormality in the imprinting process has been described in a minority of cases. However, imprinting center deletions may be familial, and if familial, the recurrence risk can be up to 50%.
- ***Imprinting Center Known Familial Mutation Analysis:*** If a mutation in the imprinting center has been identified in an affected family member, testing for just the known familial mutation in the imprinting center can be performed for at-risk relatives, including at-risk pregnancies.

## Guidelines and evidence

- The Prader-Willi Syndrome Association (2016) recommends the following test strategy when physical exam and family history suggest the diagnosis of PWS.<sup>2</sup>
  - Methylation analysis will detect greater than 99% of individuals with PWS including those with deletion, uniparental disomy, or imprinting defect.
    - If methylation testing is abnormal, it confirms the clinical diagnosis. However, to help determine whether there are risks of PWS in other family members it may be necessary to perform FISH, UPD and/or Imprinting Center testing to determine the exact cause of the abnormal methylation.
  - Deletion analysis (FISH 15q11-q13 or chromosomal microarray)
    - If deletion testing is abnormal (70% of individuals with PWS will have a deletion) chromosome analysis may be considered to rule out a familial chromosome rearrangement (rare).
    - If deletion testing is normal, it is appropriate to consider UPD analysis.
  - Uniparental Disomy (UPD) analysis of chromosome 15 determines if the patient inherited both copies of chromosome 15 from the mother.
  - If methylation analysis is abnormal, but FISH and UPD analysis are normal, it is usually assumed there is an imprinting center mutation (which carries a higher recurrence risk than other causes). There is limited clinical testing available.<sup>1,3</sup>
- The 2017 Gene Reviews article on Prader-Willi Syndrome states:<sup>1</sup>
  - “DNA methylation-specific testing is important to confirm the diagnosis of PWS in all individuals, but especially in those who have atypical findings or are too young to manifest sufficient features to make the diagnosis on clinical grounds.”
  - Abnormal methylation is sufficient to establish clinical diagnosis, but additional testing is needed to establish the mechanism of disease and recurrent risk.

## Criteria

### SNRPN Methylation Analysis

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Testing:
  - No previous SNRPN methylation analysis, AND
- Diagnostic Testing for Symptomatic Individuals:

- Developmental delay or intellectual disability, and
- Some combination of the following:
  - Neonatal hypotonia, or
  - Feeding problems (i.e., poor suck) or poor growth in infancy, or
  - Obesity and/or food-related behavior problems (i.e., hyperphagia; obsession with food), or
  - Characteristic facial features, or
  - Hypogonadism AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

### **Deletion analysis (FISH Analysis for 15q11-q13 Deletion or chromosomal microarray)**

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Testing:
  - No previous 15q11-q13 deletion analysis, and
  - No previous chromosomal microarray, AND
- Diagnostic Testing for Symptomatic Individuals:
  - Developmental delay or intellectual disability, and
  - Some combination of the following:
    - Neonatal hypotonia, or
    - Feeding problems (i.e., poor suck) or poor growth in infancy, or
    - Obesity and/or food-related behavior problems (i.e., hyperphagia; obsession with food) or
    - Characteristic facial features, or
    - Hypogonadism, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

### **Chromosome 15 Uniparental Disomy**

- Genetic Counseling:

- Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Testing:
  - SNRPN methylation analysis results are abnormal, and
  - 15q11-q13 deletion analysis is negative, and
  - No previous chromosome 15 UPD studies, AND
- Diagnostic Testing for Symptomatic Individuals:
  - Developmental delay or intellectual disability, and
  - Some combination of the following:
    - Neonatal hypotonia, or
    - Feeding problems (i.e., poor suck) or poor growth in infancy, or
    - Obesity and/or food-related behavior problems (i.e., hyperphagia; obsession with food), or
    - Characteristic facial features, or
    - Hypogonadism AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

### **Imprinting Center Defect Analysis**

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Testing:
  - SNRPN methylation analysis results are abnormal, and
  - 15q11-q13 deletion analysis is negative, and
  - Previous chromosome 15 UPD studies negative, and
  - No previous imprinting center (IC) analysis, AND
- Diagnostic Testing for Symptomatic Individuals:
  - Developmental delay or intellectual disability, and
  - Some combination of the following:
    - Neonatal hypotonia, or

- Feeding problems (i.e., poor suck) or growth failure in infancy, or
  - Obesity and/or food-related behavior problems (i.e., hyperphagia; obsession with food), or
  - Characteristic facial features, or
  - Hypogonadism AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy

### **Imprinting Center Known Familial Mutation Analysis**

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Testing:
  - No previous imprinting center defect analysis testing, AND
- Family History:
  - Familial imprinting center defect mutation known in blood relative, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

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# Prenatal Aneuploidy FISH Testing

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## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedures addressed by this guideline | Procedure codes         |
|--|-------------------------|
| FISH Analysis for Aneuploidy           | 88271<br>88274<br>88275 |

## What is a chromosome abnormality

### Definition

A chromosome abnormality is any difference in the structure, arrangement, or amount of genetic material packaged into the chromosomes.<sup>1</sup> Aneuploidy refers to an abnormal number of chromosomes (i.e. extra or missing).<sup>1</sup>

- Humans usually have 23 pairs of chromosomes. Each chromosome has a characteristic appearance that should be the same in each person.
- Chromosome abnormalities can lead to a variety of developmental and reproductive disorders. Common chromosome abnormalities that affect development include: Down syndrome (trisomy 21), trisomy 18, trisomy 13, Turner syndrome, and Klinefelter syndrome.
- About 1 in 200 newborns has some type of chromosome abnormality<sup>2</sup> and a higher percentage of pregnancies are affected but lost during pregnancy. According to the American College of Obstetricians and Gynecologists (ACOG), "Fetuses affected with Down syndrome often do not survive pregnancy; between the first trimester and full term, an estimated 43% of pregnancies end in miscarriage or stillbirth."<sup>3</sup>
- The risk of having a child with an extra chromosome, notably Down syndrome, increases as a woman gets older.<sup>3</sup> However, many babies with Down syndrome are born to women under 35 and the risk of having a child with other types of chromosome abnormalities, such as Turner syndrome, is not related to maternal age. Therefore, prenatal screening for Down syndrome and certain other chromosome abnormalities is now routinely offered to all pregnant women. Prenatal diagnostic testing via amniocentesis or chorionic villus sampling is also an option

for any woman, although it is most commonly accepted by women with recognized risk factors.<sup>3</sup>

## Test information

- Fluorescence in situ hybridization (FISH) can be used to assess how many copies of a chromosome or smaller piece of DNA is in a cell.
  - FISH uses fluorescent probes that bind only to certain regions of a chromosome.
  - After binding, these fluorescent signals can be viewed by microscopy and counted in a sample of cells to determine if the appropriate number of copies is present.
  - Because chromosomes come in pairs, most normal cells will have two fluorescent signals for each probe.
- FISH analysis of prenatal samples (amniocentesis and CVS) is widely available for the chromosomes that are most commonly involved in prenatal chromosome abnormalities: 13, 18, 21, X, and Y.<sup>4</sup>
  - FISH does not require dividing cells like conventional karyotyping. Therefore, results are generally available much more quickly (often within 2 days of the procedure) than for standard chromosome analysis (which usually takes at least 7 days).
  - While FISH results have been shown to be highly accurate, most experts recommend that no irreversible decisions be made unless the FISH results are either confirmed by karyotyping or the abnormal result fits with the remainder of the clinical findings (e.g., ultrasound anomalies are consistent with the particular chromosome abnormality).<sup>3,4</sup>

## Guidelines and evidence

- The American College of Obstetricians and Gynecologists (ACOG, 2016) issued prenatal diagnosis guidelines recommending the following:<sup>5</sup>
  - “All pregnant women should be offered prenatal assessment for aneuploidy by screening or diagnostic testing regardless of maternal age or other risk factors.”
  - ACOG recommended the following in regards to FISH testing:<sup>5</sup>
    - “When structural abnormalities are detected by prenatal ultrasound examination, chromosomal microarray will identify clinically significant chromosomal abnormalities in approximately 6% of the fetuses that have a normal karyotype. For this reason, chromosomal microarray analysis should be recommended as the primary test (replacing conventional karyotype) for



patients undergoing prenatal diagnosis for the indication of a fetal structural abnormality detected by ultra- sound examination. If a structural abnormality is strongly suggestive of a particular aneuploidy in the fetus (e.g., duodenal atresia or an atrioventricular heart defect, which are characteristic of trisomy 21), karyotype with or without FISH may be offered before chromosomal microarray analysis.”

- “An abnormal FISH result should not be considered diagnostic. Therefore, clinical decision making based on information from FISH should include at least one of the following additional results: confirmatory traditional metaphase chromosome analysis or chromosomal microarray, or consistent clinical information (such as abnormal ultrasonographic findings or a positive screening test result for Down syndrome or trisomy 18).”
- The American College of Medical Genetics (ACMG) and the American Society of Human Genetics (ASHG) issued a joint position statement on FISH in 2000. For prenatal FISH application, they state:<sup>4</sup>
  - “For management of the fetus, it is reasonable to report positive FISH test results. Clinical decision-making should be based on information from two of three of the following: positive FISH results, confirmatory chromosome analysis, or consistent clinical information.”

## Criteria

Testing with aneuploidy FISH is allowed once per pregnancy when at least one of the following indicates an increased risk for a chromosome abnormality:

- Screening result suggests aneuploidy
- Advanced maternal age
- One major or at least two minor fetal structural defects found on ultrasound
- Previous fetus or child with aneuploidy
- Parent of this pregnancy has a structural chromosome abnormality (e.g., translocation, inversion) involving chromosome 21, 13, 18, X, or Y
- Parent of this pregnancy has an extra chromosome (e.g., Down syndrome, XXX syndrome, Klinefelter syndrome)

## Billing and Reimbursement Considerations

FISH testing (procedures codes defined in this policy) is presumed to be performed for prenatal diagnosis when:

- 88271 is billed on the same date of service as procedure code 88235 (Tissue culture for non-neoplastic disorders; amniotic fluid or chorionic villus cells), and

- 88271 is billed with 5 or more units

When FISH testing is performed for prenatal diagnosis, it will be coverable when:

- The age of the person having the procedure is 34 years or older at date of service, or
- An ICD code is present that indicates an increased risk of chromosome abnormality as defined in the Increased Risk for Chromosome Abnormality ICD Codes table.

**Table: Increased Risk for Chromosome Abnormality ICD Codes**

| ICD code(s) | Description  |
|-------------|--|
| O02.X       | Other abnormal products of conception  |
| O09.5       | Supervision of elderly primigravida and multigravida                           |
| O28.X       | Abnormal findings on antenatal screening of mother                             |
| O35.0       | Maternal care for (suspected) central nervous system malformation in fetus     |
| O35.1       | Maternal care for (suspected) chromosomal abnormality in fetus                 |
| O35.2       | Maternal care for (suspected) hereditary disease in fetus                      |
| R93.8       | Abnormal findings on diagnostic imaging of body structures                     |
| Q00.0-Q99.9 | Chromosomal abnormalities, not elsewhere classified                            |
| Z82.79      | Fam hx of congenital malformations, deformations and chromosomal abnormalities |

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# Prenatal Maternal Serum Screening

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## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedures covered by this guideline                               | Procedure codes |
|--|-----------------|
| Prenatal Maternal Serum Screening, Two Markers                     | 81508           |
| Prenatal Maternal Serum Screening, Three Markers, First Trimester  | 81509           |
| Prenatal Maternal Serum Screening, Three Markers, Second Trimester | 81510           |
| Prenatal Maternal Serum Screening, Four Markers                    | 81511           |
| Prenatal Maternal Serum Screening, Five Markers                    | 81512           |

## What is prenatal maternal serum screening

### Definition

Some factors predict an increased risk for Down syndrome and NTDs, such as maternal age, family history, and maternal diabetes or seizure disorder. However, there are no recognizable risk factors to explain the vast majority of babies born with these birth defects.<sup>4,5</sup> As a result, prenatal screening to identify affected pregnancies is routinely offered to all pregnant women.<sup>5,6</sup>

- About 3% of pregnancies have some type of birth defect.<sup>1</sup> Down syndrome and neural tube defects (NTDs) are among the most common serious birth defects. Down syndrome affects about 1 in 700 live births.<sup>2</sup> NTDs, such as spina bifida and anencephaly, affect about 3000 pregnancies per year in the United States.<sup>3,4</sup>
- While not the focus of maternal serum screening programs, other birth defects (such as abdominal wall and heart defects) and general risks for poor pregnancy outcome may also be identified.

## Test information

- Prenatal screening relies on maternal serum markers, and sometimes nuchal translucency ultrasound data (ACOG recommended technique when available)<sup>6</sup> to predict a pregnancy's risk for Down syndrome, open neural tube defects, and other rarer birth defects such as trisomy 18.

Typical marker patterns for these birth defects are seen in the first and second trimesters. Measurements are provided as multiples of the median (MoM), which compare results to normal population medians. Therefore, values are higher or lower relative to 1.0. Risk assessment algorithms evaluate several factors, so pregnancies may be at-risk without each marker being abnormal.

- Screening results are generally reported as “screen positive” for Down syndrome or trisomy 18 if the predicted risk exceeds a laboratory-determined risk cut-off (often about 1 in 270 for Down syndrome and 1 in 100 for trisomy 18). A pregnancy is screen-positive for neural tube defect if the maternal serum AFP exceeds a cut-off, which is usually 2.5 MoM.<sup>4</sup> However, different MoM calculations or cut-offs may be used for those with recognized risk factors or multiple gestations.<sup>7</sup>

## Guidelines and evidence

- Practice guidelines from the American College of Obstetricians and Gynecologists (ACOG, 2016) address prenatal screening for chromosome abnormalities. ACOG recommends the following:
  - “Aneuploidy screening or diagnostic testing should be discussed and offered to all women early in pregnancy, ideally at the first prenatal visit.” [evidence level C: “consensus and expert opinion”]<sup>6</sup>
  - “All women should be offered the option of aneuploidy screening or diagnostic testing for fetal genetic disorders, regardless of maternal age.” [evidence level C]<sup>6</sup>
  - Several other level A and B recommendations are made about test effectiveness, choice, patient counseling, and follow-up.
- The American College of Medical Genetics (ACMG)<sup>7</sup> and the American Academy of Family Physicians (AAFP)<sup>5</sup> have also published prenatal screening statements similar to ACOG's recommendations.
- While the ACOG guidelines focus primarily on Down syndrome screening, they do include this recommendation about ONTD screening: “Women who undergo first-trimester screening should be offered second-trimester assessment for open fetal defects (by ultrasonography, MSAFP screening, or both) and ultrasound screening for other fetal structural defects.” [evidence level A]<sup>6</sup> A 2003 ACOG practice guideline more directly addressed NTD screening: “Maternal serum alpha-fetoprotein evaluation is an effective screening test for NTDs and should be offered to all pregnant women.” [evidence level A]<sup>4</sup>

## Criteria

Screening for aneuploidy by ONE of the following methods is covered one time per pregnancy:

- First trimester screening – Total or free beta-HCG and PAPP-A levels performed on a maternal serum sample performed in conjunction with an ultrasound measurement of fetal nuchal translucency (NT)\*\*
- Second trimester screening – human chorionic gonadotropin (hCG), alpha-fetoprotein (AFP), unconjugated estriol (uE3), and dimeric inhibin-A (DIA) performed on a maternal serum sample
- Integrated, step-wise sequential, or contingent sequential screening – combines results of first and second trimester screening in various testing algorithms.

\*\*Limits on prenatal ultrasonography will depend on the insurer's ultrasound coverage policy and are outside the scope of this program.

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# Prolaris

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## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedures addressed by this guideline | Procedure codes |
|--|-----------------|
| Prolaris                               | 81541           |

## What are gene expression profiling tests for prostate cancer

### Definition

Prostate cancer (PC) is the most common cancer and a leading cause of cancer-related deaths worldwide. It is considered a heterogeneous disease with highly variable prognosis.<sup>1</sup>

- High-risk prostate cancer (PC) patients treated with radical prostatectomy (RP) undergo risk assessment to assess future disease prognosis and determine optimal treatment strategies. Post-RP pathology findings, such as disease stage, baseline Gleason score, time of biochemical recurrence (BCR) after RP, and PSA doubling-time, are considered strong predictors of disease-associated metastasis and mortality. Following RP, up to 50% of patients have pathology or clinical features that are considered at high risk of recurrence and these patients usually undergo post-RP treatments, including adjuvant or salvage therapy or radiation therapy, which can have serious risks and complications. According to clinical practice guideline recommendations, high risk patients should undergo 6 to 8 weeks of radiation therapy (RT) following RP. However, approximately 90% of high-risk patients do not develop metastases or die of prostate cancer, and instead may be appropriate candidates for alternative treatment approaches, including active surveillance (AS). As such, many patients may be subjected to unnecessary follow-up procedures and their associated complications, highlighting the need for improved methods of prognostic risk assessment.<sup>2,3</sup>
- Several genomic biomarkers have been commercially developed to augment the prognostic ability of currently available routine clinical and pathological tests and identify those patients most and least likely to benefit from a specific treatment strategy. Prognostic genomic tests, including gene expression profiling tests, may help to avoid overtreatment by reclassifying those men originally identified as high risk, but who are unlikely to develop metastatic disease. Genomic biomarkers may

also play a role in assisting clinicians to tailor personalized and more appropriate treatments for subgroups of PC patients, and improve overall health outcomes.<sup>2,3</sup>

## Test information

- Gene expression profiles (GEPs) evaluate the expression of several genes using one sample. Gene expression is determined through RNA analysis, using either reverse transcriptase (RT) polymerase chain reaction (PCR) or DNA microarrays.<sup>4</sup>
- Prolaris<sup>®</sup> (Myriad<sup>®</sup> Genetics)<sup>5</sup>
  - According to the manufacturer, Prolaris is a genomic test developed to predict PC-specific mortality in PC patients after needle biopsy, as well as post-RP patients to assess the risk of BCR. This test is designed to assist clinicians with predicting tumor aggressiveness combined with clinical and pathologic variables (Gleason score, PSA).

## Guidelines and evidence

### National Comprehensive Cancer Network

- The National Comprehensive Cancer Network (NCCN) 2018 Clinical Practice Guidelines on Prostate Cancer state the following regarding molecular assays:<sup>6</sup>
  - “Men with low or favorable intermediate risk disease may consider the use of the following tumor-based molecular assays: Decipher, Oncotype DX Prostate, Prolaris, Promark. Retrospective studies have shown that molecular assays performed on prostate biopsy or radical prostatectomy specimens provide prognostic information independent of NCCN risk groups.”
  - According to NCCN, the Molecular Diagnostic Services Program (MoIDX) recommendations stated the following:<sup>6</sup>
    - Decipher: “Cover post-RP for 1) pT2 with positive margins; 2) any pT3 disease; 3) rising PSA (above nadir)”
    - Prolaris: “Cover post-biopsy for NCCN very-low, low-risk, and favorable intermediate-risk prostate cancer in patients with at least 10 years life expectancy who have not received treatment for prostate cancer and are candidates for active surveillance or definitive therapy.”
    - Oncotype DX Prostate: “Cover post-biopsy for NCCN very-low, low-risk, and favorable intermediate-risk prostate cancer in patients with at least 10 years life expectancy who have not received treatment for prostate cancer and are candidates for active surveillance or definitive therapy.”
    - ProMark: “Cover post-biopsy for NCCN very-low and low-risk prostate cancer in patients with at least 10 years life expectancy who have not



received treatment for prostate cancer and are candidates for active surveillance or definitive therapy.”

- “These molecular biomarker tests have been developed with extensive industry support, guidance, and involvement, and have been marketed under the less rigorous FDA regulatory pathways for biomarkers. Although full assessment of their clinical utility requires prospective randomized clinical trials, which are unlikely to be done, the panel believes that men with low or favorable intermediate disease may consider the use of Decipher, Oncotype DX Prostate, Prolaris, or ProMark during initial risk stratification.”

### **American Association of Clinical Urologists**

The American Association of Clinical Urologists has issued a position statement on genomic testing in prostate cancer that states the following:<sup>7</sup>

- “The AACU supports the use of tissue-based molecular testing as a component of risk stratification in prostate cancer treatment decision making.”

### **American Urological Association, ASTRO, and the Society of Urologic Oncology**

The AUA/ASTRO/SUO guideline for clinically localized prostate cancer states the following:<sup>8</sup>

- “Among most low-risk localized prostate cancer patients, tissue based genomic biomarkers have not shown a clear role in the selection of candidates for active surveillance.”

### **Prolaris**

Prolaris Literature Review<sup>9-21</sup>

- Clinical studies published by the manufacturer suggest that Prolaris may have potential prognostic value in patients with localized prostate cancer and following RP. However, it is not certain if use of Prolaris improves risk assessment information provided by conventional clinicopathologic variables, following conservative management or after surgery. It also remains uncertain if use of Prolaris in clinical practice leads to changes in clinically appropriate disease management strategies and subsequent improvement in patient-relevant health outcomes.
- Several limitations characterizing the evidence base weaken the strength of these findings. The available studies focused on primarily evaluating associations between results of Prolaris and the incidence of disease recurrence or mortality, which represents a preliminary stage of development of prognostic tests. The most appropriate clinical decisions to be made based on Prolaris test results have not been clearly established since there are no published studies that have reported the ability of the Prolaris test to prospectively predict patient-relevant health outcomes by virtue of prognostic risk assessment or changes made to treatment

recommendations. The evidence base may also be subject to publication bias. With one exception, the reviewed studies with consistently positive or favorable results were sponsored or funded by the test manufacturer. The single study not funded by the manufacturer, which examined the ability of Prolaris to predict tumor grade and stage following surgery, reported that 20 of 52 patients were misclassified by the Prolaris test (using clinicopathologic variables as the reference standard), suggesting that use of the test may be misleading in some cases.

- In some cases, study follow-up was very short, and may was not sufficiently long enough to capture metastatic event data, particularly among men with localized disease who have low rates of mortality. In addition, the total number of identified cases in each study was relatively small, which limits the generalizability of study results to a heterogeneous patient population usually observed in the real world.

## Clinical Trials

### Long-term Study to Evaluate and Clinical Outcomes in patients with Favorable Intermediate Risk Localized Prostate Cancer<sup>22</sup>

- “This is a long-term prospective registry study to determine whether Prolaris testing in patients with favorable intermediate risk prostate cancer influences physician management decisions toward conservative treatment in patients with Prolaris low-risk scores without negatively impacting patient oncologic outcomes, thereby sparing low-risk patients from unnecessary treatments and associated side-effects.”
- NCT03290508
- Recruiting

### Prospective Prolaris Value and Efficacy<sup>23</sup>

- “This is a prospective study to measure the impact on first-line therapy of genomic testing of biopsy tissue from recently diagnosed treatment-naïve patients with early stage localized prostate cancer.”
- NCT03152448
- Recruiting

## Criteria

- This test is considered investigational and/or experimental.
  - Investigational and experimental (I&E) molecular and genomic (MolGen) tests refer to assays involving chromosomes, DNA, RNA, or gene products that have insufficient data to determine the net health impact, which typically means there is insufficient data to support that a test accurately assesses the outcome of interest (analytical and clinical validity), significantly improves health outcomes (clinical utility), and/or performs better than an existing standard of care medical

management option. Such tests are also not generally accepted as standard of care in the evaluation or management of a particular condition.

- In the case of MolGen testing, FDA clearance is not a reliable standard given the number of laboratory developed tests that currently fall outside of FDA oversight and FDA clearance often does not assess clinical utility.

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# ProMark Proteomic Prognostic Test

MOL.TS.296.A

v1.0.2020

## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedures addressed by this guideline | Procedure codes |
|--|-----------------|
| ProMark Proteomic Prognostic Test      | 81479           |

## What are gene expression profiling tests for prostate cancer

### Definition

Prostate cancer (PC) is the most common cancer and a leading cause of cancer-related deaths worldwide. It is considered a heterogeneous disease with highly variable prognosis.<sup>1</sup>

- High-risk prostate cancer (PC) patients treated with radical prostatectomy (RP) undergo risk assessment to assess future disease prognosis and determine optimal treatment strategies. Post-RP pathology findings, such as disease stage, baseline Gleason score, time of biochemical recurrence (BCR) after RP, and PSA doubling-time, are considered strong predictors of disease-associated metastasis and mortality. Following RP, up to 50% of patients have pathology or clinical features that are considered at high risk of recurrence and these patients usually undergo post-RP treatments, including adjuvant or salvage therapy or radiation therapy, which can have serious risks and complications. According to clinical practice guideline recommendations, high risk patients should undergo 6 to 8 weeks of radiation therapy (RT) following RP. However, approximately 90% of high-risk patients do not develop metastases or die of prostate cancer, and instead may be appropriate candidates for alternative treatment approaches, including active surveillance (AS). As such, many patients may be subjected to unnecessary follow-up procedures and their associated complications, highlighting the need for improved methods of prognostic risk assessment.<sup>2,3</sup>
- Several genomic biomarkers have been commercially developed to augment the prognostic ability of currently available routine clinical and pathological tests and identify those patients most and least likely to benefit from a specific treatment strategy. Prognostic genomic tests, including gene expression profiling tests, may help to avoid overtreatment by reclassifying those men originally identified as high risk, but who are unlikely to develop metastatic disease. Genomic biomarkers may

also play a role in assisting clinicians to tailor personalized and more appropriate treatments for subgroups of PC patients, and improve overall health outcomes.<sup>2,3</sup>

## Test information

- Gene expression profiles (GEPs) evaluate the expression of several genes using one sample. Gene expression is determined through RNA analysis, using either reverse transcriptase (RT) polymerase chain reaction (PCR) or DNA microarrays.<sup>4</sup>
- ProMark Proteomic Prognostic Test (Metamark® )<sup>5</sup>
  - According to the manufacturer, ProMark uses an 8-protein signature to predict PC aggressiveness (adverse prostate pathology of Gleason > or = 4+3 and/or non-organ confined disease [T3a, T3b, N1, or M1]) in patients with biopsy Gleason Scores of 3+3 and 3+4. It is designed to provide a personalized prediction regarding if PC can be managed with or without aggressive forms of treatment.
  - ProMark scores range from 0 to 1, reflecting the probability of adverse pathology at radical prostatectomy.

## Guidelines and evidence

### National Comprehensive Cancer Network

- The National Comprehensive Cancer Network (NCCN) 2019 Clinical Practice Guidelines on Prostate Cancer state the following regarding molecular assays:<sup>6</sup>
  - “Men with low or favorable intermediate risk disease may consider the use of the following tumor-based molecular assays: Decipher, Oncotype DX Prostate, Prolaris, Promark. Retrospective studies have shown that molecular assays performed on prostate biopsy or radical prostatectomy specimens provide prognostic information independent of NCCN risk groups.”
  - “Retrospective studies have shown that molecular assays performed on prostate biopsy or radical prostatectomy specimens provide prognostic information independent of NCCN or CAPRA risk groups. These include, but are not limited to, likelihood of death with conservative management, likelihood of biochemical progression after radical prostatectomy or external beam therapy, and likelihood of developing metastasis after radical prostatectomy or salvage radiotherapy.”
  - “Although full assessment of their clinical utility requires prospective randomized clinical trials, which are unlikely to be done, the panel believes that men with with low or favorable intermediate disease may consider the use of Decipher, Oncotype DX Prostate, Prolaris, or ProMark during initial risk stratification.”

- According to NCCN, the Molecular Diagnostic Services Program (MoIDX) recommendations stated the following:<sup>6</sup>
  - ProMark: “Cover post-biopsy for NCCN very-low and low-risk prostate cancer in patients with at least 10 years life expectancy who have not received treatment for prostate cancer and are candidates for active surveillance or definitive therapy.”

### **American Association of Clinical Urologists**

The American Association of Clinical Urologists has issued a position statement on genomic testing in prostate cancer that states the following:<sup>7</sup>

- “The AACU supports the use of tissue-based molecular testing as a component of risk stratification in prostate cancer treatment decision making.”

### **American Urological Association, ASTRO, and the Society of Urologic Oncology**

The AUA/ASTRO/SUO guideline for clinically localized prostate cancer states the following:<sup>8</sup>

- “Among most low-risk localized prostate cancer patients, tissue based genomic biomarkers have not shown a clear role in the selection of candidates for active surveillance.”

### **ProMark**

#### ProMark Literature Review<sup>9</sup>

- There is insufficient evidence to draw definitive conclusions regarding the prognostic performance of ProMark to improve risk stratification in untreated prostate cancer patients relative to conventional risk assessment methods. No direct clinical utility studies or clinical decision impact studies were identified. One clinical validity study suggests that the ProMark risk score offers additional prognostic information for patients compared with NCCN risk categories alone. However, the current evidence base consists of one clinical validity study and one analytical validity study, both published by the manufacturer.
- Additional well-designed clinical validity studies are needed to replicate the prognostic performance of this assay before it can be recommended for routine use in clinical practice.

### **Criteria**

- This test is considered investigational and/or experimental.
  - Investigational and experimental (I&E) molecular and genomic (MolGen) tests refer to assays involving chromosomes, DNA, RNA, or gene products that have

insufficient data to determine the net health impact, which typically means there is insufficient data to support that a test accurately assesses the outcome of interest (analytical and clinical validity), significantly improves health outcomes (clinical utility), and/or performs better than an existing standard of care medical management option. Such tests are also not generally accepted as standard of care in the evaluation or management of a particular condition.

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# Prosigna Breast Cancer Prognostic Gene Signature Assay

MOL.TS.222.A

v1.0.2020

## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedure addressed by this guideline                  | Procedure code |
|--|----------------|
| Prosigna Breast Cancer Prognostic Gene Signature Assay | 81520          |

## What is Prosigna

### Definition

Prosigna is a gene expression test designed to predict the chance of 10 year recurrence of breast cancer.

- Prosigna is indicated in post-menopausal women with hormone receptor positive, node negative (Stage I or II) or node positive (Stage II), early stage breast cancer.<sup>1,2</sup>
- This assay is intended to assist patients and providers considering treatment with adjuvant chemotherapy.<sup>1,2</sup>

### Test information

- Prosigna is based on the 50 gene expression signature called PAM50. This assay uses RNA from formalin fixed paraffin embedded (FFPE) samples to calculate a risk score.<sup>1,2</sup>
- The algorithm used for the Prosigna score uses the 50-gene expression profile in combination with clinical variables to classify breast cancer into one of the following four types: Luminal A, Luminal B, HER2-enriched, and Basal-like.<sup>1,2</sup>
- A risk of recurrence (ROR) score is also calculated using gene expression and clinical variables (such as tumor size and degree of proliferation). This ROR score is reported as 0-100 and reflects the probability of disease recurrence at 10 years.<sup>1,2</sup>
  - A ROR score of 1-10 corresponds to a 10 year distant recurrence of 0%. This risk increases to approximately 15% and then 33.3% when the ROR score reaches 61-70 and 91-100, respectively.<sup>3</sup>

## Guidelines and evidence

- The National Comprehensive Cancer Network (NCCN) 2019 Clinical Practice Guidelines for Breast Cancer consider the 50-gene PAM50 assay suitable for prognostic purposes (with evidence category 2A) as follows:<sup>4</sup>
  - “For patients with T1 and T2 hormone receptor-positive, HER2- negative, lymph node-negative tumors, a risk of recurrence score in the low range, regardless of T size, places the tumor into the same prognostic category as T1a–T1b, N0, M0.”
  - “In patients with hormone receptor-positive, HER2-negative, 1-3 positive lymph nodes with low risk of recurrence score, treated with endocrine therapy alone, the distant recurrence risk was less than 3.5% at 10 years 12 and no distant recurrence was seen at 10 years in TransATAC study in a similar group.”
  - These guidelines consider the therapeutic predictive value of this assay to be “not determined”.
- The National Institute for Health and Care Excellence (NICE) 2018 stated the following:<sup>5</sup>
  - “EndoPredict (EPClin score), Oncotype DX Breast Recurrence Score and Prosigna are recommended as options for guiding adjuvant chemotherapy decisions for people with oestrogen receptor (RE)-positive, human epidermal growth factor receptor 2 (HER2)-negative and lymph node (LN)-negative (including micrometastatic disease; see section 5.4) early breast cancer, only if:”
    - “they have intermediate risk of distant recurrence using a validated tool such as PREDICT or the Nottingham Prognostic index”
    - “information provided by the test would help them choose, with their clinician, whether or not to have adjuvant chemotherapy taking into account their preference”
- Evidence-based clinical guidelines from the American Society of Clinical Oncology (ASCO) 2016 state:<sup>6</sup>
  - “If a patient has ER/PgR-positive, HER2-negative (node-negative) breast cancer, the clinician may use the PAM50 risk of recurrence (ROR) score (Prosigna Breast Cancer Prognostic Gene Signature Assay; NanoString Technologies, Seattle, WA), in conjunction with other clinicopathologic variables, to guide decisions on adjuvant systemic therapy. Type: evidence based. Evidence quality: high. Strength of recommendation: strong.”
  - “If a patient has ER/PgR-positive, HER2-negative (node-positive) breast cancer, the clinician should not use the PAM50-ROR to guide decisions on adjuvant systemic therapy. Type: evidence based. Evidence quality: intermediate. Strength of recommendation: moderate.”

- “If a patient has HER2-positive breast cancer, the clinician should not use the PAM50-ROR to guide decisions on adjuvant systemic therapy. Type: informal consensus. Evidence quality: insufficient. Strength of recommendation: strong.”
- “If a patient has TN breast cancer, the clinician should not use the PAM50-ROR to guide decisions on adjuvant systemic therapy. Type: informal consensus. Evidence quality: insufficient. Strength of recommendation: strong.”
- The European Society of Medical Oncology (ESMO) 2015 published new clinical practice guidelines and stated the following:<sup>7</sup>
  - “Gene expression profiles, such as MammaPrint (Agendia, Amsterdam, the Netherlands), Oncotype DX Recurrence Score (Genomic Health, Redwood City, CA), Prosigna (Nanostring Technologies, Seattle, WA) and EndoPredict (Myriad Genetics), may be used to gain additional prognostic and/or predictive information to complement pathology assessment and to predict the benefit of adjuvant chemotherapy. The three latter tests are designed for patients with ER-positive early breast cancer only.”
  - “In cases of uncertainty regarding indications for adjuvant chemotherapy (after consideration of other tests), gene expression assays, such as MammaPrint, Oncotype DX, Prosigna and EndoPredict, may be used, where available.”
  - “In cases when decisions might be challenging, such as luminal B HER2-negative and node-negative breast cancer, commercially available molecular signatures for ER-positive breast cancer, such as Oncotype DX, EndoPredict, Prosigna, and for all types of breast cancer (pN0–1), such as MammaPrint and Genomic Grade Index, may be used in conjunction with all clinicopathological factors, to help in treatment decision making.”
- The St. Gallen International Expert Consensus (2015) stated the following:<sup>8</sup>
  - “The Panel considered the role of multiparameter molecular marker assays for prognosis separately in years 1-5 and beyond 5 years, and their value in selecting patients who require chemotherapy. Oncotype DX<sup>®</sup>, MammaPrint<sup>®</sup>, PAM-50 ROR<sup>®</sup> score, EndoPredict<sup>®</sup> and the Breast Cancer Index<sup>®</sup> were all considered usefully prognostic for years 1-5. Beyond 5 years, the Panel was divided almost equally on the prognostic value of Oncotype DX (despite the available data from NSABP Trial B-14 [32]); EndoPredict<sup>®</sup> (despite the report of Dubsy et al. [36]); and Breast Cancer Index (despite the report of Zhang et al. [37]). (All these reports show the respective tests to be prognostic beyond 5 years.) PAM50 ROR<sup>®</sup> score was agreed to be clearly prognostic beyond 5 years, and a clear majority rejected the prognostic value of MammaPrint<sup>®</sup> in this time period.”
- The Molecular Oncology Advisory Committee 2013 published a comparison of Oncotype DX with MammaPrint, PAM50, Adjuvant! Online, Ki-67, and IHC. Their recommendation is as follows:<sup>9</sup>

- “In cases of breast carcinoma where Oncotype DX is indicated for clinical prognosis and treatment decisions, other assays should not currently be considered equivalent with respect to data generated or risk stratification.”
- Per review of the peer reviewed literature, there is insufficient evidence in the peer-reviewed literature regarding the use of Prosigna/PAM50 ROR in women with early stage (ER+/HER2-), node-positive, breast cancer who are considering adjuvant chemotherapy. Limited evidence from a prospective-retrospective clinical validity study suggests that the low risk Prosigna/PAM50 ROR Score is associated with a relatively low 10-year distant recurrence rates in women with node-positive invasive breast cancer; however, a relatively wide confidence interval suggests imprecise an estimate of distant recurrence at 10 years.<sup>10</sup>
- The US Food and Drug Administration (FDA) cleared Prosigna for clinical use in 2013.<sup>11</sup>

## Criteria

- Previous Testing:
  - No repeat Prosigna testing on the same sample when a result was successfully obtained, and
  - No previous gene expression assay (e.g. OncotypeDx Breast) performed on the same sample when a result was successfully obtained, AND
- Testing Multiple Samples:
  - When more than one breast cancer primary is diagnosed:
    - There should be reasonable evidence that the tumors are distinct (e.g., bilateral, different quadrants, different histopathologic features, etc.), and
    - There should be no evidence from either tumor that chemotherapy is indicated with or without knowledge of the Prosigna test result (e.g., histopathologic features or previous Gene Expression Assay result of one tumor suggest chemotherapy is indicated), and
    - If both tumors are to be tested, both tumors must independently meet the required clinical characteristics outlined below, AND
- Required Clinical Characteristics:
  - Invasive breast cancer meeting all of the following criteria:
    - Tumor size  $\geq 0.4$ cm (4mm) in greatest dimension (T1b-T3),<sup>3</sup> and
    - Hormone receptor positive (ER+/PR+), and
    - HER2 negative, and

- Patient has no regional lymph node metastasis, and
- Chemotherapy is a treatment option for the patient; results from this Prosigna test will be used in making chemotherapy treatment decisions, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

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# PTEN Hamartoma Tumor Syndromes Testing

MOL.TS.223.A

v1.0.2020

## Procedures addressed

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| Procedures addressed by this guideline | Procedure codes |
|--|-----------------|
| PTEN Known Familial Mutation Analysis  | 81322           |
| PTEN Sequencing                        | 81321           |
| PTEN Deletion/Duplication Analysis     | 81323           |

## What is PTEN hamartoma tumor syndrome

### Definition

PTEN hamartoma tumor syndrome (PHTS) is used to describe the group of conditions caused by PTEN mutations that include hamartomatous growths: Cowden syndrome, Bannayan-Riley-Ruvalcaba syndrome, Proteus syndrome and Proteus-like syndrome, and autism spectrum disorder with macrocephaly.

- Historically, these conditions have been considered clinically distinct but share an underlying genetic etiology, and show some overlap in families.<sup>1</sup>
  - **Cowden syndrome** (CS) is characterized by an increased risk for benign and malignant tumors of the breast, endometrium, and thyroid (non-medullary).<sup>1,2</sup> Other common features include macrocephaly and growths on the skin or mucous membranes (mucocutaneous lesions). Prevalence is estimated to be 1 in 200,000 individuals, although CS is believed to be underdiagnosed.<sup>1</sup> Up to 80% of people with a clinical diagnosis of CS have a PTEN mutation in the coding region.<sup>1</sup> Ten percent of individuals with CS have a PTEN mutation in the promotor region.<sup>1</sup>
  - **Lhermitte-Duclos disease** (LDD) is a rare, benign tumor of the cerebellum called dysplastic gangliocytoma that may present in childhood or adulthood.<sup>1,2</sup> Most adult-onset LDD is caused by a PTEN mutation even when no other signs of CS are present.<sup>1</sup>
  - **Bannayan-Riley-Ruvalcaba syndrome** (BRRS) is a genetic disorder characterized by macrocephaly, multiple benign intestinal polyps

(hamartomatous type), lipomas, colored spots on the tip of the penis (pigmented macules of the glans penis), and hemangiomas. Some people with BRRS have intellectual disability and/or birth defects. There may be an increased risk for several types of cancer, including breast, thyroid and endometrial.<sup>2</sup> Up to 71% of people with a clinical diagnosis of BRRS have a PTEN mutation.<sup>1</sup>

- **Proteus and Proteus-like syndromes** are highly variable conditions characterized by overgrowth of several different tissues usually in a patchy asymmetric pattern (mosaic) that is often present from birth but gets worse over time.<sup>1</sup>

Clinical signs and symptoms include connective tissue and epidermal nevi (hamartomatous growths), ovarian cystadenomas, parotid monomorphic adenomas, lipomas, capillary/venous/lymphatic malformations, and a characteristic facial dysmorphism. Up to 50% of people with Proteus-like syndrome and 20% of people with Proteus syndrome have a PTEN mutation.<sup>1</sup>

- **Autism spectrum disorder with macrocephaly**, defined as >2.5 SDs above the age mean or ≥97<sup>th</sup> percentile, may be caused by a mutation in the PTEN gene.<sup>1</sup> An estimated 3-20% of all people with ASD/macrocephaly have a PTEN mutation.<sup>1,3</sup> The likelihood may be greater if other family members have signs and symptoms in the PHTS spectrum.
- An online tool is available to estimate the likelihood of identifying a PTEN mutation based on clinical findings: <http://www.lerner.ccf.org/gmi/ccscore/>.
- People with CS need heightened cancer surveillance starting at age 18 (or earlier if warranted: “For those with a family history of a particular cancer type at an early age screening may be initiated five to ten years prior to the youngest diagnosis in the family”)<sup>1,2</sup> The exception is children should have a yearly thyroid ultrasound from the time of diagnosis and skin check with physical examination.<sup>1</sup> Because of the overlap in clinical phenotypes, people with other PTEN-related conditions are advised to follow the same heightened cancer surveillance guidelines as for CS.<sup>4,5</sup>
  - The lifetime risk for breast cancer is 25-50% with an average age at diagnosis of 38-46 years.<sup>1</sup> However, a 2012 publication by Tan et al. reports that this lifetime risk may be as high as 85%, particularly in individuals with PTEN promoter mutations.<sup>6</sup> The lifetime risk for thyroid cancer can range from 10% to as high as 35%.<sup>1,6</sup> If it occurs, thyroid cancer is usually follicular. It is rarely papillary and is never medullary. Benign thyroid growths are also found in up to 75% of people with CS.<sup>1</sup> “However, the high frequency of thyroid disease in the general population means that when taken on their own, thyroid neoplasms have a low predictive value for identifying mutations carriers.”<sup>7</sup>
  - Endometrial cancer has an estimated 5-10% lifetime risk, although this is not well-defined.<sup>1</sup> Tan et al. reports a lifetime risk of up to 28%.<sup>6</sup>
  - The gastrointestinal polyp risk (often colonic) in patients with CS may be 80% or higher and the lifetime risk for colorectal cancer is estimated to be 9%.<sup>6</sup>



- Early onset colorectal cancer has been reported in 13% of patients with PTEN associated CS indicating earlier and more frequent colonoscopy is warranted in this population.<sup>6,8,9</sup>
- Additionally, an increased lifetime risk for kidney cancer (approximately 34%) and melanoma (about 5-6%) has been reported.<sup>1,2,6</sup>
- PTEN mutations are inherited in an autosomal dominant manner, meaning that a person only needs a mutation in one copy of the gene to be affected. A child of an affected person has a 50% chance to inherit the mutation. The majority of CS cases are simplex. Ten to fifty percent of individuals with CS have an affected parent.<sup>1</sup> Nearly all people with a PTEN mutation will develop symptoms (complete penetrance).<sup>1,2</sup>

## Test information

- **PTEN Sequencing:** Evaluates each DNA nucleotide to identify mutations throughout the gene. Such testing will detect a mutation in about 80% of people with a clinical diagnosis of CS and 60% of people with a clinical diagnosis of BRRS.<sup>1</sup>
  - **Sequencing of the promoter region** will detect an additional 10% of PTEN mutations that cause CS.<sup>1</sup> NCCN recommends comprehensive testing, which should include full sequencing, gene deletion/duplication analysis, and promoter analysis of the PTEN gene.<sup>2</sup> As such, it is important to determine whether or not the selected laboratory includes PTEN promoter analysis in their testing.<sup>2</sup>
- **PTEN Deletion/Duplication Analysis:** Used in cases where a mutation is not found by sequencing. The likelihood of identifying a deletion or duplication in people with clinically diagnosed CS is unknown, but expected to be relatively low.<sup>1</sup> About 11% of people with BRRS have large PTEN gene deletions.<sup>1</sup>
- **PTEN Known Familial Mutation Analysis:** Once the familial mutation is identified, testing for that one mutation can be offered to at-risk relatives. Such testing is much less expensive than complete gene testing and the results are highly reliable.

## Guidelines and evidence

- Evidence-based guidelines (Category 2A) from the National Comprehensive Cancer Network (NCCN, 2019) support the use of PTEN genetic testing in those with clinical features or a family history. They recommend PTEN genetic testing in any of the following situations:<sup>2</sup>
  - Family history of a known PTEN mutation [PTEN known familial mutation testing is appropriate]
  - A personal history of any of the following:

- Bannayan-Riley-Ruvalcaba syndrome (BRRS)
  - Adult-onset Lhermitte Duclos disease (cerebellar dysplastic gangliocytoma)
  - Autism spectrum disorder and macrocephaly (greater than or equal to 97th percentile)
  - Two or more biopsy proven trichilemmomas
  - Macrocephaly and at least one other major\*\* criteria
  - Three major\*\* criteria without macrocephaly
  - One major\*\* and three or more minor\*\*\* criteria
  - Four or more minor\*\*\* criteria
- At-risk relative of someone clinically diagnosed with Cowden syndrome or BRRS (who has not had genetic testing), when the at-risk relative has at least one major\*\* or two minor\*\*\* criteria. Ideally, the at-risk person is a first-degree relative (parent, sibling, child) of someone clinically diagnosed, but testing more distant relatives is acceptable if closer relatives are not available or willing to have testing.

| **Major:   | ***Minor:  |
|--|--|
| <ul style="list-style-type: none"> <li>• Breast cancer</li> <li>• Endometrial cancer</li> <li>• Follicular thyroid cancer</li> <li>• Multiple GI hamartomas or ganglioneuromas</li> <li>• Macrocephaly (at least 97<sup>th</sup> percentile: 58cm in adult women and 60cm in adult men)</li> <li>• Macular pigmentation of glans penis</li> <li>• Mucocutaneous lesions: one biopsy-proven trichilemmoma, multiple palmoplantar keratoses, multifocal or extensive oral mucosal papillomatosis, multiple cutaneous facial papules (often verrucous)</li> </ul> | <ul style="list-style-type: none"> <li>• Autism spectrum disorder</li> <li>• Colon cancer</li> <li>• 3 or more esophageal glycogenic acanthoses</li> <li>• Lipomas</li> <li>• Intellectual disability (IQ less than or equal to 75)</li> <li>• Papillary or follicular variant of papillary thyroid cancer</li> <li>• Thyroid structural lesions (e.g., adenoma, nodule(s), goiter)</li> <li>• Renal cell carcinoma</li> <li>• Single GI hamartoma or ganglioneuroma</li> <li>• Testicular lipomatosis</li> <li>• Vascular anomalies (including multiple intracranial developmental venous anomalies)</li> </ul> |

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**Note** These NCCN defined major and minor criteria for genetic testing do not fully align with the major and minor criteria required for a clinical diagnosis.

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- The American College of Medical Genetics and Genomics (ACMG, 2008, updated 2013) issued consensus practice guidelines on the genetics evaluation of autism. They propose an evaluation scheme with three tiers. The first tier includes routine studies such as chromosome analysis and fragile X genetic testing. PTEN gene testing is recommended as a second tier test when the head circumference is greater than 2.5 SDs above the mean (if no diagnosis is made via first tier testing).<sup>10</sup>
- An expert-authored review (2014) of the PTEN hamartoma syndromes states:<sup>1</sup>
  - “A presumptive diagnosis of PTEN hamartoma syndrome is based on clinical signs; by definition, however, the diagnosis of PTEN hamartoma syndrome is made only when a PTEN mutation is identified.”
  - “The appropriate order of PTEN testing to optimize yield:”
    - i. “Sequence all PTEN coding exons 1-9 and flanking intronic regions. If no pathogenic variant is identified, perform:”
    - ii. “Deletion/duplication analysis. If no pathogenic variant is identified, consider:”
    - iii. “Sequence analysis of the promoter region for variants that decrease gene expression”
  - “The most serious consequences of PHTS relate to the increased risk of cancers including breast, thyroid, endometrial, and to a lesser extent, renal. In this regard, the most important aspect of management of any individual with a PTEN pathogenic variant is increased cancer surveillance to detect any tumors at the earliest, most treatable stages.”

## Criteria

PTEN gene testing may be considered in individuals with a suspected or known clinical diagnosis of Cowden syndrome, Bannayan-Riley-Ruvalcaba syndrome (BRRS), or another PTEN-related hamartoma syndrome; or who have a known family history of a PTEN mutation.

### PTEN Known Familial Mutation Analysis

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Testing:

- No previous genetic testing of PTEN, AND
- Diagnostic and Predisposition Testing:
  - Known deleterious family mutation in PTEN identified in 1<sup>st</sup>, 2<sup>nd</sup>, or 3<sup>rd</sup> degree biologic relative(s), AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

### **PTEN Sequencing with promoter analysis**

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Testing:
  - No previous genetic testing of PTEN, AND
- Diagnostic Testing for Symptomatic Individuals
  - Personal history of ANY of the following:
    - Bannayan Riley-Ruvalcaba syndrome; or
    - Adult Lhermitte-Duclos disease (LDD); or
    - Autism spectrum disorder and macrocephaly; or
    - At least two biopsy-proven trichilemmomas; or
    - At least two major criteria\*\* (one must be macrocephaly); or
    - Three major criteria\*\* without macrocephaly; or
    - One major\*\* and at least three minor criteria\*\*\*; or
    - Four or more minor criteria\*\*\*, OR
- Predisposition testing for Presymptomatic/Asymptomatic Individuals:
  - At-risk person with a family history of:
    - A relative (includes first-degree relative or more distant relatives if the first-degree relative is unavailable or unwilling to be tested ) with a clinical diagnosis of Cowden syndrome or BRR (no previous genetic testing); and
    - One major\*\* OR two minor criteria\*\*\* in the at-risk person, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

**PTEN Deletion/Duplication Analysis:**

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Testing:
  - Sequence analysis of PTEN has been performed and resulted negative, and
  - No previous deletion/duplication testing, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

**Criteria for testing purposes are:** <sup>2</sup>

| **Major:   | ***Minor:  |
|--|--|
| <ul style="list-style-type: none"> <li>• Breast cancer</li> <li>• Endometrial cancer</li> <li>• Follicular thyroid cancer</li> <li>• Multiple GI hamartomas or ganglioneuromas</li> <li>• Macrocephaly (at least 97<sup>th</sup> percentile: 58cm in adult women and 60cm in adult men)</li> <li>• Macular pigmentation of glans penis</li> <li>• Mucocutaneous lesions: one biopsy-proven trichilemmoma, multiple palmoplantar keratoses, multifocal or extensive oral mucosal papillomatosis, multiple cutaneous facial papules (often verrucous)</li> </ul> | <ul style="list-style-type: none"> <li>• Autism spectrum disorder</li> <li>• Colon cancer</li> <li>• ≥ 3 esophageal glycogenic acanthoses</li> <li>• Lipomas</li> <li>• Intellectual disability (IQ≤75)</li> <li>• Papillary or follicular variant of papillary thyroid cancer</li> <li>• Thyroid structural lesions (e.g., adenoma, nodule(s), goiter)</li> <li>• Renal cell carcinoma</li> <li>• Single GI hamartoma or ganglioneuroma</li> <li>• Testicular lipomatosis</li> <li>• Vascular anomalies (including multiple intracranial developmental venous anomalies)</li> </ul> |

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# Molecular Respiratory Infection Pathogen Panel (RIPP) Testing

MOL.CS.293.A

v1.0.2020

## Introduction

Molecular respiratory infection testing is addressed by this guideline.

## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedure(s) addressed by this guideline  | Procedure code(s) |
|---|-------------------|
| BioFire FilmArray Respiratory Panel (RP) EZ, BioFire Diagnostics  | 0098U             |
| BioFire FilmArray Respiratory Panel (RP), BioFire Diagnostics   | 0099U             |
| BioFire FilmArray Respiratory Panel 2 (RP2), BioFire Diagnostics  | 0100U             |
| ePlex Respiratory Pathogen (RP) Panel   | 0115U             |
| Infectious agent detection by nucleic acid (DNA or RNA); respiratory virus (eg, adenovirus, influenza virus, coronavirus, metapneumovirus, parainfluenza virus, respiratory syncytial virus, rhinovirus), includes multiplex reverse transcription, when performed, and multiplex amplified probe technique, multiple types or subtypes, 3-5 targets  | 87631             |
| Infectious agent detection by nucleic acid (DNA or RNA); respiratory virus (eg, adenovirus, influenza virus, coronavirus, metapneumovirus, parainfluenza virus, respiratory syncytial virus, rhinovirus), includes multiplex reverse transcription, when performed, and multiplex amplified probe technique, multiple types or subtypes, 6-11 targets | 87632             |

| Procedure(s) addressed by this guideline   | Procedure code(s) |
|--|-------------------|
| Infectious agent detection by nucleic acid (DNA or RNA); respiratory virus (eg, adenovirus, influenza virus, coronavirus, metapneumovirus, parainfluenza virus, respiratory syncytial virus, rhinovirus), includes multiplex reverse transcription, when performed, and multiplex amplified probe technique, multiple types or subtypes, 12-25 targets | 87633             |

## What is Respiratory Pathogens Panel Testing

### Definition

Respiratory pathogens panel testing is the use of molecular technologies to detect respiratory pathogens directly in a clinical sample.

- In spite of the continued utilization of conventional diagnostic methods in clinical microbiology laboratories, the expanded availability of molecular methods for detection of pathogens directly in clinical specimens is changing the paradigm for diagnosis and management of patients with infectious diseases. One of the recent reasons for these changes has been the development of syndromic-based multiplex molecular panels, in this case, for respiratory presentations, with the ability to simultaneously detect, differentiate, and even subtype viral/bacterial pathogens in patient specimens.<sup>1</sup>
- Viral pathogens are the most common cause of respiratory tract infections. Seasonal influenza contributes to substantial morbidity and mortality each year in the United States. However, in a large portion of patients with respiratory tract infections, other viruses and non-cultivable organisms have been found to cause substantial morbidity and mortality.
- The ability to detect a large number pathogens rapidly and with high sensitivity and specificity has the potential to transform clinical microbiology as a continuing critical component of laboratory medicine. However, it is important to consider whether these tests should be front-line tests used for all patients with acute respiratory illness or whether their use should be limited to specific patients.

## Test Information

### Introduction

This section of the guideline contains information about testing for respiratory



pathogens.

- Respiratory panels may provide sample-to-answer results, using integrated nucleic acid extraction, amplification and detection with testing times of as little as 1 hour, typically using nasopharyngeal swab specimens. Several test systems have received FDA-clearance for the detection of respiratory tract pathogens, which has facilitated their rapid integration into routine testing. Other test platforms may include laboratory-validated panels that are customized for clinicians at their service clinical practice networks.
- The menu of analytes on several panels is for the first time providing access to routine testing for pathogens that have previously been difficult to detect, or for which testing was only available at reference laboratories (i.e. norovirus, coronaviruses, Chlamydomphila pneumoniae, Mycoplasma pneumoniae). These assays detect 12-20 pathogens and some include pathogens that typically cause different manifestations of infection, although they infect the same organ system.
- Analytically, the molecular assays usually exhibit comparative or superior detection rates compared to conventional methods which also result in an increased rate of diagnosis for affected patients.<sup>2,3</sup> In addition, multiplex polymerase chain reaction (mPCR) molecular panels allow laboratories to consolidate testing for a broad range of pathogens from the same samples. This consolidation provides opportunities to eliminate conventional testing methodologies, including direct fluorescent antibody (DFA) and cell culture for the detection of respiratory viruses.
- However, the fixed nature of the mPCR panels raises the concern that they might include pathogens causing infections with sufficiently clinical/epidemiological diversity, such that, in turn, simultaneous testing for those pathogens should be rare. Alternatively, the differences might be detectable by rapid, accurate and inexpensive tests (e.g. the Gram stain) that are part of routine testing.<sup>4</sup> It is reasonable to assert that negative test results for common pathogens should typically precede testing for uncommon pathogens.

## Guidelines and Evidence

### Introduction

The following section includes relevant guidelines and evidence pertaining to respiratory pathogens panel testing.

### British Committee for Standards in Hematology

A joint working group established by the Hemato-oncology subgroup of the British Committee for Standards in Hematology, the British Society for Bone Marrow Transplantation and the UK Clinical Virology Network has reviewed the available literature and made recommendations in 2016 for the diagnosis and management of respiratory viral infections in patients with hematological malignancies or those undergoing hematopoietic stem cell transplantation. To illustrate:<sup>5</sup>

- “It is currently recommended that the diagnosis of respiratory viral infections is made by quantitative nucleic acid amplification tests (NAATs), generically referred to hereafter as PCR; clinicians should be able to liaise with their virology laboratory colleagues regarding the interpretation of PCR results ... A panel of viruses should be included for PCR testing, including parainfluenza type 4.”

### **German Society for Haematology and Medical Oncology**

A panel of 18 clinicians from the Infectious Diseases Working Party of the German Society for Haematology and Medical Oncology convened to assess the available literature and provide 2016 recommendations on the management of community acquired respiratory virus infections including influenza, respiratory syncytial virus (RSV), parainfluenza virus (PIV), human metapneumovirus (hMPV) and adenovirus. Two relevant excerpts include:<sup>6</sup>

- “Most data on this topic originate from patients following allogeneic stem cell transplantation (allo-SCT), and we know little about community-acquired respiratory virus (CARV) infections in cancer patients outside the setting of allo-SCT. However, in recent years increasing evidence has been gathered about other cancer patients, revealing clinical relevance of CARV infections in non-transplant patients. Therefore, this guideline discusses CARV infections in all cancer patients with ongoing relevant immunosuppression. It is left to the treating physician to assess the degree and relevance of immunosuppression in the individual patient.”
- “In the era of multiplex-test kits, it is difficult to make a definite recommendation with regard to which viruses should be looked for. In the absence of any reliable data regarding this question, the panel feels that it is wise to search for influenza, RSV, PIV and viruses currently prevalent in the local environment in all immunosuppressed cancer patients presenting with symptoms. Patients with more severe disease (for example pneumonia or critical illness) may have the panel broadened to include hMPV and adenovirus and even viruses that only rarely cause lower respiratory tract infections like rhinovirus and coronavirus. However, evidence for this approach is low and it is strongly advisable to define local guidelines on this topic.”

### **Fourth European Conference on Infections in Leukemia**

A working group of the Fourth European Conference on Infections in Leukemia (ECIL-4) 2011 reviewed the literature on community-acquired respiratory virus (CARV), graded the available quality of evidence, and made the relevant diagnostic recommendations according to the Infectious Diseases Society of America (IDSA) grading system:<sup>7</sup>

- First-line diagnostic testing should be performed for influenza A and B, RSV, and human parainfluenza viruses (HPIV) (IDSA Grade A II).

- Testing for other CARVs should be considered according to risk of exposure and the local epidemiology, or if testing for the firstline CARVs is negative (IDSA Grade B III).

### **American Society of Transplantation/Canadian Society of Transplantation**

Manuel (2013) was modified from a previous guideline published in the American Journal of Transplantation 2009; 9(Suppl 4): S166–S172, and endorsed by American Society of Transplantation/Canadian Society of Transplantation:<sup>8</sup>

- In solid organ transplant recipients, molecular tests tend to provide higher yields and can detect a wider range of viruses in a more timely fashion.

### **Expert Written and Peer Reviewed Articles**

There have been additionally referenced indications for the use of (typically viral) respiratory pathogen panels, such as for adult patients appearing acutely ill, who are potential hospital admissions, where, for example, such panel testing would be ordered in the emergency department (ED). To illustrate, two randomized controlled studies have described some possible favorable outcomes in the ED:

- Brendish (2017)<sup>9</sup> where respiratory viral panel testing did not ... “reduce the proportion of patients treated with antibiotics. However, the primary outcome measure failed to capture differences in antibiotic use because many patients were started on antibiotics before the results of point-of-care testing (POCT) could be made available. Although POCT was not associated with a reduction in the duration of antibiotics overall, more patients in the POCT group received single doses or brief courses of antibiotics than did patients in the control group. POCT was also associated with a reduced length of stay and improved influenza detection and antiviral use, and appeared to be safe.”
- Brittain-Long (2011)<sup>3</sup> found that ... “In the group of patients randomised for a rapid result, 4.5% (9 of 202) of patients received antibiotics at the initial visit, compared to 12.3% (25 of 204) (P = 0.005) of patients in the delayed result group. At follow-up, there was no significant difference between the groups: 13.9% (28 of 202) in the rapid result group and 17.2% (35 of 204) in the delayed result group (P = 0.359), respectively.” ... with the conclusion that ... “Access to a rapid method for etiologic diagnosis of acute respiratory tract infections (ARTIs) may reduce antibiotic prescription rates at the initial visit in an outpatient setting. To sustain this effect, however, it seems necessary to better define how to follow and manage the patient according to the result of the test, which warrants further investigation.”

Furthermore, critically-ill adult patients, particularly intensive care unit (ICU) patients, lack the same evidentiary level for metrics such as the reduction of unnecessary antibiotic use, which is a major cause of morbidity in hospitalized patients. However, case series studies make a convincing case that respiratory viral pathogens are of considerable relevance in the ICU setting. To illustrate, Choi (2017)<sup>10</sup> found that viral infection is common in adult patients with severe pneumonia. About one-third of

patients with severe community-acquired pneumonia or healthcare-associated pneumonia had viral infections, and the mortality from viral infection and bacterial infection was comparable. The viral agents involved in descending order of prevalence were rhinovirus, parainfluenza virus (Types 3, 1, 2, and 4, respectively), hMPV, influenza, RSV, and, more infrequently, cytomegalovirus, human coronavirus, adenovirus, and enterovirus. Furthermore, Voiriot (2016)<sup>11</sup> observed a relatively more complicated course among ICU patients with mixed bacterial and viral respiratory infections.

Finally, however, there were no substantive peer-reviewed full articles which addressed the relative clinical impact of ordering respiratory pathogen panels, with differing numbers of infectious targets.

## Criteria

### Introduction

Requests for molecular respiratory infection pathogen panel (RIPP) testing are reviewed using the following clinical criteria.

The presence of acute respiratory symptoms in members 17 years of age or younger, OR

The presence of acute respiratory symptoms in members of any age who are:

- Immunocompromised (as defined by ICD-10 codes), OR
- Immunocompetent and receiving care for their acute respiratory symptoms in a hospital setting as evidenced by the following
  - Place of service code on the claim is: 19, 22, or 23 , or
  - Bill type code on the claim is: 13X or 14X, OR

The following is a contraindication to RIPP testing in members 18 years of age or older:

- Presence of respiratory symptoms that suggest a specific respiratory pathogen in an immunocompetent adult.

Molecular RIPP testing is limited to the minimum number of targets needed for therapeutic decision making. When ordering any configuration of infectious disease targets, whether using RIPP or conventional testing, the medical record should clearly indicate the differential diagnosis of possible microorganisms based upon member history and presenting signs/symptoms.

It is not necessary to repeat a respiratory pathogen panel to ensure a causative organism is cleared. If test of cure is indicated for a particular organism, individual organism testing should be used. Therefore, repeat testing of any panel within a two week time frame will not be reimbursed.

RIPP

## Billing and reimbursement

- No more than one respiratory virus panel should be necessary on a single date of service. Therefore, only one unit of the same panel code will be reimbursable and two different panel codes (87631, 87632, 87633, 0098U, 0099U, 0100U, or 0115U) cannot be billed on the same date of service.
- More than one type of test for the same organism will not be reimbursable for the same date of service (e.g., 87631 and 87634 may not be billed together).
- A code representing only the minimum panel necessary to detect the necessary targets should be billed. If the laboratory's testing platform consists solely of a panel of multiple targets, yet only a subset of the organisms are considered medically necessary based on the above criteria, the lab may request reimbursement for that subset of organisms using a procedure code that does not represent all organisms included on the panel (e.g., bill 87632 if only 8 targets are necessary even if 12 or more targets were tested as part of a panel usually billed with 87633).

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**Note** Inpatient services are beyond the scope and domain of this guideline.

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Although outbreak investigations may sometimes require use of RIPP testing, the public health evaluations of such outbreaks are beyond the scope and domain of this guideline.

## ICD10 codes

ICD10 codes in this section may be used to support medical necessity as described in the above criteria.

### *ICD10 Codes Indicating Cancer, Transplant, or Other Immunocompromise*

| ICD10 Code or Range | Description  |
|---------------------|--|
| B20                 | Human immunodeficiency virus [HIV] disease                                       |
| B59                 | Pneumocystosis   |
| C00-C96             | Malignant neoplasms  |
| D37-D48             | Neoplasms of uncertain behavior, polycythemia vera and myelodysplastic syndromes |
| D60-D64             | Aplastic and other anemias and other bone marrow failure syndromes               |
| D70-D77             | Other disorders of blood and blood-forming organs                                |
| D80-D89             | Certain disorders involving the immune mechanism                                 |

| ICD10 Code or Range | Description  |
|---------------------|--|
| E40-E46             | Malnutrition   |
| I120                | Hypertensive chronic kidney disease with stage 5 chronic kidney disease or end stage renal disease                                   |
| I1311               | Hypertensive heart and chronic kidney disease without heart failure, with stage 5 chronic kidney disease, or end stage renal disease |
| I132                | Hypertensive heart and chronic kidney disease with heart failure and with stage 5 chronic kidney disease, or end stage renal disease |
| K912                | Postsurgical malabsorption, not elsewhere classified   |
| M359                | Systemic involvement of connective tissue, unspecified   |
| N185                | Chronic kidney disease, stage 5  |
| N186                | End stage renal disease  |
| T86                 | Complications of transplanted organs and tissue  |
| Z48.2               | Encounter for aftercare following organ transplant   |
| Z49                 | Encounter for care involving renal dialysis  |
| Z94                 | Transplanted organ and tissue status   |
| Z992                | Dependence on renal dialysis   |

## References

### Introduction

The following references are cited in the guideline.

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# Rett Syndrome Testing

MOL.TS.224.A  
v1.0.2020

## Introduction

Rett syndrome testing is addressed by this guideline.

## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedures addressed by this guideline  | Procedure codes |
|---|-----------------|
| MECP2 Known Familial Mutation Analysis  | 81303           |
| MECP2 Sequencing                        | 81302           |
| MECP2 Deletion and Duplication Analysis | 81304           |

## What is Rett syndrome

### Definition

Rett syndrome, or classic Rett syndrome, is an X-linked brain development disorder that typically affects females. Atypical Rett syndrome may be more mild or severe than classic Rett syndrome.

### Prevalence

Rett syndrome affects about 1 in 8,500 females by age 15. Males are rarely affected with less than 100 overall affected patients reported.<sup>1</sup>

### Symptoms

Girls with Rett syndrome may not show signs at birth or during infancy, but by the age of 6 to 18 months they begin to lose their motor and language skills, which eventually stabilizes.<sup>1</sup>

Signs and symptoms of Rett syndrome usually include <sup>1,2</sup>

- intellectual disability or developmental delay
- specific hand movements, like hand “wringing” and clapping for no reason
- loss of speech



- problems with sleep
- seizures
- growth failure
- autistic behaviors, and
- gait abnormalities, either impaired or complete absence of ability.

## Cause

Rett syndrome is caused by genetic changes (mutations) in the MECP2 gene, located on the X chromosome. Females have two X chromosomes and males have one X chromosome and one Y chromosome.<sup>1</sup>

## Inheritance

Rett syndrome is an X-linked condition. A female who is found to be a MECP2 mutation carrier has a 50% chance to pass the mutation to her children.

Approximately 99% of cases of Rett syndrome are the result of a new genetic change (de novo) in the affected person and are not inherited from a carrier parent.<sup>1-3</sup> Cases of minimally affected or unaffected female carriers of MECP2 mutations have been reported.<sup>1-4</sup>

Cases of MECP2 mutations in only the germline (egg or sperm) of parents of affected people have been reported.<sup>1-3</sup> In one study, prenatal diagnosis was offered to nine couples who had a previous child with Rett syndrome due to a known de novo MECP2 mutation.<sup>3</sup> One of the nine pregnancies was found to have the same MECP2 mutation as in the affected sibling.<sup>3</sup> Since germline mosaicism cannot be predicted or ruled out in families who have a child with Rett syndrome, prenatal diagnosis may be offered.

If a mutation of unclear significance is found in an affected person, testing both the mother and the father may be appropriate to help to determine whether the mutation is actually causing the disease.<sup>1</sup>

## Diagnosis

Classic Rett syndrome is generally diagnosed by established clinical diagnostic criteria.<sup>1,2</sup> Diagnostic criteria have also been suggested for atypical Rett syndrome, but diagnostic criteria are imperfect for reliably diagnosing Rett syndrome.<sup>1,2</sup>

Genetic testing may be useful to confirm a diagnosis (particularly when unclear based on clinical criteria) and to identify the mutation for genetic counseling purposes.

### MECP2 mutation

The presence of a mutation in the MECP2 gene alone does not diagnose Rett syndrome. MECP2 mutations may cause conditions other than Rett syndrome.<sup>1</sup>

Conversely, some people who meet the clinical diagnostic criteria for Rett syndrome do not have an identifiable MECP2 mutation.<sup>1</sup>

When a male has a MECP2 mutation, he has no second normal copy of the gene to help lessen the effect of the mutation. This mutation can cause a severe disease called neonatal encephalopathy and these boys usually die before 2 years of age.<sup>1</sup> Surviving males generally have an abnormal gait or truncal movements, severe speech delay, and intellectual disability.<sup>2</sup>

## Diagnostic criteria

### Typical or classic Rett (RTT)<sup>5</sup>

- A period of regression followed by recovery or stabilization\*
- All main criteria and all exclusion criteria
- Supportive criteria are not required, although often present in typical RTT

### Atypical or variant Rett<sup>5</sup>

- A period of regression followed by recovery or stabilization\*
- At least 2 out of the 4 main criteria
- 5 out of 11 supportive criteria

### Main criteria<sup>5</sup>

- Partial or complete loss of acquired purposeful hand skills.
- Partial or complete loss of acquired spoken language or language skills\*\*
- Gait abnormalities: impaired (dyspraxic) or absence of ability.
- Stereotypic hand movements such as hand wringing/squeezing, clapping/tapping, mouthing and washing/rubbing automatisms

### Exclusion criteria for typical Rett<sup>5</sup>

- Brain injury secondary to trauma (peri- or postnatally), neurometabolic disease, or severe infection that causes neurological problems\*\*\*
- Grossly abnormal psychomotor development in first 6 months of life#

### Supportive criteria for atypical RTT##<sup>5</sup>

- Breathing disturbances when awake
- Bruxism when awake
- Impaired sleep pattern
- Abnormal muscle tone

- Peripheral vasomotor disturbances
- Scoliosis/kyphosis
- Growth retardation
- Small cold hands and feet
- Inappropriate laughing/screaming spells
- Diminished response to pain
- Intense eye communication - “eye pointing”

“\*Because MECP2 mutations are now identified in some individuals prior to any clear evidence of regression, the diagnosis of “possible” RTT should be given to those individuals under 3 years old who have not lost any skills but otherwise have clinical features suggestive of RTT. These individuals should be reassessed every 6–12 months for evidence of regression. If regression manifests, the diagnosis should then be changed to definite RTT. However, if the child does not show any evidence of regression by 5 years, the diagnosis of RTT should be questioned.”

“\*\*Loss of acquired language is based on best acquired spoken language skill, not strictly on the acquisition of distinct words or higher language skills. Thus, an individual who had learned to babble but then loses this ability is considered to have a loss of acquired language.”

“\*\*\*There should be clear evidence (neurological or ophthalmological examination and MRI/CT) that the presumed insult directly resulted in neurological dysfunction.”

“#Grossly abnormal to the point that normal milestones (acquiring head control, swallowing, developing social smile) are not met. Mild generalized hypotonia or other previously reported subtle developmental alterations<sup>16</sup> during the first six months of life is common in RTT and do not constitute an exclusionary criterion.”

“##If an individual has or ever had a clinical feature listed it is counted as a supportive criterion. Many of these features have an age dependency, manifesting and becoming more predominant at certain ages. Therefore, the diagnosis of atypical RTT may be easier for older individuals than for younger. In the case of a younger individual (under 5 years old) who has a period of regression and  $\geq 2$  main criteria but does not fulfill the requirement of 5/11 supportive criteria, the diagnosis of “probably atypical RTT” may be given. Individuals who fall into this category should be reassessed as they age and the diagnosis revised accordingly.”<sup>5</sup>

## Treatment

Treatment for Rett syndrome is based on the symptoms and usually involves therapies to help with movement and communication.<sup>1</sup> Medications can control difficult behavior and seizures, when present.<sup>1</sup>

People with Rett syndrome are at risk for an irregular heart rhythm (arrhythmia). They may need heart monitoring and should avoid certain drugs that are known to affect the heart rhythm.<sup>1</sup>

## Test information

### Introduction

Testing for Rett syndrome may include MECP2 sequencing, deletion/duplication analysis, or known familial mutation analysis.

### Sequence analysis

MECP2 sequencing identifies an MECP2 gene mutation in about 80% of people with classic Rett syndrome and 40% of people with atypical Rett syndrome.<sup>1</sup>

### Deletion/duplication analysis

When MECP2 gene sequencing is normal, deletion and duplication analysis can be performed to look for other types of gene mutations. About 8% of people with classic Rett syndrome and 3% of people with atypical Rett syndrome have an MECP2 gene deletion.<sup>1</sup>

### Known familial mutation analysis

If a pathogenic MECP2 mutation is found in an affected person, then other family members may be offered testing.<sup>1</sup> Prenatal testing is available when the MECP2 mutation in the family is known.<sup>1</sup>

## Guidelines and evidence

### Introduction

This section includes relevant guidelines and evidence pertaining to Rett syndrome testing.

### National Institute for Health and Clinical Excellence

The National Institute for Health and Clinical Excellence (NICE) released evidence-based guidelines entitled *Autism spectrum disorder in under 19s: recognition, referral and diagnosis* in 2011 (updated in 2017). These guidelines state that Rett syndrome should be considered as a type of developmental regression. Genetic testing for such conditions should be considered on an individual basis.<sup>4</sup>

### American Academy of Pediatrics

The consensus guideline from the American Academy of Pediatrics (2014)<sup>6</sup> on the clinical genetic evaluation of a child with intellectual disability (ID) or global developmental delays (DD) and the American College of Medical Genetics (ACMG)<sup>7</sup> 2013 Practice Guidelines for identifying the etiology of autism spectrum disorders state that:

“If the diagnosis is unknown and no clinical diagnosis is strongly suspected, begin with a stepwise evaluation including: chromosome microarray, specific metabolic testing, and Fragile X syndrome testing. If no diagnosis is established and the patient is female, then MECP2 sequencing, deletion, and duplication testing is appropriate.”<sup>7</sup>

## Criteria

### Introduction

Requests for Rett syndrome testing are reviewed using these criteria.

### MECP2 Known Familial Mutation Analysis

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Testing:
  - No previous genetic testing of MECP2, and
  - MECP2 mutation identified in 1st degree biologic relative, OR
- Prenatal Testing for At-Risk Pregnancies:
  - MECP2 mutation identified in a previous child of either parent.

### MECP2 Sequencing

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Testing:
  - No previous MECP2 sequencing, and
  - No known MECP2 mutation in family, AND
- Diagnostic Testing for Symptomatic Individuals:
  - Member meets clinical diagnostic criteria for classic Rett syndrome, atypical Rett syndrome, or has probable Rett syndrome, or
  - Member meets all of the following:
    - Female with a formal diagnosis of autism, and
    - Previous Fragile X testing has been performed and is negative, and

- Previous chromosome microarray has been performed and is negative, and
- Genetic testing is necessary because there is uncertainty in clinical diagnosis

### MECP2 Deletion/Duplication Analysis

- Previous testing:
  - No previous deletion/duplication analysis of MECP2, and
  - No mutations detected in full sequencing of MECP2.

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These references are cited in this guideline.

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# RosettaGX Reveal

MOL.TS.283.A

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## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedures addressed by this guideline | Procedure code |
|--|----------------|
| RosettaGX Reveal                       | 81479          |

## What are thyroid nodules

### Definition

Thyroid nodules are relatively common; however, only an estimated 15% of nodules are malignant.

The standard of diagnosis of thyroid nodules is fine needle aspiration (FNA), and for a majority of patients, an analysis of FNA smears results in a definitive and accurate designation of benign or malignant. However, approximately 10-40% of FNA results yield a cytologically indeterminate diagnosis.<sup>1</sup> Approximately 6% of indeterminate diagnoses receive a malignant status, yet the majority of patients undergo diagnostic surgery in the form of a thyroid lobectomy or a total thyroidectomy.<sup>2</sup>

A post-surgery evaluation provides a conclusive diagnosis or rules out malignancy. For those with benign cytologies, thyroid excision can lead to decreased quality of life (QoL) due to issues with subsequent hypothyroidism, irreversible hormonal changes, chronic fatigue, potential laryngeal nerve injury, and life-long implementation of hormone-replacement supplements.<sup>1</sup>

Mutation analysis of molecular markers found in thyroid microRNA isolated in FNA smears can be indicative of cancer status. The analysis of microRNA in thyroid nodules has been suggested as a means of distinguishing between expression profiles that are malignant and those that are benign.<sup>1</sup>

## Test information

### Introduction

RosettaGX Reveal™ is a thyroid microRNA classifier that assesses if a suspicious thyroid nodule is benign or malignant in patients with indeterminate cytology results for thyroid cancer.

This assay seeks to diagnose indeterminate thyroid nodules utilizing stained fine-needle aspiration (FNA) smears prepared from the patient's original biopsy, potentially reducing the need for additional surgical excisions.<sup>3</sup>

RosettaGX Reveal is a diagnostic assay that utilizes quantitative reverse transcription polymerase chain reaction (qRT-PCR) to measure levels of 24 microRNA species; these levels are then analyzed with a proprietary classifier algorithm in order to predict whether the lesion is either benign or suspicious for malignancy.<sup>4</sup>

RosettaGX Reveal utilizes air-dried Romanowsky-type stained slides and alcohol-fixed Papanicolaou slides, which are the same types of slides used for standard morphologic analysis. This assay can assess thyroid cancer status regardless of latency between collection and analysis of smear, temperature in which the smear was stored, and the overall age of the smear. RosettaGX Reveal can be utilized on minute or limited RNA amounts as small as 20ng/uL.<sup>1</sup>

## Guidelines and evidence

### Introduction

The following section includes relevant guidelines and evidence pertaining to RosettaGX Reveal testing.

### National Comprehensive Cancer Network

The National Comprehensive Cancer Network (NCCN, 2019) Thyroid Carcinoma Guidelines incorporate the use of molecular tests in the evaluation of indeterminate thyroid nodules (category 2B). For FNA results consistent with Follicular or Hürthle Cell Neoplasms, or atypia of undetermined significance/follicular lesion of undetermined significance (AUS/FLUS) with a “High clinical suspicion of malignancy”, they state:<sup>5</sup>

“The diagnosis of follicular carcinoma or Hürthle cell carcinoma requires evidence of either vascular or capsular invasion, which cannot be determined by FNA. Molecular diagnostics may be useful to allow reclassification of follicular lesions (i.e. follicular neoplasm, atypia of undetermined significance (AUS), follicular lesions of undetermined significance (FLUS)) as either more or less likely to be benign or malignant based on the genetic profile....If molecular testing, in conjunction with clinical and ultrasound features, predicts a risk of malignancy comparable to the risk of malignancy seen with a benign FNA cytology (approximately 5% or less), consider active surveillance. Molecular markers should be interpreted with caution and in the context of clinical, radiographic, and cytologic features of each individual patient.”

### American Thyroid Association

The American Thyroid Association (ATA) and ATA Guidelines Task Force have released the following commentary with regards to Bethesda Category III nodules in adults:<sup>6</sup>



- “For nodules with AUS/FLUS cytology, after consideration of worrisome clinical and sonographic features, investigations such as repeat FNA or molecular testing may be used to supplement malignancy risk assessment in lieu of proceeding directly with a strategy of either surveillance or diagnostic surgery. Informed patient preference and feasibility should be considered in clinical decision-making. (Weak recommendation, Moderate-quality evidence)”
- “If repeat FNA cytology, molecular testing, or both are not performed or inconclusive, either surveillance or diagnostic surgical excision may be performed for an AUS/FLUS thyroid nodule, depending on clinical risk factors, sonographic pattern, and patient preference. (Strong recommendation, Low-quality evidence)”
- “There is insufficient evidence regarding the utility of molecular testing aids in the evaluation of indeterminate pediatric thyroid nodules.”

### **Thyroid Scientific Committee of American Association of Clinical Endocrinologists**

The Thyroid Scientific Committee of American Association of Clinical Endocrinologists (AACE) (2016) issued the following commentary regarding current assays assessing molecular diagnostic testing of thyroid nodules with indeterminate cytopathology:<sup>7</sup>

- “Only the BRAFV600E and RET/PTC rearrangement are associated with a PPV that approaches 100%...molecular testing is meant to complement and not replace clinical judgment, sonographic assessment, and visual cytopathology interpretation[.] Prospective multicenter studies are required to validate all of these tests used either singly or in tandem.”

### **American Association of Clinical Endocrinologists, American College of Endocrinology, and Associazione Medici Endocrinologi (AACE/ACE/AME) Guidelines**

The AACE/ACE/AME 2016 Clinical Practice Guidelines for the Diagnosis and Management of Thyroid Nodules state the following:<sup>8</sup>

- In nodules with indeterminate cytologic results, no single cytochemical or genetic marker is specific or sensitive enough to rule out malignancy with certainty. However the use of immunohistochemical and molecular markers may be considered together with the cytologic subcategories and data from US (ultrasound), elastography, or other imaging techniques to obtain additional information for management of these patients.
- When molecular testing should be considered:
  - To complement not replace cytologic evaluation (BEL 2, GRADE A)
  - The results are expected to influence clinical management (BEL 2, GRADE A)
  - As a general rule, not recommended in nodules with established benign or malignant cytologic characteristics (BEL 2, GRADE A)

- Molecular testing for cytologically indeterminate nodules:
  - Cytopathology expertise, patient characteristics, and prevalence of malignancy within the population being tested impact the NPV and PPV for molecular testing (BEL 3, GRADE B)
  - Consider detection of BRAF and RET/PTC and, possibly PAX8/PPARG and RAS mutations if such detection is available (BEL 2, GRADE B)
  - Because of the insufficient evidence and limited follow-up, we do not recommend either in favor of or against the use of gene expression classifiers (GECs) for cytologically indeterminate nodules (BEL 2 GRADE B)
- Role of molecular testing for deciding the extent of surgery
  - Currently, with the exception of mutations such as BRAFV600E that have a PPV approaching 100% for papillary thyroid carcinoma (PTC), the evidence is insufficient to recommend in favor of or against the use of mutation testing as a guide to determine the extent of surgery (BEL 2, GRADE )
- How should patient with nodules that are negative at mutation testing be monitored?
  - Since the false-negative rate for indeterminate nodules is 5 to 6% and the experience and follow-up for mutation negative nodules or nodules classified as benign by a GEC are still insufficient, close follow-up is recommended (BEL 3, GRADE B)

## Literature Review

The evidence is currently insufficient to support the use of RosettaGX Reveal™ when evaluating the microRNA of thyroid nodules for determining a thyroid cancer diagnosis. There is a paucity of studies demonstrating that use of the assay helps patients avoid unnecessary surgery (lobectomy or thyroidectomy) or improves overall patient-important health outcomes (avoidance of repeated FNA procedures or life-long iatrogenic complications from full or partial thyroidectomy).<sup>1,2,9</sup>

## Criteria

### Introduction

Requests for RosettaGX Reveal testing are reviewed using the following criteria.

This test is considered investigational and/or experimental.

- Investigational and experimental (I&E) molecular and genomic (MolGen) tests refer to assays involving chromosomes, DNA, RNA, or gene products that have insufficient data to determine the net health impact, which typically means there is insufficient data to support that a test accurately assesses the outcome of interest

(analytical and clinical validity), significantly improves health outcomes (clinical utility), and/or performs better than an existing standard of care medical management option. Such tests are also not generally accepted as standard of care in the evaluation or management of a particular condition.

- In the case of MolGen testing, FDA clearance is not a reliable standard given the number of laboratory developed tests that currently fall outside of FDA oversight and FDA clearance often does not assess clinical utility.

## References

### Introduction

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# SelectMDx

MOL.TS.264.A

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## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedure addressed by this guideline | Procedure code |
|---------------------------------------|----------------|
| SelectMDx                             | 81479          |

## What is SelectMDx

### Definition

SelectMDx is a proprietary test that is designed to identify an individual's risk of prostate cancer without the need for a biopsy.

- Prostate cancer is the most common cancer among men, with over 200,000 new cases identified each year in the United States.<sup>1,2</sup> The median age at diagnosis is 66 years.<sup>3</sup> Older men are more likely to be affected than younger men, and African American men have higher rates compared to men of other ethnic backgrounds.<sup>3</sup>
- Screening programs for prostate cancer allow for its early detection. Screening is typically performed by prostate-specific antigen (PSA) test and digital rectal examination (DRE).<sup>2</sup>
- Diagnosis is confirmed by prostate biopsy.<sup>4,6</sup> Biopsy is typically performed by collection of approximately 12 needle biopsy cores.<sup>6</sup>
- Initial biopsies only detect 65-77% of prostate cancers, and repeat biopsies are frequently performed.<sup>7,8</sup> The false negative rate of biopsy may be as high as 25%.<sup>9</sup>

### Test information

- SelectMDx is a urine based assay that measures mRNA levels of DLX1 and HOXC6 to determine an individual's risk of prostate cancer. KLK3 expression is used as an internal reference.<sup>10</sup>
  - Higher levels of DLX1 and HOXC6 are associated with an increased risk of prostate cancer.
- This test is performed on first-void urine samples in patients post-digital rectal exam.
- Individuals with a high risk score on SelectMDx may need a biopsy.<sup>10</sup>

- Individuals with a low risk score on this test may be able to avoid a biopsy.<sup>10</sup>

## Guidelines and evidence

### National Comprehensive Cancer Network

The National Comprehensive Cancer Network (NCCN, 2019) considers SelectMDx to be investigational, citing lack of additional validation of the scoring model in independent populations/cohorts and lack of long-term follow-up in the studied population.<sup>6</sup>

### American Urological Association

The American Urological Association issued a Guideline Statement (2013, reaffirmed 2018) and stated:<sup>11</sup>

- “While the benefits of PSA-based prostate cancer screening have been evaluated in randomized-controlled trials, the literature supporting the efficacy of digital rectal exam (DRE), PSA derivatives and isoforms (e.g. free PSA, -2proPSA, prostate health index, hK2, PSA velocity or PSA doubling time) and novel urinary markers and biomarkers (e.g. PCA3) for screening with the goal of reducing prostate cancer mortality provide limited evidence to draw conclusions.”

### Literature Review

- 2 clinical studies were identified for SelectMDx, which detail the results of a total of 3 studies describing the development and initial clinical validation of SelectMDx. One of the studies detailed the process with which the genetic markers utilized in the test were discovered, and the second study described the development of a risk score that incorporates the genetic markers with traditional risk factors, and the subsequent clinical validation of the risk score.<sup>12,13</sup>
- Though the initial results are encouraging, there is an overall paucity of sufficient evidence currently available in the peer-reviewed literature to evaluate the clinical utility of this test. Only two studies have been published to date regarding the performance of SelectMDx. Of the three cohorts studied among the two publications, the first two were utilized to establish analytical validity, and the third was utilized for clinical validity. Furthermore, studies were conducted in the Netherlands; thus the results are not generalizable to men living in other countries, including the United States.<sup>1,2</sup>
- Given that most of the results were focused on test validation, SelectMDx needs to be tested in larger and heterogeneous populations in diverse clinical settings to further assess the clinical validity and clinical utility of this test.<sup>1,2</sup>

## Criteria

- This test is considered investigational and/or experimental.
  - Investigational and experimental (I&E) molecular and genomic (MolGen) tests refer to assays involving chromosomes, DNA, RNA, or gene products that have insufficient data to determine the net health impact, which typically means there is insufficient data to support that a test accurately assesses the outcome of interest (analytical and clinical validity), significantly improves health outcomes (clinical utility), and/or performs better than an existing standard of care medical management option. Such tests are also not generally accepted as standard of care in the evaluation or management of a particular condition.
  - In the case of MolGen testing, FDA clearance is not a reliable standard given the number of laboratory developed tests that currently fall outside of FDA oversight and FDA clearance often does not assess clinical utility.

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# SensiGene Fetal RHD Genotyping

MOL.TS.275.A

v1.0.2020

## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedure addressed by this guideline | Procedure code |
|---------------------------------------|----------------|
| SensiGene RhD Assay                   | 81403          |

## What is RhD

### Definition

Women who are Rhesus D negative (RhD-) exposed to fetal RhD+ erythrocytes can develop anti-Rh antibodies (Rh-isoimmunization), which have the potential to permeate the placenta and cause hemolytic disease. If proper screening and treatment procedures are not carried out, Rh-isoimmunization has the potential to result in fetal/neonatal morbidity and mortality. Early identification of pregnancies with high risk for isoimmunization can better prepare both mothers and physicians for proper course of treatment, which may include fetal transfusion (during early pregnancy) or scheduled labor induction (for later pregnancies). The prevalence of RhD- blood type varies by race, where 15% of Caucasians, 5%-8% of African Americans, and 1%- 2% of Asians and Native Americans are RhD-.<sup>1</sup>

- Amniocentesis is the chief modality employed to assess fetal blood type; however, invasive procedures such as amniocentesis and chorionic villus sampling (CVS) have the potential to increase risk of fetal-maternal hemorrhage, which can lead to severe complications. An estimated 50% of previously sensitized women are considered low-risk for fetal RhD incompatibility; therefore, an alternative, non-invasive assay may reduce the number of unnecessary invasive procedures, as well as parental stress in cases involving sensitized women.<sup>2</sup> In cases where RhD hemolytic antibodies are not produced, identification of the fetus as RhD- is necessary to manage the use of anti-D prophylaxis immune globulin, and it may reduce need for intensive prenatal monitoring to predict and treat fetal anemia.<sup>3</sup>

## Test information

- SensiGene® RHD Assay is a non-invasive, prenatal blood-test developed to evaluate circulating cell-free fetal DNA (ccffDNA) derived from the placenta. This ccffDNA in maternal plasma is used to detect the incompatibility between the mother's blood-type and the fetus' blood-type. It can determine the presence of the

fetal RhD genotype in RhD- mothers, as early as 10 weeks' gestation. This assay may reduce the utilization of invasive procedures that may increase maternal sensitization.<sup>4</sup>

- SensiGene® Fetal RHD Genotyping assay, produced by Sequenom, isolates exons 4, 5, and 7 of the RhD gene (locus chromosome 1), psi-pseudogene in exon 4, and 3 targets on the Y chromosome (SRY, TTTY, DBY), all of which are known to be the directly related to the genetic basis for RhD- phenotypes.
- Reasons to determine fetal RhD status utilizing cffDNA in maternal blood may include
  - avoiding the cost of paternity test/paternal genotyping
  - identifying fetal RhD status in the absence of maternal anti-Rh antigens
  - determining fetal RhD- status for parents opposed to immunization/vaccination
  - reducing the need for CVS or amniocentesis in RhD-sensitized patients
- This assay analyzes cffDNA, which comprises approximately 3% to 6% of cell-free DNA in maternal plasma. Identification of specific exons of the RhD gene, not generally present in RhD- patients, has been found to predict an RhD+ fetus.<sup>4</sup>

## Guidelines and evidence

### The American College of Obstetricians and Gynecologists

In 2017 the American College of Obstetricians and Gynecologists (ACOG) published a practice bulletin stating, "However, at current costs, noninvasive assessment of fetal Rh D status is not recommended for routine use at present".<sup>5</sup>

### Society of Obstetricians and Gynaecologists of Canada

In 2017, the Society of Obstetricians and Gynaecologists of Canada (SOGC) issued a consensus statement in collaboration with the Canadian National Rh Working Group stating that "optimal management of the D-negative pregnant woman is based on the prediction of the fetal D-blood group by cell-free DNA in maternal plasma with targeted antenatal anti-D prophylaxis. This approach should be adopted in Canada (II-2A)."<sup>6</sup>

### The Society for Maternal-Fetal Medicine

In 2015 The Society for Maternal-Fetal Medicine (SMFM) issued a Clinical Guideline regarding the diagnosis and management of the fetus at risk for anemia. The guideline makes no specific recommendations about the use of cell-free DNA in maternal plasma to determine fetal RhD status. However, Figure 2 of their Clinical Guideline, "Algorithm for clinical management of the red cell alloimmunized pregnancy," allows for "Free fetal DNA testing for Rh(D) status or amniocentesis for fetal Rh(D) genotyping."<sup>7</sup>

## Peer Reviewed Literature

- The available evidence suggests that the clinical validity of the fetal RHD genotyping using the SensiGene® assay is relatively high since the test demonstrates good accuracy in correctly predicting fetal RHD status.<sup>1,2,8-11</sup> However, there is a risk of false positive results with use of the test.
- There is limited evidence regarding the clinical utility of the SensiGene RHD Assay to change clinician treatment decisions and ultimately improve patient-health outcomes compared with conventional modes of testing. Additional research is required to determine the performance of RhD genotyping with maternal plasma in improving patient health outcomes compared with amniocentesis and CVS.

## Criteria

- This test is considered investigational and/or experimental.
  - Investigational and experimental (I&E) molecular and genomic (MolGen) tests refer to assays involving chromosomes, DNA, RNA, or gene products that have insufficient data to determine the net health impact, which typically means there is insufficient data to support that a test accurately assesses the outcome of interest (analytical and clinical validity), significantly improves health outcomes (clinical utility), and/or performs better than an existing standard of care medical management option. Such tests are also not generally accepted as standard of care in the evaluation or management of a particular condition.
  - In the case of MolGen testing, FDA clearance is not a reliable standard given the number of laboratory developed tests that currently fall outside of FDA oversight and FDA clearance often does not assess clinical utility.

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# Sept9 Methylation Analysis for Colorectal Cancer

MOL.TS.164.A

v1.0.2020

## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedure addressed by this guideline | Procedure code |
|---------------------------------------|----------------|
| SEPT9 Methylation Analysis            | 81327          |

## What is Sept9 methylation analysis for colorectal cancer

### Definition

Colorectal cancer (CRC) is one of the most common types of cancers, with over 140,000 new cases identified each year in the United States.<sup>1</sup> It typically affects adults over 55 years old, with a median age at diagnosis of 67 years.<sup>1</sup>

- Screening programs for CRC allow for its early detection. The earlier CRC is caught, the better chance a person has of surviving. Five year survival rates are 89.8% for localized cancer, 71.1% for cancer that has spread regionally, and 13.8% for CRC with distant metastasis.<sup>1</sup>
- Standard recommended screening for CRC includes guaiac-based fecal occult blood test (gFOBT), fecal immunochemical test (FIT), multitargeted stool DNA test (FIT-DNA), colonoscopy, CT colonography, and flexible sigmoidoscopy. Screening begins at age 50 years and continues until at least age 75 for people at average risk for CRC.<sup>2</sup>
- Although several screening tests have been endorsed and found to be cost-effective, compliance with CRC screening recommendations is limited. According to 2010 data from the Centers for Disease Control and Prevention (CDC), the percentage of adults over 50 years who reported their CRC screening was up to date ranged from 58.92% to 75.03%, depending on the state. The CDC estimates that 28 million Americans are not up-to-date on CRC screening.<sup>3</sup>
- Two tests designed to detect colorectal cancer by analyzing Sept9 methylation will be addressed in this guideline: Epi proColon and ColoVantage.
- The Epi proColon Test (Epigenomics) is a Septin 9 assay that measures the presence of methylated Septin 9 DNA in a blood sample. It is intended to identify early stage colorectal cancer.<sup>4</sup> It offers an alternative to current screening options.

- The ColoVantage Test (Quest Diagnostics) is a Septin 9 assay that measures the presence of methylated Septin 9 DNA in a blood sample. This test “aids in the detection of colorectal cancer in patients non-adherent to current testing approaches.”<sup>5</sup>

## Test information

- Both Epi proColon and ColoVantage are performed on a blood sample. No bowel preparation or dietary or medication restrictions are required to complete either test.<sup>4,5</sup>
- Both tests measure methylation of Septin 9 DNA.<sup>4,5</sup> Tumors may have increased methylation of Septin 9. When tumor DNA is shed into the bloodstream, this increase in methylation of Septin 9 may be found in the blood.<sup>4,6</sup>
- Epi proColon provides a qualitative result: positive or negative. People who receive positive results should be referred for a diagnostic colonoscopy. Those with negative results can continue with standard CRC screening recommendations.<sup>4,6</sup>

## Guidelines and evidence

- There are currently no US guidelines that specifically address the use of either Epi proColon or ColoVantage testing.
- Current CRC cancer screening guidelines from the U.S. Preventive Services Task Force (USPSTF, 2016) recommend the use of gFOBT, FIT, FIT-DNA, colonoscopy, CT colonography, and flexible sigmoidoscopy for individuals ages 50 years to 75 years at average risk of colorectal cancer.<sup>2</sup> These guidelines specifically state the following regarding Septin DNA testing:
  - “Although a serology test to detect methylated **SEPT9** DNA was included in the systematic evidence review, this screening method currently has limited evidence evaluating its use (a single published test characteristic study met inclusion criteria, which found it had a sensitivity to detect colorectal cancer of <50%). It is therefore not included in this table.”<sup>2</sup>
- For other age groups, the USPSTF guidelines recommend the following:
  - “For older adults aged 76 to 85 years, the benefits of screening for colorectal cancer decline, and the risk of experiencing serious associated harms increases. The most important consideration for clinicians and patients in this age group is whether the patient has previously been screened. Patients in this age group who have never been screened for colorectal cancer are more likely to benefit than those who have been previously screened.”<sup>2</sup>
  - “Screening [in adults aged 76 to 85 years] would be most appropriate among adults who 1) are healthy enough to undergo treatment if colorectal cancer is

- detected and 2) do not have comorbid conditions that would significantly limit their life expectancy.”<sup>2</sup>
- “The USPSTF does not recommend routine screening for colorectal cancer in adults 86 years and older. In this age group, competing causes of mortality preclude a mortality benefit that would outweigh the harms.”<sup>2</sup>
  - The U.S. Food and Drug Administration approved Epi proColon in 2016 as an in vitro diagnostic.<sup>6</sup>
    - “The Epi proColon test is indicated to screen adults of either sex, 50 years or older, defined as average risk for CRC, who have been offered and have a history of not completing CRC screening.”
    - “The Epi proColon test is not intended to replace colorectal cancer screening tests that are recommended by appropriate guidelines (e.g., 2008 USPSTF guidelines) such as colonoscopy, sigmoidoscopy and high sensitivity fecal occult blood testing.”
    - “The Epi proColon test is not intended for patients who are willing and able to undergo routine colorectal cancer screening tests that are recommended by appropriate guidelines.”
    - “Tests that are available and recommended in the USPSTF 2008 CRC screening guidelines should be offered and declined prior to offering the Epi proColon test.”
  - The National Comprehensive Cancer Network guidelines on colorectal cancer screening (version 1.2018) include the following footnote regarding methylated SEPT9 DNA testing:<sup>7</sup>
    - “A blood test that detects circulating methylated SEPT9 DNA was recently FDA-approved and may provide an option for screening for those who refuse other screening modalities, but its ability to detect CRC and advanced adenoma is inferior to other recommended screening modalities. The interval for repeating testing is unknown.”
  - ***Epi proColon***
    - “The performance of Epi proColon has been established in cross-sectional (i.e., single point in time) studies. Programmatic performance of Epi proColon (i.e., benefits and risks with repeated testing over an established period of time) has not been studied. Performance has not been evaluated for patients who have been previously tested with Epi proColon. Non-inferiority of Epi proColon programmatic sensitivity as compared to other recommended screening methods for CRC has not been established.”<sup>7</sup>
    - “Screening with Epi proColon in subsequent years following a negative test result should be offered only to patients who after counseling by their healthcare provider, again decline CRC screening methods according to appropriate guidelines. The screening interval for this follow-up has not been established.”<sup>6</sup>

- The frequency interval that follow up Epi proColon testing should be performed has yet to be established.<sup>6,7</sup>
- A large, prospective multicenter trial (PRESEPT) evaluated men and women over the age of 50 years who were at average risk for colorectal cancer.<sup>8</sup>
  - Clinical performance of the Epi proColon test in terms of sensitivity and specificity was based on 1544 samples from subjects whose colorectal cancer status was determined by colonoscopy.
  - Sensitivity was determined to be 68.2% with a specificity of 78.8%. Positive predictive value (PPV) was 2.4% with a negative predictive value (NPV) of 99.7%.
- In 6 clinical validation studies, values of sensitivity and specificity of the Epi proColon test were reported.<sup>10-15</sup> Sensitivity ranged from 72% to 82%, and specificity ranged from 81% to 97%. One study showed increasing sensitivity for higher CRC stages (~89% at Stage IV). In a comparative clinical validation trial, Epi proColon showed better sensitivity but worse specificity, when compared with gFOBT or FIT. Another study showed that the performance of the test is negatively impacted by risk factors frequently observed in CRC screening populations, such as early-stage disease, age > 65 years, diabetes, arthritis, and arteriosclerosis. Specifically, increased age was associated with increased rates of false positive and false negative results.
  - Results of a recent meta-analysis/systematic review indicate that the area under the receiver operating curve (AUC) for the pooled diagnostic accuracy results for Epi proColon test was 0.8709. In head-to-head comparisons, the AUC of the combined results of 1) Epi proColon and mSEPT 9 tests and 2) FOBT for CRC diagnosis were 0.7857 and 0.6571, respectively.<sup>16</sup>
- **ColoVantage**
  - The analytical validity, clinical validity, and clinical utility of the ColoVantage test for detecting CRC has not been established.
    - ColoVantage Plasma is currently undergoing clinical trials in Australia.<sup>17</sup>

## Criteria

- **Epi proColon and ColoVantage testing** are considered investigational and/or experimental.
  - Investigational and experimental (I&E) molecular and genomic (MolGen) tests refer to assays involving chromosomes, DNA, RNA, or gene products that have insufficient data to determine the net health impact, which typically means there is insufficient data to support that a test accurately assesses the outcome of interest (analytical and clinical validity), significantly improves health outcomes



(clinical utility), and/or performs better than an existing standard of care medical management option. Such tests are also not generally accepted as standard of care in the evaluation or management of a particular condition.

- In the case of MolGen testing, FDA clearance is not a reliable standard given the number of laboratory developed tests that currently fall outside of FDA oversight and FDA clearance often does not assess clinical utility

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# Somatic Mutation Testing-Hematological Malignancies

MOL.TS.313.A

v1.0.2020

## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedures addressed by this guideline                        | Procedure codes |
|---|-----------------|
| ASXL1 Full Gene Sequencing                                    | 81175           |
| ASXL1 Mutation Analysis                                       | 81176           |
| ABL1 Mutation Analysis  | 81170           |
| CALR Exon 9 Mutation Analysis                                 | 81219           |
| CEBPA Mutation Analysis                                       | 81219           |
| CEBPA Full Gene Sequencing                                    | 81218           |
| EZH2 Common Variant(s) (e.g. codon 646)                       | 81237           |
| EZH2 Full Gene Sequencing                                     | 81236           |
| FLT3 Mutation Analysis (internal tandem duplication variants) | 81245           |
| FLT3 Mutation Analysis (tyrosine kinase domain variants)      | 81246           |
| IDH1 Mutation Analysis  | 81120           |
| IDH2 Mutation Analysis  | 81121           |
| JAK2 V617F Mutation Analysis                                  | 81270           |
| KIT Targeted Mutation Analysis                                | 81272           |
| KIT Mutation Analysis (D816 variants)                         | 81273           |
| NPM1 Mutation Analysis  | 81310           |
| NRAS Mutation Analysis  | 81311           |
| RUNX1 Mutation Analysis                                       | 81334           |
| TERT Targeted Sequence Analysis                               | 81345           |
| Hematolymphoid Neoplasm Molecular Profiling                   | 81450           |

| Procedures addressed by this guideline                                | Procedure codes |
|---|-----------------|
| Solid Organ or Hematolymphoid Neoplasm Molecular Profiling - Expanded | 81455           |
| Miscellaneous Molecular Tumor Marker Test                             | 81400           |
| Miscellaneous Molecular Tumor Marker Test                             | 81401           |
| Miscellaneous Molecular Tumor Marker Test                             | 81402           |
| Miscellaneous Molecular Tumor Marker Test                             | 81403           |
| Miscellaneous Molecular Tumor Marker Test                             | 81404           |
| Miscellaneous Molecular Tumor Marker Test                             | 81405           |
| Miscellaneous Molecular Tumor Marker Test                             | 81406           |
| Miscellaneous Molecular Tumor Marker Test                             | 81407           |
| Miscellaneous Molecular Tumor Marker Test                             | 81408           |
| Miscellaneous Molecular Tumor Marker Test                             | 81479           |
| Miscellaneous Molecular Tumor Marker Test                             | 88271           |

## What are somatic mutation tests

### Definition

A somatic mutation test for hematologic malignancies is broadly defined here as any test that measures changes in DNA, RNA, or chromosomes and is used to make cancer management decisions.

- Somatic mutation tests are increasingly useful for therapy selection. Many cancer therapies are targeted at particular gene functions (therapeutic targets) and some require information about the genetics of the malignancy to use the therapies effectively (companion diagnostics). In these cases, National Comprehensive Cancer Network (NCCN) as well as the U.S. Food and Drug Administration (FDA)

have outlined tumor testing that is recommended for specific cancers and the associated treatment implications.<sup>1-4</sup>

## Test information

- The specific methodology used to identify somatic mutations is dependent upon the type of mutation being investigated. For hematological malignancies, this testing is typically performed in a bone marrow or blood sample.
  - DNA mutations are generally detected through direct analysis of individual mutations, portions of a gene, a whole gene, panels of genes, or the entire exome.
  - Chromosome abnormalities, such as translocations or deletions, may be detected through direct visualization of the chromosomes (karyotyping), in situ hybridization of probes (e.g., FISH) to detect deletions or duplications that are too small to see directly, or by DNA-based methods (hybridization arrays or sequencing) that identify deletions or translocation breakpoints.
  - Gene expression profiling simultaneously measures the amount of RNA being made by many genes. Expression patterns may be used to predict the type of cancer present, the aggressiveness of the malignancy, and therapies that are likely to be effective.
- The efficiency of next generation sequencing (NGS) has led to an increasing number of large, multi-gene somatic mutation panels. Given that malignancies can have multiple and unexpected genetic changes, these panels may provide physicians with information about therapeutic targets that would not otherwise be considered.

## Guidelines and evidence

- The National Comprehensive Cancer Network (NCCN) provides the following guidance:
  - NCCN Guidelines for Treatment of Cancer by Site provide detailed guidelines on the use of individual markers for each cancer type addressed.<sup>4</sup>
  - NCCN also maintains a biomarker compendium stating “the goal of the NCCN Biomarkers Compendium is to provide essential details for those tests which have been approved by NCCN Guideline Panels and are recommended by the NCCN Guidelines.”<sup>3</sup> Biomarkers for specific cancer types that are listed in the NCCN Biomarker Compendium have a level of evidence associated with their clinical utility.
- Some FDA labels require results from biomarker tests to effectively or safely use the therapy for a specific cancer type.<sup>2</sup> A list of all Pharmacogenomic Biomarkers included in FDA labeling and associated implications can be found [here](#). While

these tests generally consist of a single biomarker, some larger panels of biomarkers are also included in the FDA labeling.

## Criteria

This guideline applies to all molecular somatic mutation testing intended for use in hematological malignancies. This guideline does not apply to somatic mutation testing for solid tumors; please see *Somatic Mutation Testing – Solid Tumors*. This guideline does not apply to tumor markers assayed by liquid biopsy; please see *Liquid Biopsy Testing – Solid Tumors*. This guideline also does not apply when testing for germline (inherited) mutations in genes related to hereditary cancer syndromes (e.g., Hereditary Breast and Ovarian Cancer, Lynch syndrome, etc.). Although some of the same genes may be tested for inherited or acquired (somatic) mutations, this guideline addresses only testing for acquired mutations from hematological malignancies.

Medical necessity criteria differ based on the type of testing being performed (i.e., tests for individual genes separately chosen based on the cancer type versus pre-defined panels of genes) and how that testing will be billed (one or more individual gene-specific procedure codes, specific panel procedure codes or unlisted procedure codes).

When separate procedure codes will be billed for individual biomarkers (e.g., Tier 1 MoPath codes 81200-81355 or Tier 2 MoPath codes 81400-81408), each individually billed test will be evaluated separately. The following criteria will be applied:

- The member has a malignancy type that will benefit from information provided by the requested test based on at least one of the following:
  - All criteria are met from a test-specific guideline, if available, or
  - An oncology therapy FDA label requires results from the test to effectively or safely use the therapy for the member's cancer type, or
  - NCCN guidelines include the test in the management algorithm for that particular cancer type and all other requirements are met (specific pathology findings, staging, etc.); however, the marker must be explicitly recommended in the guidelines and not simply included in a footnote as an intervention that may be considered, or
  - The NCCN Biomarker Compendium has a level of evidence of at least 2A for the biomarker's application to the member's specific cancer type

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**Note** If five or more individually billed biomarker tests are under review together (a "panel") and the member meets the below criteria for a multi-gene panel, the panel will be approved. However, the laboratory will be redirected to use an appropriate panel CPT code for billing purposes (e.g. 81450).

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When a multi-gene panel is being requested and will be billed with a single panel CPT code (e.g. 81450), the panel will be considered medically necessary when the following criteria are met:

- All criteria for a multi-gene panel are met from a test-specific guideline, if available, OR
- At least 5 markers included in the panel individually meet criteria for the member's cancer type based on one of the following:
  - All criteria are met from a test-specific guideline, if available, or
  - An oncology therapy FDA label requires results from the test to effectively or safely use the therapy for the member's cancer type, or
  - NCCN guidelines include the test in the management algorithm for that particular cancer type and all other requirements are met (specific pathology findings, staging, etc.); however, the marker must be explicitly included in the guidelines and not simply included in a footnote as an intervention that may be considered, or
  - The NCCN Biomarker Compendium has a level of evidence of at least 2A for the marker's application to the member's specific cancer type

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**Note** If the member meets criteria for less than 5 of the individual biomarkers in the panel, the panel will not be reimbursed. The laboratory will be redirected to billing for individual tests for which the member meets criteria.

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### **Billing and reimbursement consideration**

Panels over 50 genes, typically billed with CPT code 81455, are not considered medically necessary, as they are excessive for use in evaluating hematological malignancies and, therefore, are not eligible for reimbursement.

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# Somatic Mutation Testing-Solid Tumors

MOL.TS.230.A  
v1.0.2020

## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedures addressed by this guideline                                | Procedure codes |
|---|-----------------|
| APC Sequencing  | 81201           |
| BRAF V600 Targeted Mutation Analysis                                  | 81210           |
| BRCA1/2 Sequencing  | 81163           |
| BRCA1 Sequencing  | 81165           |
| BRCA2 Sequencing  | 81216           |
| EGFR Targeted Mutation Analysis                                       | 81235           |
| KIT Targeted Sequence Analysis  | 81272           |
| KIT D816 Targeted Mutation Analysis                                   | 81273           |
| KRAS Exon 2 Targeted Mutation Analysis                                | 81275           |
| KRAS Targeted Mutation Analysis, Additional Variants                  | 81276           |
| MGMT Promoter Methylation Analysis                                    | 81287           |
| MLH1 Sequencing   | 81292           |
| MSH2 Sequencing   | 81295           |
| MSH6 Sequencing   | 81298           |
| NRAS Exon 2 and Exon 3 Analysis                                       | 81311           |
| PDGFRA Targeted Sequence Analysis                                     | 81314           |
| PMS2 Sequencing   | 81317           |
| PTEN Sequencing   | 81321           |
| Solid Organ Neoplasm Molecular Profiling                              | 81445           |
| Solid Organ or Hematolymphoid Neoplasm Molecular Profiling - Expanded | 81455           |
| TERT Targeted Sequence Analysis                                       | 81345           |
| Miscellaneous Molecular Tumor Marker Test                             | 81400           |

| Procedures addressed by this guideline    | Procedure codes |
|---|-----------------|
| Miscellaneous Molecular Tumor Marker Test | 81401           |
| Miscellaneous Molecular Tumor Marker Test | 81402           |
| Miscellaneous Molecular Tumor Marker Test | 81403           |
| Miscellaneous Molecular Tumor Marker Test | 81404           |
| Miscellaneous Molecular Tumor Marker Test | 81405           |
| Miscellaneous Molecular Tumor Marker Test | 81406           |
| Miscellaneous Molecular Tumor Marker Test | 81407           |
| Miscellaneous Molecular Tumor Marker Test | 81408           |
| Miscellaneous Molecular Tumor Marker Test | 81479           |
| Miscellaneous Molecular Tumor Marker Test | 88271           |
| Oncomine Dx Target Test                   | 0022U           |
| MSK-IMPACT                                | 0048U           |
| Praxis Extended RAS Panel                 | 0111U           |

## What are somatic mutation tests

### Definition

Somatic mutation tests are broadly defined here as any test that measures changes in DNA, RNA, or chromosomes found in tumor tissue that is used to make cancer management decisions.

- Somatic mutation tests are increasingly useful for therapy selection. Many cancer therapies are targeted at particular gene functions (therapeutic targets) and some require information about tumor genetics to use the therapies effectively (companion diagnostics). In these cases, NCCN as well as the FDA have outlined tumor testing that is recommended for specific cancers and the associated treatment implications.<sup>1-5</sup>

## Test information

- The specific methodology used to identify somatic mutations is dependent upon the type of mutation being investigated.
  - DNA mutations are generally detected through direct analysis of individual mutations, portions of a gene, a whole gene, panels of genes, or the entire exome.
  - Chromosome abnormalities, such as translocations or deletions, may be detected through direct visualization of the chromosomes (karyotyping), in situ hybridization of probes (e.g., FISH) to detect deletions or duplications that are too small to see directly, or by DNA-based methods (hybridization arrays or sequencing) that identify deletions or translocation breakpoints.
  - Gene expression profiling simultaneously measures the amount of RNA being made by many genes. Expression patterns may be used to predict the type of cancer present, tumor aggressiveness, and therapy needs.
- The efficiency of next generation sequencing (NGS) has led to an increasing number of large, multi-gene somatic mutation panels. Given that tumors can have multiple and unexpected genetic changes, these panels may provide physicians with information about therapeutic targets that would not otherwise be considered. The following are examples of somatic mutation panels (not intended to be a complete list):
  - Foundation Medicine: FoundationOne tumor marker panel
  - Caris Life Sciences: Caris Tumor profiling
  - Paradigm: Paradigm Cancer Diagnostic (PCDx) Panel
  - GenPath Diagnostics: OnkoMatch
  - University of Washington: UW-OncoPlex-Cancer Gene Panel

## Guidelines and evidence

- The National Comprehensive Cancer Network (NCCN) provides the following guidance:
  - NCCN Guidelines for Treatment of Cancer by Site provide detailed guidelines on the use of individual tumor markers for each cancer type addressed.<sup>5</sup>
  - NCCN also makes the following recommendations specifically for using multi-gene panels in the evaluation of non-small cell lung cancer (NSCLC): “The NCCN NSCLC Guidelines Panel strongly endorses broader molecular profiling with the goal of identifying rare driver mutations for which effective drugs may already be available, or to appropriately counsel patients regarding the availability of clinical trials. Broad molecular profiling is a key component of the improvement of care of patients with NSCLC.”<sup>2</sup>

- NCCN also maintains a biomarker compendium stating “the goal of the NCCN Biomarkers Compendium is to provide essential details for those tests which have been approved by NCCN Guideline Panels and are recommended by the NCCN Guidelines.”<sup>4</sup> Biomarkers for specific cancer types that are listed in the NCCN Biomarker Compendium have a level of evidence associated with their clinical utility.
- Some FDA labels require results from biomarker tests to effectively or safely use the therapy for a specific cancer type.<sup>3</sup> A list of all Pharmacogenomic Biomarkers included in FDA labeling and associated implications can be found [here](#). While these tumor marker tests generally consist of a single biomarker, some larger panels of biomarkers are also included in the FDA labeling.
  - In 2017, the FDA approved FoundationOne CDx panel testing, which includes 324 genes, for particular individuals with NSCLC, melanoma, breast cancer, colorectal cancer, or ovarian cancer. See FDA document [here](#).<sup>6</sup> A list of cleared or approved companion diagnostic devices, including FoundationOne CDx can be found [here](#).<sup>7</sup>
  - In 2016, the FDA approved Oncomine Dx Target Test for individuals with non-small cell lung cancer (NSCLC). “The Oncomine™ Dx Target Test is a qualitative in vitro diagnostic test that uses targeted high throughput, parallel-sequencing technology to detect single nucleotide variants (SNVs) and deletions in 23 genes from DNA and fusions in ROS1 from RNA isolated from formalin fixed, paraffin-embedded (FFPE) tumor tissue samples from patients with non-small cell lung cancer (NSCLC) using the Ion PGM™ Dx System.”<sup>8</sup>

## Criteria

This guideline applies to all molecular somatic mutation testing intended for use in solid tumors. This guideline does not apply to testing for hematologic malignancies. This guideline also does not apply to tumor markers found by liquid biopsy. Please see *Liquid Biopsy Testing – Solid Tumors*. This guideline also does not apply when testing for germline (inherited) mutations in genes related to hereditary cancer syndromes (e.g., Hereditary Breast and Ovarian Cancer, Lynch syndrome, etc.). Although some of the same genes may be tested for inherited or acquired mutations, this guideline addresses only testing for acquired mutations from tumor tissue.

Medical necessity criteria differ based on the type of testing being performed (i.e., tests for individual genes separately chosen based on the cancer type, versus pre-defined panels of genes) and how that testing will be billed (one or more individual gene-specific procedure codes, specific panel procedure codes, or unlisted procedure codes).

When separate procedure codes will be billed for individual tumor markers (e.g., Tier 1 MoPath codes 81200-81355 or Tier 2 MoPath codes 81400-81408), each individually billed tumor marker test will be evaluated separately. The following criteria will be applied:

- The member has a tumor type that will benefit from information provided by the requested tumor marker test based on at least one of the following:
  - All criteria are met from a test-specific guideline if one is available (See *Common cancer types and associated tumor markers* table below for tumor marker tests that have separate test-specific guidelines.), or
  - An oncology therapy FDA label requires results from the tumor marker test to effectively or safely use the therapy for the member's cancer type (See *Common cancer types and associated tumor markers* table below for examples of currently recognized companion diagnostics), or
  - NCCN guidelines include the tumor marker test in the management algorithm for that particular cancer type and all other requirements are met (specific pathology findings, staging, etc.); however, the tumor marker must be explicitly included in the guidelines and not simply included in a footnote as an intervention that may be considered, or
  - The NCCN Biomarker Compendium has a level of evidence of at least 2A for the tumor marker's application to the member's specific cancer type

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**Note** If five or more individually billed tumor marker tests are under review together (a "panel") and the member either has non-small cell lung cancer OR meets criteria for 5 or more individual tumor markers, the panel will be approved. However, the laboratory will be redirected to use a panel CPT code for billing purposes (e.g. 81445 or 81455).

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When a multi-gene panel is being requested and will be billed with a single panel CPT code (e.g. 81445 or 81455), the panel will be considered medically necessary when the following criteria are met:

- The member has a diagnosis of non-small cell lung cancer, OR
- At least 5 tumor markers included in the panel individually meet criteria for the member's tumor type based on one of the following:
  - All criteria are met from a test-specific guideline if one is available (See *Common cancer types and associated tumor markers* table below for tumor marker tests that have separate test-specific guidelines.), or
  - An oncology therapy FDA label requires results from the tumor marker test to effectively or safely use the therapy for the member's cancer type (See *Common cancer types and associated tumor markers* table below for examples of currently recognized companion diagnostics for available therapies.), or
  - NCCN guidelines include the tumor marker test in the management algorithm for that particular cancer type and all other requirements are met (specific pathology findings, staging, etc.); however, the tumor marker must be explicitly included in the guidelines and not simply included in a footnote as an intervention that "may be considered", or

- The NCCN Biomarker Compendium has a level of evidence of at least 2A for the tumor marker's application to the member's specific cancer type

**Note** If the member meets criteria for less than 5 of the individual tumor markers in the panel, the panel will not be reimbursed. The laboratory will be redirected to billing for individual tests for which the member meets criteria.

### FoundationOne CDx

When FoundationOne CDx testing is being requested, please see the guideline *FoundationOne CDx* for criteria.

### Common cancer types and associated tumor markers

This list not all inclusive.

#### Examples of common cancer types and associated tumor markers

| Cancer Type  | Tumor Marker | CPT   | Claim Code | Associated Treatments*   | Applicable Guideline |
|--|--------------|-------|------------|--|----------------------|
| Colorectal <sup>9</sup><br>(Metastatic, stage IV. Prognostic purposes only.) | BRAF         | 81210 | N/A        | N/A  | MOL.TS.133           |
| Colorectal <sup>9</sup><br>(Metastatic)                                      | KRAS         | 81275 | N/A        | cetuximab <sup>9</sup> , panitumumab <sup>9</sup>  | MOL.TS.191           |
| Colorectal <sup>9</sup><br>(Metastatic)                                      | NRAS         | 81311 | N/A        | N/A  | MOL.TS.230           |
| Melanoma <sup>10</sup><br>(Metastatic)                                       | BRAF         | 81210 | N/A        | vemurafenib <sup>10</sup> , dabrafenib <sup>10</sup> , trametinib/dabrafenib <sup>10</sup> , vemurafenib/cobimetinib <sup>10</sup> | MOL.TS.134           |
| Non-small cell lung <sup>2</sup>   | EGFR         | 81235 | N/A        | erlotinib <sup>2</sup> , afatinib <sup>2</sup> , gefitinib, osimertinib (T790M)  | MOL.TS.163           |

| Cancer Type                      | Tumor Marker | CPT   | Claim Code | Associated Treatments*   | Applicable Guideline |
|----------------------------------|--------------|-------|------------|--|----------------------|
| Non-small cell lung <sup>2</sup> | ALK          | 81401 | EML4ALK    | crizotinib <sup>2</sup> ,<br>ceritinib <sup>2</sup> ,<br>alectinib | MOL.TS.230           |
| Non-small cell lung <sup>2</sup> | ALK          | 81479 | ALK        | crizotinib <sup>2</sup> ,<br>ceritinib <sup>2</sup> ,<br>alectinib | MOL.TS.230           |

**Note** \*\* In general, when there is an associated treatment, results from the referenced tumor marker are necessary for the safe or effective use of that therapy (companion diagnostics). The therapies and tumor markers are only included for cancer types approved for treatment according to FDA labeling.

## References

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# Spinal Muscular Atrophy Testing

MOL.TS.225.A

v1.0.2020

## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedures addressed by this guideline   | Procedure codes |
|--|-----------------|
| SMN1 Gene Analysis; Dosage/Deletion Analysis (eg, carrier testing), includes SMN2 Analysis, if performed | 81329           |
| SMN1 Full Gene Sequencing  | 81336           |
| SMN1 Known Familial Mutation Analysis  | 81337           |
| SMN2 Dosage/Deletion Analysis  | 81479           |
| SMN2 Targeted Mutation Analysis (c.859G>C)   | 81479           |

## What is spinal muscular atrophy

### Definition

Spinal muscular atrophy (SMA) is a severe, autosomal recessive neuromuscular disease that affects 1 in 8000 to 1 in 10,000 people.<sup>1,2</sup>

- SMA is caused by loss of lower motor neurons (anterior horn cells) in the spinal cord, resulting in progressive symmetrical muscle weakness and atrophy.<sup>1-3</sup>
- The diagnosis of SMA is established in a proband with a history of motor difficulties, evidence of motor unit disease on physical examination, and identification of biallelic pathogenic variants in survival motor neuron 1 (*SMN1*) on molecular genetic testing.<sup>3</sup>
- SMA has historically been divided into three to five clinical subtypes based on age of onset and clinical course. While genetic testing has shown these clinical subtypes are not completely distinct, they are still widely used, and include:<sup>1-3</sup>
  - Prenatal onset form ("Type 0" proposed) is characterized by polyhydramnios, decreased fetal movements, breech presentation, arthrogryposis multiplex congenita, respiratory failure at birth, and life span of less than 6 months.
  - Type I (infantile or Werdnig-Hoffmann type) is the most common form (60-70% of cases). It presents before 6 months of age and median age of survival is 24

- months with cause of death due to respiratory failure. Affected children have severe, generalized weakness and do not ever sit without support.
- Type II (intermediate type) causes muscle weakness with onset after 6 months, although children often are able to sit alone and can survive through childhood. Approximately 70% of affected individuals are alive at 25 years. Intelligence is normal.
  - Type III (juvenile, Kugelberg-Welander type) is milder. Onset ranges from infancy to youth, but affected people usually walk unassisted albeit with frequent falls or trouble with stairs. Survival is prolonged and intelligence is normal.
  - Type IV (adult type) has much later onset with muscle weakness generally presenting at 20-30 years of age. People may or may not become wheelchair dependent, have normal lifespan and normal intelligence.
- SMA is caused by mutations in the SMN1 gene.
    - Large gene deletions (exon 7 +/- exon 8) cause SMA in the vast majority (95-98%) of affected individuals.<sup>3</sup>
    - The remaining 2-5% of individuals with SMA have a deletion in one SMN1 gene and a different mutation in the other.<sup>3</sup>
  - The survivor motor neuron 2 (SMN2) gene is almost identical to SMN1 and located on the same chromosome. SMN2 gene mutations do not cause SMA. In fact, about 15% of unaffected people have no copies of the SMN2 gene. However, SMN2 has been shown to modify the disease severity in people with SMA. More copies (usually 3 or more) of SMN2 are associated with milder disease course. Individuals may have between 0-5 copies of SMN2.<sup>3</sup>
  - SMA is inherited in an autosomal recessive manner.
    - An affected person has two SMN1 gene mutations.<sup>2,3</sup> Most do not have a known family history of the condition.
    - People with only one mutation in the SMN1 gene are called carriers. Carriers do not show symptoms of SMA, but have a 50% chance of passing on their mutation to their children.
    - SMA is present in all ethnic groups. About 1 in 40 to 1 in 60 people are carriers.<sup>2</sup>
    - Two carriers of SMA have a 25% chance of having a child with the disorder.
    - About 2% of SMA patients have a de novo (new) mutation in one of their two SMN1 genes. In this case, only one parent is a carrier of SMA.<sup>3</sup>

## Test information

- **SMN1 Deletion/Dosage Analysis:** Diagnostic testing in an affected individual begins with deletion or copy number analysis, which will identify a deletion of exon

7 in the SMN1 gene. For most affected individuals, both SMN1 genes will be missing exon 7. If one or both SMN1 genes do not have an exon 7 deletion, SMN1 gene sequencing can be considered.

- **SMN1 Sequencing Analysis** is typically performed in reflex, when one or no deletions are identified by deletion/dosage analysis. About 2-5% of affected individuals fall into this group. Sequencing detects the other mutation in virtually all cases.<sup>2,3</sup>
- **Carrier testing** is usually performed by quantitative analysis that determines the dosage, or copy number, of exon 7-containing SMN1 genes.<sup>3-5</sup>
  - Gene dosage ranges from one to three copies in most people. Asymptomatic carriers typically have one intact copy of the SMN1 gene and one SMN1 gene with the common deletion.
  - However, some unaffected carriers have two intact copies of the SMN1 gene. These may be on the same chromosome with no intact SMN1 gene on the other chromosome. Rare mutations and those carrying two SMN1 genes on the same chromosome will not be detected by gene dosage analysis. Therefore, a negative gene dosage analysis reduces the carrier risk but cannot completely rule out that a person is an SMA carrier.<sup>3-5</sup>
  - The detection rate of carrier screening varies based on ethnicity, ranging from 71% in African Americans to 95% in Caucasians.<sup>2</sup>
    - An addendum issued in 2016 updated the Carrier Testing section of the ACMG guidelines (see below), stating "Recently, the presence of an extended haplotype block specific to SMN1 duplications was reported in individuals of Ashkenazi Jewish and African American ethnicities [Luo et al.; 2014]. Testing for haplotype specific markers in conjunction with carrier testing offers an incremental improvement in residual risk estimates following a negative carrier test result".<sup>6</sup>
- **SMN2 Deletion/Dosage Analysis** is performed by quantitative PCR to determine the number of copies of the SMN2 gene.
  - Most people have 0-3 copies of SMN2, although copy numbers as high as 5 have been reported.<sup>3</sup>
  - The clinical severity of SMA can be influenced by the number of copies a person has of the SMN2 gene.<sup>3</sup> Although a higher copy number of SMN2 is generally associated with a milder phenotype, SMA is still a highly variable disease. It is difficult to use SMN2 copy number to reliably predict the clinical manifestations of SMA in an affected person because sequence variation in SMN2 may also influence disease course regardless of copy number.<sup>4</sup>
  - Of note, SMN2 dosage is often paired with SMN1 dosage as concurrent test.
- **Known Familial Mutation Testing:** Once mutations have been identified in carriers or affected individuals, family members can be tested for the known familial

mutation(s). Preimplantation diagnosis and prenatal testing can be considered when both parents are known SMA carriers.

## Guidelines and evidence

### Diagnostic Testing

- The International Standard of Care Committee for Spinal Muscular Atrophy issued a consensus statement in 2007 that stated the following:<sup>5</sup>
  - “The first diagnostic test for a patient suspected to have spinal muscular atrophy should be the SMN [SMN1] gene deletion test.”<sup>5</sup>
  - “The current literature suggests SMN2 copy numbers correlate with spinal muscular atrophy clinical phenotypes. However, although a higher copy number of SMN2 is correlated with milder phenotype, phenotypes can vary substantially given SMN2 copy number. Therefore, predicting clinical phenotype using SMN2 copy number can be risky and is not currently recommended.”<sup>5</sup>
- The European Federation of Neurological Societies (EFNS, 2011) published guidelines on the molecular diagnosis of various neuromuscular disorders.<sup>1</sup> Regarding SMA testing they state:
  - “Screening for SMN1 deletions is indicated in SMA I-III to confirm the diagnosis and provide genetic counseling (Level B).”<sup>1</sup>
  - “In adult-onset SMA, genetic testing for SBMA should be considered in males with bulbar manifestations, gynecomastia and X-linked inheritance (Level B).”<sup>1</sup>
  - “As nearly all of these studies have a retrospective design and look for a specific mutation in a previously ascertained and clinically diagnosed cohort of patients, the highest achievable recommendation level will be B.”<sup>1</sup>

### Carrier Testing

- The American College of Obstetricians and Gynecologists (ACOG, 2017)<sup>7</sup> stated the following in regards to carrier testing for SMA in an updated Committee Opinion:
  - “Screening for spinal muscular atrophy should be offered to all women who are considering pregnancy or are currently pregnant.”
- The American College of Medical Genetics (ACMG, 2008; reaffirmed 2013)<sup>4</sup> state the following regarding carrier testing for SMA:
  - “Because SMA is present in all populations, carrier testing should be offered to all couples regardless of race or ethnicity. Ideally, the testing should be offered before conception or early in pregnancy. The primary goal is to allow carriers to make informed reproductive choices.”

- In 2011 the Association of Molecular Pathology issued their statement on SMA carrier screening stating that it is “a technology on the threshold of feasibility.”<sup>8</sup> They outlined 6 concerns, 2 of which related to population carrier frequencies, another regarding the need for pilot programs, need for genotype/phenotype research, and another about technical issues with SMN1/SMN2 as outlined above.

### **Spinraza (nusinersen)**

- In 2016, the FDA approved the use of Spinraza (nusinersen) in individuals with SMA. While the FDA label does not require SMN2 copy number analysis, the study of 121 patients on which FDA approval was based used the following inclusion criteria:<sup>9</sup>
  - 5q SMN1 homozygous gene deletion or mutation or compound heterozygous mutation
  - 2 copies of the SMN2 gene (98% of enrolled patients had 2 copies of SMN2)
  - Onset of SMA symptoms at or before 6 months of age
  - No hypoxemia at baseline screening at age 7 months or younger
  - A review of nusinersen clinical trials for patients with SMA by the Guideline Development, Dissemination, and Implementation Subcommittee of the American Academy of Neurology in 2018 identified two complete studies in which nusinersen was administered after 6 months of age with improvement in participant symptoms.<sup>10</sup>

### **Zolgensma (onasemnogene abeparvovec-xioi)**

- In 2019, the FDA approved the use of Zolgensma (onasemnogene abeparvovec-xioi) in individuals with SMA. While the FDA label does not require SMN2 copy number analysis, the study of the 21 patients on which FDA approval was based used the following inclusion criteria:<sup>11</sup>
  - Confirmed bi-allelic SMN1 gene deletions
  - 2 copies of the SMN2 gene
  - Onset of SMA symptoms before 6 months of age
  - Absence of the c.859G>C modification in exon 7 of the SMN2 gene

## **Criteria**

### **SMN1 Exon 7 Deletion**

- Genetic Counseling:

- Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - No previous genetic testing of the SMN1 gene, AND
- Diagnostic Testing for Symptomatic Individuals:
  - Child with hypotonia and weakness (generally symmetrical, proximal more than distal), or
  - Young adult (through twenties) onset of weakness more severely affecting the legs than arms (may be associated with frequent falls, difficulty with stairs), and
  - No obvious signs of a different neurological disorder, OR
- Carrier Screening:
  - SMN1 exon 7 deletion testing is not suitable for carrier screening. SMN1/SMN2 dosage analysis is necessary, OR
- Prenatal Testing:
  - Both parents are carriers of an SMA mutation (at least one of which is an exon 7 deletion mutation), AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

### **SMN1/SMN2 Deletion/Dosage Analysis**

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Testing:
  - No previous genetic testing of the SMN1 gene in the carrier testing setting, AND
- Diagnostic Testing for Symptomatic Individuals:
  - Index of suspicion for SMA remains high based on:
    - Proximal greater than distal weakness, and
    - Normal creatine kinase (CK), and
    - Neurogenic EMG, OR
- Carrier Screening:
  - Be of reproductive age, and
  - Have potential and intention to reproduce, OR

- Prenatal Testing:
  - SMN1/SMN2 Dosage Analysis is not suitable for preimplantation/prenatal diagnosis. Other forms of SMA testing may be indicated based on the mutation status of parents. See those sections for guidance, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

### **SMN1 Known Familial Mutation Analysis**

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy) , AND
- Previous Genetic Testing:
  - No previous genetic testing for known SMN1 family mutation(s), AND
- Diagnostic Testing for Symptomatic Individuals:
  - Known family SMN1 point mutation(s) in biological relative, OR
- Carrier Screening
  - Known family SMN1 point mutation(s) in biological relative, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

### **SMN1 Sequencing**

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - SMN1 exon 7 deletion testing did not reveal a homozygous SMN1 deletion or SMN1/SMN2 gene dosage analysis identified a single copy of SMN1 exon 7 in the diagnostic setting, or
  - SMN1/SMN2 gene dosage analysis did not confirm carrier status of an exon 7 deletion in the carrier testing setting, AND
- Diagnostic Testing for Symptomatic Individuals:
  - Individual suspected to have compound heterozygous SMA based previous test results and:
    - Proximal greater than distal weakness, and
    - Normal creatine kinase (CK), and

- Neurogenic EMG, OR
- Carrier Screening:
  - Have one of the following increased risk indications with a noninformative SMN1/SMN2 gene dosage analysis result:
    - Have a reproductive partner who is a carrier of SMA, or
    - Have a reproductive partner with SMA, OR
- Prenatal Testing:
  - SMN1 full gene sequencing is not generally necessary for preimplantation/prenatal diagnosis as parental mutation status should have already been determined with SMN1 exon 7 deletion testing +/- SMN1 known familial variant analysis, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

### **SMN2 Deletion/Dosage Analysis**

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Member meets the following criteria:
  - Member has a genetically confirmed diagnosis of SMA, and
  - Member has a diagnosis of either SMA Type 1 or SMA Type 2, and
  - Member has not had previous SMN2 copy number analysis performed, and
  - Treatment with Spinraza or Zolgensma is being considered, and
  - Documentation is provided that SMN2 copy number is needed to obtain insurance approval for either Spinraza or Zolgensma, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

### **SMN2 Targeted Mutation Analysis (c.859G>C)**

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Member meets the following criteria:
  - Member has a genetically confirmed diagnosis of SMA, and



- Member has a diagnosis of either SMA Type 1 or SMA Type 2, and
  - Member has not had previous c.859G>C analysis performed, and
  - Treatment with Zolgensma is being considered, and
  - Documentation is provided that c.859G>C analysis is needed to obtain insurance approval for Zolgensma, AND
- Rendering laboratory laboratory is a qualified provider of service per the Health Plan policy.

### Exclusions

Genetic testing is not approved for SMN2 gene copy analysis for the purpose of predicting SMA prognosis because it is currently considered experimental, investigational, or is unproven.

Genetic testing is not approved for c.859G>C analysis only for the purpose of predicting SMA prognosis because it is currently considered experimental, investigational, or is unproven.

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# Spinocerebellar Ataxia Genetic Testing

**MOL.TS.311.A**  
**v1.0.2020**

## Introduction

Spinocerebellar ataxia (SCA) genetic testing is addressed by this guideline.

## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedures addressed by this guideline                                      | Procedure codes |
|---|-----------------|
| ATXN1 gene analysis, evaluation to detect abnormal (eg,expanded) allele     | 81178           |
| ATXN2 gene analysis, evaluation to detect abnormal (eg,expanded) allele     | 81179           |
| ATXN3 gene analysis, evaluation to detect abnormal (eg,expanded) allele     | 81180           |
| ATXN7 gene analysis, evaluation to detect abnormal (eg,expanded) allele     | 81181           |
| ATXN8 gene analysis, evaluation to detect abnormal (eg, expanded) alleles   | 81182           |
| ATXN10 gene analysis, evaluation to detect abnormal (eg, expanded) alleles  | 81183           |
| CACNA1A gene analysis; evaluation to detect abnormal (eg, expanded) alleles | 81184           |
| CACNA1A gene analysis; full gene sequence                                   | 81185           |
| CACNA1A gene analysis; known familial variant                               | 81186           |
| PPP2R2B gene analysis, evaluation to detect abnormal (eg, expanded) alleles | 81343           |
| TBP gene analysis, evaluation to detect abnormal (eg, expanded) alleles     | 81344           |
| Unlisted molecular pathology procedure                                      | 81479           |

## What is spinocerebellar ataxia

### Definition

Spinocerebellar ataxias (SCA) are a group of autosomal dominant ataxias that have a range of phenotypes. There are various subtypes of SCA, which are denoted by numbers (e.g. SCA1, SCA3, etc.)

### Incidence and Prevalence

The prevalence of autosomal dominant cerebellar ataxias, as a whole, is 1-5:100,000.<sup>1</sup> SCA3 is the most common autosomal dominant form of ataxia. This is followed by SCA1, SCA2, SCA6, and SCA7.<sup>1</sup> The prevalence of specific subtypes of SCA vary by region. SCA3 is most common in Portugal.<sup>1</sup>

### Symptoms

Although the specific phenotype of each subtype varies, most individuals with SCA have “progressive adult-onset gait ataxia (often with hand dysmetria) and dysarthria associated with cerebellar atrophy on brain imaging.”<sup>1</sup> The age of onset for the different subtypes also overlaps, which makes it difficult to distinguish between subtypes based on clinical phenotype only.<sup>1,2</sup> See the table below for the various subtypes of SCA and the associated clinical features.

### Cause

SCAs are caused by mutations in one of numerous genes. See the table below for the various subtypes of SCA and the associated genes.

### Inheritance

SCAs are inherited in an autosomal dominant pattern. Children of an individual with an SCA have a 50% chance of inheriting the mutation. Anticipation is also observed in some of the SCAs. This means that as the disease passes through generations, the severity can increase and the age of onset can decrease.

### Diagnosis

Molecular genetic testing can be used to establish a specific diagnosis, which aids in understanding the prognosis and risk assessment for family members.<sup>1</sup>

### Treatment

Treatment of ataxia is largely supportive, and includes the use of canes and walkers for ambulation, speech therapy, and other assistive devices.<sup>1</sup>

| SCA subtype | Gene Associated | Clinical Features  |
|-------------|-----------------|--|
| SCA1        | ATXN1           | Progressive cerebellar ataxia, dysarthria, deterioration of bulbar functions, pyramidal signs, peripheral neuropathy <sup>2,3</sup>  |
| SCA2        | ATXN2           | Progressive ataxia and dysarthria, nystagmus, slow saccadic eye movements, peripheral neuropathy, decreased DTRs, dementia <sup>2,4</sup>  |
| SCA3        | ATXN3           | Gait problems, speech difficulties, clumsiness, visual blurring, diplopia, hyperreflexia, progressive ataxia, nystagmus, dysarthria, pyramidal and extrapyramidal signs; lid retraction, nystagmus, decreased saccade velocity; amyotrophy fasciculations, sensory loss <sup>2,5</sup> |
| SCA4        | 16q22.1         | Sensory axonal neuropathy, deafness; may be allelic with 16q22-linked SCA <sup>2</sup>   |
| SCA5        | SPTBN2          | Early onset, slow course <sup>2</sup>  |
| SCA6        | CACNA1A         | Progressive cerebellar ataxia, dysarthria, nystagmus, sometimes episodic ataxia, very slow progression <sup>2,6</sup>  |
| SCA7        | ATXN7           | Progressive cerebellar ataxia, dysarthria, dysphagia, cone-rod and retinal dystrophy with progressive central visual loss resulting in blindness <sup>2,7</sup>  |

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| SCA subtype | Gene Associated | Clinical Features  |
|-------------|-----------------|--|
| SCA8        | ATXN8           | Principally cerebellar ataxia, slowly progressing ataxia, scanning dysarthria, truncal instability, hyperactive tendon reflexes, decreased vibration sense; rarely, cognitive impairment <sup>2,8</sup>      |
| SCA10       | ATXN10          | Progressive cerebellar ataxia, scanning dysarthria, dysphagia, upper-limb ataxia, generalized motor seizures and/or complex partial seizures, most families are of Native American background <sup>2,9</sup> |
| SCA11       | TTBK2           | Progressive cerebellar ataxia, abnormal eye signs (jerky pursuit, horizontal and vertical nystagmus), mild, remain ambulatory <sup>2,10</sup>  |
| SCA12       | PPP2R2B         | Slowly progressive ataxia; action tremor in the 30s; hyperreflexia; subtle Parkinsonism possible; cognitive/psychiatric disorders including dementia <sup>2</sup>  |
| SCA13       | KCNC3           | Ranges from progressive childhood-onset cerebellar ataxia, cerebellar dysarthria, occasional seizures to adult-onset progressive ataxia, mild intellectual disability, short stature <sup>2,11</sup>         |

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| SCA subtype | Gene Associated | Clinical Features  |
|-------------|-----------------|--|
| SCA14       | PRKCG           | Progressive cerebellar ataxia, dysarthria, nystagmus, axial myoclonus, cognitive impairment, tremor, sensory loss, Parkinsonian features including rigidity and tremor <sup>2,12</sup>           |
| SCA15       | ITPR1           | Progressive gait and limb ataxia, ataxic dysarthria, titubation, upper limb postural tremor, mild hyperreflexia, gaze-evoked nystagmus, and impaired vestibuloocular reflex gain <sup>2,13</sup> |
| SCA16       | ITPR1           | Head tremor; reported in one Japanese family <sup>2</sup>  |
| SCA17       | TBP             | Ataxia, dementia, mental deterioration; occasional chorea, dystonia, myoclonus, epilepsy; Purkinje cell loss, intranuclear inclusions with expanded polyglutamine <sup>2,14</sup>                |
| SCA18       | 7q22-q32        | Ataxia with early sensory/motor neuropathy, nystagmus, dysarthria, decreased tendon reflexes, muscle weakness, atrophy, fasciculations, Babinski responses <sup>2</sup>                          |
| SCA19/22    | KCND3           | Slowly progressive, rare cognitive impairment, myoclonus, hyperreflexia <sup>2</sup>   |

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| SCA subtype | Gene Associated | Clinical Features  |
|-------------|-----------------|--|
| SCA20       | 11q12           | Progressive ataxia, dysarthria, palatal tremor (myoclonus), and/or abnormal phonation clinically resembling spasmodic adductor dysphonia, hyperreflexia, bradykinesia; calcification of the dentate nucleus. <sup>2,15</sup>         |
| SCA21       | TMEM240         | Mild cognitive impairment <sup>2</sup>   |
| SCA23       | PDYN            | Dysarthria, abnormal eye movements, reduced vibration and position sense; reported in one Dutch family; neuropathology <sup>2</sup>  |
| SCA25       | SCA25           | Sensory neuropathy; reported in one French family <sup>2</sup>   |
| SCA26       | EEF2            | Dysarthria, irregular visual pursuits; reported in one Norwegian-American family; MRI: cerebellar atrophy <sup>2</sup>   |
| SCA27       | FGF14           | Early-onset tremor; dyskinesia, cognitive deficits; reported in one Dutch family <sup>2</sup>  |
| SCA28       | AFG3L2          | Young-adult onset, progressive gait and limb ataxia resulting in coordination and balance problems, dysarthria, ptosis, nystagmus, and ophthalmoparesis, increased tendon reflexes; reported in two Italian families <sup>2,16</sup> |
| SCA29       | ITPR1           | Learning deficits <sup>2</sup>   |
| SCA30       | 4q34.3-q35.1    | Hyperreflexia <sup>2</sup>   |
| SCA31       | BEAN1           | Normal sensation <sup>2</sup>  |

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| SCA subtype | Gene Associated | Clinical Features   |
|-------------|-----------------|---|
| SCA35       | TGM6            | Hyperreflexia, Babinski responses; spasmodic torticollis <sup>2</sup>   |
| SCA36       | NOP56           | Late-onset, slowly progressive cerebellar syndrome typically associated with sensorineural hearing loss, muscle atrophy and denervation, especially of the tongue, as well as pyramidal signs, muscle fasciculations, hyperreflexia <sup>2,17</sup> |
| SCA37       | 1p32            | Abnormal vertical eye movements <sup>1</sup>  |
| SCA38       | ELOVL5          | Adult onset, axonal neuropathy <sup>1</sup>   |
| SCA40       | CCDC88C         | Adult onset, brisk reflexes, spasticity <sup>1</sup>  |
| SCA42       | CACNA1G         | Mild pyramidal signs, saccadic pursuit <sup>1</sup>   |

## Test Information

### Introduction

Testing for SCA may include known familial mutation analysis, expansion analysis, sequencing, deletion/duplication analysis, or multi-gene panel testing. Test methods vary by gene.

### Expansion analysis

Several of the SCAs are caused by triplet repeat expansions. Testing for these conditions is performed by expansion analysis to identify the number of repeats. Expansion analysis can be performed for diagnostic testing, presymptomatic testing, as well as prenatal testing.

### Sequencing

Sequence analysis detects single nucleotide substitutions and small (several nucleotide) deletions and insertions. Regions analyzed typically include the coding

sequence and intron/exon boundaries. Promoter regions and intronic sequences may also be sequenced if disease-causing mutations are known to occur in these regions of a gene.

### **Deletion/duplication**

Analysis for deletions and duplications can be performed using a variety of technical platforms including exon array, MLPA, and NGS data analysis.

These assays detect gains and losses too large to be identified through sequencing technology, often single or multiple exons or whole genes.

### **Known familial mutation analysis**

Analysis for known familial mutations is typically performed by trinucleotide repeat expansion analysis. Some mutations may require Sanger sequencing or deletion/duplication analysis.

Known familial mutation analysis is performed when a causative mutation has been identified in a close relative of the individual requesting testing.

## **Guidelines and Evidence**

### **Introduction**

This section includes relevant guidelines and evidence pertaining to SCA testing.

### **European Federation of Neurological Sciences**

The European Federation of Neurological Sciences (EFNS, 2014) stated the following with regards to testing for autosomal dominant cerebellar ataxia:<sup>18</sup>

- “In the case of a family history that is compatible with an autosomal dominant cerebellar ataxia, screening for SCA1, SCA2, SCA3, SCA6, SCA7, and SCA17 is recommended (Level B). In Asian patients, DRPLA should also be tested for.”
- “If mutation analysis is negative, we recommend contact with or referral to a specialized clinic for reviewing the phenotype and further genetic testing (good practice point)”
- “In the case of sporadic ataxia and independent from onset age, we recommend routine testing for SCA1, SCA2, SCA3, SCA6, and DRPLA (in Asian patients) (level B), the step one panel of the recessive ataxia workup, i.e. mutation analysis of the FRDA gene (level B), and biochemical testing that includes cholestanol, vitamin E, cholesterol, albumin, CK, and alpha-fetoprotein.”

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## American College of Medical Genetics

The American College of Medical Genetics (ACMG, 2013) stated the following regarding establishing the diagnosis of hereditary ataxias:

- "Detection on neurological examination of typical clinical signs including poorly coordinated gait and finger/hand movements, dysarthria (incoordination of speech), and eye movement abnormalities such as nystagmus, abnormal saccade movements, and ophthalmoplegia."
- "Exclusion of nongenetic causes of ataxia."
- "Differential diagnosis of hereditary ataxia includes acquired, nongenetic causes of ataxia, such as alcoholism, vitamin deficiencies, multiple sclerosis, vascular disease, primary or metastatic tumors, and paraneoplastic diseases associated with occult carcinoma of the ovary, breast, or lung, and the idiopathic degenerative disease multiple system atrophy (spinal muscular atrophy). The possibility of an acquired cause of ataxia needs to be considered in each individual with ataxia because a specific treatment may be available."
- "Documentation of the hereditary nature of the disease by finding a positive family history of ataxia, identifying an ataxia-causing mutation, or recognizing a clinical phenotype characteristic of a genetic form of ataxia."<sup>2</sup>

For testing when the family history suggests autosomal dominant inheritance, ACMG recommends the following:

- "An estimated 50–60% of the dominant hereditary ataxias can be identified with highly accurate and specific molecular genetic testing for SCA1, SCA2, SCA3, SCA6, SCA7, SCA8, SCA10, SCA12, SCA17, and DRPLA; all have nucleotide repeat expansions in the pertinent genes."
- "Because of the broad clinical overlap, most laboratories that test for the hereditary ataxias have a battery of tests including testing for SCA1, SCA2, SCA3, SCA6, SCA7, SCA10, SCA12, SCA14, and SCA17. Many laboratories offer them as two groups in stepwise fashion based on population frequency, testing first for the more common ataxias, SCA1, SCA2, SCA3, SCA6, and SCA7. Although pursuing multiple genes simultaneously may seem less optimal than serial genetic testing, it is important to recognize that the cost of the battery of ataxia tests often is equivalent to that of an MRI. Positive results from the molecular genetic testing are more specific than MRI findings in the hereditary ataxias. Guidelines for genetic testing of hereditary ataxia have been published."
- "Testing for the less common hereditary ataxias should be individualized and may depend on factors such as ethnic background (SCA3 in the Portuguese, SCA10 in the Native American population with some exceptions<sup>73</sup>); seizures (SCA10); presence of tremor (SCA12, fragile X-associated tremor/ataxia syndrome); presence of psychiatric disease or chorea (SCA17); or uncomplicated ataxia with long duration (SCA6, SCA8, and SCA14). Dysphonia and palatal myoclonus are associated with calcification of the dentate nucleus of cerebellum (SCA20).

- "If a strong clinical indication of a specific diagnosis exists based on the affected individual's examination (e.g., the presence of retinopathy, which suggests SCA7) or if family history is positive for a known type, testing can be performed for a single disease."

For testing simplex cases, ACMG recommends the following:

- "If no acquired cause of the ataxia is identified, the probability is ~13% that the affected individual has SCA1, SCA2, SCA3, SCA6, SCA8, SCA17, or FRDA, and mutations in rare ataxia genes are even less common."
- "Other possibilities to consider are a de novo mutation in a different autosomal dominant ataxia, decreased penetrance, alternative paternity, or a single occurrence of an autosomal recessive or X-linked disorder in a family such as fragile X-associated tremor/ataxia syndrome."
- "Although the probability of a positive result from molecular genetic testing is low in an individual with ataxia who has no family history of ataxia, such testing is usually justified to establish a specific diagnosis for the individual's medical evaluation and for genetic counseling."
- "Always consider a possible nongenetic cause such as multiple system atrophy, cerebellar type in simplex cases."

### Peer-reviewed literature

Hadjivassiliou M, Martindale J, Shanmugarajah P, et al (2017) stated the following with regard to testing for hereditary ataxias:

- "We have shown that patients with early onset idiopathic ataxia (irrespective of family history) are much more likely to have a genetic aetiology (81%) than those with late onset idiopathic ataxia (55%). One possible selection criterion for genetic testing is early onset ataxia. Additional selection criteria may include the presence of other clinical features, for example, 1% of patients with histologically suspected/genetically confirmed mitochondrial disease had ataxia with other clinical features (eg, deafness, diabetes, myoclonus, etc) and only 9% pure ataxia."<sup>19</sup>
- "Furthermore, the presence of severe cerebellar atrophy without any clinical correlation and with well-preserved spectroscopy of the cerebellum often suggests that the ataxia is long standing (maybe even early onset) and slowly progressive. Patients should therefore be offered genetic testing. The pattern of cerebellar involvement on MR spectroscopy may also direct to a particular diagnosis. Most genetic ataxias involve both the hemispheres and the vermis while the majority of immune-mediated acquired ataxias (eg, gluten ataxia, anti-GAD ataxia and primary autoimmune cerebellar ataxia) have a predilection for the vermis."<sup>19</sup>

## Criteria

### Introduction

Requests for SCA testing are reviewed using these criteria.

#### Known familial mutation analysis

- Genetic Counseling:
  - Pre- and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - No previous gene analysis of requested gene that would have identified the mutation, AND
- Presymptomatic Testing for Asymptomatic Individuals:
  - Member is 18 years of age or older, and
  - Known disease-causing mutation in SCA gene identified in 1<sup>st</sup> or 2<sup>nd</sup> degree relative(s), OR
- Diagnostic Testing for Symptomatic Individuals:
  - Known disease-causing mutation in SCA gene identified in 1<sup>st</sup> or 2<sup>nd</sup> degree relative(s), AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy

#### Single gene testing

- Genetic Counseling:
  - Pre- and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - No previous testing of requested gene(s), and
  - No mutation identified by previous analysis, if performed, and
  - No known familial mutation in a gene known to cause ataxia, AND
- Diagnostic Testing for Symptomatic Individuals:
  - Individual has been diagnosed with cerebellar ataxia, and
  - Medical history points to the specific subtype of SCA requested (e.g. age of onset, distinguishing features present, etc), AND

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- Documentation from ordering provider indicating how test results will be used to directly impact medical care for the individual (e.g. change in surveillance or treatment plan), AND
- The member does not have a known underlying cause for their ataxia (e.g. alcoholism, vitamin deficiencies, multiple sclerosis, vascular disease, tumors, known mutation, etc), AND
- Family history is consistent with an autosomal dominant inheritance pattern (including simplex cases), AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy

### **Multigene panel testing**

- Genetic counseling:
  - Pre- and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - No previous testing of requested genes, and
  - No mutation identified by previous analysis, if performed, and
  - No known familial mutation in a gene known to cause ataxia, AND
- Diagnostic Testing for Symptomatic Individuals:
  - Individual has been diagnosed with cerebellar ataxia, regardless of age of onset, AND
- Documentation from ordering provider indicating how test results will be used to directly impact medical care for the individual (e.g. change in surveillance or treatment plan), AND
- The member does not have a known underlying cause for their ataxia (e.g. alcoholism, vitamin deficiencies, multiple sclerosis, vascular disease, tumors, known mutation, etc), AND
- Family history is consistent with an autosomal dominant inheritance pattern (including simplex cases), AND
- Medical history does not point to a specific genetic diagnosis for which a more focused test or panel would be appropriate, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy

### **Billing and reimbursement considerations**

For broader hereditary ataxia panel testing requests, see *Hereditary Ataxia Multigene Panel Testing* guideline.

Gene panels that are specific to SCA will be eligible for reimbursement according to the criteria outlined in this guideline. When multiple CPT codes are billed for components of a panel and there is a more appropriate CPT code representing the panel, eviCore will redirect to the panel code(s).

If the laboratory will not accept redirection to a panel code, the medical necessity of each billed component procedure will be assessed independently.

- In general, only a limited number of panel components that are most likely to explain the member's presentation will be reimbursable. The remaining panel components will not be reimbursable.
- When the test is billed with multiple stacked procedure codes, only the following genes may be considered for reimbursement:
  - ATXN1 (SCA1)
  - ATXN2 (SCA2)
  - ATXN3 (SCA3)
  - CACNA1A (SCA6)
  - ATXN7 (SCA7)
  - TBP (SCA17)

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# Tay-Sachs Disease Testing

MOL.TS.226.A

v1.0.2020

## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedures addressed by this guideline | Procedure codes |
|--|-----------------|
| HEXA Known Familial Mutation Analysis  | 81403           |
| HEXA Targeted Mutation Analysis        | 81255           |
| HEXA Sequencing                        | 81406           |
| Beta-Hexosaminidase A Enzyme Analysis  | 83080           |

## What is Tay-Sachs disease

### Definition

Tay-Sachs disease is a neurodegenerative genetic disorder.<sup>1</sup> Affected individuals typically present in infancy with progressive weakness, loss of motor skills, decreased attentiveness, and increased startle response between 3-6 months of age. Eventually they develop seizures and blindness, with death in early childhood. There is no cure for Tay-Sachs disease and treatment is supportive.<sup>1,2</sup>

- Rare, less severe, Tay-Sachs variants exist that are associated with later onset, and less progressive symptoms, and cause more variable neurological problems. These variants include juvenile, chronic, and adult-onset forms.<sup>1</sup>
- Tay-Sachs disease is caused by mutations in the HEX A gene. HEX A gene mutations lead to reduced activity of the  $\beta$ -hexosaminidase A enzyme, allowing toxic substances to build up in the cells of the brain and spinal cord. Eventually, neurons are destroyed, causing the signs and symptoms of Tay-Sachs disease.<sup>1</sup>
- Before widespread carrier screening, Tay-Sachs disease affected about 1 in 3,600 Ashkenazi Jewish births.<sup>1</sup>
- Tay-Sachs disease is an autosomal recessive disorder. An affected individual must inherit a HEX A gene mutation from both parents.<sup>1,2</sup>
  - Individuals who inherit only one mutation are called carriers. Carriers do not show symptoms of Tay-Sachs disease, but have a 50% chance of passing on the mutation to their children.
  - About 1 in 30 Ashkenazi Jewish individuals are carriers for Tay-Sachs disease.<sup>1-3</sup>

- Two carriers of Tay-Sachs disease have a 25% chance of having a child with the disorder.
- Individuals at increased risk to have a child with Tay-Sachs should routinely be offered carrier screening. This includes those with:<sup>1-6</sup>
  - Ashkenazi Jewish, French Canadian, or Cajun ancestry
  - A family history of Tay-Sachs disease (regardless of ethnicity)
  - A partner who is a known carrier of Tay-Sachs (or affected with a late-onset variant)
- Carrier screening for Tay-Sachs disease is widely available as part of an “Ashkenazi Jewish Panel” that includes several other genetic diseases that are more common in this population (See the **Ashkenazi Jewish Carrier Screening**).

## Test information

- **Hexosaminidase A (HEXA) enzyme analysis** measures the activity of HEXA in the serum or white blood cells. This test is used both for diagnostic testing of symptomatic individuals, and carrier screening.
  - Individuals with classic Tay-Sachs have little to no HEX A enzyme activity in the presence of normal or elevated activity of the beta-hexosaminidase B (HEX B) isoenzyme. HEX A enzyme activity levels correctly diagnose the vast majority of people with all forms of Tay-Sachs disease.
  - Carriers have about 50% of the normal level of HEX A activity.<sup>1,2</sup> HEX A enzyme analysis detects 97%-98% of carriers, regardless of ethnicity.<sup>3,4</sup> Enzyme analysis is recommended as the first step for all people being screened.<sup>5</sup>
  - A small percentage of individuals will get a false positive result by enzyme analysis. This means that they have enzyme activity that appears to be in the carrier range, but they are not actually carriers of a disease-causing mutation. These individuals carry a “pseudodeficiency allele.”<sup>1</sup> Inconclusive enzyme analysis results are also possible where enzyme activity is in the overlap range between carrier and normal levels.<sup>1</sup> If HEXA enzyme analysis is abnormal or inconclusive, HEXA mutation analysis may be considered.<sup>1,3</sup>
- **HEXA mutation panel.** This genetic test looks for the most common HEXA gene mutations (such as +TATC1278, +1 IVS 12, +1 IVS 9, G269, R247W, and R249W), which account for up to 98% of all Ashkenazi Jewish Tay-Sachs mutations.<sup>1</sup> The detection rate of standard HEXA mutation panels is much lower in other ethnicities. Some panels include mutations more common in other at-risk ethnic groups (e.g., a 7.6kb deletion more common in French Canadians).<sup>1</sup> If using mutation panels in non-Ashkenazi Jewish, providers should confirm those mutation panels include any ethnicity-specific mutations.

- **HEXA sequencing** analyzes the entire coding region of the HEXA gene and finds the vast majority of HEXA mutations that cause Tay-Sachs disease. Sequencing is most useful for individuals diagnosed by enzyme analysis, but for whom mutation panels found only one or no disease-causing mutations.<sup>1</sup>
- **HEXA known familial mutation analysis:** Once the disease-causing mutations have been identified in an affected family member or known carriers, other at-risk relatives can be tested for just those mutations. Prenatal diagnosis can be performed by mutation analysis if both parental mutations are known.

## Guidelines and evidence

- Professional guidelines support population-based Tay-Sachs carrier screening for those at increased risk. They do not generally recommend a specific testing strategy (enzyme and/or mutation analysis) for Ashkenazi Jewish individuals, but do recommend enzyme analysis as a first-line test for non-Jewish individuals.<sup>2,3</sup>
- Consensus guidelines from the American College of Obstetricians and Gynecologists (ACOG, 2017) recommend:<sup>3</sup>
  - “Screening for TSD should be offered when considering pregnancy or during pregnancy if either member of a couple is of Ashkenazi Jewish, French–Canadian, or Cajun descent. Those with a family history consistent with TSD also should be offered screening.”
  - “When one member of a couple is at high risk (i.e., of Ashkenazi Jewish, French–Canadian, or Cajun descent or has a family history consistent with TSD) but the other partner is not, the high-risk partner should be offered screening...If the high-risk partner is determined to be a carrier, the other partner also should be offered screening. If the woman is already pregnant, it may be necessary to offer screening to both partners simultaneously to ensure that results are obtained promptly and that all options are available to the couple.”
  - “Enzyme testing in pregnant women and women taking oral contraceptives should be performed using leukocyte testing because serum testing is associated with an increased false-positive rate in these populations.”
  - “If Tay-Sachs disease screening is performed as part of pan-ethnic expanded carrier screening, it is important to recognize the limitations of the mutations screened in detecting carriers in the general population. In the presence of a family history of Tay-Sachs disease, expanded carrier screening panels are not the best approach to screening unless the familial mutation is included on the panel.”
- Consensus guidelines from the American College of Medical Genetics (ACMG, 2008) recommend carrier screening for a group of disorders that includes Tay-Sachs disease when at least one member of the couple is Ashkenazi Jewish and that couple is pregnant or planning pregnancy.<sup>2</sup>

- No evidence-based U.S. testing guidelines that address Tay-Sachs diagnostic testing have been identified.
- A 2006 comprehensive literature review states that: “The diagnosis of hexosaminidase A deficiency relies upon the demonstration of absent to near-absent beta-hexosaminidase A (HEX A) enzymatic activity.”<sup>1</sup> HEXA mutation analysis can be used in follow-up to resolve inconclusive results or to identify the familial mutations for reproductive purposes.<sup>1</sup>
- Professional guidelines generally recommend prenatal testing for Tay-Sachs disease in any of the following situations:<sup>1-4</sup>
  - HEX A enzyme activity testing revealed both parents to be carriers of Tay-Sachs disease and pseudodeficiency alleles have been ruled out.
  - Disease-causing mutations in HEXA have been identified in both parents.
  - One parent is a known carrier and HEX A enzyme activity testing in the other parent was inconclusive.
  - The mother is a known carrier and the father is unknown or unavailable for testing.
- Guidelines do not generally recommend a specific testing strategy (HEX A enzyme activity and/or mutation analysis). However, the clinical circumstances may deem one strategy more accurate than the other. For instance, mutation analysis is most accurate if both of the parental mutations are known.
- The American College of Obstetricians and Gynecologists (ACOG, 2017) guidelines for Tay-Sachs disease state: “If both partners are determined to be carriers of Tay-Sachs disease, genetic counseling and prenatal diagnosis should be offered.”<sup>3</sup>
- The American College of Obstetricians and Gynecologists (ACOG, 2009) guidelines for Ashkenazi Jewish carrier screening state: “Carrier screening for TSD, Canavan disease, cystic fibrosis, and familial dysautonomia should be offered to Ashkenazi Jewish individuals before conception or during early pregnancy so that a couple has an opportunity to consider prenatal diagnostic testing options. If the woman is already pregnant, it may be necessary to screen both partners simultaneously so that the results are obtained in a timely fashion to ensure that prenatal diagnostic testing is an option... Carrier couples should be informed of the disease manifestations, range of severity, and available treatment options. Prenatal diagnosis by DNA-based testing can be performed on cells obtained by chorionic villus sampling and amniocentesis.”<sup>6</sup>

## Criteria

### HEXA Known Familial Mutation Analysis

- Genetic Counseling:

- Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - No previous molecular genetic testing of HEXA, AND
- Carrier Screening:
  - Known family mutation in HEXA identified in 1<sup>st</sup>, 2<sup>nd</sup>, or 3<sup>rd</sup> degree biologic relative(s), OR
- Prenatal Testing for At-Risk Pregnancies:
  - HEXA mutation identified in both biologic parents, and
  - Pseudodeficiency allele mutation has been ruled out, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

#### **HEXA Targeted Mutation Analysis for Common Mutations and Pseudodeficiency Alleles**

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - This same test has not been performed previously, and
  - No known HEXA mutation in family, AND
- Diagnostic Testing:<sup>6</sup>
  - Abnormal or indeterminate HEX A enzymatic activity in serum, white blood cells, or other tissues, and clinical symptoms of TSD, but diagnosis remains uncertain, or
  - Asymptomatic individual with abnormal HEX A enzymatic activity in order to test for a pseudodeficiency allele, or
  - Children under the age of 6 months with
    - Progressive weakness and loss of motor skills, or
    - Decreased attentiveness, or
    - Increased startle response, or
    - Macular cherry red spot, or
    - Seizures, or

- Blindness, or
- Young children with
  - Ataxia and incoordination, or
  - Speech, life skills and cognition decline, or
  - Spasticity and seizures, or
  - Loss of vision, sometimes with:
    - Cherry red spot, or
    - Optic atrophy, or
    - Retinitis pigmentosa, or
- Adolescent/adult (and SMA type Kugelberg-Welander disease or early onset ALS has been ruled out) with
  - Progressive dystonia, or
  - Spinocerebellar degeneration, or
  - Motor neuron disease, or
  - Cognitive dysfunction, dementia, recurrent psychotic depression or bipolar symptoms, or
- French Canadian, Cajun, or Old Order Amish descent regardless of symptoms, OR
- Preconception/Prenatal Carrier testing
  - Ashkenazi Jewish descent, and
  - Intention to reproduce, AND
- Carrier testing for Individuals with Family History or Partners of Carriers:
  - 1<sup>st</sup>, 2<sup>nd</sup>, or 3<sup>rd</sup> degree biologic relative with Tay-Sachs clinical diagnosis, family mutation unknown, and affected relative unavailable for testing, or
  - Partner is monoallelic or biallelic for HEXA mutation, and
  - Have the potential and intention to reproduce, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

### HEXA Sequencing

- Genetic Counseling:

- Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - No mutations found on targeted mutation analysis, and
  - No previous full sequencing of HEXA, AND
- Diagnostic Testing:<sup>6</sup>
  - Abnormal or indeterminate HEX A enzymatic activity in serum, white blood cells, or other tissues, and clinical symptoms of TSD, but diagnosis remains uncertain, OR
  - Children under the age of 6 months with one or more of the following:
    - Progressive weakness and loss of motor skills,
    - Decreased attentiveness
    - Increased startle response
    - Macular cherry red spot
    - Seizures
    - Blindness, or
  - Young children, with one or more of the following:
    - Ataxia and incoordination
    - Speech, life skills and cognition decline
    - Spasticity and seizures
    - Loss of vision, sometimes with:
      - Cherry red spot
      - Optic atrophy
      - Retinitis pigmentosa, or
  - Adolescence/adult (and SMA type Kugelberg-Welander disease or early onset ALS has been ruled out), with one or more of the following:
    - Progressive dystonia
    - Spinocerebellar degeneration
    - Motor neuron disease
    - Cognitive dysfunction, dementia, recurrent psychotic depression or bipolar symptoms, and



- Carrier testing for Individuals with Family History or Partners of Carriers:
  - 1<sup>st</sup>, 2<sup>nd</sup>, or 3<sup>rd</sup> degree biologic relative with Tay-Sachs clinical diagnosis, and familial mutation unknown, and affected relative unavailable for testing, or
  - Partner is monoallelic or biallelic for a HEXA mutation, and
  - Have the potential and intention to reproduce, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

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# Thoracic Aortic Aneurysms and Dissections (TAAD) Panel Testing

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## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedures addressed by this guideline   | Procedure codes |
|--|-----------------|
| ACTA2 Known Familial Mutation Analysis   | 81403           |
| CBS Known Familial Mutation Analysis     | 81403           |
| COL3A1 Known Familial Mutation Analysis  | 81403           |
| COL5A1 Known Familial Mutation Analysis  | 81403           |
| COL5A2 Known Familial Mutation Analysis  | 81403           |
| EFEMP2 Known Familial Mutation Analysis  | 81403           |
| FBN1 Known Familial Mutation Analysis    | 81403           |
| MYH11 Known Familial Mutation Analysis   | 81403           |
| FBN2 Known Familial Mutation Analysis    | 81403           |
| FLNA Known Familial Mutation Analysis    | 81403           |
| MED12 Known Familial Mutation Analysis   | 81403           |
| MYLK Known Familial Mutation Analysis    | 81403           |
| NOTCH1 Known Familial Mutation Analysis  | 81403           |
| PLOD1 Known Familial Mutation Analysis   | 81403           |
| PRKG1 Known Familial Mutation Analysis   | 81403           |
| SKI Known Familial Mutation Analysis     | 81403           |
| SLC2A10 Known Familial Mutation Analysis | 81403           |
| SMAD3 Known Familial Mutation Analysis   | 81403           |
| SMAD4 Known Familial Mutation Analysis   | 81403           |

| Procedures addressed by this guideline                             | Procedure codes |
|--|-----------------|
| TGFB2 Known Familial Mutation Analysis                             | 81403           |
| TGFB3 Known Familial Mutation Analysis                             | 81403           |
| TGFBR1 Known Familial Mutation Analysis                            | 81403           |
| TGFBR2 Known Familial Mutation Analysis                            | 81403           |
| Aortic Dysfunction or Dilation Genomic Sequence Analysis Panel     | 81410           |
| Aortic Dysfunction or Dilation Duplication/Deletion Analysis Panel | 81411           |

## What are thoracic aortic aneurysms and dissections (TAAD)

### Definition

The major cardiac problems seen in individuals with Thoracic Aortic Aneurysms and Dissections (TAAD) include “a permanent, localized dilation...involving the aortic root and/or ascending aorta and dissections involving the ascending aorta (Stanford Type A) or that originate at the descending aorta (Stanford Type B)”<sup>1</sup>

- TAAD can be diagnosed by various imaging studies, including echocardiography, computed tomography (CT) and MRI.<sup>1</sup>
- The age of aortic dissection and the severity of the disease can range.<sup>1</sup> Treatment for TAAD may involve medications. Surgical repair of the aorta may be necessary in some cases to help prevent aortic dissection.<sup>1</sup>
- Genetic testing can be helpful to determine if there is an underlying genetic condition causing the TAAD. There are many genes which can predispose someone to TAAD. Some of these genes are associated with specific genetic conditions which may require additional management or surveillance. Medical management, including timing of surgery, may differ based on the underlying genetic etiology.<sup>2,3</sup> In many cases, a careful clinical examination by a specialist familiar with clinical features of these conditions can help to point toward one condition. In these cases, testing for gene(s) associated with a single condition would be most appropriate.
- Specific genetic conditions that have TAAD as a clinical manifestation:
  - **Marfan syndrome (MFS)** – MFS is an autosomal dominant disorder that affects connective tissue in many parts of the body.<sup>4</sup> MFS is caused by mutations in the FBN1 gene. Approximately 70-93% of people meeting diagnostic criteria for

Marfan will have a mutation in this gene. Diagnostic criteria, called the Ghent criteria, exists for Marfan syndrome. Major manifestations of the disease include aortic enlargement and ectopia lentis. Other features include, but are not limited to, bone overgrowth and joint laxity, long arms and legs, scoliosis, sternum deformity (pectus excavatum or carinatum), long thin fingers and toes, dural ectasia (stretching of the dural sac), hernias, stretch marks on the skin, and lung bullae. Symptoms can present in males or females at any age. Symptoms typically worsen over time. Infants who present with symptoms typically have the most severe disease course.<sup>4</sup>

- **Loeys-Dietz syndrome (LDS)** - LDS is an autosomal dominant disorder that affects many parts of the body.<sup>5</sup> LDS is mostly caused by mutations in either the TGFBR1 gene (20%) or TGFBR2 gene (60%). However, a small percentage of people with LDS may have mutations in the SMAD2 (5%), SMAD3 gene (5%), TGFB2 gene (5%), or TGFB3 (5%). Major manifestations of this condition include “vascular findings (cerebral, thoracic, and abdominal arterial aneurysms and/or dissections), skeletal manifestations (pectus excavatum or pectus carinatum, scoliosis, joint laxity, arachnodactyly, talipes equinovarus, cervical spine malformation and/or instability), craniofacial features (widely spaced eyes, strabismus, bifid uvula/ cleft palate, and craniosynostosis that can involve any sutures), and cutaneous findings (velvety and translucent skin, easy bruising, and dystrophic scars).”<sup>5</sup> Given that there is no clinical diagnostic criteria established for LDS, genetic testing can help with the diagnosis.<sup>5</sup>
- **Vascular Ehlers-Danlos syndrome (vEDS or EDS type IV)** – EDS type IV is an autosomal dominant condition. It is caused by mutations in the COL3A1 gene. Major manifestations of this condition include “arterial, intestinal, and/or uterine fragility; thin, translucent skin; easy bruising; characteristic facial appearance (thin vermilion of the lips, micrognathia, narrow nose, prominent eyes); and an aged appearance to the extremities, particularly the hands.”<sup>6</sup> Many adults present with the following symptoms: vascular dissection or rupture, gastrointestinal perforation, or organ rupture. Infants and children may present with congenital dislocation of the hips, clubfoot, pneumothorax, and/or recurrent joint subluxation or dislocation.<sup>6</sup>
- **Heritable Thoracic Aortic Disease (HTAD)** – HTAD describes those with TAAD who have absence of a known syndrome (e.g., Marfan syndrome, vEDS, LDS) and have a positive family history of TAAD.<sup>1</sup> 30% of those with HTAD will have a causative pathogenic variant identified in one of the 16 known HTAD-related genes (ACTA2, BGN, COL3A1, FBN1, FOXE3, LOX, MAT2A, MFAP5, MYH11, MYLK, PRKG1, TGFB2, TGFB3, TGFBR1, TGFBR2, SMAD3).<sup>1</sup>

## Test information

- Many laboratories offer testing for at least 9 genes that have been associated with TAAD in their panels, including the genes that cause MFS, LDS, EDS type IV and HTAD. Detection rates of expanded panels vary by laboratory and depend on the

genes included and the methods used for testing.<sup>1</sup> If features of TAAD and ectopia lentis are present, targeted testing for Marfan syndrome (FBN1) is most appropriate.<sup>1</sup> Testing multiple genes, without supporting clinical features, has the potential to obtain results which may be hard to interpret. The chance that a variant of uncertain significance will be found increases as more genes are tested. However, given that many of the symptoms of conditions associated with TAAD overlap, if a person presents with overlapping features of more than one condition, a panel approach may be considered.

- Without symptoms of a specific genetic condition associated with TAAD, mutations in the ACTA2 gene are the most common. Mutations in this gene account for approximately 12-21% of HTAD.<sup>1</sup>
- Once a mutation is identified in a family member, the known familial mutation can be specifically identified in asymptomatic or symptomatic family members.

## Guidelines and evidence

- Cardiac Society of Australia and New Zealand (CSANZ) Cardiovascular Genetic Disease Council (2017) states:<sup>7</sup>
  - “A definitive molecular genetic diagnosis can clarify an equivocal clinical picture or result in a diagnosis in an apparently phenotypically normal individual. It is unknown at this stage what proportion of patients with these different genetic mutations will develop aortic dilatation or dissection. Identification of a causal mutation allows for the provision of accurate genetic counseling, the screening of at-risk family members and offers the possibility of accurate prenatal or preimplantation genetic diagnosis.”
  - “Molecular confirmation of a suspected clinical diagnosis is increasingly important for guiding patient management. As an example, an individual who looks marfanoid will have more extensive arterial imaging screening if identified to have a SMAD3 mutation as opposed to an FBN1 mutation.”
  - “Many clinical laboratories offer a multi-gene MFS/LDS/ familial TAAD panel that includes FBN1 and numerous other genes associated with aortic aneurysm and dissection disorders. This approach may be advantageous, given the known clinical and genetic heterogeneity of these disorders.”
  - “The clinical picture of non-syndromic aortopathies remains to be fully elucidated, and therefore the optimal extent and frequency of vascular imaging is unclear. We would err on the side of caution and suggest imaging the entire vasculature, at least at baseline, in non-syndromic individuals with a genetic mutation.”
  - “If there is a clear genetic diagnosis, then first-degree relatives should be offered predictive testing. If the screened relative does not have the familial mutation they can be released from screening. We advocate erring on the side of caution

with respect to screening echocardiography of at-risk relatives.” Screening is advised in the following relatives:

- i. “All family members who share the familial mutation and who therefore should be under clinical care, not screening”
  - ii. “At-risk family members where a clinical genetic diagnosis exists”
  - iii. “At-risk family members where no clinical genetic diagnosis is made but the dissection occurred in a young individual without an apparent risk factor e.g. long standing hypertension.”
- The European Society of Cardiology (ESC, 2014) stated the following:<sup>8</sup>
    - “Once a familial form of TAAD is highly suspected, it is recommended to refer the patient to a geneticist for family investigation and molecular testing.” (Class I, Level C)
  - The Canadian Cardiovascular Society (2014) stated the following:<sup>9</sup>
    - “We recommend screening for TAD-associated genes in non-BAV aortopathy index cases to clarify the origin of disease and improve clinical and genetic counseling (Strong recommendation, moderate quality evidence).”
    - “We recommend complete aortic imaging at initial diagnosis and at 6 months for patients with LDS or a confirmed genetic aortopathy (e.g., TGFBR1/2, TGFB, SMAD3, ACTA2, or MYH11) to establish if enlargement is occurring (Strong Recommendation, Moderate-Quality Evidence).”
    - “We recommend that genetic counseling and testing be offered to first-degree relatives of patients in whom a causal mutation of a TAD-associated gene is identified. We recommend that aortic imaging be offered only to mutation carriers (Strong Recommendation, Low-Quality Evidence).”
  - Joint evidence-based guidelines from ACCF/AHA/AATS/ACR/ASA/SCA/SCAI/SIR/STS/SVM (2010) for the diagnosis and management of thoracic aortic disease.<sup>10</sup>
    - Predictive genetic testing for at-risk relatives is addressed in the following guidelines statement:
      - “If the mutant gene (FBN1, TGFBR1, TGFBR2, COL3A1, ACTA2, MYH11) associated with aortic aneurysm and/or dissection is identified in a patient, first-degree relatives should undergo counseling and testing. Then, only the relatives with the genetic mutation should undergo aortic imaging.”<sup>7</sup>  
[Evidence level I: “Evidence from only expert opinion, case studies, or standard of care.” Recommendation classification C: “Recommendation that procedure or treatment is useful/effective.”]<sup>10</sup>
    - ACTA2 sequencing is addressed in the following guidelines statement:

- “Sequencing of the ACTA2 gene is reasonable in patients with a family history of thoracic aortic aneurysms and/or dissections to determine if ACTA2 mutations are responsible for the inherited predisposition (Pannu et al., 2005; Guo et al., 2007; Zhu et al., 2006; Loeys et al., 2006; Stheneur et al., 2008; Guo et al., 2009).” [Evidence level IIa: “Only diverging expert opinion, case studies, or standard of care.” Recommendation classification B: “Recommendation in favor of treatment or procedure being useful/effective.”]⁹
- Additional genetic testing is addressed in the following guidelines statement:
  - “Sequencing of other genes known to cause familial thoracic aortic aneurysms and/or dissection (TGFB1, TGFB2, MYH11) may be considered in patients with a family history and clinical features associated with mutations in these genes (Pannu et al., 2005; Guo et al., 2007; Zhu et al., 2006; Loeys et al., 2006; Stheneur et al., 2008; Guo et al., 2009).” [Evidence level IIb: “Greater conflicting evidence from single randomized trial or nonrandomized studies.” Recommendation classification B: “Recommendation's usefulness/efficacy less well established.”]⁹
  - “Patients with Loeys-Dietz syndrome or a confirmed genetic mutation known to predispose to aortic aneurysms and aortic dissections (TGFB1, TGFB2, FBN1, ACTA2, or MYH11) should undergo complete aortic imaging at initial diagnosis and 6 months thereafter to establish if enlargement is occurring. (Evidence level I: "Evidence from only expert opinion, case studies, or standard of care." Recommendation classification C: "Recommendation that procedure or treatment is useful/effective.”

## Criteria

### Known Familial Mutation(s) for TAAD

- Genetic Counseling
  - Pre and post-test counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - No previous genetic testing for TAAD inclusive of known family mutation, AND
- Diagnostic or Predisposition Testing for Symptomatic or Presymptomatic Individuals:\*\*
  - TAAD family mutation in 1<sup>st</sup> degree biological relative, AND
- Rendering laboratory is a qualified provider for service per the Health Plan policy

**\*\*NOTE:** Since symptoms may occur in childhood, testing of children who are at-risk for a pathogenic mutation may be considered.

### **TAAD Genetic Testing Sequencing Panel**

Gene panels that are specific to TAAD that include the following genes will be eligible for coverage according to the criteria outlined in this policy: FBN1, TGFBR1, TGFBR2, COL3A1, MYH11, ACTA2, SLC2A10, SMAD3, and MYLK. This sequencing panel will only be considered for coverage when billed under the appropriate panel CPT code: 81410. For criteria specific to Marfan syndrome, please see the policy ***Marfan Syndrome Genetic Testing***.

- Genetic Counseling
  - Pre and post-test counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - No previous panel testing for TAAD, AND
- Diagnostic Testing for Symptomatic Individuals:
  - Cardiology examination consistent with a diagnosis of TAAD, and
  - Clinical features are not sufficiently specific to suggest a single condition, and
  - The results of the test will directly impact the diagnostic and treatment options that are recommended for the patient, AND
- Rendering laboratory is a qualified provider for service per Health Plan policy

### **TAAD Genetic Testing Duplication/Deletion Panel**

This duplication/deletion panel will only be considered for coverage when billed under the appropriate panel CPT code: 81411.

- Criteria for TAAD Genetic Testing Sequencing panel met, AND
- No mutations found in TAAD Sequencing panel, AND
- No previous deletion/duplication analysis for TAAD

### **Billing and reimbursement considerations**

- This guideline addresses testing specifically for TAAD. Additional indications are addressed in the *Hereditary Connective Tissue Disorder Testing* guideline.
- When multiple CPT codes are billed for components of a panel and there is a more appropriate CPT code representing the panel, eviCore will redirect to the panel code(s).



- If the laboratory will not accept redirection to a panel code, the medical necessity of each billed component procedure will be assessed independently.
  - In general, only a limited number of panel components that are most likely to explain the member's presentation will be reimbursable. The remaining panel components will not be reimbursable.
  - When a TAAD multi-gene panel is billed with multiple stacked codes, only the following genes may be considered for reimbursement:
    - TGFBR2
    - TGFBR1
    - ACTA2
    - SMAD3

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# ThyGeNEXT and ThyraMIR miRNA Gene Expression Classifier

MOL.TS.259.A

v1.0.2020

## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedures addressed by this guidelines   | Procedure codes |
|---|-----------------|
| ThyGeNEXT                                 | 81445           |
| ThyraMIR miRNA Gene Expression Classifier | 0018U           |

## What are thyroid nodules

### Definition

Thyroid nodules are a common occurrence, especially in an aging population. Fine-needle aspiration (FNA) with accompanying cytology examination is the standard method for distinguishing between benign and malignant nodules and subsequent removal of tumors. Approximately 15 to 30% of thyroid nodules examined using FNA and traditional cytology examination are considered indeterminate. Clinicians are then faced with the decision to either remove the nodule unnecessarily or leave a potentially malignant nodule in place.<sup>1</sup>

Additional diagnostic procedures have been developed to help further classify indeterminate nodules as either benign or malignant. These procedures usually involve assessment of known genetic point mutations or through the expression activity of microRNA.<sup>1</sup>

### Test information

- Thyroid nodules are traditionally assessed through inspection of cell cytology; however, some aspirate samples may be indeterminate. ThyraMIR uses an algorithm of 10 microRNAs previously validated using nodules with known malignancy to assist in determining if indeterminate cytology is malignant. It is used in conjunction with ThyGeNEXT. The ThyGeNEXT panel identifies DNA mutations (ALK, BRAF, GNAS, HRAS, KRAS, NRAS, PIK3CA, PTEN, RET, and TERT), and the RNA panel identifies the number of fusions: ALK (2), BRAF (2), NTRK (8), PPARg (6), RET (14), THADA (5). This test was previously called ThyGenX.

Specifically, the manufacturer reports that TERT and ALK mutations have been “newly added” to “predict aggressive biological features of thyroid cancer.”<sup>3-5</sup>

- Specimens for testing with the combination of ThyGeNEXT + ThyraMIR are obtained when performing FNA.<sup>5</sup> When a thyroid fine needle aspirate sample is found to be indeterminate, the ThyGeNEXT test is run on the sample. If the ThyGeNEXT test result is negative for malignancy, the ThyraMIR miRNA classifier test is then used to increase the overall sensitivity and specificity of the test combination. The overall test result is either positive or negative for malignancy.

## Guidelines and evidence

### National Comprehensive Cancer Network

The National Comprehensive Cancer Network (NCCN, 2018) Thyroid Carcinoma Guidelines state the following:<sup>3</sup>

- “The diagnosis of follicular carcinoma or Hürthle cell carcinoma requires evidence of either vascular or capsular invasion, which cannot be determined by FNA. Molecular diagnostics may be useful to allow reclassification of follicular lesions (i.e. follicular neoplasm, atypia of undetermined significance (AUS), follicular lesions of undetermined significance (FLUS)) as either more or less likely to be benign or malignant based on the genetic profile. ...If molecular testing, in conjunction with clinical and ultrasound features, predicts a risk of malignancy comparable to the risk of malignancy seen with a benign FNA cytology (approximately 5% or less), consider active surveillance. Molecular markers should be interpreted with caution and in the context of clinical, radiographic, and cytologic features of each individual patient.”

### American Thyroid Association

The American Thyroid Association (2016) makes the following statement regarding molecular testing and FNA-indeterminate thyroid nodules:<sup>4</sup>

- “For nodules with AUS/FLUS cytology, after consideration of worrisome clinical and sonographic features, investigations such as repeat FNA or molecular testing may be used to supplement malignancy risk assessment in lieu of proceeding directly with a strategy of either surveillance or diagnostic surgery. Informed patient preference and feasibility should be considered in clinical decision-making. (Weak recommendation, Moderate-quality evidence)”
- “If repeat FNA cytology, molecular testing, or both are not performed or inconclusive, either surveillance or diagnostic surgical excision may be performed for an AUS/FLUS thyroid nodule, depending on clinical risk factors, sonographic pattern, and patient preference. (Strong recommendation, Low-quality evidence)”

## American Association of Clinical Endocrinologists, American College of Endocrinology, and Associazione Medici Endocrinologi (AACE/ACE/AME) Guidelines

The AACE/ACE/AME 2016 Clinical Practice Guidelines for the Diagnosis and Management of Thyroid Nodules state the following:<sup>5</sup>

- “In nodules with indeterminate cytologic results, no single cytochemical or genetic marker is specific or sensitive enough to rule out malignancy with certainty. However the use of immunohistochemical and molecular markers may be considered together with the cytologic subcategories and data from US (ultrasound), elastography, or other imaging techniques to obtain additional information for management of these patients.”
- When molecular testing should be considered:
  - “To complement not replace cytologic evaluation (BEL 2, GRADE A).”
  - “The results are expected to influence clinical management (BEL 2, GRADE A).”
  - “As a general rule, not recommended in nodules with established benign or malignant cytologic characteristics (BEL 2, GRADE A).”
- Molecular testing for cytologically indeterminate nodules:
  - “Cytopathology expertise, patient characteristics, and prevalence of malignancy within the population being tested impact the NPV and PPV for molecular testing (BEL 3, GRADE B).”
  - “Consider detection of BRAF and RET/PTC and, possibly PAX8/PPARG and RAS mutations if such detection is available (BEL 2, GRADE B).”
  - “Because of the insufficient evidence and limited follow-up, we do not recommend either in favor of or against the use of gene expression classifiers (GECs) for cytologically indeterminate modules (BEL 2 GRADE B).”
- Role of molecular testing for deciding the extent of surgery
  - “Currently, with the exception of mutations such as BRAFV600E that have a PPV approaching 100% for papillary thyroid carcinoma (PTC), the evidence is insufficient to recommend in favor of or against the use of mutation testing as a guide to determine the extent of surgery (BEL 2, GRADE A).”
- How should patient with nodules that are negative at mutation testing be monitored?
  - “Since the false-negative rate for indeterminate nodules is 5 to 6% and the experience and follow-up for mutation negative nodules or nodules classified as benign by a GEC are still insufficient, close follow-up is recommended (BEL 3, GRADE B).”

## Literature review

- The evidence base of the combined ThyGenX and ThyraMIR is currently insufficient to assess the effects of this combined test on patient health outcomes.<sup>6-10</sup>
  - Clinical validity studies reported area under the receiver operator curve (AUC) values ranging from 0.89 to 0.94; the test correctly classified 92% of benign lesions as low risk or negative and correctly classified 92% of malignant lesions as high risk or positive.<sup>9</sup>
  - Another study reported that ThyraMIR correctly identified 64% of malignant lesions and 96% of benign lesions. The sensitivity and specificity of the test was reported as 89% (95% confidence interval [CI], 73-97%) and 85% (95% CI, 75-92%), respectively. With a 32% prevalence rate, 61% of the results were considered benign, generating a negative predictive value (NPV) of 94% (95% CI, 85-98%).
- The evidence for clinical validity and clinical utility of the combined ThyGenX and ThyraMIR is sparse and consists of 2 retrospective studies. Direct evidence for clinical utility was not identified in the peer-reviewed literature. Overall, the evidence is insufficient to assess the effects of this combined test on patient health outcomes.
- There is no evidence of analytical validity, clinical validity or clinical utility of the newly named and expanded test, ThyGeNEXT. There is no published evidence evaluating the diagnostic accuracy or clinical utility of the combination test, ThyGeNEXT +ThyraMIR. Therefore, the evidence is insufficient to assess the effects of this combined test on patient health outcomes.

## Criteria

- This test is considered investigational and/or experimental.
  - Investigational and experimental (I&E) molecular and genomic (MolGen) tests refer to assays involving chromosomes, DNA, RNA, or gene products that have insufficient data to determine the net health impact, which typically means there is insufficient data to support that a test accurately assesses the outcome of interest (analytical and clinical validity), significantly improves health outcomes (clinical utility), and/or performs better than an existing standard of care medical management option. Such tests are also not generally accepted as standard of care in the evaluation or management of a particular condition.
  - In the case of MolGen testing, FDA clearance is not a reliable standard given the number of laboratory developed tests that currently fall outside of FDA oversight and FDA clearance often does not assess clinical utility.

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# ThyroSeq

MOL.TS.270.A

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## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedure addressed by this guideline | Procedure code |
|---------------------------------------|----------------|
| ThyroSeq                              | 0026U          |

## What are thyroid nodules

### Definition

Thyroid nodules are a common occurrence, especially in an aging population. Fine-needle aspiration (FNA) with accompanying cytology examination is the standard method for distinguishing between benign and malignant nodules and subsequent removal of tumors. Approximately 25% of thyroid nodules examined using FNA and traditional cytology examination are considered indeterminate. Clinicians are then faced with the decision to either remove the nodule unnecessarily or leave a potentially malignant nodule in place.<sup>1</sup>

- Additional diagnostic procedures have been developed to help further classify indeterminate nodules as either benign or malignant. These procedures usually involve assessment of known genetic mutations, gene fusions, or the expression activity of microRNA.<sup>1</sup>

### Test information

- ThyroSeq is designed to aid in the classification of thyroid nodules with indeterminate cytology as either malignant or benign.
- ThyroSeq is a gene sequencing panel used on thyroid cells obtained using fine needle aspiration (FNA) to detect genetic mutations known to be associated with thyroid cancer. ThyroSeq detects gene fusions and point mutations in 112 genes related to thyroid cancer. The test is used when cytological examination of cells obtained by FNA are indeterminate, thus helping to either identify malignant nodules and guide therapy (with positive test results) or avoid surgery for those with benign nodules (with negative test results).
- The ThyroSeq test has an overall negative predictive value of 97% and a positive predictive value of 66% in a tested population with a 28% prevalence of thyroid



cancer.<sup>2</sup> Results from the sequencing test give a prediction as either positive or negative for malignancy.

- Depending on the Bethesda thyroid cancer grade the following risks of malignancy are observed with negative and positive ThyroSeq results:<sup>2</sup>
  - Bethesda III Atypia of Undetermined Significance/Follicular Lesion of Undetermined Significance (AUS/FLUS) with 14% risk of malignancy:
    - ThyroSeq Negative: 3% risk of malignancy
    - ThyroSeq Positive: 64% risk of malignancy
  - Bethesda IV Follicular neoplasm/suspicion for a follicular neoplasm (FN/SFN) with 27% risk of malignancy:
    - ThyroSeq Negative: 2% risk of malignancy
    - ThyroSeq Positive: 68% risk of malignancy

## Guidelines and evidence

### National Comprehensive Cancer Network

The National Comprehensive Cancer Network (NCCN, 2018) Thyroid Carcinoma Guidelines incorporate the use of molecular tests in the evaluation of indeterminate thyroid nodules (category 2B). For FNA results consistent with Follicular or Hürthle Cell Neoplasms, or atypia of undetermined significance/follicular lesion of undetermined significance (AUS/FLUS) with a “High clinical suspicion of malignancy”, they state:<sup>3</sup>

“The diagnosis of follicular carcinoma or Hürthle cell carcinoma requires evidence of either vascular or capsular invasion, which cannot be determined by FNA. Molecular diagnostics may be useful to allow reclassification of follicular lesions (i.e. follicular neoplasm, atypia of undetermined significance (AUS), follicular lesions of undetermined significance (FLUS)) as either more or less likely to be benign or malignant based on the genetic profile....If molecular testing, in conjunction with clinical and ultrasound features, predicts a risk of malignancy comparable to the risk of malignancy seen with a benign FNA cytology (approximately 5% or less), consider active surveillance. Molecular markers should be interpreted with caution and in the context of clinical, radiographic, and cytologic features of each individual patient.”

### American Thyroid Association

The American Thyroid Association (2016) makes the following statement regarding molecular testing and FNA-indeterminate thyroid nodules:<sup>4</sup>

- “For nodules with AUS/FLUS cytology, after consideration of worrisome clinical and sonographic features, investigations such as repeat FNA or molecular testing may be used to supplement malignancy risk assessment in lieu of proceeding directly

with a strategy of either surveillance or diagnostic surgery. Informed patient preference and feasibility should be considered in clinical decision-making. (Weak recommendation, Moderate-quality evidence)”

- “If repeat FNA cytology, molecular testing, or both are not performed or inconclusive, either surveillance or diagnostic surgical excision may be performed for an AUS/FLUS thyroid nodule, depending on clinical risk factors, sonographic pattern, and patient preference. (Strong recommendation, Low-quality evidence)”

### **American Association of Clinical Endocrinologists, American College of Endocrinology, and Associazione Medici Endocrinologi (AACE/ACE/AME) Guidelines**

The AACE/ACE/AME 2016 Clinical Practice Guidelines for the Diagnosis and Management of Thyroid Nodules state the following:<sup>5</sup>

- In nodules with indeterminate cytologic results, no single cytochemical or genetic marker is specific or sensitive enough to rule out malignancy with certainty. However the use of immunohistochemical and molecular markers may be considered together with the cytologic subcategories and data from US (ultrasound), elastography, or other imaging techniques to obtain additional information for management of these patients.
- When molecular testing should be considered:
  - To complement not replace cytologic evaluation (BEL 2, GRADE A)
  - The results are expected to influence clinical management (BEL 2, GRADE A)
  - As a general rule, not recommended in nodules with established benign or malignant cytologic characteristics (BEL 2, GRADE A)
- Molecular testing for cytologically indeterminate nodules:
  - Cytopathology expertise, patient characteristics, and prevalence of malignancy within the population being tested impact the NPV and PPV for molecular testing (BEL 3, GRADE B)
  - Consider detection of BRAF and RET/PTC and, possibly PAX8/PPARG and RAS mutations if such detection is available (BEL 2, GRADE B)
  - Because of the insufficient evidence and limited follow-up, we do not recommend either in favor of or against the use of gene expression classifiers (GECs) for cytologically indeterminate modules (BEL 2 GRADE B)
- Role of molecular testing for deciding the extent of surgery
  - Currently, with the exception of mutations such as BRAFV600E that have a PPV approaching 100% for papillary thyroid carcinoma (PTC), the evidence is insufficient to recommend in favor of or against the use of mutation testing as a guide to determine the extent of surgery (BEL 2, GRADE )

- How should patient with nodules that are negative at mutation testing be monitored?
  - Since the false-negative rate for indeterminate nodules is 5 to 6% and the experience and follow-up for mutation negative nodules or nodules classified as benign by a GEC are still insufficient, close follow-up is recommended (BEL 3, GRADE B)

### Literature review

The current published peer-reviewed literature is comprised of several analytical and clinical validity studies that report wide variation among the diagnostic accuracy values (sensitivity, specificity, PPV and NPV). These are likely due to the heterogeneity of the included sample sets, such as malignancy prevalence in the overall population; overall sample size; the Bethesda Type; the proportions of each Bethesda Type; and variable definitions used for benign nodule classification.<sup>6-18</sup>

Evidence derived from randomized, multicenter trials evaluating direct clinical utility of the impact of ThyroSeq v2 on health outcomes, such as survival and quality of life, is still lacking. Early evidence suggests a potential for clinical utility, but additional well-designed studies of ThyroSeq v2 are needed to evaluate the impact of clinical decisions on health outcomes. Given the heterogeneity across the available studies as well as other the inherent study limitations, it is difficult to draw definitive conclusions regarding the clinical usefulness of the ThyroSeq v2 panel to ascertain initially indeterminate FNA cytology results.

Larger, prospective studies are needed to evaluate the behavior of cytologically indeterminate thyroid nodules that are deemed to be negative on the ThyroSeq v2 panel (ruling out disease) and to substantiate positive ThyroSeq v2 results (ruling in disease) with surgical biopsy. Clinical utility studies that follow up benign cases are also necessary to determine the degree of influence ThyroSeq v2 may have on disease management and if changes in disease management lead to clinically relevant improved outcomes, such as sparing patients from future invasive surgery, reducing the incidence of morbidity, and improving disease-specific survival over the long term.

### Criteria

- This test is considered investigational and/or experimental.
  - Investigational and experimental (I&E) molecular and genomic (MolGen) tests refer to assays involving chromosomes, DNA, RNA, or gene products that have insufficient data to determine the net health impact, which typically means there is insufficient data to support that a test accurately assesses the outcome of interest (analytical and clinical validity), significantly improves health outcomes (clinical utility), and/or performs better than an existing standard of care medical management option. Such tests are also not generally accepted as standard of care in the evaluation or management of a particular condition.

- In the case of MolGen testing, FDA clearance is not a reliable standard given the number of laboratory developed tests that currently fall outside of FDA oversight and FDA clearance often does not assess clinical utility.

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# Tissue of Origin Testing for Cancer of Unknown Primary

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v1.0.2020

## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedures addressed by this guideline | Procedure codes |
|--|-----------------|
| Miscellaneous Tissue of Origin Test    | 81479           |
| Miscellaneous Tissue of Origin Test    | 84999           |
| Tissue of Origin Testing               | 81504           |
| CancerTYPE ID                          | 81540           |

## What is cancer of unknown primary testing

### Definition

In order to determine the most effective treatment regimen for a patient with cancer it is important to identify the cancer cell type.<sup>1</sup>

- When a cancer is found in one or more metastatic sites but the primary site is not known, it is called a cancer of unknown primary (CUP) or an occult primary cancer.<sup>2</sup> This happens in a small portion of cancers.
- The most commonly used techniques to identify tissue of origin (TOO) for CUP include light microscopy, immunohistochemistry (IHC) staining and computed tomography (CT) or positron emission tomography (PET) imaging.<sup>1</sup>
- With advances in technology, some laboratory tests utilize gene expression profiling or other molecular techniques in cancer cells. Ramaswamy et al. found that a cancer-intrinsic gene expression pattern distinguished primary from metastatic adenocarcinomas.<sup>3</sup> By comparing the pattern of gene expression in the CUP sample to the patterns seen with other known types of cancer, a CUP may be identified as belonging to a particular cancer type.

## Test information

- A number of different companies and approaches are being utilized to diagnose metastatic neoplasms for patients with CUP. These include but are not limited to:
  - Tissue of Origin Test from Cancer Genetics Incorporated - uses microarray analysis to measure the expression of over two thousand genes.<sup>4</sup>
  - CancerType ID from Biotheranostics analyzes the expression of 92 genes and requires only 300 cells.<sup>5</sup>
  - Cancer Origin Test from Rosetta Genomics- uses a RT-PCR platform to analyze the expression levels of 64 microRNAs (miRNAs).<sup>6</sup>

## Guidelines and evidence

- Under 2019 NCCN guidelines for CUP (occult primary), gene signature profiling for tissue of origin is not recommended for standard management at this time. The panel states that "As noted, outcomes data are not currently available to recommend routine use of molecular profiling in the working of CUP." Also, "the clinical benefit that might be derived from the use of these molecular assays, if any, remains to be determined."<sup>7</sup>
- In a systematic review of cancer of unknown primary site in Lancet, gene-profiling diagnosis was noted to have high sensitivity, but additional prospective studies were deemed necessary to establish whether patients' outcomes are improved by its clinical use.<sup>8</sup>

## Criteria

This test is considered investigational and/or experimental.

- Investigational and experimental (I&E) molecular and genomic (MolGen) tests refer to assays involving chromosomes, DNA, RNA, or gene products that have insufficient data to determine the net health impact, which typically means there is insufficient data to support that a test accurately assesses the outcome of interest (analytical and clinical validity), significantly improves health outcomes (clinical utility), and/or performs better than an existing standard of care medical management option. Such tests are also not generally accepted as standard of care in the evaluation or management of a particular condition.
- In the case of MolGen testing, FDA clearance is not a reliable standard given the number of laboratory developed tests that currently fall outside of FDA oversight and FDA clearance often does not assess clinical utility.

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# TPMT Testing for Thiopurine Drug Response

MOL.TS.229.A

v1.0.2020

## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedures addressed by this guideline       | Procedure codes |
|--|-----------------|
| TPMT Genotyping                              | 81335           |
| Thiopurine Methyltransferase Enzyme Analysis | 82542           |
| Thiopurine Methyltransferase Enzyme Analysis | 83789           |
| Thiopurine Methyltransferase Enzyme Analysis | 82657           |

## What is thiopurine drug toxicity

### Definition

These drugs have a relatively narrow therapeutic window and adverse drug reactions are frequent, with estimates ranging from 5% to 40%.<sup>1-2</sup> Drug toxicity can result in myelosuppression or hepatotoxicity, and can be life-threatening.<sup>3</sup> People taking thiopurine should have regular complete blood cell count (CBC) monitoring.<sup>4</sup>

- The thiopurine drugs – azathioprine (AZA), 6-mercaptopurine (6-MP), and 6-thioguanine (6-TG) – are commonly used to treat hematological malignancies, autoimmune conditions, inflammatory bowel disease, and solid organ transplant rejections.<sup>1</sup>
- These drugs are metabolized by the enzyme TPMT (thiopurine methyltransferase). Genetic variants in the TPMT gene are associated with lower enzyme activity, leading to an increased risk for drug toxicity.<sup>3</sup>
- TPMT enzyme activity is largely influenced by polymorphisms (changes) in the TPMT gene. About 29 TPMT variants have been identified. TPMT\*2, TPMT\*3A, TPMT\*3C and account for 85-90% of intermediate or low TPMT enzyme activity.<sup>5</sup>
- About 1 in 300 (0.3%) people have deficient or undetectable TPMT activity, 11% have low (intermediate) activity and 89% have normal activity. Evidence of a fourth

group of ultra-high TPMT activity has recently been found in about 2% of the population.<sup>4-6</sup>

- The overall distribution of low, intermediate and normal TPMT activity does not appear to vary among Caucasians, Asians or African-Americans. However, the TPMT variants are not equally distributed among ethnic populations. The frequency of the variant alleles for which commercial genetic testing is currently available is highest in Caucasians and African-Americans. These variants are less common in Southeast (Indonesian, Thai, Filipino, Taiwanese) and Southwest (Indian, Pakistani) Asians.<sup>4,7</sup>
- TPMT activity can account for up to 75% of the cases of neutropenia associated with thiopurines. People with absent TPMT activity treated with normal doses of thiopurines are at approximately 100% risk of developing severe or fatal myelosuppression.<sup>7</sup> People with low TPMT activity have a 30-40% risk of developing adverse reactions to thiopurines when treated with standard doses.<sup>6</sup>

## Test information

- **Phenotyping** quantifies TPMT enzyme activity. Testing laboratories generally interpret results as normal, intermediate, or low. Some also report a high enzyme activity level. Phenotyping will detect any lowered enzyme activity, regardless of the specific underlying genetic variation. However, phenotyping results may not be accurate for:
  - People who have received recent blood transfusions (within the last four months).<sup>4</sup>
  - People currently treated with thiopurine drugs.<sup>4</sup>
  - People currently taking drugs that inhibit TPMT, including: naproxen, ibuprofen, ketoprofen, furosemide, sulfasalazine, mesalamine, olsalazine, mefenamic acid, thiazide diuretics, and benzoic acid inhibitors. Patients should abstain from these drugs for at least 48 hours prior to blood collection.<sup>2</sup>
- **Genotyping** for TPMT sensitivity is done by targeted analysis for the most common variant alleles. TPMT\*1 is the normal (wild-type) allele; the TPMT\*2, \*3A, \*3B, and \*3C alleles are variants common in the general population. Genetic test results are not affected by medication use or blood transfusion.<sup>15</sup>

## Guidelines and evidence

- The US Food and Drug Administration (2004) revised the labeling for azathioprine, 6-mercaptopurine and 6-thioguanine:
  - Azathioprine: “It is recommended that consideration be given to either genotype or phenotype patients for TPMT. Phenotyping and genotyping methods are commercially available. The most common non-functional alleles associated

with reduced levels of TPMT activity are TPMT\*2, TPMT\*3A and TPMT\*3C. Patients with two non-functional alleles (homozygous) have low or absent TPMT activity and those with one non-functional allele (heterozygous) have intermediate activity. Accurate phenotyping (red blood cell TPMT activity) results are not possible in patients who have received recent blood transfusions. TPMT testing may also be considered in patients with abnormal CBC results that do not respond to dose reduction. Early drug discontinuation in these patients is advisable.”<sup>4</sup>

- 6-mercaptopurine (6-MP): “Several published studies indicate that patients with reduced TPMT... activity receiving usual doses of Mercaptopurine, accumulate excessive cellular concentrations of active 6-TGns, and are at higher risk for severe myelosuppression. TPMT genotyping or phenotyping (red blood cell TPMT activity)... can identify patients who have reduced activity of these enzymes. Patients with homozygous TPMT require substantial dosage reductions.”<sup>8</sup>
- 6 thioguanine (6-TG): “There are individuals with an inherited deficiency of the enzyme thiopurine methyltransferase (TPMT) who may be unusually sensitive to the myelosuppressive effects of Thioguanine and prone to developing rapid bone marrow suppression following the initiation of treatment. Substantial dosage reductions may be required to avoid the development of life-threatening bone marrow suppression in these patients. Prescribers should be aware that some laboratories offer testing for TPMT deficiency.”<sup>9</sup>
- Guidelines from the American College of Gastroenterology (2010) and the American Gastroenterological Association (2017) mirror the FDA recommendations and support testing of TPMT activity for people treated with thiopurines.<sup>10,11</sup>
- Ideally, TPMT activity testing should occur prior to initiating treatment with thiopurines, so that alternative treatment strategies can be considered in those at higher risk for toxicity.<sup>5-7,10-13,15</sup>
  - Thiopurine use in patients with deficient TPMT activity is contraindicated.<sup>4,6</sup>
  - Patients with intermediate TPMT activity should be treated with a reduced dose. Some guidelines have suggested a reduction of 50-67%.<sup>6,13</sup>
- TPMT testing may also be considered in patients with abnormal blood cell counts or when clinical evidence of severe toxicity does not respond to dose reduction.<sup>4</sup>
- The TARGET trial (TPMT: Azathioprine Response to Genotyping and Enzyme Testing) was a randomized controlled trial evaluating TPMT genotyping prior to treatment with azathioprine. Results from this trial indicated that individuals with homozygous TPMT variants were at risk for severe neutropenia whereas heterozygotes were not at increased risk when taking standard doses of azathioprine.<sup>14</sup>

## Criteria

TPMT testing by phenotyping or genotyping is indicated in individuals considering treatment with any thiopurine drug:

- azathioprine (AZA, Imuran<sup>®</sup> , Azasan<sup>®</sup> )
- 6-mercaptopurine (6-MP, Mercaptopurinum<sup>®</sup> , Purinethol<sup>®</sup> )
- thioguanine (6-TG, Tabloid<sup>®</sup> , Thioguanine<sup>®</sup> )

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# UGT1A1 Variant Analysis for Irinotecan Response

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v1.0.2020

## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedure addressed by this guideline | Procedure code |
|---------------------------------------|----------------|
| UGT1A1 Targeted Variant Analysis      | 81350          |

## What are UGT1A1 and irinotecan

### Definition

Irinotecan is a chemotherapy drug often prescribed together with other standard agents for treating patients with metastatic and recurrent colorectal cancer. Irinotecan is metabolized in the liver by a gene called UGT1A1.<sup>1</sup>

- Variations in TA repeat number in the TATAAA element of the 5' UGT1A1-promoter affects transcription efficiency and can lead to reduced enzyme activity. The common number of repeats is six [(TA)<sub>6</sub>, \*1 allele], while seven repeats [(TA)<sub>7</sub>, \*28 allele] called UGT1A1\*28, can lead to reduced enzyme activity. This can cause a buildup of drug metabolites, resulting in toxicity.<sup>1,2</sup>
- Several studies have confirmed an increased risk of having reduced white blood cell count, or neutropenia, in people with UGT1A1 genetic variants. Studies have also shown an increased risk of severe diarrhea at doses of irinotecan > 125 mg/m.<sup>2,3,4</sup>
- About 10% of North Americans have two copies of the UGT1A1\*28 variant (homozygous, also referred to as UGT1A1 7/7) and 40% have just one copy (heterozygous).<sup>2</sup>
- Not all people with UGT1A1\*28 variants will experience increased toxicity.<sup>3</sup> Individuals homozygous for the \*28 variant are 3.5 times more likely to develop severe neutropenia than those with the wild genotype.<sup>1</sup>

## Test information

- Targeted variant analysis of the UGT1A1 gene by polymerase chain reaction (PCR) identifies variations in TA repeat number in the TATAAA element of the 5' *UGT1A1* region. <sup>1,2</sup>
  - **Wild-type** = UGT1A1 6/6 (\*1/\*1) genotype; Wild-type genotype; No UGT1A1\*28 variant is identified. Low risk of severe toxicity from standard initial dosages of irinotecan.
  - **Heterozygous** = UGT1A1 6/7 (\*1/\*28) genotype; One wild-type allele and one UGT1A1\*28 allele identified. Increased risk for irinotecan toxicity, but initial standard doses may be still be tolerated.
  - **Homozygous** = UGT1A1 7/7 (\*28/\*28) genotype. Increased risk for severe toxicity from standard initial doses of irinotecan, thus irinotecan product labeling recommends considering a reduced initial dose.<sup>5</sup>

## Guidelines and evidence

- In May 2010, the FDA announced a safety change to the prescribing information for Camptosar<sup>®</sup> (irinotecan) Injection:<sup>2,6</sup>
  - “When administered in combination with other agents, or as a single-agent, a reduction in the starting dose by at least one level of Camptosar<sup>®</sup> should be considered for patients known to be homozygous for the UGT1A1\*28 allele. However, the precise dose reduction in this patient population is not known and subsequent dose modifications should be considered based on individual patient tolerance to treatment.”
  - “A laboratory test is available to determine the UGT1A1 status of patients. Testing can detect UGT1A1 6/6, 6/7, 7/7 genotypes.”
- UGT1A1 \*28 testing for irinotecan is included on the FDA's table for therapeutic products with pharmacogenomic data on the drug label. <sup>7</sup>
- Guidelines for genetic testing have not been established by organizations such as the National Comprehensive Cancer Network (NCCN) and the Evaluation of Genomic Applications in Practice and Prevention (EGAPP) Working Group. However, both organizations recognize the availability and utility of testing UGT1A1 \*28 prior to treatment with irinotecan.<sup>4,8</sup>
- The NCCN states the following:
  - “Also, a warning was added to the label for irinotecan indicating that a reduced starting dose of the drug should be used in patients known to be homozygous for UGT1A1\*28.”

- “A practical approach to the use of UGT1A1\*28 allele testing with respect to patients receiving irinotecan has been presented, although guidelines for the use of this test in clinical practice have not been established.”
- “UGT1A1 testing on patients who experience irinotecan toxicity is not recommended, because they will require a dose reduction regardless of the UGT1A1 test result.”<sup>8</sup>

## Criteria

UGT1A1 variant analysis is indicated in individuals with metastatic and/or recurrent colorectal cancer prior to the initiation of irinotecan therapy.

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# UroVysion FISH for Bladder Cancer

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## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedures addressed by this guideline                          | Procedure codes |
|---|-----------------|
| FISH Analysis for Bladder Cancer (UroVysion), Manual            | 88120           |
| FISH Analysis for Bladder Cancer (UroVysion), Computer-Assisted | 88121           |

## What is UroVysion FISH testing for bladder cancer

### Definition

UroVysion™ was developed to be used with current standard diagnostic tools to aid in initial diagnosis of bladder cancer and monitoring for tumor recurrence in previously diagnosed patients.<sup>4</sup>

- Bladder cancer is one of the most common types of cancer in the U.S., especially among men. Approximately 76,960 new cases of bladder cancer are projected for 2016 (58,950 in men and 18,010 in women).<sup>1</sup> Older individuals (median age, 65 years) are most often affected.
- Bladder cancer is categorized as non-muscle invasive disease (NMID) or muscle invasive disease (MID).<sup>2</sup> The majority (≈80%) of bladder cancers are NMID.<sup>3</sup>
- Urothelial carcinoma (UC) accounts for most cases of bladder cancer.<sup>2,4</sup>
  - Most cases of UC are low-grade and easily treated.<sup>4</sup>
  - However, UC has a high risk of recurrence (70%), and patients must be monitored for several years after treatment.<sup>4</sup>
- Diagnostic monitoring usually consists of regular testing of cells in the urine (cytology).<sup>2,5</sup> UroVysion FISH (fluorescence in situ hybridization) testing is an alternative to cytology.<sup>4,5</sup>

## Test information

- UroVysion testing detects extra or missing chromosomes 3, 7 or 17 and gene changes to a piece of chromosome 9 often found in UC patients.<sup>4</sup>
- Cytology is the standard procedure for diagnosing and monitoring of UC. UroVysion testing can be performed if the cytology returns negative or atypical results.<sup>2,4,5</sup>
- One study showed UroVysion testing to have 85% sensitivity for low-grade UC, and nearly 100% sensitivity for the more rare but serious high-grade UC.<sup>4</sup>

## Guidelines and evidence

- UroVysion testing is FDA approved,<sup>6</sup> but reviews and guidelines call for additional study before it becomes standard procedure.<sup>5</sup>
- The National Cancer Center Network (NCCN, 2016)<sup>2</sup> has published clinical guidelines on bladder cancer, and state that urinary urothelial tumor markers are classified as category 2B, and an option for follow-up assessment in patients with low to high grade disease who have undergone adjuvant intravesical treatment. They report that urothelial tumor markers have better sensitivity, but lower specificity, for detecting bladder cancer compared with urinary cytology. It is not known whether urinary marker tests add useful clinical information for the detection and management of non-muscle invasive bladder tumors.
- The American Urological Association and the Society of Urologic Oncology (AUA, 2016)<sup>7</sup> recently published clinical practice guidelines regarding microscopic hematuria and the management of non-muscle invasive bladder cancer (NMIB). For urinary markers utilized after diagnosis of bladder cancer, they state the following:
  - “In surveillance of NMIC, a clinician should not use urinary biomarkers in place of cystoscopic evaluation (Strong Recommendation; Evidence Strength: Grade B)”
  - “In a patient with a history of low-risk cancer and a normal cystoscopy, a clinician should not routinely use a urinary biomarker or cytology during surveillance. (Expert Opinion)”
  - “In a patient with NMIBC, a clinician may use biomarkers to assess response to intravesical BCG (UroVysion FISH) and adjudicate equivocal cytology (UroVysion FISH and ImmunoCyt). (Expert Opinion)”
- The National Institute for Health and Care Excellence (NICE, 2015)<sup>8</sup> published a guideline regarding the diagnosis and management of bladder cancer. They stated that urinary biomarker tests (such as UroVysion using FISH, ImmunoCyt or a nuclear matrix protein 22 (NMP22) test may be used for the diagnosis of individuals with suspected bladder cancer.
- The American Urological Association (AUA, 2012)<sup>9</sup> stated the following regarding the management of asymptomatic microhematuria:

- “The use of urine cytology and urine markers (NMP22, BTA-stat, and UroVysion FISH) is NOT recommended as a part of routine evaluation of the asymptomatic microhematuria patient. (Recommendation: Evidence Strength C).”
- American Urological Association (2007) guidelines for diagnosis and management of bladder cancer consider techniques like UroVysion to “hold promise” in future assessment of risk, prognosis, and targeted treatment.<sup>5</sup>
- A systematic review of UroVysion was conducted by the Agency for Healthcare Research and Quality (AHRQ).<sup>10</sup> Based on 11 studies that were reviewed, the following were noted by authors:
  - Diagnostic testing:
    - The sensitivity of UroVysion to detect bladder cancer among undiagnosed patients with clinical signs and symptoms was 63% (95% CI, 50% to 75%) and specificity was 87% (95% CI, 79% to 93%).
    - The positive likelihood ratio was 5.02 (95% CI 2.93 to 8.60) (moderate increase in the likelihood of disease). The negative likelihood ratio was 0.42 (95% CI 0.30 to 0.59) (small decrease in likelihood of disease).
  - Surveillance testing:
    - For individuals being monitored for cancer recurrence the sensitivity was 55% (95% CI, 36% to 72%; 7 studies) and specificity was 80% (95% CI, 66% to 89%; 6 studies).
    - For evaluation of symptoms, sensitivity was 73% (95% CI, 50% to 88%), based on two studies.
  - The sensitivity of the test increased with higher tumor stage and grade.
- A prior systematic review and meta-analysis provided a comparison of UroVysion with cystoscopy for diagnosing UC.<sup>11</sup>
  - Pooled sensitivity of UroVysion was 72% (95% CI, 69% to 75%) compared with 42% (95% CI, 38% to 45%) for cytology.
  - Specificity of UroVysion was 83% (95% CI, 82% to 85%) and 96% (95% CI, 95% to 97%) for cytology.
  - Sensitivity increased if earlier stage tumors (Ta) were excluded: UroVysion, 86% (95% CI, 82% to 89%) and cytology, 61% (95% CI, 56% to 66%).
  - The overall diagnostic performance was higher for UroVysion (area under the curve [AUC], 0.867, standard error [SE], 0.021) versus cytology (AUC, 0.626 (SE 0.091). However, these differences in diagnostic accuracy disappeared when Ta cases were excluded, indicating that the tests may be more comparable for accurately detecting later stage disease.

- A prospective, blinded study compared results of ImmunoCyt and UroVysion with urine cytology to detect recurrent UC.<sup>12</sup> A total of 100 patients with a history of bladder cancer submitted a single voided urine sample, immediately followed by cystoscopy and biopsy.
  - The overall results of sensitivity for low- and high-grade UC for the various tests were as follows: Cytology, 15% and 27%; Immunocyt, 62% and 91%; and UroVysion, 8% and 18%.
  - Specificity of the tests were: Cytology, 97%; ImmunoCyt, 63%; UroVysion, 90%.
  - Positive predictive value (PPV) and negative predictive value (NPV) of each test demonstrated: Cytology 71% and 78%; ImmunoCyt 43% and 88%; UroVysion 33% and 72%
  - ImmunoCyt had significantly better sensitivity at detecting low-grade tumors compare with cytology or UroVysion (76% vs 21% and 13%, respectively;  $P < 0.001$  for both). However, specificity for ImmunoCyt was significantly lower than for cytology and UroVysion (63% vs 97% and 90%;  $P < 0.001$  for both).
  - The authors concluded that UroVysion and cytology had comparably high rates of specificity for patients with negative cystoscopy, and as a result, UroVysion may have value to confirm cytology or ImmunoCyt.
- A retrospective study evaluating the ability of UroVysion to predict recurrence and progression in NMIBC with suspicious cytology and negative cystoscopy results was undertaken due to the high rate of recurrence and frequent cystoscopic monitoring required in this disease. Authors evaluated predictors for recurrence, progression and findings on subsequent cystoscopy.<sup>13</sup>
  - A positive UroVysion result was a significant predictor for recurrence (hazard ratio [HR], 2.35; 95% CI, 1.42 to 3.90;  $P = 0.001$ ) and for progression (HR, 3.01; 95% CI, 1.10 to 8.21;  $P = 0.03$ ).
  - A positive UroVysion result, however, was not significantly associated with evidence of tumor on subsequent surveillance cystoscopy compared with negative UroVysion results (odds ratio [OR], 0.80; 95% VI, 0.26 to 2.74; NS).
  - The authors concluded that an “anticipatory positive” UroVysion result can predict recurrence and progression in patients with NMIBC when cystoscopy results are negative and cytology results are suspicious.
  - The lack of association between UroVysion results and tumor recurrence on cystoscopy during the immediate follow-up period, however, suggests that UroVysion has limited value to modify surveillance regimens in these patients.

## Criteria

- Previous Testing:

- No repeat UroVysion® testing on the same sample when a result was successfully obtained, AND
- Diagnosis
  - UroVysion is not indicated for the routine evaluation of hematuria or microhematuria and will not be reimbursed when billed with an ICD10 code in the R31 Hematuria range. Exceptions may be made for uncertain or equivocal results on standard diagnostic assessments, such as cytology, OR
- Surveillance
  - UroVysion is indicated when the individual has a personal history of bladder cancer defined by ICD10 code of Z85.51 (Personal history of malignant neoplasm of bladder) or C67.0-C67.9 (Malignant neoplasm of the bladder, range), AND
  - The member is being monitored for cancer recurrence, AND
    - Member had been diagnosed with low grade bladder cancer and the results of cytology are equivocal, or
    - Member had been diagnosed with high grade bladder cancer and the results of cytology are negative or equivocal, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy

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# VeriStrat Testing for NSCLC TKI Response

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v1.0.2020

## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedure addressed by this guideline | Procedure code |
|---------------------------------------|----------------|
| VeriStrat                             | 81538          |

## What is VeriStrat testing for non-small cell lung cancer

### Definition

The aim of the VeriStrat® test is to assess prognosis for outcomes in advanced NSCLC and predict survival benefit between EGFR TKIs and single agent chemotherapy.<sup>1</sup>

- NSCLC is any type of cancer of the epithelial cells of the lungs that is not classified as small-cell lung cancer.<sup>2</sup>
- Although associated with cigarette use and smoke exposure, NSCLC can be diagnosed in individuals who have never smoked.<sup>2</sup>
- About 80-85% of NSCLC tumors express EGFR.<sup>3</sup> EGFR is a cell surface receptor that causes activation of the intracellular tyrosine kinase domain. Overexpression of EGFR results in increased proliferation and survival of cells, leading to the growth of tumors.<sup>3,4</sup>
- Treatment selection in NSCLC may be guided by molecular genetic testing:
  - Approximately 15-25% of patients with NSCLC have activating mutations in the EGFR gene. These patients display improved progression-free survival following treatment with EGFR TKI therapy, such as erlotinib.<sup>5,6</sup>
  - Another 5-7% of patients with NSCLC have the ALK or ROS-1 rearrangements and are treated with crizotinib (Xalkori).<sup>7</sup>
- For the remaining 75-85% of patients, who are negative for both EGFR mutations and ALK or ROS-1 rearrangements, other therapies are used as first-line treatment. However, some of these patients who fail platinum-based chemotherapy or targeted therapies may still benefit from EGFR TKI therapy with erlotinib, which is generally

well-tolerated.<sup>8</sup> This applies in particular to patients whose tumors express an increased number of copies of EGFR (even without EGFR mutations).<sup>9</sup>

## Test information

- VeriStrat is a proprietary, serum-based proteomic test designed to be an adjunct to a conventional clinical workup, combined with the patient's clinical history, other diagnostic tests, and clinicopathologic factors.<sup>1</sup>
- The VeriStrat test result is reported as good, poor, or indeterminate.<sup>1</sup> The results are also intended to set patient expectations, facilitate a discussion about prognosis, improve knowledge to potentially reduce anxiety, and improve quality of life.<sup>1</sup>
  - **VSGood results:** A good result indicates that a patient is more likely to benefit from standard of care (SOC) treatment and have better overall survival (OS).<sup>1</sup>
  - **VSPoor results:** A poor result indicates that a patient will likely have decreased OS and may benefit from alternative treatment strategies such as novel combination of therapies, NGS testing for rare mutations, non-platinum based regimens, and/or palliative care.<sup>1</sup>
  - **Indeterminate results:** In rare instances (< 2%), a test result of indeterminate is reported, indicating that a VSGood or VSPoor classification could not be confirmed.
- VeriStrat is not a replacement for an EGFR mutation test.

## Guidelines and evidence

Previous National Comprehensive Cancer Network (NCCN) guidelines for the treatment of NSCLC supported the use of proteomic tests to evaluate potential therapies in advanced NSCLC. However, likely due to technical advances, availability of next generation sequencing testing for solid tumors, and treatment options, available current NCCN guidelines no longer incorporate these proteomic tests into their NSCLC evaluation algorithms.<sup>10</sup>

- Previous eviCore criteria (VeriStrat Testing for NSCLC TKI Response) were largely based on the 2015 NCCN Guidelines. These recommended proteomic testing for patients with advanced NSCLC who were either EGFR wild type or had an unknown mutation status. For these patients, the NCCN stated that those with a "Poor" result should not be offered second-line erlotinib therapy.
- In contrast, the current 2019 NCCN guidelines for NSCLC no longer include specific recommendations for proteomic testing; there is no mention of proteomic testing or the use of VeriStrat for NSCLC.

Literature Review<sup>11-24</sup>



- The available peer-reviewed clinical validity studies assess the predictive performance of VeriStrat-directed erlotinib therapy compared with chemotherapy in patients who were either EGFR wild type or had an unknown EGFR mutation status and had progressed after first-line treatment. However, 2019 NCCN treatment pathway for patients with NSCLC do not include erlotinib as recommended therapy for patients whose disease has progressed after first-line treatment and are either EGFR wild type or have an unknown mutational status. Since erlotinib is not a recommended therapy, there is no longer a need for a test that can identify it as a treatment option.
- The overall evidence base for predictive use is also characterized by several study design limitations. For example, VeriStrat was not used to determine treatment in the available studies and the majority of the study authors reported that treatment selection was based on standard of care. In addition, a “VSGood” result claims to identify NSCLC patients who are EGFR wild-type but still likely to benefit from EGFR-TKI therapy. Yet the clinical validity studies did not consistently test for EGFR variants and, consequently, the true relationship between VeriStrat results, EGFR status, and survival cannot be definitively understood.
- For VeriStrat to demonstrate clinical validity in patients with NSCLC in light of the NCCN guideline changes and some of the original design limitations, additional studies supporting its performance are required.
- Direct clinical utility studies were not identified in the scientific literature. Examples of these would include prospective studies comparing survival outcomes in patients who had EGFR-TKI treatment selected either by VeriStrat classification or through other standard EGFR variant analysis methods (such as next-generation sequencing).
- Regarding the prognostic ability of VeriStrat, the majority of the available evidence predicting disease outcomes included retrospective clinical validity studies which evaluated the test in patients with advanced NSCLC who were treatment-naïve or had either failed first-line treatment or had a recurrence. To infer how well VeriStrat performed as a prognostic test, these studies examined the degree of association between VSGood or VSPoor scores and survival outcomes. Overall, this evidence base demonstrating the performance of VeriStrat as a prognostic test is of low quality.
- A number of individual study limitations were observed that weakened the strength of the evidence base. This includes the VeriStrat score not being used to determine treatment and the variability in testing for EGFR variants. Also, the adjustments for EGFR status in survival analyses were inconsistently reported and the relationship between VeriStrat scores and overall survival (OS) as well as progression-free survival (PFS) in study populations with unknown EGFR status was not clear.

## Criteria

Given that VeriStrat Testing is not currently supported in clinical practice guidelines for the treatment of advanced NSCLC and the published evidence does not independently

meet the criteria for coverage for this indication, the use of VeriStrat is not considered medically necessary.

- VeriStrat is not considered medically necessary and therefore, not eligible for reimbursement.

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# Von Hippel-Lindau Disease Testing

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## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedures addressed by this guideline | Procedure codes |
|--|-----------------|
| VHL Known Familial Mutation Analysis   | 81403           |
| VHL Sequencing                         | S3842           |
|  | 81404           |
| VHL Deletion/Duplication Analysis      | 81403           |

## What is Von Hippel-Lindau (VHL) syndrome

### Definition

Von Hippel-Lindau (VHL) syndrome is a hereditary cancer syndrome.

- Von Hippel-Lindau (VHL) syndrome is a hereditary cancer syndrome whose main clinical features include hemangioblastomas of the central nervous system (CNS) and retina, renal cysts and renal cell carcinoma, pheochromocytoma, and endolymphatic sac tumors.<sup>1</sup>
  - The cardinal feature of VHL syndrome is hemangioblastoma. CNS hemangioblastomas present in 60%-80% of individuals, and retinal hemangioblastomas present in about 70-80% of individuals.<sup>1,2</sup>
  - The risk to develop clear cell renal carcinoma by age 60 is as high as 70%, and is the leading cause of death for individuals with VHL syndrome.<sup>1,2</sup>
  - Pheochromocytomas and endolymphatic sac tumors are less commonly seen in VHL syndrome than other manifestations.
  - Epididymal tumors have also been reported in VHL. Males with bilateral epididymal tumors may have infertility.<sup>1</sup>
  - Clinical findings of VHL may include vision loss, hearing loss, gait disturbance, pain and sensory motor loss depending on the location of the tumor.<sup>1</sup>
- The incidence of VHL is 1 in 36,000 people.<sup>1</sup>

- VHL syndrome is caused by mutations in the VHL gene. More than 1500 germline and sporadic VHL gene mutations have been identified. The VHL gene is a tumor suppressor whose normal role is to control cell growth and proliferation.<sup>1</sup> VHL mutations lead to a loss of function of the gene and an increased risk for uncontrolled growth of tumors and cysts.<sup>1</sup>
- Most (80%) of VHL mutations are inherited (germline), and about 20% are new (de novo) mutations.<sup>1</sup> VHL syndrome is an autosomal dominant condition with children of affected individuals having a 50% chance of inheriting the disease-causing mutation.<sup>1</sup>
- Almost 100% of individuals with a VHL gene mutation show symptoms of the disease by age 65.<sup>1</sup> Age of onset, disease severity, and tumor types vary between and within affected families.
- Surveillance recommendations for individuals diagnosed with or at-risk for inheriting VHL syndrome include annual ophthalmologic exams, MRI of the brain and total spine every two years starting at age 16 years, annual abdominal ultrasound, MRI of the abdomen every two years starting at 16 years, annual blood pressure monitoring, annual blood or urinary fractionated metanephrines starting at 5 years, and audiologic evaluation. Some of the screenings should begin at one year of age in at-risk/affected individuals.<sup>3,4</sup> Early detection of VHL tumors may lead to improved outcome.<sup>1</sup> However, at-risk individuals can forego screening if genetic testing for a known familial mutation is performed and they have a normal (negative) result.<sup>1,2</sup>

## Test information

- **VHL full gene sequence analysis** checks all three exons and will detect about 89% of mutations.<sup>1</sup> Some laboratories perform only sequencing, while others do sequencing with reflex to deletion/duplication analysis or perform sequencing and deletion/duplication analysis concurrently.
- **VHL deletion/duplication analysis** detects partial or complete gene deletions which account for about 11% of VHL mutations.<sup>1</sup>
- **VHL known familial mutation analysis:** Once a VHL mutation is identified in an affected person, predictive testing is available for at-risk family members, as is prenatal or preimplantation genetic diagnosis.<sup>1</sup> Family members should be tested using the method that can accurately identify the familial mutation. This testing is typically less expensive than a full gene evaluation and provides clear results about whether the family member is predisposed to developing VHL syndrome.<sup>1</sup>

## Guidelines and evidence

- A 2018 expert-authored review states the following with regard to diagnosing VHL:<sup>1</sup>
  - "The diagnosis of von Hippel-Lindau (VHL) syndrome is established in a proband with... clinical features... and/or by identification of a heterozygous

- germline pathogenic variant in VHL on molecular genetic testing. Identification of a heterozygous germline pathogenic variant in VHL by molecular genetic testing establishes the diagnosis and supports periodic follow up even if clinical and radiographic features are nonconclusive."
- "The clinical sensitivity of molecular genetic testing of VHL makes it possible to effectively rule out von Hippel-Lindau (VHL) syndrome with a high degree of certainty in individuals with (1) isolated hemangioblastoma, retinal angioma, or clear cell renal cell carcinoma and (2) no detectable germline VHL pathogenic variant. Somatic mosaicism or a VHL pathogenic variant could still be considered in such individuals."
  - Diagnostic testing can be accomplished through single gene testing when the phenotype, laboratory analysis and imaging suggest the diagnosis of VHL.
  - At-Risk Relatives: "If the VHL pathogenic variant in the family is known, molecular genetic testing can be used for early identification of at-risk family members to improve diagnostic certainty and reduce the need for screening procedures in those at-risk family members who have not inherited the pathogenic variant."
- Consensus-based clinical diagnostic guidelines state that the diagnosis of VHL can be made in the following circumstances:<sup>5</sup>
    - "Patients with a family history, and a CNS haemangioblastoma (including retinal haemangioblastomas), pheochromocytoma, or clear cell renal carcinoma are diagnosed with the disease."
    - "Those with no relevant family history must have two or more CNS haemangioblastomas, or one CNS haemangioblastoma and a visceral tumour (with the exception of epididymal and renal cysts, which are frequent in the general population) to meet the diagnostic criteria."
  - The American Society of Clinical Oncologists (ASCO) position statement on genetic testing (originally published 1996<sup>6</sup>; revised/affirmed in 2003<sup>7</sup>, 2010<sup>8</sup>, and 2015<sup>9</sup>) considers VHL syndrome a Group 1 disorder: "Tests for families with well defined hereditary syndromes for either a positive or negative result will change medical or prenatal management, and for whom genetic testing may be utilized as part of the routine medical care."
    - The 2003 update specifically addresses issues around genetic testing in affected and at-risk children:
      - "ASCO recommends that the decision to offer testing to potentially affected children should take into account the availability of evidence-based risk-reduction strategies and the probability of developing a malignancy during childhood. Where risk-reduction strategies are available or cancer predominantly develops in childhood, ASCO believes that the scope of parental authority encompasses the right to decide for or against testing."

- A peer reviewed 2016 article recommends: “Although the average age of onset of VHL tumors is in the third decade of life, some patients develop tumors at age younger than 10 years and as early as infancy; therefore, presymptomatic genetic testing for VHL is justified, and also may identify those children who did not inherit the familial VHL mutation, thus sparing them from a lifetime of clinical screening. It is strongly recommended that genetic counseling for presymptomatic genetic testing be conducted by a genetics professional in a comfortable environment and with the option of having multiple genetic counseling sessions as necessary.”<sup>10</sup>

## Criteria

### VHL Known Familial Mutation Analysis

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Testing:
  - No previous VHL gene testing that would have detected the family mutation, AND
- Diagnostic and Predisposition Testing:\*\*
  - Known family mutation in VHL identified in 1st degree relative(s). (Note: 2nd or 3rd degree relatives may be considered when 1st degree relatives are unavailable or unwilling to be tested), AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy

\*\* Includes prenatal testing for at-risk pregnancies.

### VHL Sequencing

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Testing:
  - No previous VHL gene sequencing, and
  - No known familial mutation, AND
- Diagnostic Testing for Symptomatic Individuals:
  - A positive family history of VHL, and



- Spinal or cerebellar hemangioblastoma, or
- Retinal hemangioblastoma, or
- Renal cell carcinoma, or
- Pheochromocytoma, or
- Multiple renal and/or pancreatic cysts, OR
- No known family history of VHL-related findings, and
  - Two or more hemangioblastomas involving the retina, spine, and/or brain, or
  - A single hemangioblastoma and a characteristic visceral mass (such as renal cell carcinoma, pheochromocytoma, endolymphatic sac tumors, papillary cystadenomas of the epididymis or broad ligament, or neuroendocrine tumors of the pancreas), OR
- Predisposition Testing for Presymptomatic/Asymptomatic Individuals:
  - A first-degree relative of someone with a clinical diagnosis of VHL who has had no previous genetic testing (Note that testing in the setting of a more distant affected relative will only be considered if the first-degree relative is unavailable or unwilling to be tested ); AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy

### **VHL Deletion/Duplication Analysis**

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - There is no known familial mutation, and
  - No previous deletion/duplication analysis of the VHL gene has been performed, and
  - Above criteria for VHL full gene sequence analysis are met, and
  - VHL sequencing was previously performed and no mutations were found, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

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# Whole Exome Sequencing

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## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedures addressed by this guideline   | Procedure codes |
|--|-----------------|
| Whole Exome Sequencing (e.g., unexplained constitutional or heritable disorder or syndrome)    | 81415           |
| Whole Exome Sequencing, Comparator (e.g., parent(s), sibling(s))                               | 81416           |
| Whole Exome Sequencing Re-evaluation (e.g., updated knowledge or unrelated condition/syndrome) | 81417           |

## What is whole exome sequencing

### Definition

Whole exome sequencing (WES) utilizes DNA-enrichment methods and massively parallel nucleotide sequencing to identify disease-associated variants throughout the human genome.

- WES has been proposed for diagnostic use in individuals who present with complex genetic phenotypes suspected of having a rare genetic condition, who cannot be diagnosed by standard clinical workup, or when features suggest a broad differential diagnosis that would require evaluation by multiple genetic tests.
- The standard approach to the diagnostic evaluation of an individual suspected of having a rare genetic condition may include combinations of radiographic, biochemical, electrophysiologic, and targeted genetic testing such as a chromosomal microarray, single-gene analysis, and/or a targeted gene panel.<sup>1</sup>
- WES is typically not an appropriate first-tier test, but can be appropriate if initial testing is unrevealing, or if there is no single-gene or panel test available for the particular condition.<sup>2</sup>
- Identifying a molecularly confirmed diagnosis in a timely manner for an individual with a rare genetic condition can have a variety of health outcomes,<sup>2-9</sup> including:

- guiding prognosis and improving clinical decision-making, which can improve clinical outcome by
  - application of specific treatments as well as withholding of contraindicated treatments for certain rare genetic conditions
  - surveillance for later-onset comorbidities
  - initiation of palliative care
  - withdrawal of care
- reducing the financial & psychological impact of diagnostic uncertainty and the diagnostic odyssey (e.g., eliminating lower-yield testing and additional screening testing that may later be proven unnecessary once a diagnosis is achieved)
- informing genetic counseling related to recurrence risk and prenatal or preconceptual (utilizing in-vitro fertilization with preimplantation genetic diagnosis) diagnosis options
- allowing for more rapid molecular diagnosis than a sequential genetic testing approach

### Test information

- WES is limited to the DNA sequence of coding regions (exons) and flanking intronic regions of the genome, which is estimated to contain 85% of heritable disease-causing variants.
- Pathogenic variants that can be identified by WES include missense, nonsense, splice-site, and small deletions or insertions.
- At the present time, WES typically fails to detect certain classes of disease-causing variants, such as structural variants (e.g., translocations, inversions), abnormal chromosome imprinting or methylation, copy-number variants, some mid-size insertions and deletions (ca. 10-500 bp), trinucleotide repeat expansion mutations, deeper intronic mutations, and low-level mosaicism.
- WES has the advantage of decreased turnaround time and increased efficiency relative to Sanger sequencing of multiple genes.
- WES is associated with technical and analytical variability, including uneven sequencing coverage, gaps in exon capture before sequencing, as well as variability in variant classification based on proprietary filtering algorithms and potential lack of critical clinical history or family samples.<sup>10</sup>

## Guidelines and evidence

### American College of Medical Genetics and Genomics

- The American College of Medical Genetics (ACMG, 2012) states the following regarding the clinical application of whole exome and whole genome testing:<sup>11</sup>
  - “WGS/WES should be considered in the clinical diagnostic assessment of a phenotypically affected individual when:”
    - “The phenotype or family history data strongly implicate a genetic etiology, but the phenotype does not correspond with a specific disorder for which a genetic test targeting a specific gene is available on a clinical basis.”
    - “A patient presents with a defined genetic disorder that demonstrates a high degree of genetic heterogeneity, making WES or WGS analysis of multiple genes simultaneously a more practical approach.”
    - “A patient presents with a likely genetic disorder, but specific genetic tests available for that phenotype have failed to arrive at a diagnosis.”
    - “A fetus with a likely genetic disorder in which specific genetic tests, including targeted sequencing tests, available for that phenotype have failed to arrive at a diagnosis.”
    - “Prenatal diagnosis by genomic (i.e., next-generation whole-exome or whole-genome) sequencing has significant limitations. The current technology does not support short turnaround times, which are often expected in the prenatal setting. There are high rates of false positives, false negatives, and variants of unknown clinical significance. These can be expected to be significantly higher than seen when array CGH is used in prenatal diagnosis.”
  - The following are recommended pretest considerations:
    - “Pretest counseling should be done by a medical geneticist or an affiliated genetic counselor and should include a formal consent process.”
    - “Before initiating WGS/WES, participants should be counseled regarding the expected outcomes of testing, the likelihood and type of incidental results that could be generated, and what results will or will not be disclosed.”
    - “As part of the pretest counseling, a clear distinction should be made between clinical and research-based testing. In many cases, findings will include variants of unknown significance that might be the subject for research; in such instances a protocol approved by an institutional review board must be in place and appropriate prior informed consent obtained from the participant.”
- The American College of Medical Genetics (ACMG, 2012) states the following regarding informed consent for whole exome and whole genome testing:<sup>12</sup>

- “Before initiating GS/ES, counseling should be performed by a medical geneticist or an affiliated genetic counselor and should include written documentation of consent from the patient.”
- “Incidental/secondary findings revealed in either children or adults may have high clinical significance for which interventions exist to prevent or ameliorate disease severity. Patients should be informed of this possibility as a part of the informed consent process.”
- “Pretest counseling should include a discussion of the expected outcomes of testing, the likelihood and type of incidental results that may be generated, and the types of results that will or will not be returned. Patients should know if and what type of incidental findings may be returned to their referring physician by the laboratory performing the test.”
- “GS/ES is not recommended before the legal age of majority except for:”
  - “Phenotype-driven clinical diagnostic uses;”
  - “Circumstances in which early monitoring or interventions are available and effective; or”
  - “Institutional review board–approved research.”
- “As part of the pretest counseling, a clear distinction should be made between clinical and research-based testing.”
- “Patients should be informed as to whether individually identifiable results may be provided to databases, and they should be permitted to opt out of such disclosure.”
- “Patients should be informed of policies regarding re-contact of referring physicians as new knowledge is gained about the significance of particular results.”
- The American College of Medical Genetics (ACMG, Updated 2016) published guidelines for the reporting of incidental findings in clinical exome and genome sequencing.<sup>13,14</sup> They state the following:
  - “We continue to support the reporting of known or expected pathogenic variants, but we do not recommend reporting variants of uncertain significant as secondary findings (SFs).”
  - This 2016 ACMG guideline includes a table of “ACMG SF v2.0 genes and associated phenotypes recommended for return of secondary findings in clinical sequencing.”
- Evidence for the clinical utility of WES in individuals with multiple congenital anomalies and/or a neurodevelopmental phenotype includes numerous large case series. Relevant outcomes include improved clinical decision-making (e.g., application of specific treatments, withholding of contraindicated treatments, changes to surveillance), changes in reproductive decision making, and resource

utilization. WES serves as a powerful diagnostic tool for individuals with rare genetic conditions in which the specific genetic etiology is unclear or unidentified by standard clinical workup.<sup>7,15,16</sup>

- The average diagnostic yield of WES is 20-40% depending on the individual's age, phenotype, previous workup, and number of comparator samples analyzed.<sup>5,15,17</sup> Among individuals with a pathogenic or likely pathogenic findings by WES, 5-7% received a dual molecular diagnosis (i.e., two significant findings associated with non-overlapping clinical presentations).<sup>15,17</sup>
- The use of family trio WES reduces the rate of uncertain findings, adds to the clinical sensitivity with regard to the interpretation of clinically novel genes, and increases the diagnostic utility of WES. For example, in three publications the positive rate ranges from 31-37% in patients undergoing trio analysis compared to 20-23% positive rate among proband-only WES.<sup>15,18,19</sup>
- Re-evaluation of previously obtained exome sequence has the potential for additional diagnostic yield because of constant expansions of existing variant databases, as well as periodic novel gene discovery.<sup>20</sup>

### **American College Obstetricians and Gynecologists and Society for Maternal Fetal Medicine**

In a joint statement, the American College of Obstetricians and Gynecologists and the Society for maternal Fetal Medicine (2016) state the following regarding prenatal WES.<sup>21</sup>

- “The routine use of whole-genome or whole-exome sequencing for prenatal diagnosis is not recommended outside of the context of clinical trials until sufficient peer-reviewed data and validation studies are published.”

### **International Society for Prenatal Diagnosis, Society for Maternal Fetal Medicine, and Perinatal Quality Foundation**

A joint statement from the International Society for Prenatal Diagnosis, the Society for Maternal Fetal Medicine, and the Perinatal Quality Foundation on prenatal WES states:<sup>22</sup>

- “The routine use of prenatal [genome wide] sequencing as a diagnostic test cannot currently be supported due to insufficient validation data and knowledge about its benefits and pitfalls. Prospective studies with adequate population numbers for validation are needed.... Currently, it is ideally done in the setting of a research protocol. Alternatively, sequencing may be performed outside a research setting on a case-by-case basis when a genetic disorder is suspected for which a confirmatory genetic diagnosis can be obtained more quickly and accurately by sequencing. Such cases should be managed after consultation with and under the expert guidance of genetic professionals working in multidisciplinary teams with expertise in the clinical diagnostic application of sequencing, including interpretation of

genomic sequencing results and how they translate to the prenatal setting, as well as expertise in prenatal imaging and counseling.”

- “There is currently limited genotype-phenotype correlation for the genetic disorders identified in the fetal period because ultrasound imaging is frequently limited, and the fetal phenotypes of many conditions have not been well described.”

### Peer Reviewed Literature

- The clinical utility of prenatal exome is currently lacking. According to one review, although analyses of the clinical utility of prenatal WES are beginning to be published, it is too soon to “determine the extent to which prenatal genomic sequencing results actually alter perinatal care and result in benefits or harm to families.”<sup>23</sup>
- Potential promises of fetal WES include early diagnosis for informed decision-making, potential in utero or early perinatal treatment or therapy, and improved knowledge of prenatal presentations and development.<sup>24</sup>
- Potential pitfalls include the need for extensive pre- and post-test counseling, long turn-around times and the need for a well-defined phenotype to provide the most informative and rapid results, difficulty in interpreting variants of uncertain clinical significance in the context of a phenotype defined by prenatal ultrasound findings, and the ethical issues inherent in discovering secondary and incidental findings in the prenatal period.<sup>24</sup>
- Technical issues prenatal WES include gaps in sequence coverage, the extended time required when secondary methods are used to fill these gaps, and the inability to detect copy number variations, trinucleotide repeat mutations, or low level mosaicism.<sup>24</sup>
- It is essential that additional data on the clinical utility and risks of prenatal WES be collected.<sup>23</sup>

### Criteria

- Whole exome sequencing (WES) is considered medically necessary when ALL of the following criteria are met:
  - The patient and family history have been evaluated by a Board-Certified or Board-Eligible Medical Geneticist, AND
    - A clinical letter detailing the evaluation by a Geneticist is provided which includes ALL of the following information:
      - Differential diagnoses, and
      - Testing algorithm, and
      - Previous tests performed and results, and



- A genetic etiology is the most likely explanation, and
  - Recommendation that whole exome sequencing is the most appropriate test, and
  - Predicted impact on member's plan of care, AND
- Patient is <21 years of age, AND
  - A genetic etiology is considered the most likely explanation for the phenotype, based on EITHER of the following:
    - Multiple congenital abnormalities defined by ONE of the following:
      - Two or more major anomalies affecting different organ systems\*, or
      - One major and two or more minor anomalies affecting different organ systems\*, OR
    - TWO of the following criteria are met:
      - major abnormality affecting at minimum a single organ system\*, and/or
      - formal diagnosis of autism, significant developmental delay, or intellectual disability (e.g., characterized by significant limitations in both intellectual functioning and in adaptive behavior), and/or
      - symptoms of a complex neurodevelopmental disorder (e.g., self-injurious behavior, reverse sleep-wake cycles, dystonia, ataxia, alternating hemiplegia, neuromuscular disorder), and/or
      - severe neuropsychiatric condition (e.g., schizophrenia, bipolar disorder, Tourette syndrome), and/or
      - period of unexplained developmental regression, and/or
      - biochemical findings suggestive of an inborn error of metabolism, AND
  - Alternate etiologies have been considered and ruled out when possible (e.g., environmental exposure, injury, infection), AND
  - Clinical presentation does not fit a well-described syndrome for which first tier testing (e.g., single gene testing, comparative genomic hybridization [CGH]/chromosomal microarray analysis [CMA]) is available, AND
  - Multiple targeted panels are appropriate based on the member's clinical presentation, AND
  - There is a predicted impact on health outcomes including:
    - Application of specific treatments, or
    - Withholding of contraindicated treatments, or

- Surveillance for later-onset comorbidities, or
  - Initiation of palliative care, or
  - Withdrawal of care, AND
- A diagnosis cannot be made by standard clinical work-up, excluding invasive procedures such as muscle biopsy

\* Major structural abnormalities are generally serious enough as to require medical treatment on their own (such as surgery) and are not minor developmental variations that may or may not suggest an underlying disorder.

### **Prenatal diagnosis by whole exome sequencing**

This test is considered investigational and/or experimental.

- Investigational and experimental (I&E) molecular and genomic (MolGen) tests refer to assays involving chromosomes, DNA, RNA, or gene products that have insufficient data to determine the net health impact, which typically means there is insufficient data to support that a test accurately assesses the outcome of interest (analytical and clinical validity), significantly improves health outcomes (clinical utility), and/or performs better than an existing standard of care medical management option. Such tests are also not generally accepted as standard of care in the evaluation or management of a particular condition.
- In the case of MolGen testing, FDA clearance is not a reliable standard given the number of laboratory developed tests that currently fall outside of FDA oversight and FDA clearance often does not assess clinical utility.

### **Exclusions and other considerations**

- Whole exome deletion/duplication analysis (typically billed with 81228 or 81229) is considered experimental/investigational and therefore, not reimbursable.
- WES is considered experimental/investigational for screening for genetic disorders in asymptomatic or pre-symptomatic individuals.

### **Billing and reimbursement**

- WES will be considered for reimbursement when it is deemed more efficient and economical than the separate single-gene tests or panels that would be recommended based on the differential diagnosis (e.g., genetic conditions that demonstrate a high degree of genetic heterogeneity).
- WES will be considered for reimbursement only when billed with an appropriate CPT code:
  - 81415 should be billed for the proband. 81415 should only be billed when analyzing the entire whole exome sequence, rather than a targeted set of

genes. At a minimum, genes associated with the clinical presentation and those constitutional mutations in genes listed on the ACMG minimum list entitled “Conditions, genes, and variants recommended for return of incidental findings in clinical sequencing”<sup>13</sup>, when requested, should be reported by the laboratory to the ordering clinician, regardless of the indication for which the exome sequence was ordered.

- 81416 should be billed when a comparator exome is performed. A trio of the proband and both parents is generally preferred, although other family members may be more informative based on the clinical presentation. A maximum of two units of 81416 will be considered for reimbursement.
- 81415 is not reimbursable for a targeted exome analysis (e.g. XomeDxSlice custom gene panel completed on a single exome platform). The appropriate GSP panel code, unlisted code (e.g. 81479), or Tier 1 or Tier 2 code(s) must be billed.
- 81415 will be reimbursable once per lifetime.
- When a single exome platform is used for more than one test (e.g., XomeDxSlice reflex to full exome analysis), all tests reported from the same exome analysis may be:
  - Billed together under one unit of 81415, or
  - Billed separately, but 81415 cannot be used. When billed separately, studies may be billed using Tier 1 codes, Tier 2 codes, or 81479 at an amount that does not exceed the cost of full exome analysis.
- 81417 is not an appropriate code for reflex from targeted to full exome.
- Re-evaluation of a previously obtained exome due to updated knowledge or for the purpose of evaluating a patient for an unrelated condition/syndrome on a different date of service will be considered for reimbursement only when billed using 81417.

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# Whole Genome Sequencing

MOL.TS.306.A

v1.0.2020

## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedures addressed by this guideline   | Procedure codes |
|--|-----------------|
| Genome (eg, unexplained constitutional or heritable disorder or syndrome); sequence analysis   | 81425           |
| Genome (eg, unexplained constitutional or heritable disorder or syndrome); sequence analysis, each comparator genome (eg, parents, siblings) (List separately in addition to code for primary procedure) | 81426           |
| Genome (eg, unexplained constitutional or heritable disorder or syndrome); re-evaluation of previously obtained genome sequence (eg, updated knowledge or unrelated condition/syndrome)                  | 81427           |
| RCIGM Rapid Whole Genome Sequencing  | 0094U           |

## What is whole genome sequencing

### Definition

Whole genome sequencing (WGS or GS) utilizes DNA-enrichment methods and massively parallel nucleotide sequencing to identify disease-associated variants throughout the human genome.

- WGS has been proposed for diagnostic use in individuals who present with complex genetic phenotypes suspected of having a rare genetic condition, who cannot be diagnosed by standard clinical workup, or when features suggest a broad differential diagnosis that would require evaluation by multiple genetic tests.
- The standard approach to the diagnostic evaluation of an individual suspected of having a rare genetic condition may include combinations of radiographic, biochemical, electrophysiologic, and targeted genetic testing such as a chromosomal microarray, single-gene analysis, and/or a targeted gene panel.<sup>1</sup>

- WGS is typically not an appropriate first-tier test, but can be appropriate if initial testing is unrevealing, or if there is no single-gene or panel test available for the particular condition.<sup>2</sup>
- Identifying a molecularly confirmed diagnosis in a timely manner for an individual with a rare genetic condition can have a variety of health outcomes,<sup>2-9</sup> including:
  - guiding prognosis and improving clinical decision-making, which can improve clinical outcome by
    - application of specific treatments as well as withholding of contraindicated treatments for certain rare genetic conditions
    - surveillance for later-onset comorbidities
    - initiation of palliative care
    - withdrawal of care
  - reducing the financial and psychological impact of diagnostic uncertainty and the diagnostic odyssey (e.g., eliminating lower-yield testing and additional screening testing that may later be proven unnecessary once a diagnosis is achieved)
  - informing genetic counseling related to recurrence risk and prenatal or preconceptual (utilizing in-vitro fertilization with preimplantation genetic diagnosis) diagnosis options
  - allowing for more rapid molecular diagnosis than a sequential genetic testing approach

## Test information

- Both coding (exons) and noncoding (introns) regions are analyzed by WGS.<sup>10</sup> Often, coding regions are first analyzed by WGS. If no pathogenic mutations are found, the noncoding regions are then analyzed.<sup>10</sup>
- Pathogenic variants that can be identified by WGS include missense, nonsense, splice-site, and small deletions or insertions. “Data can also be examined for copy-number variants (CNVs) or structural variants that may either be outside of the coding regions or more easily detected using GS due to increased quantitative accuracy.”<sup>10</sup>
- WGS currently is “the most costly technology with the least average depth of coverage, although these limitations are likely to diminish in the future.”<sup>10</sup>

## Guidelines and evidence

- The American College of Medical Genetics (ACMG, 2012) states the following regarding the clinical application of whole exome and whole genome testing:<sup>11</sup>

- “WGS/WES should be considered in the clinical diagnostic assessment of a phenotypically affected individual when:”
  - “The phenotype or family history data strongly implicate a genetic etiology, but the phenotype does not correspond with a specific disorder for which a genetic test targeting a specific gene is available on a clinical basis.”
  - “A patient presents with a defined genetic disorder that demonstrates a high degree of genetic heterogeneity, making WES or WGS analysis of multiple genes simultaneously a more practical approach.”
  - “A patient presents with a likely genetic disorder, but specific genetic tests available for that phenotype have failed to arrive at a diagnosis.”
  - “A fetus with a likely genetic disorder in which specific genetic tests, including targeted sequencing tests, available for that phenotype have failed to arrive at a diagnosis.”
  - “Prenatal diagnosis by genomic (i.e., next-generation whole-exome or whole-genome) sequencing has significant limitations. The current technology does not support short turnaround times, which are often expected in the prenatal setting. There are high rates of false positives, false negatives, and variants of unknown clinical significance. These can be expected to be significantly higher than seen when array CGH is used in prenatal diagnosis.”
- The following are recommended pretest considerations:
  - “Pretest counseling should be done by a medical geneticist or an affiliated genetic counselor and should include a formal consent process.”
  - “Before initiating WGS/WES, participants should be counseled regarding the expected outcomes of testing, the likelihood and type of incidental results that could be generated, and what results will or will not be disclosed.”
  - “As part of the pretest counseling, a clear distinction should be made between clinical and research-based testing. In many cases, findings will include variants of unknown significance that might be the subject for research; in such instances a protocol approved by an institutional review board must be in place and appropriate prior informed consent obtained from the participant.”
- The American College of Medical Genetics (ACMG, 2012) states the following regarding informed consent for whole exome and whole genome testing:<sup>12</sup>
  - “Before initiating GS/ES, counseling should be performed by a medical geneticist or an affiliated genetic counselor and should include written documentation of consent from the patient.”
  - “Incidental/secondary findings revealed in either children or adults may have high clinical significance for which interventions exist to prevent or ameliorate



- disease severity. Patients should be informed of this possibility as a part of the informed consent process.”
- “Pretest counseling should include a discussion of the expected outcomes of testing, the likelihood and type of incidental results that may be generated, and the types of results that will or will not be returned. Patients should know if and what type of incidental findings may be returned to their referring physician by the laboratory performing the test.”
  - “GS/ES is not recommended before the legal age of majority except for:”
    - “Phenotype-driven clinical diagnostic uses;”
    - “Circumstances in which early monitoring or interventions are available and effective; or”
    - “Institutional review board–approved research.”
  - “As part of the pretest counseling, a clear distinction should be made between clinical and research-based testing.”
  - “Patients should be informed as to whether individually identifiable results may be provided to databases, and they should be permitted to opt out of such disclosure.”
  - “Patients should be informed of policies regarding re-contact of referring physicians as new knowledge is gained about the significance of particular results.”
- The American College of Medical Genetics (ACMG, Updated 2016) published guidelines for the reporting of incidental findings in clinical exome and genome sequencing.<sup>13,14</sup> They state the following:
    - “We continue to support the reporting of known or expected pathogenic variants, but we do not recommend reporting variants of uncertain significance as secondary findings (SFs).”
    - This guideline includes a table of “ACMG SF v2.0 genes and associated phenotypes recommended for return of secondary findings in clinical sequencing.”
  - Peer reviewed literature<sup>15-22</sup>
    - There is limited evidence regarding the accuracy, reliability, and clinical utility of WGS to identify a genetic basis for suspected genetic disorders in children and young adults, with indeterminate findings on conventional diagnostic testing. Additional well-conducted research is necessary to examine the accuracy, reliability, and clinical utility of WGS before its role can be established in a clinical setting.

## Criteria

### Introduction

Requests for WGS are reviewed using the following criteria.

This test is considered investigational and/or experimental.

- Investigational and experimental (I&E) molecular and genomic (MolGen) tests refer to assays involving chromosomes, DNA, RNA, or gene products that have insufficient data to determine the net health impact, which typically means there is insufficient data to support that a test accurately assesses the outcome of interest (analytical and clinical validity), significantly improves health outcomes (clinical utility), and/or performs better than an existing standard of care medical management option. Such tests are also not generally accepted as standard of care in the evaluation or management of a particular condition.
- In the case of MolGen testing, FDA clearance is not a reliable standard given the number of laboratory developed tests that currently fall outside of FDA oversight and FDA clearance often does not assess clinical utility.

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# Administrative Guidelines

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# Medical Necessity Review Information Requirements

MOL.AD.304.A

v1.0.2020

## Introduction

This guideline addresses the minimum information needed to perform a medical necessity review of laboratory testing.

## Description

In order to accurately and effectively conduct medical necessity reviews, certain information is necessary when the case is submitted. This guideline outlines the information that is required to conduct a medical necessity review.

This information must be provided before applicable medical necessity criteria can be applied. If the below information is not received, the testing will be denied, as medical necessity cannot be determined.

## Criteria

The following information must be submitted to perform a medical necessity review for any test:

- Details about the test being performed (test name, description, and/or unique identifier), and
- Laboratory that will be performing the test, and
- All CPT codes and units that will be billed related to the entire test, and
- Clinical information, which may include:
  - All information required by applicable policy, or
  - Test indication, including any applicable signs and symptoms or other reasons for testing, and
  - Any applicable test results (laboratory, imaging, pathology, etc.), and
  - Any applicable family history, and
  - How test results will impact patient care

# Medicare: Hierarchy for Applying Coverage Decisions for Laboratory Testing

MOL.AD.101.B

v1.0.2020

## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedures addressed by this guideline  | Procedure codes   |
|---|---|
| Molecular Pathology   | 81105 - 81479   |
| Multianalyte Assays with Algorithmic Analyses (MAAA)                                  | 81490 – 81599; 0002M - 0013M  |
| Molecular HCPCS Codes   | S3800 - S3870<br>G0452, G9143   |
| Proprietary Laboratory Analyses (PLA)   | Molecular tests* within range (currently 0001U-0138U)                             |
| Molecular Infectious Testing  | Molecular tests* within range<br>87149 – 87912, G0476                             |
| Molecular Cytopathology Procedures (Flow Cytometry, In Situ Hybridization)            | 88120 - 88121<br>88182 - 88199  |
| Cytogenetics  | 88230 - 88299   |
| Molecular Surgical Pathology Procedures (Immunohistochemistry, In Situ Hybridization) | 88341 - 88344<br>88360 - 88361<br>88364 – 88369<br>88373 - 88377<br>88380 - 88388 |
| Other Molecular Codes   | 84999<br>86152<br>86153   |

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**Note** \* Generally defined as codes that include “DNA”, “RNA”, “nucleic acid”, “genotype”, “phenotype” or related language in the code description.

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## Medicare background

### Medicare Administrative Contractors (MACs)

eviCore healthcare follows Medicare rules when applying coverage decisions for laboratory testing under Medicare Advantage plans. Medicare has devised a system where various contractors, called Medicare Administrative Contractors (MAC), set coverage policy for various jurisdictions, which cover specific states. A complete list of MACs by state can be found [here](#).

### National Coverage Determinations (NCDs)

NCDs are developed by Centers for Medicare and Medicaid Services (CMS). They apply to Medicare coverage nationwide for a specific medical service, procedure, or device.

### Local Coverage Determinations (LCDs)

LCDs are written by the different MACs. LCDs outline whether a certain medical service, procedure, or device is covered.<sup>‡</sup> However, the coverage guidelines outlined in LCDs are enforceable only in the states under the specific MAC’s jurisdiction.

- In the case of independent laboratories that perform their own billing, the MAC jurisdiction will be based on the state in which the performing lab is located.\*
- In the case of a reference lab, the MAC jurisdiction will be based on the state in which the billing is performed.<sup>†</sup>
- For purposes of prior authorization case reviews, eviCore healthcare will ask a series of questions to determine if there is a reference lab relationship that would impact jurisdiction. If a reference lab relationship is not disclosed or cannot be determined with the information provided, eviCore healthcare will default to the state in which the performing laboratory is located to determine MAC jurisdiction.

### Articles

Medicare contractors may issue Medicare Coverage Articles. Articles are non-LCD documents that contain coverage statements, coding guidelines or medical review related billing or claims considerations. They may include specific coding instructions and/or clarify existing medical review policies. Articles typically include guidance by ICD10 codes only; however, Articles may sometimes include verbiage containing test-specific medical review coverage criteria. Articles are posted, along with LCDs, in the Medicare Coverage Database.<sup>2</sup>



## MolDX program

Medicare contractors may choose to create their own LCDs or to defer to the MolDX<sup>®</sup> Program administered by the Palmetto GBA MAC for the development of LCDs for genetic tests.<sup>1</sup> In addition to Palmetto GBA, the following contractors have implemented the MolDX Program's coverage criteria in their LCDs for genetic tests: Noridian, CGS, and Wisconsin Physicians Service (WPS).<sup>3,4,5</sup>

The MolDX Program maintains a list of tests that can be considered for coverage if the patient meets either test-specific criteria (if available) or general Medicare coverage criteria of a reasonable and necessary service. Inclusion on this list is not a guarantee of coverage. A complete list of MolDX "covered" tests is available [here](#). The MolDX Program also maintains a list of tests that are excluded from coverage. A list of MolDX excluded tests and associated articles is available [here](#).

## Reasonable and necessary service

Medicare defines services as reasonable and necessary if they lead to "the diagnosis or treatment of illness or injury or to improve the functioning of a malformed body member." § 6

## Medicare Criteria Hierarchy

When performing case reviews for genetic tests for Medicare members, eviCore will employ the following strategy:<sup>6</sup>

- Identify any NCDs that apply to the test by searching the Medicare coverage database [here](#);
  - When applying an NCD, verify that the effective date of the NCD includes the date of service of the test.
  - If an applicable NCD is identified, then the test-specific coverage criteria from the NCD along with a determination that the service is reasonable and necessary (as described below), will be applied.
- If no NCD exists, identify any LCDs that apply to the test by searching the Medicare coverage database [here](#);
  - To identify the applicable LCD, first look at the LCDs established by the appropriate MAC.<sup>7</sup>
  - In the case of independent laboratories that perform their own billing, the MAC jurisdiction will be based on the state in which the performing lab is located.
  - In the case of a reference lab, the MAC jurisdiction will be the state in which the billing is performed.
    - For purposes of prior authorization case reviews, eviCore healthcare will ask a series of questions to determine if there is a reference lab relationship that

would impact jurisdiction. If a reference lab relationship is not disclosed or cannot be determined with the information provided, eviCore healthcare will default to the state in which the performing laboratory is located to determine MAC jurisdiction.

- If there are no appropriate LCDs in the MAC jurisdiction, determine if the MAC has an existing relationship with Palmetto GBA and has implemented the MoIDX criteria. If the MAC defers to the MoIDX Program, then identify any policies from the MoIDX Program that apply to the test.
  - Under the MoIDX Program, “covered” tests can be considered for coverage if the patient meets either test-specific criteria including any ICD10 code restrictions (if available) or general Medicare coverage criteria of a reasonable and necessary service. Inclusion on this list is not a guarantee of coverage. A complete list of MoIDX “covered” tests is available [here](#).
  - The MoIDX Program also maintains a list of tests that are excluded from coverage because they do not meet a Medicare statutory benefit. A list of MoIDX excluded tests and associated articles is available [here](#). If the test is listed as excluded or not covered on an applicable article or LCD, this testing will not be covered for MACs that defer to MoIDX .
- When applying an LCD, verify that the effective date of the LCD includes the date of service of the test.
- If an applicable LCD is identified, then the test-specific coverage criteria, from the LCD along with a determination that the service is reasonable and necessary (as described below), will be applied.
- If there are no applicable LCDs, or the applicable LCD does not include test-specific coverage criteria, then any applicable Article(s) containing test-specific coverage criteria will be applied.
- If the test is listed on an Article or LCD as being not covered, the testing will not be covered.
- If there are no applicable LCDs or Articles, or the applicable LCD or Article does not include test-specific coverage criteria, then eviCore evidence-based criteria for diagnostic testing will be applied.\*\*8
  - In addition to eviCore diagnostic testing criteria, patient-specific coverage approval or denial decisions may be made in accordance with the Medicare requirement that a service be reasonable and necessary for the treatment of an injury or illness. To determine whether a service is reasonable and necessary, specific criteria will be applied that may include, but are not limited to, the following:<sup>1,9,10</sup>
    - The beneficiary must display clinical features of an associated disease, noting that coverage of molecular testing for carrier status or family studies (often referred to as pre-symptomatic or pre-disposition testing) is considered screening and is statutorily excluded from coverage; and

- The result of the test will directly impact the treatment being delivered to the beneficiary; and
  - If, after history, physical examination, pedigree analysis, genetic counseling, and completion of conventional diagnostic studies, a definitive diagnosis remains uncertain, then such testing can be considered for coverage.
- If application of eviCore criteria has resulted in an “investigational/experimental” coverage determination for the test(s), but there is an applicable Article containing specific guidance including only ICD10 diagnosis codes, then that Article should be applied.
  - If application of eviCore criteria has resulted in a “not medically necessary” coverage determination for the test(s) for a member, that decision will not be altered based on an Article containing guidance including only ICD10 diagnosis codes.

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\* Per Section 50.5 of the Medicare Claims Processing Manual, Chapter 16 (*Rev. 4000, 03-16-18*), “jurisdiction of payment requests for laboratory services furnished by an independent lab ... lies with the A/B MAC (B) serving the area in which the laboratory test is performed. Jurisdiction is not affected by whether or not the independent laboratory uses a central billing office and whether or not the laboratory provides services to customers outside its A/B MAC (B)’s service area. The location where the independent laboratory performed the test determines the appropriate billing jurisdiction.”

‡ Per Chapter 13 of Medicare Program Integrity Manual “An LCD, as defined in §1869(f)(2)(B) of the Social Security Act (SSA), is a determination by a Medicare Administrative Contractor (MAC) respecting whether or not a particular item or service is covered on a contractor–wide basis in accordance with section 1862(a)(1)(A) of the Act.”

† Per Section 50.5.1 of the Medicare Claims Processing Manual, Chapter 16 (*Rev. 4000, 03-16-18*), Regardless of whether the laboratory that bills Medicare is the referring or reference laboratory, the laboratory that does the billing may bill only the A/B MAC (B) that services the jurisdiction in which the billing laboratory is physically located. The location of the draw station, when a separate draw station is employed, never determines claims filing jurisdiction.

§ Per Chapter 3 of the Medicare Program Integrity Manual (*Rev. 825, 09-21-18*), “CMS issues national coverage determinations (NCDs) that specify whether certain items, services, procedures or technologies are reasonable and necessary under §1862(a) (1) (A) of the Act. In the absence of an NCD, Medicare contractors are responsible for determining whether services are reasonable and necessary. If no local coverage determination (LCD) exists for a particular item or service, the MACs, CERT, Recovery Auditors, and ZPICs shall consider an item or service to be reasonable and necessary if the item or service meets the following criteria: It is safe and effective; It is not experimental or investigational; and It is appropriate, including the duration and

frequency in terms of whether the service or item is: Furnished in accordance with accepted standards of medical practice for the diagnosis or treatment of the beneficiary's condition or to improve the function of a malformed body member..."

\*\* Per section 90.5 of the Medicare Managed Care Manual, Chapter 4 (*Rev. 121, 04-22-16*), "In coverage situations where there is no NCD, LCD, or guidance on coverage in original Medicare manuals, an MAO (Medicare Advantage Organization) may adopt the coverage policies of other MAOs in its service area. However, if the MAO decides not to use coverage policies of other MAOs in its service area, the MAO: Must make its own coverage determination; ...Must provide CMS an objective evidence-based rationale relying on authoritative evidence..."

# Molecular Pathology Tier 2 Molecular CPT Codes

MOL.AD.102.A

v1.0.2020

## Introduction

The administrative handling of Tier 2 Molecular Pathology CPT codes 81400-81408 is addressed by this guideline. The assessment of medical necessity of tests billed with Tier 2 molecular pathology codes is addressed separately.

## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedures addressed by this policy  | Procedure codes |
|--|-----------------|
| Molecular pathology procedure, Level 1, (e.g., identification of single germline variant [e.g. SNP] by techniques such as restriction enzyme digestion or melt curve analysis)   | 81400           |
| Molecular pathology procedure, Level 2, (e.g., 2-10 SNPs, 1 methylated variant, or 1 somatic variant [typically using nonsequencing target variant analysis], or detection of a dynamic mutation disorder/triplet repeat)  | 81401           |
| Molecular pathology procedure, Level 3, (e.g., greater than 10 SNPs, 2-10 methylated variants, or 2-10 somatic variants [typically using non-sequencing target variant analysis], immunoglobulin and T-cell receptor gene rearrangements, duplication/deletion variants of 1 exon, loss of heterozygosity [LOH], uniparental disomy [UPD]) | 81402           |

| Procedures addressed by this policy   | Procedure codes |
|---|-----------------|
| Molecular pathology procedure, Level 4, (e.g., analysis of single exon by DNA sequence analysis, analysis of >10 amplicons using multiplex PCR in 2 or more independent reactions, mutation scanning or duplication/deletion variants of 2-5 exons)           | 81403           |
| Molecular pathology procedure, Level 5, (e.g., analysis of 2-5 exons by DNA sequence analysis, mutation scanning or duplication/deletion variants of 6-10 exons, or characterization of a dynamic mutation disorder/triplet repeat by Southern blot analysis) | 81404           |
| Molecular pathology procedure, Level 6, (e.g., analysis of 6-10 exons by DNA sequence analysis, mutation scanning or duplication/deletion variants of 11-25 exons)  | 81405           |
| Molecular pathology procedure, Level 7, (e.g., analysis of 11-25 exons by DNA sequence analysis, mutation scanning or duplication/deletion variants of 26-50 exons, cytogenomic array analysis for neoplasia)   | 81406           |
| Molecular pathology procedure, Level 8, (e.g., analysis of 26-50 exons by DNA sequence analysis, mutation scanning or duplication/deletion variants of >50 exons, sequence analysis of multiple genes on one platform)  | 81407           |
| Molecular pathology procedure, Level 9, (e.g., analysis of >50 exons in a single gene by DNA sequence analysis)   | 81408           |

## What are molecular pathology Tier 2 molecular CPT codes

### Definition

Tier 2 codes are intended to report a wide range of molecular pathology procedures for which Tier 1 or other test-specific CPT codes have not been assigned.

## Tier 2 code use

Tier 2 codes are organized and assigned based on level of technical and interpretive effort required. Each code includes examples of analytes that might be appropriate for assignment to that code in parentheses, but this is not meant to be complete.

The 2019 AMA CPT Professional codebook states the following regarding use of the Tier 2 codes:

- “Use the appropriate molecular pathology procedure level code that includes the specific analyte listed after the code descriptor. If the analyte tested is not listed under one of the Tier 2 codes or is not represented by a Tier 1 code, use the unlisted molecular pathology procedure code, 81479.”<sup>1</sup>

## Criteria

### Authorization Requirements

- The following information must be submitted for medical necessity review of CPT codes 81400 through 81408:
  - Details about the test being performed (test name, description, and/or unique identifier), and
  - Laboratory that will be performing the test, and
  - All CPT codes and units that will be billed related to the entire test, and
  - Clinical information, which may include:
    - All information required by test-specific policy, or
    - Test indication, including any applicable signs and symptoms or other reasons for testing, and
    - Any applicable test results (laboratory, imaging, pathology, etc.), and
    - Any applicable family history, and
    - How test results will impact patient care if available

### Claims Review and Payment Rules for 81400-81408

- A Tier 2 code should only be used when the AMA has specifically assigned the performed test to a Tier 2 code (i.e., laboratory self-assigned Tier 2 codes will not be accepted). Laboratories may not self-assign tests to Tier 2 codes that are not specifically listed as analytes by the AMA.
- Claims submitted for 81400 through 81408 may require a unique test identifier. Please refer to the *Unique Test Identifiers for Non-Specific Procedure Codes* guideline for additional information.



- All claims received for 81400 through 81408 are subject to the applicable authorization requirements regardless of the specific test performed.

## References

### Introduction

These references are cited in this guideline.

1. American Medical Association. CPT 2019 Professional Edition. Chicago, IL; 2019.

# Unique Test Identifiers for Non-Specific Procedure Codes

MOL.AD.107.A

v1.0.2020

## Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

| Procedures addressed by this guideline | Procedure codes |
|--|-----------------|
| MOPATH PROCEDURE LEVEL 1               | 81400           |
| MOPATH PROCEDURE LEVEL 2               | 81401           |
| MOPATH PROCEDURE LEVEL 3               | 81402           |
| MOPATH PROCEDURE LEVEL 4               | 81403           |
| MOPATH PROCEDURE LEVEL 5               | 81404           |
| MOPATH PROCEDURE LEVEL 6               | 81405           |
| MOPATH PROCEDURE LEVEL 7               | 81406           |
| MOPATH PROCEDURE LEVEL 8               | 81407           |
| MOPATH PROCEDURE LEVEL 9               | 81408           |
| UNLISTED MOLECULAR PATHOLOGY           | 81479           |
| UNLISTED MAAA                          | 81599           |
| CLINICAL CHEMISTRY TEST                | 84999           |

## Description

This policy provides instruction on how to submit a unique test identifier when a procedure code is billed that does not adequately describe the performed molecular or genomic test referred to here as “non-specific procedure codes.”

Given the large and rapidly increasing number of molecular and genomic tests, many tests do not have unique procedure codes and are instead billed with non-specific procedure codes. These non-specific procedure codes generally fall into one of the following categories.

### Tier 2 codes

Tier 2 Molecular Pathology codes (81400-81408) are a set of CPT codes designed to represent the level of technical and interpretive effort required for a large number of molecular and genomic tests that have not been assigned a unique CPT code

(i.e., are not addressed by Tier 1, GSP, MAAA, PLA, etc. codes). Specific tests, or analytes, are assigned to these Tier 2 codes by the AMA a few times yearly and cannot be self-assigned by the laboratory.

The AMA publishes a set of gene abbreviations or analyte identifiers, called claim designation codes, for each test assigned to a Tier 2 code. These codes are intended to provide billing transparency such that the combination of a Tier 2 code and the applicable claim designation code on a claim form are reasonably specific to the test performed. Where the test is specific to a gene, the claim designation code is generally the standard gene name. The claim designation codes are published in the annual AMA CPT Professional codebook.<sup>1</sup>

### **Unlisted codes**

If a molecular or genomic test has not been assigned to any test-specific or Tier 2 CPT code, those tests are generally billed under one of the following unlisted codes:

- 81479: Unlisted molecular pathology procedure
- 81599: Unlisted multianalyte assay with algorithmic analysis
- 84999: Unlisted chemistry procedure

The proper unlisted code depends on the nature of the test, but most molecular tests are best described by 81479 or 81599.

There is no publicly-available, widely-adopted source of unique codes for tests billed under unlisted codes.

The Palmetto MoIDX program requires that most molecular tests be registered with the program and obtain a unique identifier (McKesson Z-Code or Palmetto Test Indicator) for the purposes of claim processing.<sup>2</sup> However, this identifier is both lab and test-specific and is currently primarily utilized by only certain Medicare jurisdictions.

## **Criteria**

### **Unique test identifier assignment**

#### **Tier 2 AMA claim designation codes**

For tests billed under a Tier 2 CPT code, the unique test identifier is the same as the original claim designation code published by the AMA when available, provided the claim designation code described only a single test assigned to that Tier 2 code. In the event that the same claim designation code described more than one test assigned to the same Tier 2 code, eviCore assigned a unique code (not the original AMA claim designation code) to at least one of these tests. When the AMA has not published a claim designation code, a unique code is developed by eviCore. No separate registration or notification process is required on the part of the laboratory.

### Tier 2 special cases

Tier 2 code 81403 allows for known familial variant testing to be billed without specific gene assignment. The unique test identifier for known familial variants not otherwise specified is generally either: "KFMNOS" or the AMA assigned claim designation code for the gene if one exists with the addition of "KFM" (e.g., ATM and ATMKFM).

### Unlisted codes

For tests billed under unlisted procedure codes, a unique code will be developed unrelated to the Tier 2 claim designation codes. No separate registration or notification process is required on the part of the laboratory.

### Obtaining a unique test identifier

When a medical necessity review is performed for a test that will be billed under a non-specific procedure code, billing instructions will include the appropriate unique test identifier if required in the determination communication.

If a medical necessity review is not performed for a test that will be billed under a non-specific procedure code, a unique test identifier can be obtained by contacting eviCore through the phone number provided by the health plan. However, most non-specific procedure codes require medical necessity determination. If pre-service medical necessity determination is required and not obtained, that requirement will take precedence over any other billing requirements.

### Billing tests using non-specific procedure codes

When a unique test identifier is provided in the medical necessity determination communication, it must be included on the claim regardless of medical necessity review requirements or determination outcome. Enter the unique test identifier in one of the following narrative fields based on the type of claim being submitted:

| Claim type          | Electronic claim   | Paper claim   |
|---------------------|--|---|
| <b>Professional</b> | 837P: Enter in the 2400 SV101-7 field (Line Item Description) associated with the non-specific CPT code. Each non-specific CPT code should have a unique identifier in the associated field. | CMS-1500: Enter in box 24 in the shaded line above the service line that contains the non-specific CPT code. Each non-specific CPT code should have a unique identifier entered above it. Each test identifier should have the qualifier "ZZ" appended at the beginning (e.g., ZZBRAFF) to assist in recognition of the code. |

| Claim type                  | Electronic claim   | Paper claim   |
|-----------------------------|--|---|
| <b><i>Institutional</i></b> | 837I: Enter in the 2400 SV202-7 field (Line Item Description) associated with the non-specific CPT code. Each non-specific CPT code should have a unique identifier in the associated field. | UB-04: Enter in box 80 (Remarks). Only a single non-specific CPT code should be billed per claim form due to the limitations of a single descriptive field. The test identifier should have the qualifier "ZZ" appended at the beginning (e.g., ZZBRAf) to assist in recognition of the code. |

## References

1. American Medical Association. CPT 2018 Professional Edition. Chicago IL: American Medical Association; 2018.
2. Palmetto GBA. MoIDX Program Information. Available at: <http://www.palmettogba.com/palmetto/MoIDX.nsf/DocsCatHome/MoIdx>.

# Glossary

v1.0.2020

| Term                              | Definition   |
|-----------------------------------|--|
| <b>adenoma</b>                    | An ordinarily benign neoplasm of epithelial tissue. If an adenoma becomes cancerous, it is known as an adenocarcinoma.   |
| <b>adenomatous polyposis coli</b> | Adenomatous polyposis coli (APC) is a gene located on chromosome 5q. Inherited APC gene mutations are associated with Familial Adenomatous Polyposis (FAP) and Attenuated FAP. Most colorectal cancer polyps have mutations in both copies of the APC gene, even in people that don't have FAP.  |
| <b>adjuvant therapy</b>           | When discussing cancer treatment, adjuvant therapy is given after a primary treatment (like surgery) to increase the chances of a cure. Adjuvant therapy may include chemotherapy, radiation therapy, hormone therapy, or biological therapy.  |
| <b>adverse drug reaction</b>      | A harmful or unpleasant reaction to a drug that generally means the drug should be prescribed differently or avoided.  |
| <b>aerobic exercise</b>           | Any physical activity that causes the heart to pump faster and harder and breathing to quicken. Strengthens the heart muscle and may also help lower high blood pressure and increase good cholesterol.  |
| <b>AFAP</b>                       | Attenuated FAP (AFAP) is a form of FAP characterized by a less dramatic proliferation of polyps (between 20-99 cumulative polyps) and age of onset for colorectal cancer of approximately 50 years. Polyps generally localize to the proximal (right-sided) colon. The American Gastroenterological Association (AGA) recommends genetic testing once a person has developed 20 or more cumulative polyps. |
| <b>AFP</b>                        | Short for "alpha-fetoprotein", a substance found in pregnant women's blood. High levels of AFP are associated with risk for spina bifida and abdominal wall defects.   |
| <b>amniotic fluid</b>             | The protective fluid that surrounds the developing baby. This fluid fills the amniotic sac, or "bag of water" inside the mother's uterus.  |
| <b>ancestry</b>                   | Can be represented by a family tree showing how biological family members are related to each other. It is sometimes used interchangeably with "lineage."  |
| <b>anemia</b>                     | A condition caused by too little oxygen in the blood, usually caused by too little hemoglobin or too few red blood cells   |

| Term                       | Definition   |
|----------------------------|--|
| <b>angina</b>              | Pain, pressure, or a feeling of indigestion in the chest caused by too little oxygen-rich blood reaching the heart. Usually caused by coronary artery disease.   |
| <b>anticipation</b>        | A way certain genetic diseases are inherited that causes them to get worse over the generations.   |
| <b>anticoagulant</b>       | Medications that prevent the blood from clotting -- often call "blood thinners."   |
| <b>anticonvulsant drug</b> | Medications used to prevent or treat seizures. Common anticonvulsant drugs include Dilantin, Zarontin, Klonopin, Valium, Tegretol, Depakote and others.  |
| <b>antidepressant</b>      | A medication used to prevent or treat depression. Current antidepressants categories include SSRIs, MAOIs, tricyclics, tetracyclics, and others.   |
| <b>antipsychotic</b>       | Medications used to treat schizophrenia, schizoaffective disorder, bipolar disorder and other conditions that distort a person's grasp of reality  |
| <b>antiretroviral</b>      | A medication used to treat a retrovirus infection, such as HIV   |
| <b>APOB</b>                | A gene for the protein that normally helps deliver LDL cholesterol to the liver to be broken down. An APOB gene mutation causes a person not to clear LDL from the body as well as usual and it builds up. APOB mutations are one cause of familial hypercholesterolemia, although LDLR mutations are the most common. |
| <b>Apolipoprotein B100</b> | ApoB100 is short for apolipoprotein B100. It is a normal protein that is a major part of "bad" cholesterol. High ApoB100 is a strong risk factor for heart disease.  |
| <b>aromatase inhibitor</b> | A class of drugs used to treat postmenopausal women who have hormone-dependent breast cancer. Als work by blocking the enzyme aromatase responsible for converting androgen to estrogen. This limits the amount of estrogen available to promote breast cancer growth.   |
| <b>arrhythmia</b>          | Any variation from the normal heart rate or rhythm. The heart might beat faster than usual (tachycardia), slower than usual (bradycardia), or with an unusual pattern.   |
| <b>artery</b>              | Blood vessels that carry oxygen-rich blood throughout the body. The coronary arteries carry blood to the heart muscle.   |

| Term                       | Definition   |
|----------------------------|--|
| <b>Ashkenazi Jewish</b>    | Jewish people whose ancestors are from Eastern Europe -- mostly Germany, Poland, Russia, and some parts of France. Whereas Sephardic Jewish people have ancestry from Spain, Portugal, parts of France, Italy, North Africa, and the Middle East. Most American Jews are Ashkenazi.  |
| <b>atherosclerosis</b>     | A disease caused by plaque buildup inside the arteries that limits blood flow. Also called hardening of the arteries.  |
| <b>autosomal dominant</b>  | A pattern of inheritance where only one gene from a pair isn't working properly and causes the condition. Anyone with an autosomal dominant condition has a 50% chance of passing on the nonworking gene -- and, therefore, the condition -- to each child.  |
| <b>autosomal recessive</b> | Describes a pattern of inheritance where both genes from a pair must be working abnormally to cause the condition. People with one abnormal and one normally working gene don't have the condition and are called carriers. When both parents are unaffected carriers of a condition, there is a 25% chance to have an affected child with each pregnancy. |
| <b>average woman</b>       | The "average woman" is someone picked at random from the general public.   |
| <b>Beta-thalassemia</b>    | An inherited blood disorder that causes anemia, which is a shortage of red blood cells. This disorder causes lower than usual amounts of oxygen in the blood.  |
| <b>b-hCG</b>               | Short for "beta-human chorionic gonadotropin", this substance is known as the pregnancy hormone. It is produced by the placenta.   |
| <b>biopsy</b>              | The process of removing tissue from living patients for diagnostic evaluation.   |
| <b>black box warning</b>   | A warning required by the U.S. Food and Drug Administration (FDA) on the package inserts of some prescription drugs. These are the strongest warnings from the FDA about a significant risk for serious or life-threatening complications of a drug. Black box refers to the heavy black line surrounding the warning.                                     |
| <b>blood clot</b>          | Proteins change liquid blood into a solid blood clot usually in response to an injury to prevent further blood loss. Imbalance in the clotting proteins can lead to too little or too much clotting (thrombosis). When an abnormal clot forms, it can block blood flow and cause tissue damage or death.   |



| Term                          | Definition  |
|-------------------------------|---|
| <b>blood clotting factor</b>  | Proteins and enzymes in the blood that control changing liquid blood into a solid blood clot. Imbalance of these factors can cause too little or too much clotting.   |
| <b>blood transfusion</b>      | Transferring blood or components of blood, such as blood plasma, into a patient.  |
| <b>blood vessel</b>           | The channels that carry blood throughout the body: arteries, veins and capillaries  |
| <b>bone marrow transplant</b> | A procedure that replaces diseased or damaged bone marrow with healthy bone marrow. The damaged bone marrow may be destroyed by chemotherapy or radiation. The healthy bone marrow can come from the patient or a donor.  |
| <b>bowel preparation</b>      | Purging and cleansing of the bowel of fecal and other matter to assure clear evaluation of the bowel.   |
| <b>BRCA1</b>                  | A gene located on chromosome 17 that normally produces a protein to help restrain cell growth. A harmful change in BRCA1 may predispose a person toward developing breast and/or ovarian cancer.  |
| <b>BRCA2</b>                  | A gene located on chromosome 13 that normally produces a protein to help to restrain cell growth. A harmful change in BRCA2 may predispose a person toward developing breast and/or ovarian cancer.   |
| <b>breast MRI</b>             | MRI uses powerful magnets and radio waves to create detailed pictures of the breast and surrounding tissues. It provides clear pictures of parts of the breast that are difficult to see clearly on ultrasound or mammogram, but it's not a replacement for mammography.          |
| <b>cancer</b>                 | A disease where abnormal cells grow and divide without control. Cancer cells can invade nearby tissues and spread through the bloodstream and lymphatic system to other parts of the body (called metastasis).  |
| <b>carbohydrate</b>           | Carbohydrates are the most abundant nutrients we eat and are broken down by the liver into glucose (sugar) to provide energy.   |
| <b>carcinoma</b>              | A cancer that begins in the skin or tissues that line or cover internal organs.   |
| <b>cardiomyopathy</b>         | A heart muscle disease that usually leads to a weakened heart muscle and a reduced ability to pump blood effectively. Any damage to the heart muscle can cause cardiomyopathy. Recognized causes include genetic factors, heart attack, alcoholism, and certain viral infections. |

| Term                            | Definition  |
|---------------------------------|---|
| <b>carrier</b>                  | A person who has one copy of a changed gene and one normal copy of that gene.   |
| <b>CBC</b>                      | An abbreviation for “complete blood count”. A standard test that provides information including the white blood cell count, red blood cell count, amount of hemoglobin, platelet count and more.  |
| <b>CCR5-tropic</b>              | A form of HIV virus that uses a protein on the outside of a cell, called the CCR5 receptor, to enter and infect the cell.   |
| <b>CD4 cells</b>                | A kind of white blood cell, also called “helper T cells”, which help protect the body against infection. These are the cells that the HIV virus infects.  |
| <b>cell</b>                     | The basic building block of the tissues and organs in the body. Most cells have a complete copy of our genetic code and all cells are made by copying existing cells.   |
| <b>chelation therapy</b>        | Treatment to remove iron from the body using a chemical that attaches to heavy metals inside the body to remove them.   |
| <b>chemoprevention</b>          | The administration of any chemical or drug to treat a disease or condition and limit its further progress, or to prevent the condition from ever occurring.   |
| <b>cholesterol</b>              | A waxy, fat-like substance used by the body to make hormones, vitamin D, and other important substances. Eating too much cholesterol increases the risk of heart disease.   |
| <b>chromosome</b>               | A threadlike strand of DNA that carries genes and transmits hereditary information. Each chromosome can contain hundreds or thousands of individual genes. The number of chromosomes in the normal human cell is 46 (23 pairs).                                       |
| <b>chromosome translocation</b> | A genetic condition where material from one chromosome breaks off and sticks to another chromosome, or switches places with a part of another chromosome. There are different types of translocations, and they can have different effects on health and development. |
| <b>CHRPE</b>                    | Congenital Hypertrophy of Retinal Pigmented Epithelium - a benign eye abnormality common in those with FAP.   |
| <b>close relative</b>           | A close relative is defined as a mother, father, sister, brother or child.  |
| <b>colectomy</b>                | The surgical removal of the colon. A total colectomy is the surgical removal of the colon and rectum. A subtotal colectomy is the surgical removal of the colon or portions of the colon only (not rectum).   |

| Term                           | Definition  |
|--------------------------------|---|
| <b>colon</b>                   | Another name for the large intestine; the section of the large intestine extending from the cecum to the rectum. An adult colon is approximately five to six feet in length and is responsible for absorbing water and forming, storing, and expelling waste.   |
| <b>colonoscopy</b>             | A procedure that examines the entire rectum and colon. A colonoscope is a long, flexible, lighted tube with a tiny lens on the end used to directly examine the whole colon and look for the presence of growths. Colonoscopy is the most effective way to evaluate the inside of your entire colon for the presence of colorectal cancer or polyps. This procedure is considered “invasive,” because it requires sedation and the insertion of the colonoscope through the rectum. |
| <b>colorectal cancer</b>       | Cancer that occurs in the rectum or the colon.  |
| <b>Comprehensive Analysis</b>  | Comprehensive Analysis is the most complete BRCA test. It looks at all the coding DNA of the BRCA1 and BRCA2 genes, to see if there are any changes or mutations. It can find: changes that are known to cause cancer, changes that are harmless, and changes whose link to cancer is unknown.  |
| <b>congenital heart defect</b> | A problem with the structure of the heart, or the vessels connected to it, which is present from birth. Many types of heart defects exist. They can affect how the blood flows through the heart, or its rhythm.  |
| <b>corneal arcus</b>           | Also called “arcus cornealis”. An accumulation of cholesterol around the cornea (the clear front surface of the eye) that causes a grey ring around the colored part of the eye. May be a normal feature of aging, but may also be a sign of unusually high cholesterol levels.   |
| <b>CXCR4-tropic</b>            | A form of HIV virus that uses a protein on the outside of a cell, called the CXCR4 receptor, to enter and infect the cell.  |
| <b>CYP1A2</b>                  | An enzyme involved in the metabolism of many drugs, including caffeine. Some people have a form of CYP1A2 that is particularly susceptible to tobacco smoke and may have adverse reactions when taking drugs metabolized by CYP1A2 while smoking.   |
| <b>CYP2C19</b>                 | An enzyme involved in the metabolism of many drugs, including several ulcer and reflux drugs. Variants in the gene can cause adverse reactions to drugs metabolized by CYP2C19.   |

| Term                    | Definition  |
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| <b>CYP2C9</b>           | An enzyme involved in the metabolism of many drugs, including warfarin and celecoxib. and several anti-inflammatories. Variants in the gene can cause adverse reactions to drugs metabolized by CYP2C9.   |
| <b>CYP2D6</b>           | An enzyme involved in the metabolism of many drugs, including codeine, tamoxifen, and several antidepressants. Variants in the gene can cause adverse reactions to drugs metabolized by CYP2D6.   |
| <b>cytochrome P450</b>  | Cytochrome P450, abbreviated CYP450, is a large family of drug metabolizing enzymes, including CYP1A2, CYP2C9, CYP2C19, and CYP2D6.   |
| <b>de novo mutation</b> | A mutation that is not running in the family yet, but occurs when a gene is damaged at conception. A de novo mutation can also then be passed on to one's children.   |
| <b>Desmoid tumor</b>    | Fibrous growth identified generally in the abdominal area associated with FAP and AFAP.   |
| <b>detection rate</b>   | Also called “sensitivity”. Refers to the likelihood that a test will actually find the condition that it is looking for. If a test has a 90% detection rate, it will find 90% (9 out of 10) of people with the condition. Most tests don't have a 100% detection rate, so you should pay attention to detection rates to understand the limitations of any test you consider. |
| <b>diabetes</b>         | A disease that causes you to have too much glucose (sugar) in your blood because of a problem with the hormone insulin. People with diabetes either can't make insulin (type I) or they can't use it well enough (type II).   |
| <b>DNA</b>              | Stands for “deoxyribonucleic acid”. The chemical inside the nucleus of the cell that encodes the genetic instructions passed from generation to generation. Genes are made of DNA.  |
| <b>DNA replication</b>  | The duplication process of genetic material.  |
| <b>drug interaction</b> | When a drug reacts with another drug (prescribed, over-the-counter, herbs, supplements, etc.), food, or other environmental exposure to cause an altered response. The effect may be an increased or decreased response or an adverse drug reaction.  |
| <b>environment</b>      | When talking about what causes disease, environment refers to basically everything that isn't controlled by genetics. Environment can include what we eat, physical activity, medications we take, chemicals we are exposed to, our physical surroundings, and countless other factors.   |

| Term                                  | Definition   |
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| <b>enzyme</b>                         | A protein made by the body that encourages a biochemical reaction. Humans make hundreds of different enzymes from the instructions in our genes. If any one enzyme isn't working normally, it can cause a disease.   |
| <b>epithelium</b>                     | Membranous tissue constructed of one or more layers of cells that cover the internal and external surfaces of the body and its organs.   |
| <b>ethnic background</b>              | The geographical and racial identity of a person's ancestors   |
| <b>ethnic group</b>                   | A group of people whose ancestors lived in the same region of the world, and thus, who share a common genetic background   |
| <b>ethnicity</b>                      | A group of people who frequently share some common ancestry and are, therefore, more likely to share certain genetic traits or mutations. May be based on descending from the same geographical location, a shared religion, a tribal connection, or other cultural practices. People often belong to more than one ethnic group.        |
| <b>extensive metabolizer</b>          | Extensive metabolizers have two “normal” drug metabolism genes. They make the average amount of enzyme and usually have normal drug response. Most people are extensive metabolizers. People have many drug metabolism genes and can be different kinds of metabolizers for each.  |
| <b>false negative</b>                 | A test result that is read as negative when the disease is present.  |
| <b>false positive</b>                 | A test result that is read as positive when the disease is not present.  |
| <b>familial adenomatous polyposis</b> | Familial Adenomatous Polyposis (FAP) is an inherited condition that causes the formation of hundreds to thousands of precancerous polyps within the colon, often before age 20. FAP is usually caused by an inherited mutation in one copy of the APC gene.  |
| <b>familial hypercholesterolemia</b>  | An inherited condition that causes people to have very high levels of LDL, or “bad”, cholesterol and a high risk for heart disease if not aggressively treated with cholesterol-lowering drugs.  |
| <b>family history</b>                 | Family history may refer to whether or not you have any biological relative with a specific condition. It may also refer to the collective medical histories of all of your biological relatives. An accurate family history is one of the most important tools available to predict and prevent conditions that you may be at risk for. |

| Term                             | Definition   |
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| <b>FDA</b>                       | U.S. Food and Drug Administration, a department of the federal government, that regulates drugs, foods, some tests, medical devices, and other things that may impact public health and safety.  |
| <b>fecal immunochemical test</b> | Fecal immunochemical test (FIT) is a test, similar to FOBT, to check for hidden blood in the stool. Blood may signal cancer or one of many non-cancer related causes of bleeding.  |
| <b>fecal occult blood test</b>   | Fecal occult blood test (FOBT) is a test to check for hidden blood in the stool. The presence of blood in stool may be a sign of cancer or one of the many non-cancer related causes of bleeding (e.g. hemorrhoids).   |
| <b>fibrate</b>                   | A group of drugs that work to lower your “bad” (LDL) cholesterol by reducing your triglycerides (another type of fat) and raising your “good” (HDL) cholesterol. Commonly prescribed fibrates include fenofibrate (brand name examples include: Antara, Fenoglide, Lipofen, Lofibra, TriCor, Triglide, and Lipidil) and gemfibrozil (brand name: Lopid). |
| <b>flexible sigmoidoscopy</b>    | Procedure used to examine the rectum and lower third of the colon. A sigmoidoscope is a long, flexible, slender tube with a lens on the end used to visualize a portion of the colon to look for the presence of growths.  |
| <b>functional</b>                | Functional refers to genes or proteins that are not affected by genetic changes that disrupt their normal structure or behavior.   |
| <b>gastrointestinal tract</b>    | The digestive system, consisting of the esophagus, stomach, small intestine and large intestine.   |
| <b>gene</b>                      | A piece of DNA that acts as an instruction to the body for how to make a specific protein (enzyme, hormone, etc.). Genes are inherited, passed from parent to child.   |
| <b>gene sequencing</b>           | A genetic test that is considered the gold standard for finding genetic changes known as mutations.  |
| <b>genetic</b>                   | Refers to any trait that is inherited, or passed from generation to generation through genes. These traits may range from having specific diseases to our response to certain drugs to simply our physical characteristics, like eye and hair color.   |
| <b>genetic condition</b>         | A genetic condition is any disease, disorder, syndrome, or trait that is caused, at least in part, from alterations in genes or chromosomes.   |

| Term                          | Definition  |
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| <b>genetic counseling</b>     | Genetic counseling is a process to help people learn about, cope with, and manage their risk of genetic disorders. This risk may be uncovered because the person is diagnosed with a condition, has a family history, has an affected child, and/or has an abnormal genetic test result.  |
| <b>genetic counselor</b>      | A healthcare professional with specialized training in how the science of genetics relates to medical care. A genetic counselor can evaluate your personal and family history, identify any risk factors for birth defects or genetic conditions, and help you understand and make decisions about testing or other options you may have. |
| <b>genetic discrimination</b> | Treatment or consideration based on genetic status or category rather than individual merit or actual conditions.   |
| <b>genetic modifier</b>       | A gene that changes how another gene is expressed.  |
| <b>genetic predisposition</b> | Any condition in which genetic make-up leaves the individual more susceptible to disease.   |
| <b>genetic test</b>           | A specific type of laboratory test that is designed to find out if a person has a genetic disorder, is a carrier of a genetic disease, or has a predisposition to develop a genetic problem. Genetic testing can look at chromosomes, genes, or proteins -- depending on the specific condition being tested.                             |
| <b>genomics</b>               | The study of the genome and its significance to pathology and disease.  |
| <b>genotype</b>               | The version of genes a person, organism, or cancer has.   |
| <b>genotyping</b>             | Tests that look specifically at the genetic information of a person, organism, or cancer. These tests may predict a certain characteristic (“phenotype”) but don't actually test for that characteristic.   |
| <b>glucose</b>                | A form of sugar made from carbohydrates we eat that the body uses for energy. Too much glucose in their blood may be a sign of diabetes.  |
| <b>HBB</b>                    | A gene involved in making a piece of a protein called hemoglobin. Genetic changes, or mutations, in the HBB gene can cause sickle cell disease and beta-thalassemia.  |
| <b>HDL</b>                    | High density lipoprotein cholesterol. Also called the “good” cholesterol. High HDL lowers the risk for heart disease.   |
| <b>HDL2</b>                   | A subtype of HDL (the “good” cholesterol). HDL2 is the “best” cholesterol because high levels give you the most protection against heart disease -- even more than just high total HDL.   |

| Term   | Definition   |
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| <b>HDL3</b>                                      | A subtype of HDL (the “good” cholesterol). HDL3 is not as good for you as other types of HDL. Some studies show that high levels of HDL3 may actually increase your risk for heart disease.  |
| <b>heart</b>                                     | A muscular organ whose primary job is to pump blood to all parts of the body.  |
| <b>heart attack</b>                              | When the blood supply to part of the heart muscle is suddenly blocked. The heart muscle may be damaged or start to die if blood doesn't return quickly.  |
| <b>heart disease</b>                             | A general term for any condition that threatens the heart's ability to function normally. Because coronary artery disease (plaque buildup that may cause a heart attack) is by far the most common type, it is often just called heart disease.  |
| <b>hemochromatosis</b>                           | A condition in which too much iron builds up in the body, which can lead to organ damage.  |
| <b>hemoglobin</b>                                | A protein found in red blood cells that carries oxygen throughout the body   |
| <b>hemoglobin analysis</b>                       | A test that measures the different types of hemoglobin in the blood. It is used to diagnose diseases caused by abnormal hemoglobin, such as sickle cell anemia.  |
| <b>hereditary</b>                                | Genetically transmitted -- or capable of being transmitted -- from parent to child.  |
| <b>hereditary nonpolyposis colorectal cancer</b> | Hereditary non-polyposis colorectal cancer (HNPCC) is an inherited disorder in which there is a tendency to develop colorectal cancer without a significant number of polyp precursors. HNPCC is specifically associated with inherited mutations in five mismatch repair genes.                                   |
| <b>HFE gene</b>                                  | The HFE gene makes a protein that regulates how much iron your body absorbs from your diet.  |
| <b>high performance liquid chromatography</b>    | A laboratory procedure that can separate a liquid mixture into its individual compounds. As an example, this procedure is used is to separate different kinds of hemoglobins in a person's blood.  |
| <b>HNPCC-related cancer</b>                      | Other primary cancers included in an inherited cancer syndrome because of the increased prevalence in syndrome carriers. In addition to colon cancer, HNPCC-related cancers include cancer of the endometrium, ovary, stomach, kidney/urinary tract, brain, biliary tract, central nervous system and small bowel. |



| Term                                | Definition   |
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| <b>hormone</b>                      | Chemical messengers made mostly in our glands that influence our growth and development, sexual function, reproduction, mood, and metabolism. Hormone medications include oral contraceptive pills, patches or rings; hormonal treatments for infertility; hormone replacement therapy; or serum estrogen modifiers (sometimes taken to treat or prevent certain forms of cancer). |
| <b>human immunodeficiency virus</b> | A retrovirus that attacks the human immune system, thus affecting the body's ability to fight off the organisms that cause disease. HIV is the cause of acquired immune deficiency syndrome or AIDS.   |
| <b>hypertension</b>                 | Blood pressure that stays at 140/90 mmHg or higher over a period of time. Average blood pressure is about 120/80 mmHg.   |
| <b>IDL</b>                          | Intermediate density lipoprotein -- a type of "bad" cholesterol. High IDL increases the risk for heart disease even more than just high total LDL levels. IDL is under strong genetic control so close relatives of someone with high IDL should also consider testing.  |
| <b>in vitro fertilization</b>       | A laboratory procedure in which sperm fertilize eggs outside the body in a laboratory setting to facilitate pregnancy. The fertilized egg is then placed in the woman's uterus for implantation.   |
| <b>inherited</b>                    | Any trait that is passed from generation to generation through our genes. These traits may range from having a specific disease to how we respond to certain drugs to simply our physical characteristics, like eye and hair color.  |
| <b>inhibin A</b>                    | A substance made by the placenta during pregnancy and found in the mother's blood. Also abbreviated "DIA."   |
| <b>insulin</b>                      | A hormone that helps glucose, the sugar used by the body for energy, get into the cells that need it. When you don't make enough insulin or you can't use insulin effectively, you are likely to develop diabetes.   |
| <b>intermediate metabolizer</b>     | Intermediate metabolizers have a drug metabolism gene that doesn't work properly. They make less of the enzyme coded for by those genes, but usually make enough to process most drugs. People have many drug metabolism genes and can have be different kinds of metabolizers for each.   |

| Term                    | Definition   |
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| <b>iron overload</b>    | A condition in which higher-than-usual amounts of iron collect in the tissues of the body. Over time, iron overload can damage organs like the liver and cause problems like diabetes.   |
| <b>K-RAS</b>            | A gene that when mutated contributes to converting a normal cell into a cancerous cell.  |
| <b>LDL</b>              | Low-density lipoprotein cholesterol. Also called the “bad” cholesterol. High LDL increases the risk of heart disease.  |
| <b>LDLR</b>             | Stands for low density lipoprotein receptor. The LDLR gene normally makes a protein that helps to remove LDL ((bad≈ cholesterol) from the blood. An LDLR gene mutation causes a person not to get rid of LDL as quickly and it builds up. LDLR mutations are the most common cause of familial hypercholesterolemia.   |
| <b>leukemia</b>         | A cancer that starts in blood-forming tissue, such as the bone marrow, and causes large numbers of abnormal blood cells to be produced and enter the bloodstream.  |
| <b>lifestyle</b>        | In talking about health conditions, lifestyle generally refers to factors within your control like diet, physical activity, smoking, alcohol use, and use of other preventive health measures.   |
| <b>lipid</b>            | A fat that acts as a source of energy and helps the body use certain vitamins. Cholesterol and triglycerides are examples of lipids. High lipid levels increase the risk for heart disease and diabetes and may be caused by eating too much fat, alcohol use, inactivity, inherited conditions, and certain medications and disease.                              |
| <b>lipoprotein a</b>    | Lp(a) stands for lipoprotein a -- a type of “bad” cholesterol. High Lp(a) increases the risk of heart disease 2 to 10 times more than just high total LDL levels and may cause heart disease earlier than usual. Drug therapy is usually needed. Lp(a) is under strong genetic control so close relatives of someone with high Lp(a) should also consider testing. |
| <b>liver</b>            | An organ involved in a wide range of functions, including helping with digestion and the detoxification of chemicals.  |
| <b>liver biopsy</b>     | A surgical procedure that removes a small piece of liver so it can be examined in a lab.   |
| <b>lymphoma</b>         | Cancer that begins in the cells of the immune system.  |
| <b>maintenance dose</b> | The amount of drug that is needed over the long-term to reach a stable, therapeutic response.  |

| Term                                 | Definition  |
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| <b>malignant</b>                     | Cancerous. Malignant tumors, or cancer, have the ability to invade adjacent tissues and spread throughout the body. Thus, malignant tumors can become life threatening.   |
| <b>mammogram</b>                     | An X-ray picture of the breast. The x-ray images make it possible to detect tumors that cannot be felt. They can also find microcalcifications that may signal the presence of cancer.  |
| <b>maraviroc</b>                     | The generic name of Selzentry, a drug used to treat HIV infection that only works in people whose HIV uses a specific receptor (CCR5) to infect the cell.   |
| <b>maternal serum screening test</b> | A blood test that looks at the levels of certain substances in a pregnant woman's blood. These tests are used to find the risk for having certain birth defects. They can't tell for sure whether a pregnancy has a birth defect.                     |
| <b>MCH</b>                           | An abbreviation for "mean corpuscular hemoglobin". The average amount of hemoglobin in the average red blood cell. The normal range for the MCH is 27 - 32 picograms. MCH is a standard part of a CBC (complete blood count) test.                    |
| <b>MCV</b>                           | An abbreviation for "mean corpuscular volume". The average size of a red blood cell. The normal range for the MVC is 80 - 100 femtoliters. MVC is a standard part of the CBC (complete blood count) test.   |
| <b>Mediterranean</b>                 | Someone whose ancestors come from one of the countries bordering the Mediterranean Sea. These countries include but are not limited to: Spain, southern France, Italy, and Greece.  |
| <b>metabolic syndrome</b>            | Also called "insulin resistance". A combination of factors (like abnormal cholesterol, abdominal obesity, high blood sugar, and high blood pressure) that increases the risk of getting both heart disease and diabetes.                              |
| <b>metabolism or metabolize</b>      | The way drugs and other substances are broken down for use in the body and elimination.   |
| <b>metastasis</b>                    | The spread of cancer from one part of the body to another.  |
| <b>methylation</b>                   | A process by which a methyl group is added to the DNA base cytosine. This process often decreases the amount of gene product that is made. For example, tumor suppressor genes are often methylated which decrease their function and lead to cancer. |
| <b>mlh1</b>                          | A mismatch repair (MMR) gene located on chromosome 3. Mutations in MLH1 are associated with Lynch syndrome (also called HNPCC) and greatly increase the chance of cancer -- especially colon.   |

| Term                              | Definition   |
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| <b>MMR gene</b>                   | Mismatch repair gene, a gene that functions as a part of the “spell check” system of a cell. Mutations in MMR genes are involved in causing some hereditary cancer syndromes.  |
| <b>morbidity</b>                  | A diseased state.  |
| <b>MSH2</b>                       | A mismatch repair (MMR) gene located on chromosome 2. Mutations in MLH1 are associated with Lynch syndrome (also called HNPCC) and greatly increase the chance of cancer -- especially colon.  |
| <b>multifactorial inheritance</b> | Conditions that are caused by an interaction between more than one gene and environmental (non-genetic) factors. Most common human diseases seem to be multifactorial, including diabetes, heart disease, mental illness, and most birth defects. A family history of a multifactorial condition usually increases the risk for other relatives. |
| <b>multiple myeloma</b>           | Cancer that begins in the cells of the immune system.  |
| <b>multisite</b>                  | Multisite Testing looks for the three BRCA gene mutations that cause 80% to 90% of all hereditary breast and ovarian cancers in Ashkenazi Jewish people. This test gives you a clear result: either you have one of these three mutations, or you don't. If you don't, it is possible to have a different BRCA mutation that was not tested for. |
| <b>mutation</b>                   | A change in the DNA code that may cause a gene not to function in the normal way.  |
| <b>newborn screening</b>          | Testing that is done routinely after birth, to look for serious developmental, genetic and metabolic disorders. This testing is done so that important medical treatments or other actions can start before symptoms develop.  |
| <b>niacin</b>                     | Also called “nicotinic acid”. Part of vitamin B3 found in foods like meat, fish, milk, eggs, green vegetables, and grains. Niacin supplements increase HDL, lower Lp(a), and to a lesser degree, lower LDL cholesterol. Common brand names include: Niacor, Niaspan, Nicolar, Nicotinex Elixir, and Slo-Niacin.                                  |
| <b>non-invasive procedure</b>     | Procedures that do not require insertion of an instrument or device through the skin or a bodily orifice for diagnosis or treatment.   |
| <b>Noonan syndrome</b>            | A genetic disorder that causes abnormal development of many parts of the body. It can be caused by a defect in one of four different genes (KRAS, PTPN11, RAF1, SOS1). Noonan syndrome may be inherited from a parent who has the condition, or may happen by chance in a pregnancy.   |

| Term                      | Definition  |
|---------------------------|---|
| <b>obesity</b>            | Having a high amount of body fat. Usually defined by a body mass index (BMI) of 30 or higher.   |
| <b>omega 3-fatty acid</b> | Also called “fish oil”. Omega-3 fatty acids from eating oily fish or taking fish oil supplements may lower triglycerides, slow the buildup of plaque in the arteries, and raise HDL (“good”) cholesterol. Too much omega-3 fatty acid is dangerous, so you should always talk to your doctor before starting supplements. |
| <b>organs</b>             | A grouping of tissue that works together to perform a common function. Examples of organs include: stomach, lungs, and liver.   |
| <b>osteoma</b>            | Benign, bony tumors often on the skull or mandible (sometimes a clinical finding with FAP patients).  |
| <b>over-the-counter</b>   | OTC or over-the-counter drugs can be bought without a prescription. OTC drugs still carry certain risks and may interact with other drugs.  |
| <b>P-53</b>               | A gene which normally regulates the cell cycle and protects the cell from damage to its genome. Mutations in this gene cause cells to develop cancer.   |
| <b>PAPP-A</b>             | Short for “pregnancy-associated plasma protein A”, a substance found in pregnant women's blood. Low levels of PAPP-A at 8-14 weeks of pregnancy have been associated with risk for Down syndrome and pregnancy complications.   |
| <b>pedigree</b>           | A diagram of biological relationships that usually includes information on each relative's medical history.   |
| <b>premenopausal</b>      | The time when a women is entering menopause until it is complete -- often defined as from the time periods become irregular until 12 months after the last period.  |
| <b>phenotype</b>          | Characteristics that can be seen or measured and are often the result of genes and environment working together. Examples include things like eye color, weight, IQ, cholesterol levels, or drug response.  |
| <b>phenotyping</b>        | Tests that measure specific traits or characteristics that can be caused by genes and/or environmental factors. This is in contrast to genotype testing that only looks at genetic information.   |
| <b>placebo</b>            | A phony treatment or “sugar pill”. Researchers often compare people taking a drug with those taking a placebo to better measure the real effects of the drug.   |

| Term                    | Definition   |
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| <b>placenta</b>         | Also called the afterbirth, the placenta is the tissue that connects the developing baby to the mother's uterus. It develops as part of the pregnancy and has the same DNA as the developing baby. The placenta allows for the exchange of nutrients, waste and gases between the developing baby and the mother.                  |
| <b>plaque</b>           | Related to heart disease, plaque is the buildup of cholesterol, calcium, and other substances on the inside walls of the arteries causing the arteries to be more narrow and less flexible.  |
| <b>plasma</b>           | The liquid part of the blood that carries blood cells and other components   |
| <b>polymorphism</b>     | Natural differences in a DNA sequence that are usually common and do not cause disease   |
| <b>polyp</b>            | A usually non-cancerous growth or tumor protruding from the lining of an organ, such as the colon. Left untreated, polyps have an increased risk of becoming cancerous.  |
| <b>poor metabolizer</b> | Produce inactive drug metabolism enzyme or no enzyme at all. Poor metabolizers may have a reduced response or no response and may have increased side effects  |
| <b>poor metabolizer</b> | Poor metabolizers have a pair of drug metabolism genes that don't work properly. They make very little or none of the enzyme coded for by that pair of genes. This causes slower metabolism or the inability to process certain drugs. People have many drug metabolism genes and can be different kinds of metabolizers for each. |
| <b>postmenopausal</b>   | The time in a woman's life after menopause is complete -- often defined as starting 12 months after the last period.   |
| <b>pre-cancerous</b>    | Condition of the tissue, such as a polyp, that can turn into a cancer if not treated or removed.   |
| <b>preconception</b>    | Generally considered the period of time when a person is planning pregnancy but has not yet conceived (become pregnant).   |
| <b>pre-diabetes</b>     | Diagnosed when glucose (sugar) levels are higher than normal, but not high enough to make the diagnosis of diabetes -- usually a fasting glucose of 100 to 125 mg/dL or a glucose of 140 to 199 mg/dL after glucose tolerance test.  |
| <b>predisposition</b>   | Any condition, genetic or other, that renders an individual more susceptible to disease.   |

| Term                                       | Definition   |
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| <b>preimplantation genetic diagnosis</b>   | A technique used with in vitro fertilization to test early-stage embryos for disease-causing genes, so that embryos without the disease-causing genes can be implanted in the mother's uterus.   |
| <b>prenatal diagnosis</b>                  | Testing for diseases in the fetus or embryo before it is born.   |
| <b>presymptomatic</b>                      | The stage prior to an individual presenting with symptoms that are clinically relevant to the disease in question.   |
| <b>prophylactic bilateral mastectomy</b>   | A risk-reducing treatment where both breasts, as well as some of the surrounding tissue, are surgically removed in order to keep cancerous cells from forming.   |
| <b>prophylactic bilateral oophorectomy</b> | A risk-reducing treatment where ovaries are surgically removed in order to keep cancerous cells from forming; recommended after childbearing is complete.  |
| <b>protein</b>                             | Large, complex molecules made of amino acids that form body structures, enzymes, hormones, and antibodies. Proteins are all made based on the instructions in our genes. The amino acids we need to make new proteins are consumed in the protein we eat or made by the body.  |
| <b>protein(s)</b>                          | The molecules that form the body, allow it to grow, and regulate how it works. Our bodies make the proteins we need using the instructions from our genes.   |
| <b>receptor</b>                            | A protein on the surface of a cell that only binds with certain other molecules. When this happens, a cellular process can occur.  |
| <b>rectum</b>                              | The last portion of the digestive tract, at the end of the colon.  |
| <b>red blood cells</b>                     | A cell in the blood that carries oxygen to all parts of the body. Also called an erythrocyte.  |
| <b>risk factor</b>                         | Anything that increases the chance of developing a certain disease or having a child with a specific condition. Risk factors might include your family history, lifestyle, other health conditions, blood test results, age, gender, and countless other factors.  |
| <b>sarcoma</b>                             | A cancer that begins in bone, cartilage, fat, muscle, blood vessels, or other connective or supportive tissues.  |
| <b>screening</b>                           | In medicine, screening generally refers to a test or exam that is reasonably simple, inexpensive, and harmless that can be given to a large group of people in order to find a smaller group with a higher-than-average chance for a certain condition. These people will sometimes have more specific testing or be treated early before symptoms appear. |

| Term   | Definition   |
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| <b>selective estrogen receptor modulator</b> | Selective Estrogen Receptor Modulator (SERM) is a hormone-like drug that affects multiple tissues by interacting with receptors for the hormone estrogen. A particular SERM may have estrogen-like effects in some tissues and anti-estrogen effects in others.              |
| <b>Selzentry</b>                             | The brand name of maraviroc, a drug used to treat HIV infection that only works in people whose HIV uses a specific receptor (CCR5) to enter the cell.   |
| <b>sequencing</b>                            | A lab method that looks at each DNA nucleotide (A,T,G, and C) in a piece of DNA for differences (mutations) from the usual DNA sequence. A more labor intensive and expensive test that is often used when the specific mutations that cause a disease aren't known.         |
| <b>serum CA-125</b>                          | A blood test used in an effort to detect ovarian cancer.   |
| <b>serum ferritin</b>                        | A protein your body makes when it stores iron.   |
| <b>siblings</b>                              | Brothers and/or sisters.   |
| <b>sickle cell disease</b>                   | An inherited disorder in which the red blood cells have an abnormal crescent shape that affects blood flow. This disorder causes anemia because the abnormal blood cells don't survive long.   |
| <b>sickle/beta-thalassemia</b>               | A disease that occurs when someone inherits a sickle-cell anemia gene mutation from one parent and a beta-thalassemia gene mutation from the other parent. Symptoms are usually very similar to sickle cell disease.   |
| <b>side effect</b>                           | An unintended and usually undesired reaction to a drug or treatment.   |
| <b>Single Site</b>                           | Single Site Testing looks for just one BRCA mutation. This test can only be done for people who know the DNA sequence of a BRCA mutation that is running in their family. This test gives you a clear result: Either you have the mutation that was tested for or you don't. |
| <b>southeast Asian</b>                       | Someone whose ancestors come from one of the countries south of China and east of India. These countries include but are not limited to: Vietnam, Cambodia, Laos, Burma, or Indonesia.   |
| <b>spleen</b>                                | An organ in the abdomen that supports the immune system, destroys and filters out old blood cells, and holds a reserve of blood cells. People can live without a spleen.   |
| <b>sporadic</b>                              | In reference to cancer, this means a cancer not caused by hereditary genetic mutations. Most cancers are sporadic.   |



| Term                              | Definition   |
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| <b>statin</b>                     | A group of drugs that lower the amount of cholesterol made naturally by the liver. When diet and exercise changes aren't enough, statins are often the first choice for drug therapy. Commonly prescribed statins include: Lovastatin (Mevacor, Altoprev), Pravastatin (Pravachol), Simvastatin (Zocor), Fluvastatin (Lescol), Atorvastatin (Lipitor), and Rosuvastatin (Crestor). |
| <b>Stevens-Johnson syndrome</b>   | An allergic reaction to a drug or infection that causes flu-like symptoms, skin wounds, and may affect other organs like the eyes and mouth.   |
| <b>stroke</b>                     | Caused by a sudden lack of blood supply and oxygen to the brain. Usually happens because either a blood clot blocks a blood vessel in the brain (ischemic stroke) or a blood vessel breaks and bleeds into the brain (hemorrhagic stroke).   |
| <b>symptom</b>                    | Any sign that a person has a condition or disease. Symptoms, like headache, fever, fatigue, nausea, vomiting, and pain, may not be specific but together point to an underlying cause.   |
| <b>symptoms</b>                   | Changes or signs that are caused by or accompany a disease or condition. Symptoms are the evidence of that underlying disease or condition. Symptoms can be used to help diagnose a problem.   |
| <b>tamoxifen</b>                  | A drug commonly used to treat patients with breast cancer, certain other cancers, and those at high risk for breast cancer. It works by interfering with the activity of the hormone estrogen, which feeds the growth of many, but not all breast cancers.   |
| <b>toxic epidermal necrolysis</b> | A life-threatening allergic reaction started by certain drugs, infections, illnesses, and unknown factors. TEN can cause large areas of the skin to peel away, flu-like symptoms, and other complications. The condition gets worse quickly and usually requires hospitalization.  |
| <b>transferrin saturation</b>     | The percentage of transferrin (a protein that carries iron in the blood) that is currently carrying iron.  |
| <b>translocation</b>              | A genetic condition where material from one chromosome breaks off and sticks to another chromosome, or switches places with a part of another chromosome. There are different types of translocations, and they can have different effects on health and development.  |
| <b>transvaginal ultrasound</b>    | A type of ultrasound done by inserting an ultrasound probe into the vagina. This allows a view of a woman's reproductive organs, including the uterus, ovaries, cervix, and vagina.  |

| Term                          | Definition   |
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| <b>triglycerides</b>          | A type of energy-rich fat. High triglycerides (over 200mg/dL) increase the risk for heart disease and stroke.  |
| <b>tropism</b>                | The specific cell types that a virus can recognize and infect.   |
| <b>tumor</b>                  | An abnormal mass of tissue that results from excessive cell division. Tumors may be benign (not cancerous) or malignant (cancerous).   |
| <b>Turner syndrome</b>        | A genetic condition in which a girl or woman does not have the usual pair of two X chromosomes. Instead, some or all of her cells are missing an X chromosome, or part of an X chromosome. Symptoms are variable but usually include short stature and infertility.  |
| <b>ultra metabolizer</b>      | Have more than two functional copies of a drug metabolism gene, and produce a larger-than-normal amount of enzyme. Ultra metabolizers may have a reduced or no response and may have increased side effects  |
| <b>ultrarapid metabolizer</b> | Ultrarapid metabolizers have extra copies of a gene involved in drug metabolism, so they make more enzyme than the average person. This results in faster metabolism of drugs processed by that enzyme.  |
| <b>umbilical cord</b>         | The cord that connects the developing baby to the placenta, which is attached to the mother's uterus. The umbilical cord carries oxygen- and nutrient-rich blood to the developing baby.   |
| <b>unconjugated estriol</b>   | One of the three main estrogens produced by the body. Low levels of this substance are associated with risk for certain birth defects, including Down syndrome and trisomy 18. Also abbreviated "uE3."   |
| <b>variant</b>                | Gene variations contribute to diversity and make people unique. When a certain form of a gene is seen in at least 1% of people, but not most people, it is called a variant. Variants may also increase or decrease a person's risk for certain genetic diseases but usually don't cause the disease themselves. |
| <b>vein</b>                   | Blood vessels that carry blood low in oxygen back to the heart.  |
| <b>virtual colonoscopy</b>    | A method of examining the colon by taking a series of X-rays (called a CT scan) and using a high-powered computer to reconstruct 2-D and 3-D pictures of the interior surfaces of the colon from these X-rays.   |

| Term                     | Definition  |
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| <b>VKORC1</b>            | A gene that tells the body how to make vitamin K epoxide reductase (VKOR), an enzyme important in forming blood-clotting factors. A common VKORC1 gene variant (-1639G>A) puts people at increased risk for complications when taking warfarin at standard doses. |
| <b>VLDL</b>              | Very low density lipoprotein -- a type of “bad” cholesterol. High VLDL increases the risk for plaque buildup in the arteries and heart disease.   |
| <b>VLDL3</b>             | A subtype of VLDL (a “bad” cholesterol). High VLDL3 increases heart disease risk the most and is a risk factor even when total cholesterol levels are normal. Diet and exercise changes are very effective for lowering VLDL3.                                    |
| <b>warfarin</b>          | The most commonly prescribed drug for preventing harmful blood clots from forming or from growing larger. Belongs to a class of drugs called anticoagulants or “blood thinners.”  |
| <b>white blood cells</b> | A cell found in the blood whose primary job is to defend the body against infection.  |
| <b>xanthoma</b>          | Fat buildup that looks like a yellow lump under the skin, most commonly on the heels, hands, elbows, other joints, feet, and buttocks. Especially common in people with inherited high cholesterol like familial hypercholesterolemia.'                           |