Rapid Sequencing of Known Mendelian Genes in Neonatal ICU Patients (NICU)

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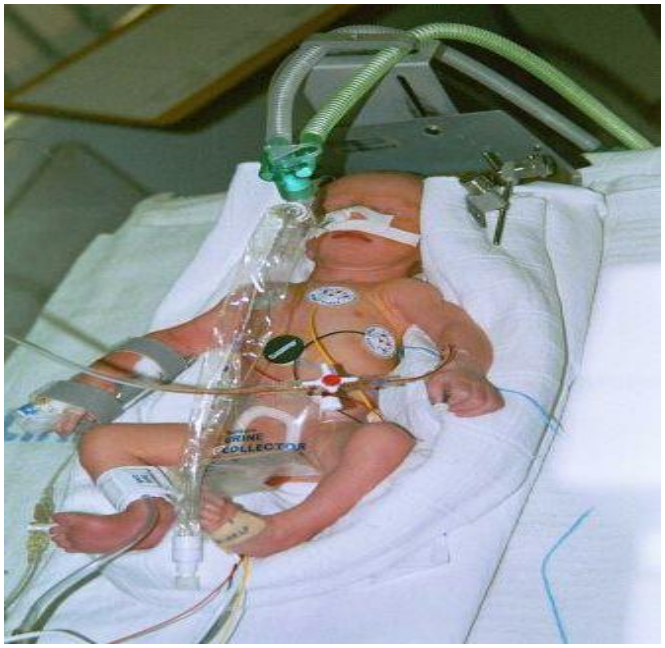
Objectives

- Describe the state-of-the-art next-generation sequencing technology improving the precision medicine in NICU patients.
- Describe the collaboration between ARUP and Primary Children Medical Center (PCMC) NICU on the rapid sequencing of known Mendelian genes panel (RapidSeq Panel)
- Explain the advantages of rapid turnaround RapidSeq Panel in relation to whole exome and whole genome sequencing.
- Discuss the limitations, case selections, and present the interesting cases.

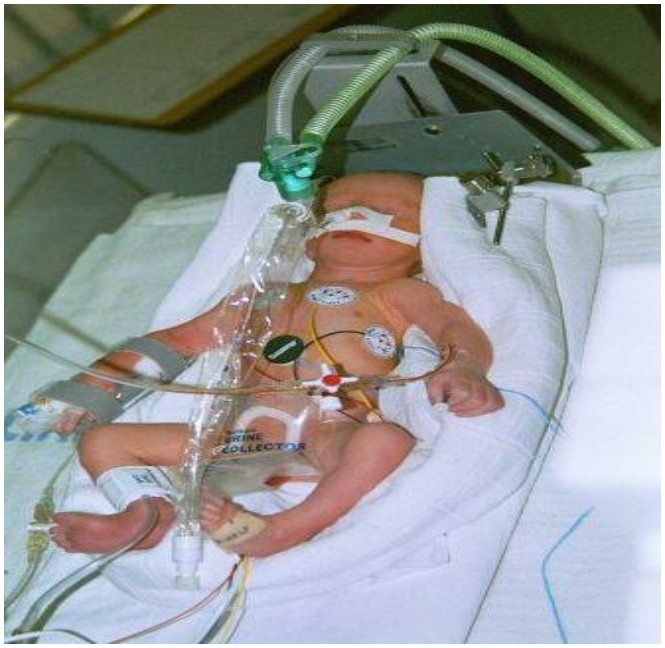
Case 1

- Two weeks old, Caucasian male
- Respiratory failure, incubated at delivery
- Hypotonia, absence of intentional movements, absence of cranial nerve reflexes, contractures of shoulder, upper and lower limbs
- mildly dilated hypertrophied right ventricle



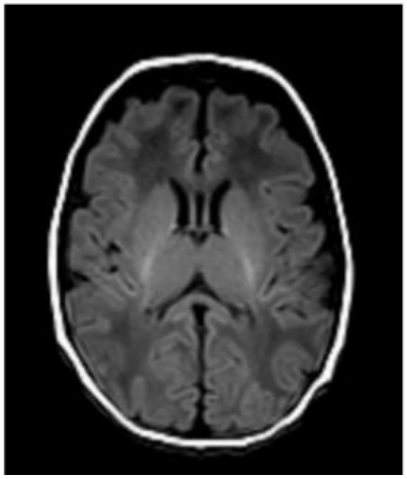
Case 1

- Dysmorphic facial features: sparse eyebrows, sloping forehead, thickened nares, recessed jaw
- Small penis and undescended testes
- Born at term with birth weight of 3520 grams (42%) and length at 75th percentile and HC at 17th %
- First child, no family history of the same condition



Case 1

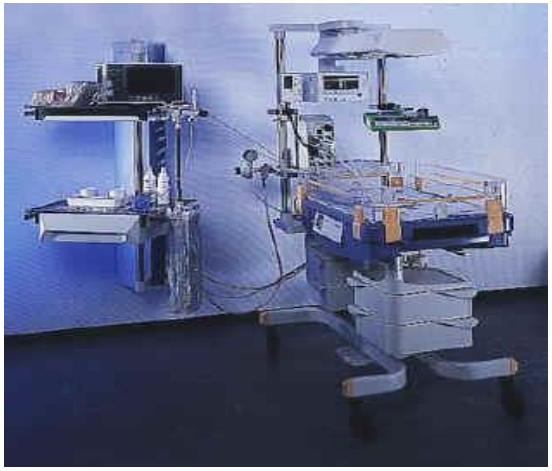
- Normal Laboratory testing
  - brain MRI
  - SMA testing (spinal muscular atrophy)
  - carbohydrate deficient transferrin (CDG)
  - SNP microarray



Neonatal ICU (NICU)

- SOME BABIES NEED SPEICAL CARE
  - If a child is premature or has health problems at birth, such as an infection or respiratory stress, he or she may need to spend some time in the special area with facilities for special care …..

THE NICU



Most Common Diseases in NICU

- Respiratory Distress Syndrome (RDS)
- Transient Tachypnea of the Newborn (TTN)
- Patent Ductus Arterious (PDA)
- Meconium Aspiration Syndrome (MAS)
- Persistent Pulmonary Hypertension (PPHN)
- Wilson-Mikity Syndrome (Pulmonary Dysmaturity)
- Bronchopulmonary Dysplasia (BPD)
- Diaphragmatic Hernia
- Pulmonary Barotrauma and Air Leak Syndromes
- Trans-illumination
- Necrotizing Enterocolitis (NEC)
- Congenital Cardiac Anomalies

10-15% of patients are due to monogenic genetic diseases

NICU - High Mortality

- According to the March of Dimes, about 150,000 babies are born with birth defects each year in the United States.
- 3 out of every 100 babies born in the United States have some kind of major birth defect, severe, NICU
- Birth defects can be caused by genetic, environmental, or unknown factors. For most birth defects, the cause is believed to be an interaction of a number of genetic and environmental factors.
- High mortality

Costs analysis - PCMC at UU

- The median variable cost per day is $1,379, with the 75th percentile at $1,662
- **Median stay in NICU is 35 days**
- Looking at 2013-2016, we average ~38 deaths per year in the NICU
- ~35 per year have support withdrawn
- The reasons given: (table)

Courtesy to Steven Bleyl

Age when support withdrawn (2013-2016)

Mortality Primary Cause of	Patients	Average Age (Days) at death
Anomaly or Syndrome	35	22
Central Nervous System(CNS) Injury	23	13
GI/Intra-abdominal Catastrophe	23	19
Inborn Error	1	7
Infection	6	57
Massive Hemorrhage/Coagulopathy	2	41
Multi-organ System failure	8	20
Other	7	45
Renal Failure	1	28
Respiratory Failure	33	59
Grand Total	139	32

Courtesy to Steven Bleyl

Critical for NICU

- The quick and precise diagnosis of disease
  - Neonatal presentation atypical: hypotonia, respiratory distress, abnormal metabolism
- Effective treatment
  - Many diseases are treatable: metabolic diseases
- Reduce mortality rate

ARUP+PCMC Spring, 2015

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- Pinar Bayrak-Toydemir
- Karl Voelkerding
- Brendan O'Fallon
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- Steven Bleyl (director of medical stewardship)
- Luca Brunelli (chief of NICU)
- Ted Pysher
- Lorenzo Botto
- Robert Christensen
- Christian Con Yost
- Elizabeth O'Brien
- Stephen Guthery
- Joshua Bonkowsky
- Betsy Ostrander
- Susan Morelli
- Seth Andrews
- Jim Gudgeon
- Roger Faiz

Rapid Genomic Sequencing (RapidSeq Panel) for NICU

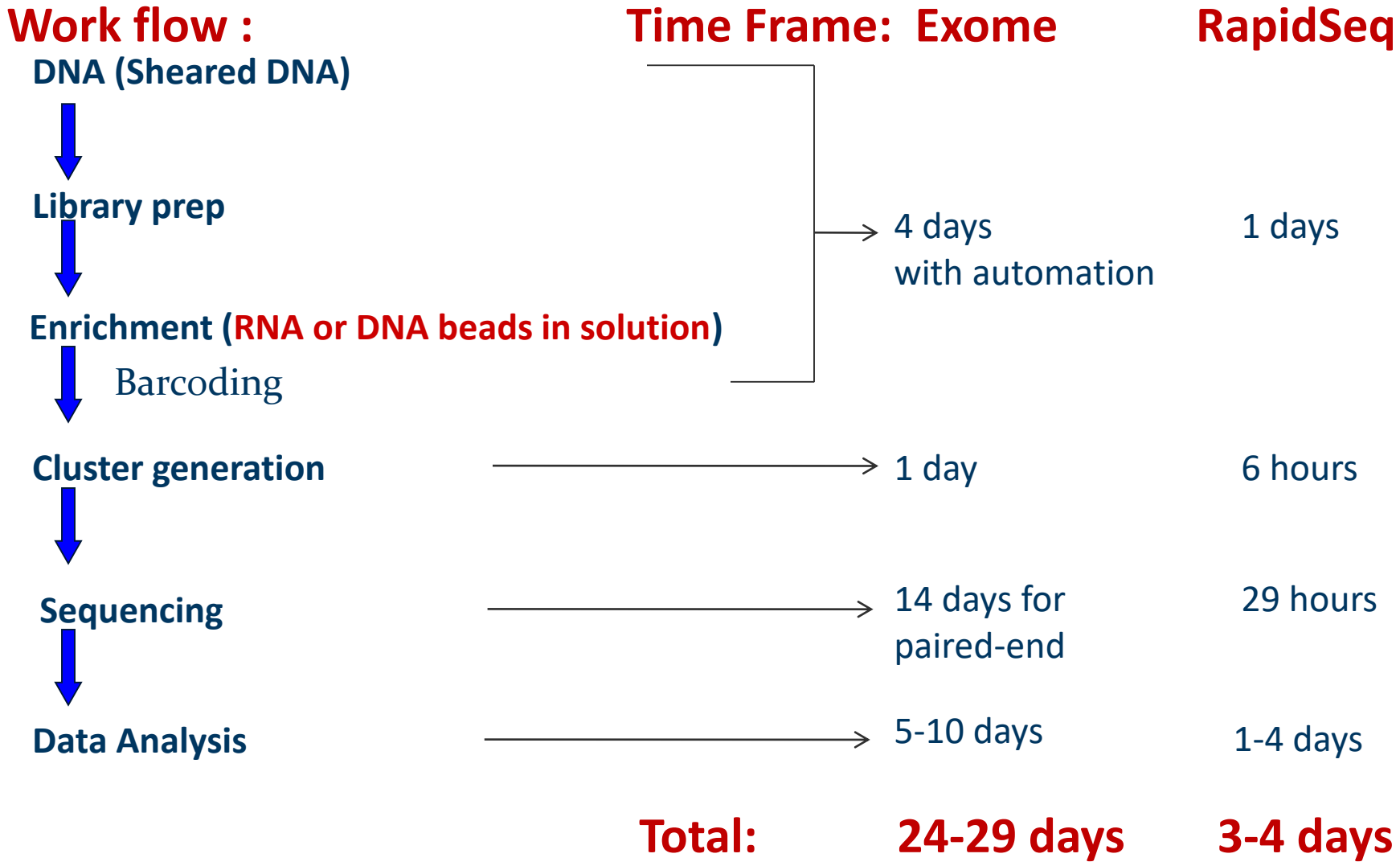
- Content:
  - 4,503 HGMD genes for inherited diseases
  - 98% Overlapping with OMIM 4000 known disease-causing genes

- Advantage
  - Targeted 15 Mb design
  - Consistent performance and high uniformity
  - Cost: 4 days in NICU

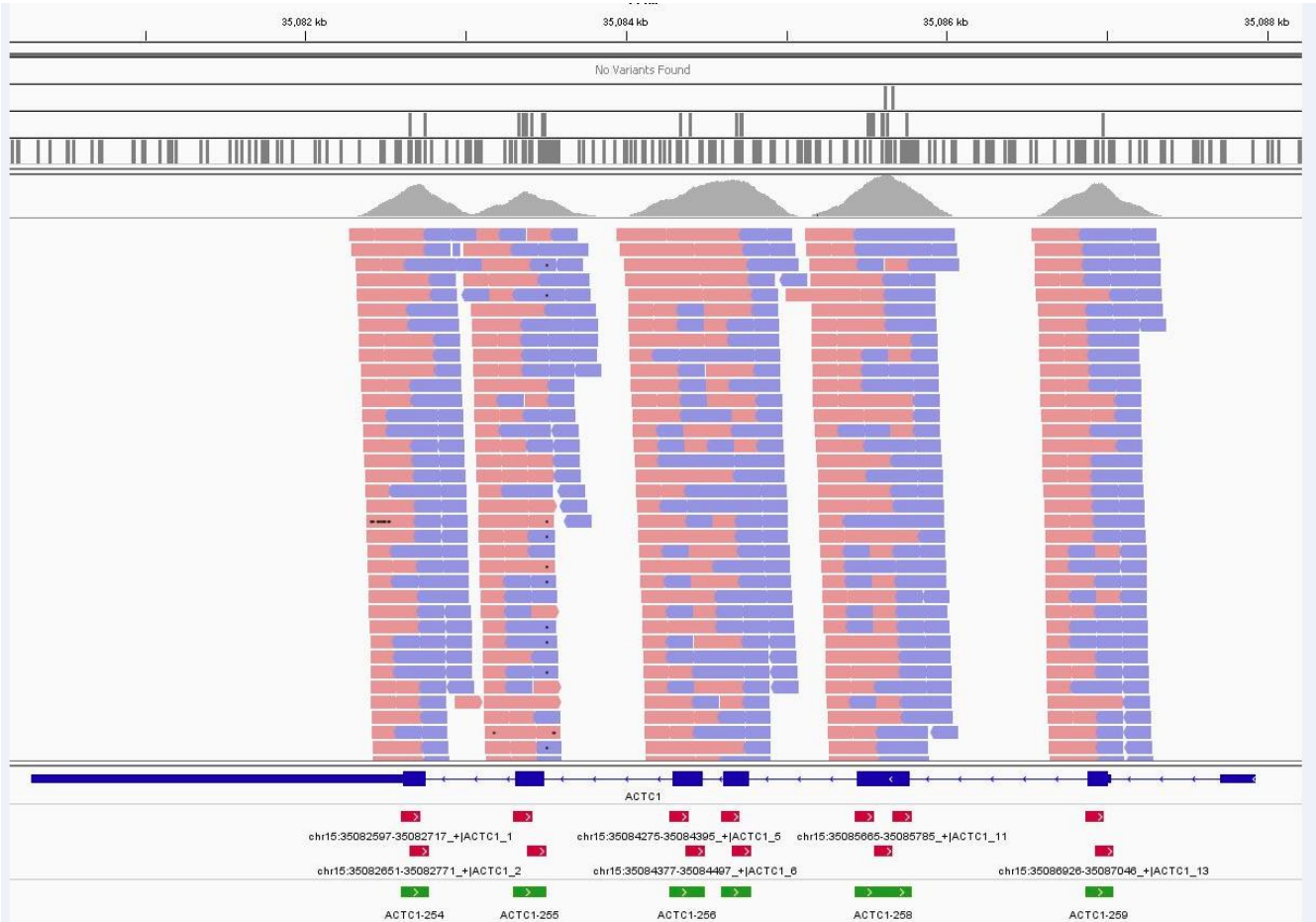
Why 4503 Gene Panel?

- Compare 4500 gene panel vs Exome vs whole genome
  - Includes most known disease-causing genes
  - Rapid TAT: 3-4 days of 4500 gene panel vs 24-29 days for exome
  - Cost effective: 1/6 of whole genome sequencing cost
  - Focus on targeted region, deep sequencing 300-500X mean

Rapid Turnaround Time



RapidSeq Coverage >99% for the targeted regions with 20X



Comparison RapidSeq vs Exome Tests

Test	Rapid Seq Panel	Exome Sequencing
Genes	4500+	~20,000
TAT	14-28 days	8-12 weeks
Family Members	Parental samples required (trio)	Proband only, Trio, Trio + other informative family members
ACMG Secondary Variants	Reported if desired for proband only	Reported if desired for proband and family members
Sanger Confirmation	When necessary	Yes

Courtesy to Tatiana Tvrdik

Process Overview

High acuity candidate
patient as judged by NICU staff and consultants

NICU/Consultant:
- Do **Pre-test counseling**
- Consultant fills out forms:
  - **patient phenotype**
  - **consent documents**
- Database (REDCAP database) entry by ordering CGI

Testing

Preliminary results (7 days):
- Counseling
- Management changes
- Data entry by CGI

Final results (3-4 weeks):
- Counseling
- Management changes
- Data entry

NICU RTIDS committee review of data and results quarterly

- NICU, <1 year only (to start)
- Trio (parents/child)
- Consent and intake done by ordering MD

Rapid Seq Test Inclusion Criteria

- Patient's disease is plausibly monogenic
- Making diagnosis will alter acute decision-making
- Alternative testing is not available, more costly or protracted
- Trio (patient and both parents) available

Brunelli et al.2018, submitted manuscript

Sample Collection

- Submit everything together
  - Trio: proband and parents' samples
  - Signed consent
  - Completed patient history form
  - Abnormal genomic microarray or MRI results
  - Clinical and genetics summary notes
- Turnaround time won't start until everything received

Courtesy to Chris Miller

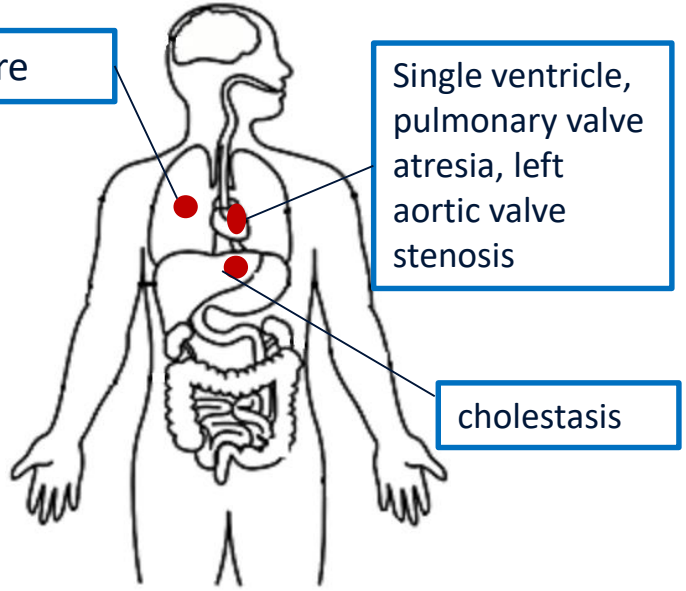
Consent

- Genetic counselor, NICU attending, nurse
- Should be face to face
- We are only examining 4500 genes with known function out of over 20,000 that exist
- Even if genes have known function, some mutations are not detectable with this technology
- Some conditions are not caused by DNA changes
- Not report ACMG secondary finding **(Revised)**

Courtesy to Chris Miller

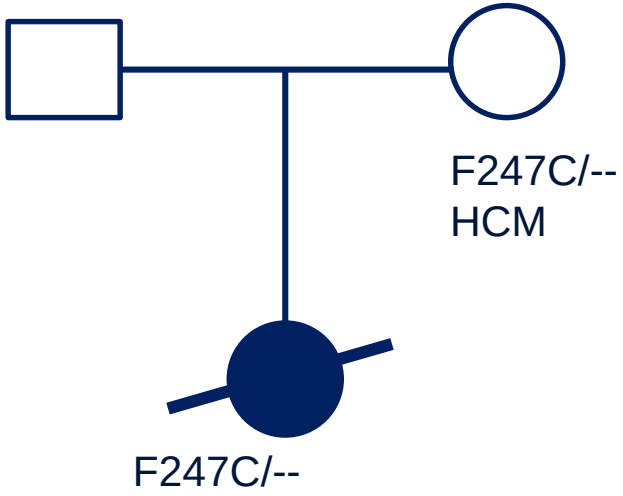
Case 2

- 38-day old female
- Hydrops and respiratory failure
- Heart defect: prenatal ultrasound found single ventricle, pulmonary valve atresia, left aortic valve stenosis
- Recently progressive cholestasis, suspect Dx of Alagille syndrome
- Family hx: mother has HCM and carrying *MHY7* variant c. 740T>G, p.Phe247Cys (LP)
- Died at 3 months, from congenital heart defect leading to respiratory failure

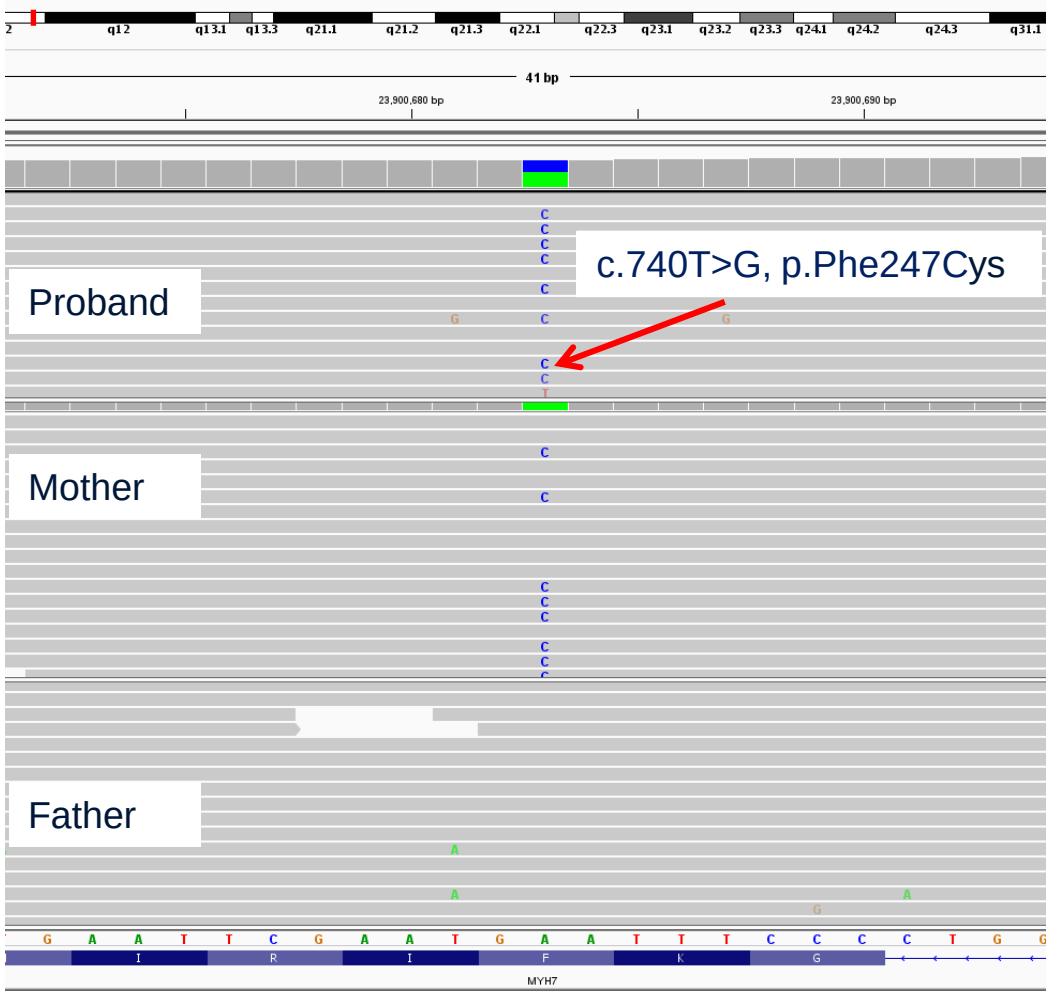


Case 2

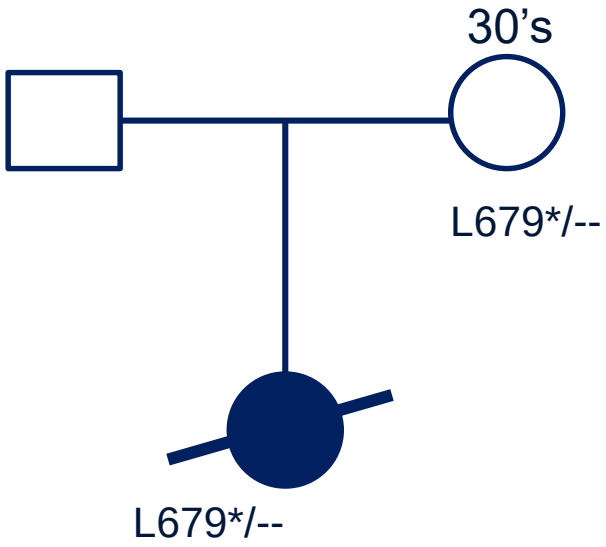
- Results: Negative



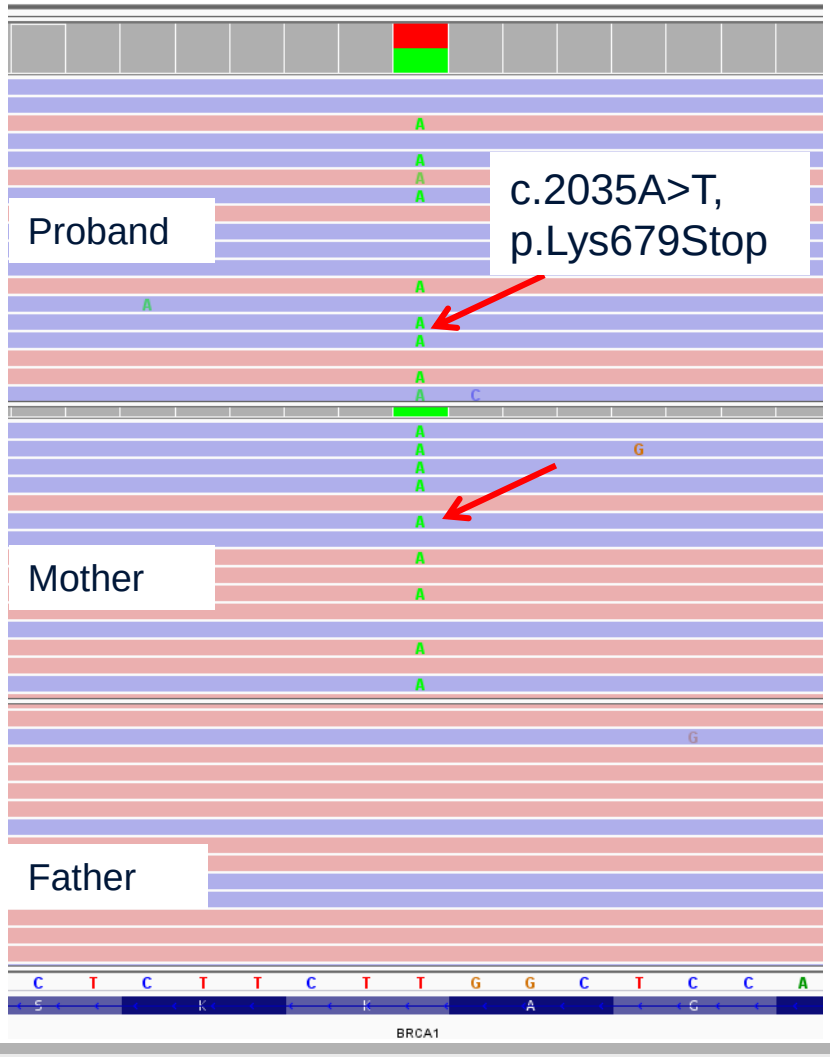
MYH7 Variant



Case 2: Secondary Finding



BRCA1 Variant



Results Could Be Upsetting

- Results may not be informative
- Child may have a condition that is not treatable
- A parent could carry or be at risk for the same medical condition as child
- Parents may have an increased risk to have additional children with the same condition

Courtesy to Chris Miller

Parental Samples

- Must accompany infant's sample
- Must know if parent has any birth defects, learning problems, other conditions, etc.
- Full sequencing is performed
- Used to determine significance of variant
  - Is variant de novo?
  - Is variant in healthy adult?
  - Confirm autosomal recessive

Courtesy to Chris Miller

Non-Paternity

- Will be detected
- May render result uninterpretable
- If this is a possibility, tell GC before sequencing

Data Analysis and Interpretation

- Preliminary Report: 7-14 days
  - Disease-causing mutation responsible for patient phenotype
    - Recessive disease: one loss of function mutation and one variant
  - De novo mutations
  - Sanger confirmation when necessary (deletion/insertions)
- Follow-up call to make sure someone received

Data Analysis and Interpretation

- Final Report: 21-28 days
- Positive report: lists gene and variant(s) believed related to phenotype
- Negative for preliminary report, 4500 genes analysis and report based on proband phenotype
  - Uncertain report: several gene variants will be reported that may or may not be causative for phenotype
  - Negative report: no variants detected believed related to phenotype; does not mean genetic cause has been excluded

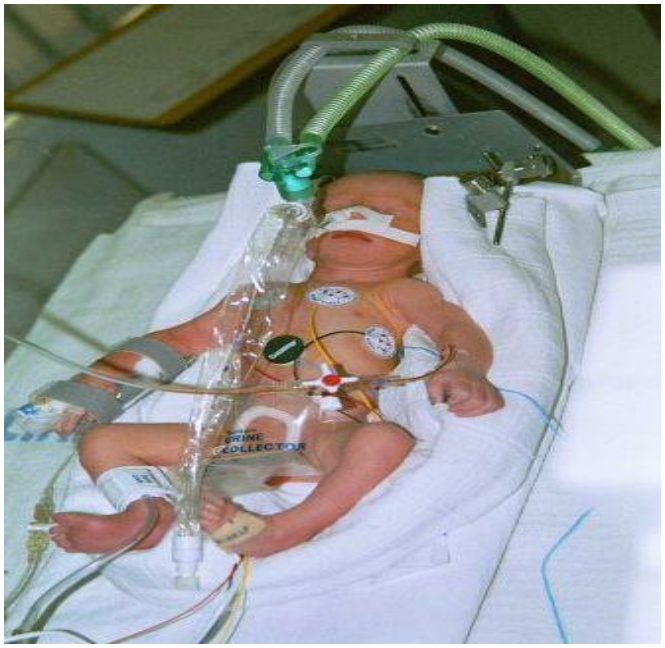
Storage and Information Sharing

- De-identified DNA stored indefinitely
  - For test validation purposes
- De-identified data shared with national/international databases
  - Aids clinicians understanding of significance of variants

Courtesy to Chris Miller

Case 1

- Perform RapidSeq in two weeks after the baby admitted to NICU
- Trio (proband and parents)



Case 1

- HGMD Matches: 1% frequency, 27 variants

ruplab.net:8080/VarViewer/

wer

Sample : 15281404284_RAP_MiSeq

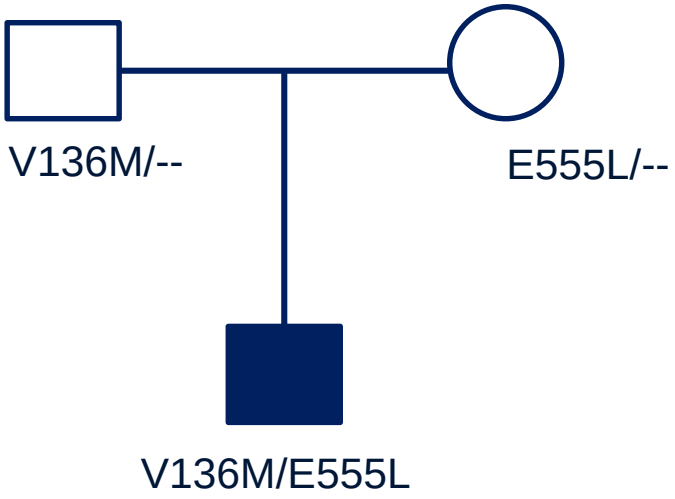
CHAT

Gene	Exon effect	Zygosity	c.dot	p.dot	Pop. Freq. MAF	HGMD & OMIM	dbSNP #	IGV	15281404293-OME	15281404295-OME
CHAT	missense_variant	Het	c.406G>A	p.Val136Met	0	HG OM	rs201479289		het	Ref
CHAT	missense_variant	Het	c.1663G>A	p.Glu555Lys	0	HG OM	-		Ref	het

Father

Mother

CHAT gene

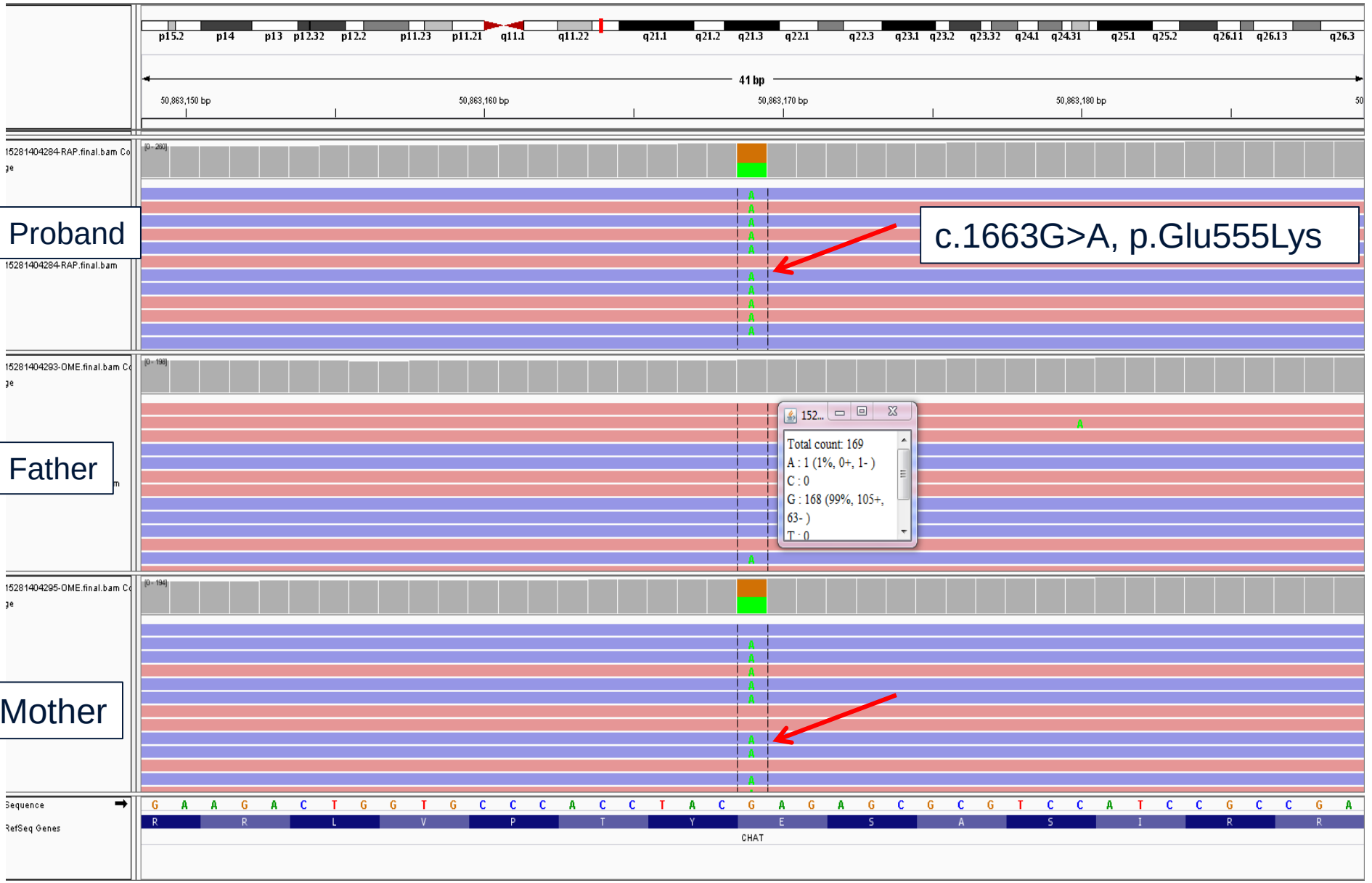


Case 1

Mutation 1 in *CHAT*

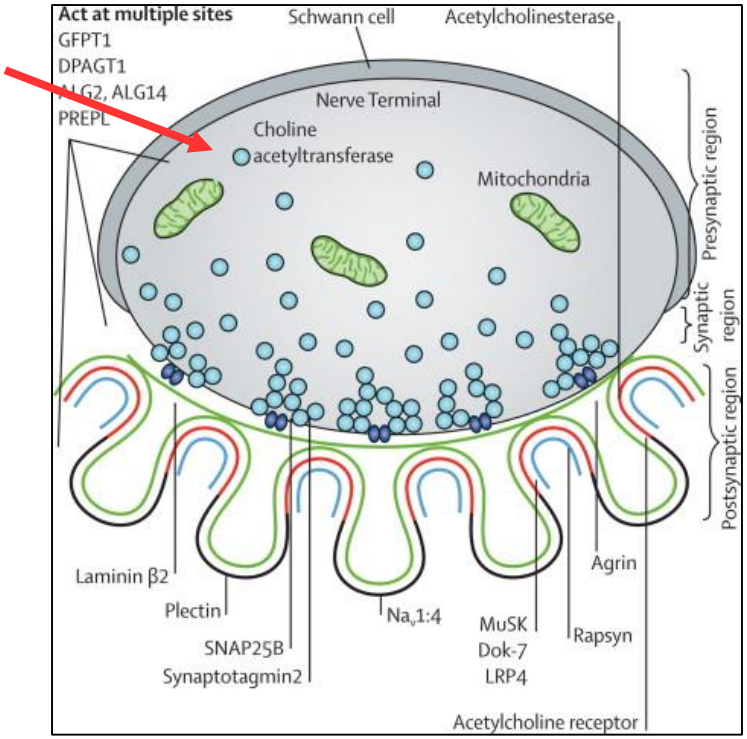
Case 1

Mutation 2 in *CHAT*



Case 1 *CHAT* Gene

- *CHAT*: Choline Acetyltransferase (OMIM:254310). It is the biosynthetic enzyme for the neurotransmitter acetylcholine in the central and peripheral nervous systems



Case 1 *CHAT* Gene Mutations

- Congenital Myasthenic Syndrome (CMS)
- Autosomal Recessive
- Affect the neuromuscular junction. Presents with muscle weakness between infancy and adulthood: hypotonia, respiratory distress/insufficiency due to muscle weakness, arthrogryposis, etc.
- Treatment: acetylcholinesterase inhibitors: Neostigmine (anitcholinesterase agent)

Case 1

- Preliminary report within 5 days
- Final report in 7 days
- Patient had a trial treatment with Neostigmine, and improved motor function. Discharged from NICU a week later.

Quick diagnosis of the disease can make differences in patient care.

Brunelli, et al, AJMG 201712:1002-1005

Case 3

- A PICU patient
- Clinical information
  - Four months old female, Native American ancestry
  - Acute liver failure and cholestasis
  - No family history of any birth defects, intellectual deficiency
  - Purpose for testing: rule out genetic causes of liver failure. Physician request to pay special attention to *CIRH1A (UPT4)* and *MPV17* genes; autosomal recessive childhood cirrhosis and progressive liver failure

Case 2

- PICU Trio to test:
  - Proband
  - Parental samples

Case 3

- Data review and interpretation
  - *CIRH1A* and *MPV17* gene well-covered by NGS, no rare variants detected
  - A panel of cholestasis and liver failure, well-covered and no rare variants

AGL, ABCB11, ABCB4, ABCB11, ABCC2, ABCG5, ABCG8, AKR1D1, ALDOB, ARG1, ASL, ASS1, ATP7B, ATP8B1, BAAT, BCS1L, CC2D2A, CCR5, CFTR, CLDN1, CPT1A , CYP27A1, CYP7A1, CYP7B1, DGUOK, DHCR7, **EPHX1,** FAH, FBP1, G6PC, GAA, GBE1, GYS2, HFE,HNF1B, HSD3B7, IFNG, INVS, JAG1, LIPA, MBOAT7, MKS1, MPV17, NOS3, NOTCH2, NPC1, NPC2, NPHP1, NPHP3, NPHP4, NR1H4, PCK1, PEX1, PEX10, PEX11B, PEX12, PEX13, PEX14, PEX16, PEX19, PEX2, PEX26, PEX3, PEX5, PEX6, PEX7, PHKA2, PHKB, PHKG2, PKHD1, PRKCSH, POLG, PTPRC, PYGL, SERPINA1, SLC2A2, **SLC10A1,** SLC25A13, SLC27A5, SLC37A4, SLCO1B3, SLCO1B1, SMPD1, TJP2, TMEM216, TRMU, TYR, UGT1A1, VIPAS39, VPS33B.

Negative result is important prior liver transplantation.

Limitation of RapidSeq Panel

- Regions missing
  - deep intronic region, 5’ and 3’ UTRs, repetitive regions, and pseudogenes
- Genes not included
  - trinucleotide repeats, imprinting genes, etc.
- Detect CNVs: in development, not ready yet

Case 4

- 15 days old, baby girl, Caucasian
- Delivered by emergent C-section at 32 weeks gestation due to non-immune hydrops
- Cyanotic and without respiratory effort
- Thrombocytopenia, possible contractures
- No family history
- In NICU after birth

Courtesy to Tatiana Tvrdik

Case 4

Previous testing

- Normal karyotype
- Normal CMA SNP
- MPS Screen – suggestive of Mucopolysaccharidosis type VII

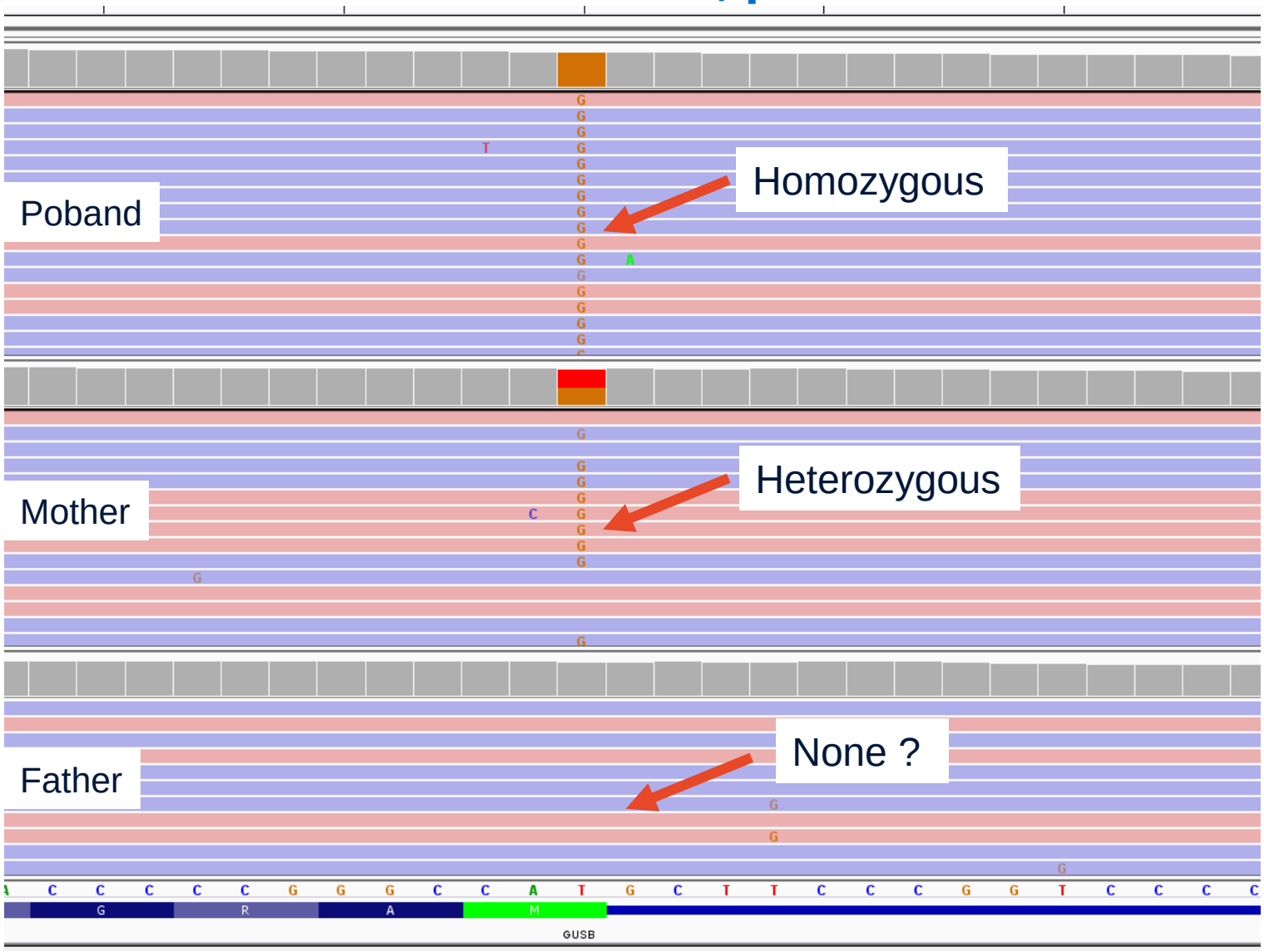
RapidSeq

- Trio (proband+parents)

Courtesy to Tatiana Tvrdik

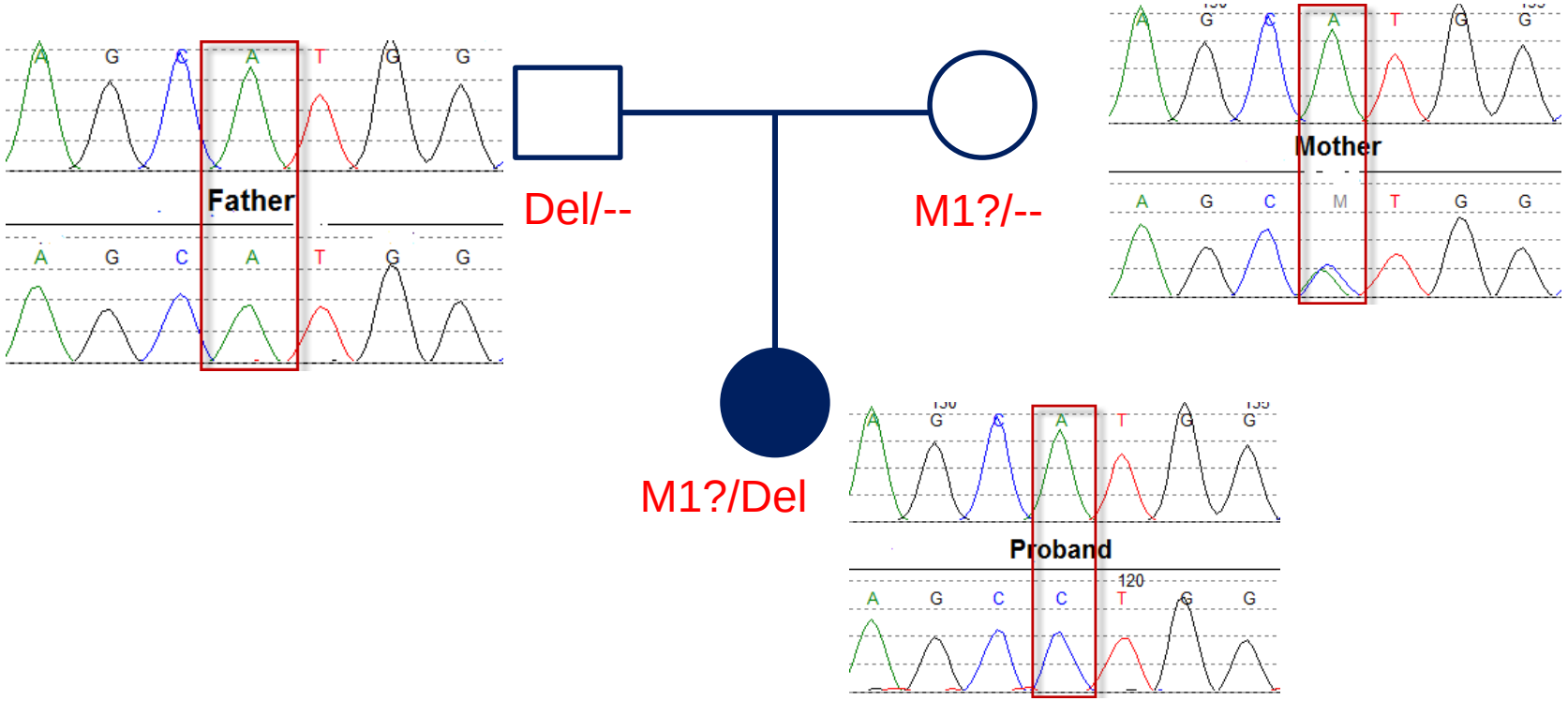
Case 4

GUSB c.1A>C, p.Met1? Variant



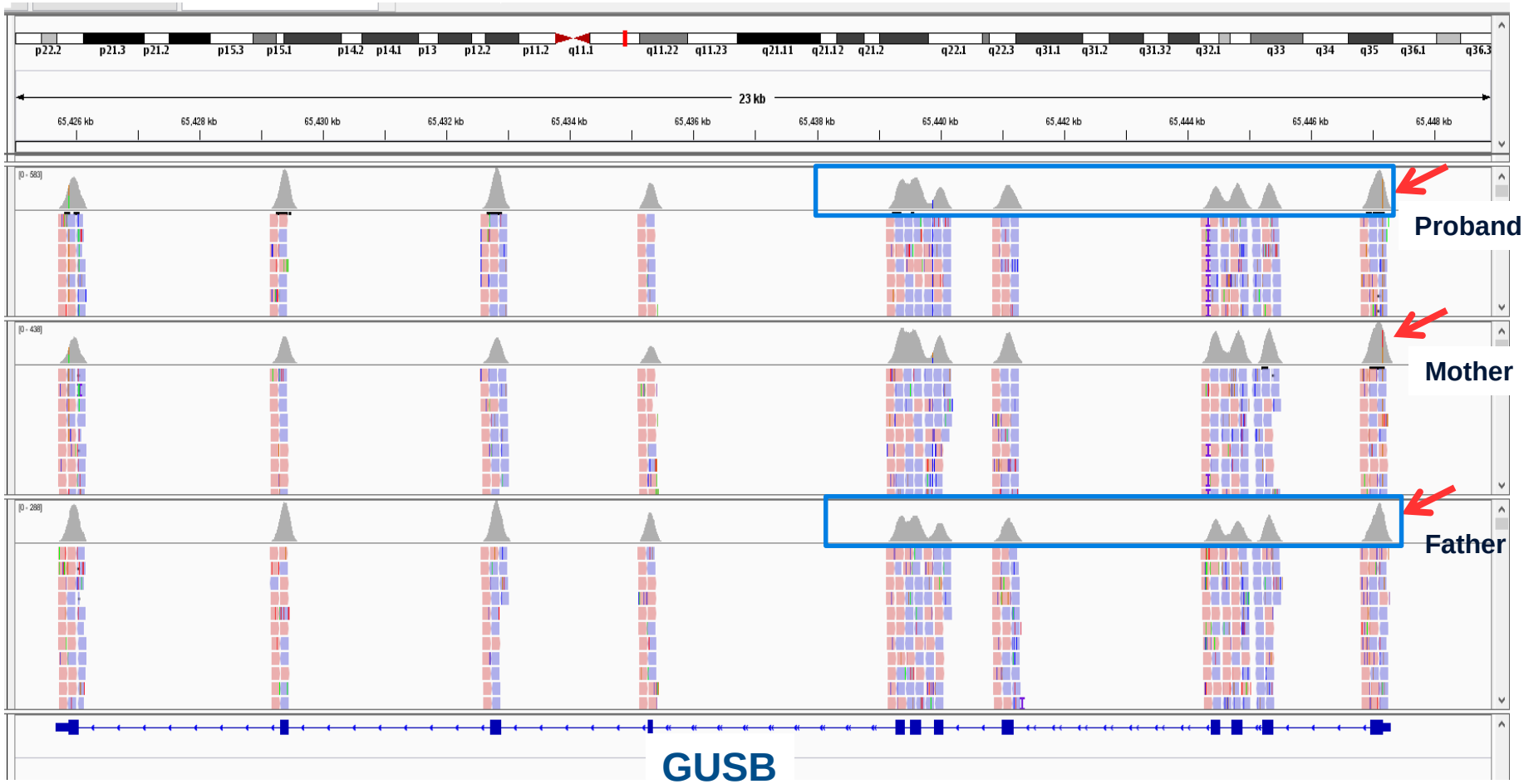
Case 4

GUSB: Autosomal recessive, Mucopolysaccharidosis VII (MPS7, OMIM:611499)



Case 4

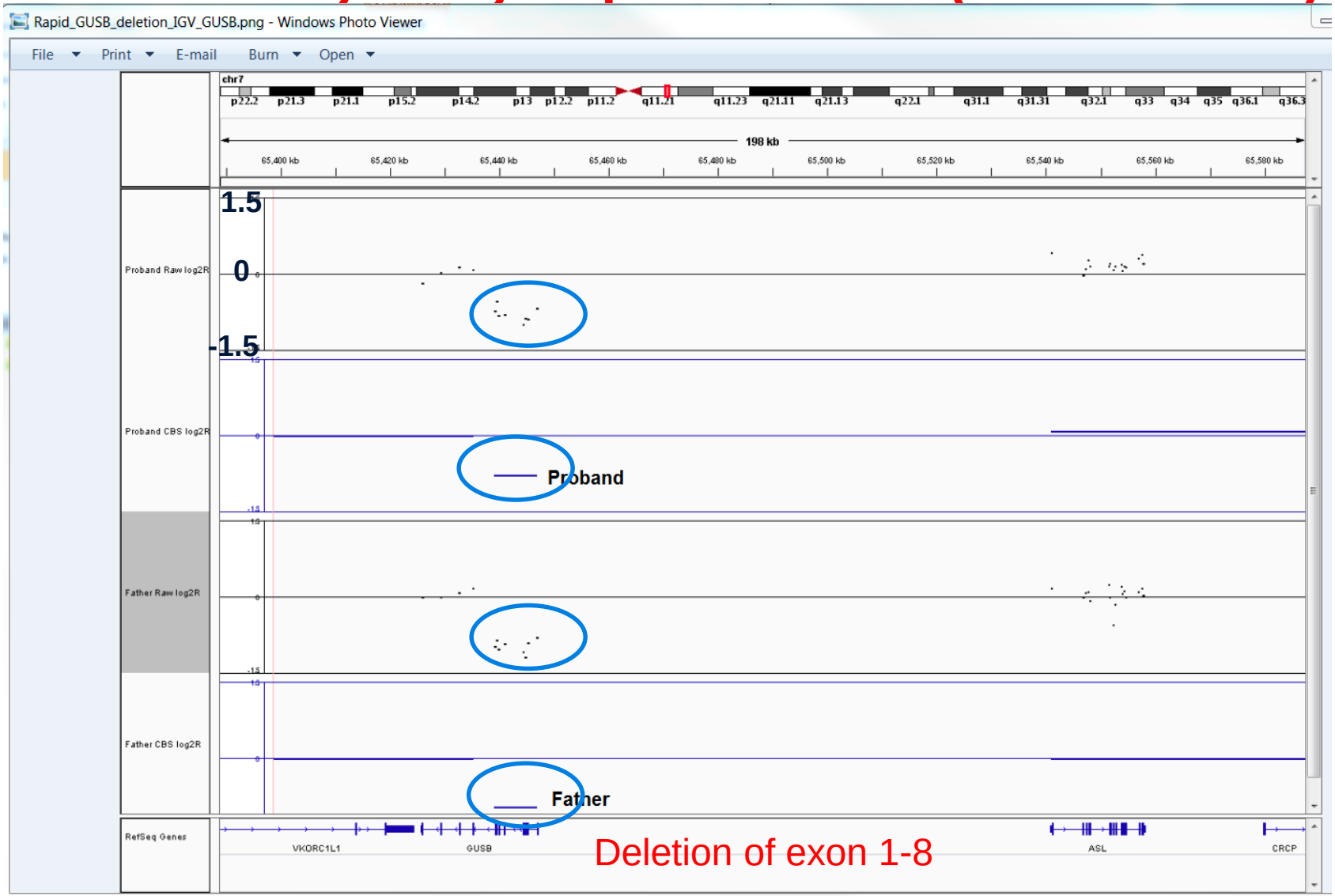
IGV GUSB



Courtesy to Tatiana Tvrdik

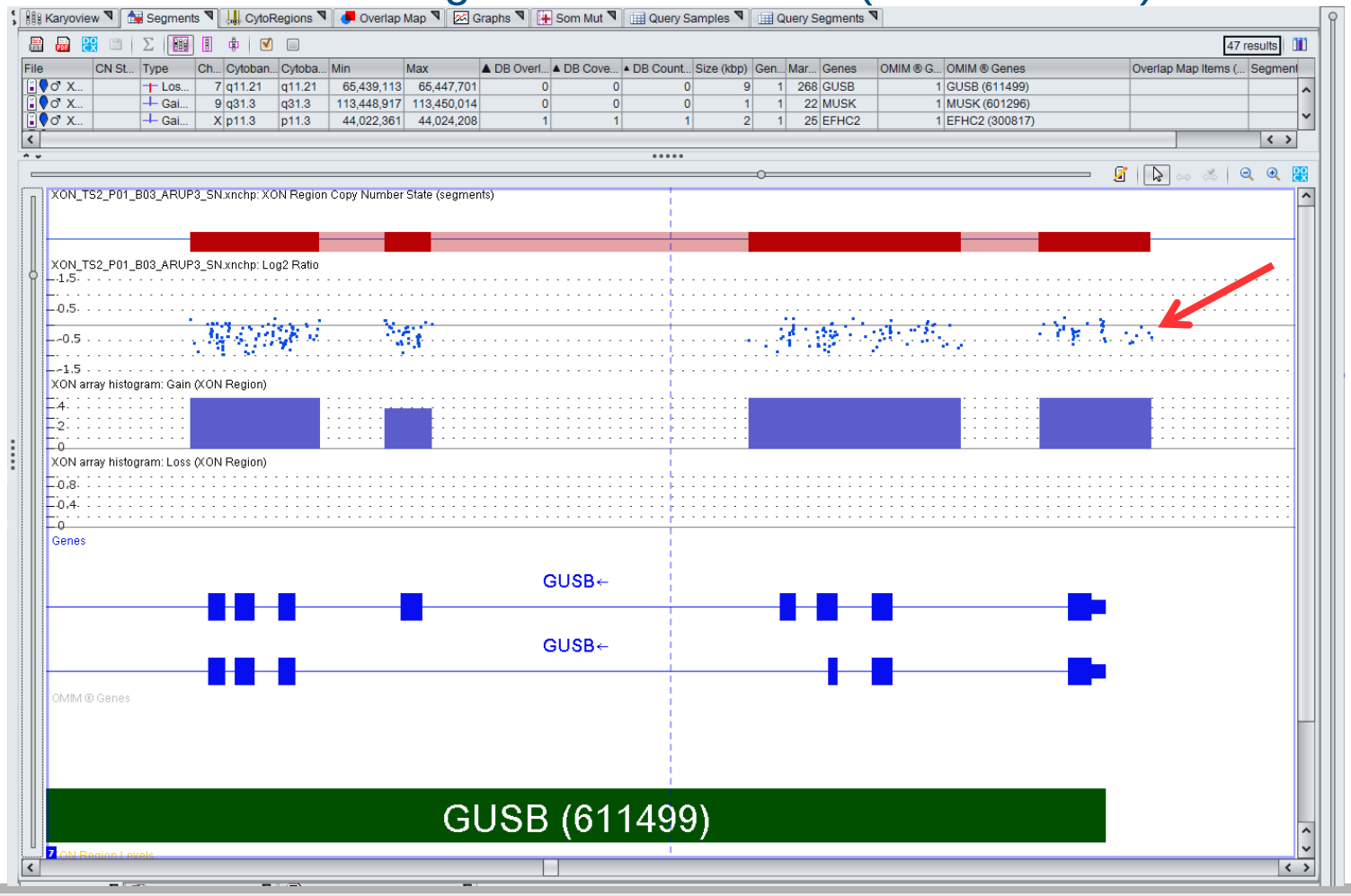
Case 4

CNV Analysis by Depth of Reads (Dr. Wei Shen)



Confirmed by CytoScan XON (Exome Microarray)

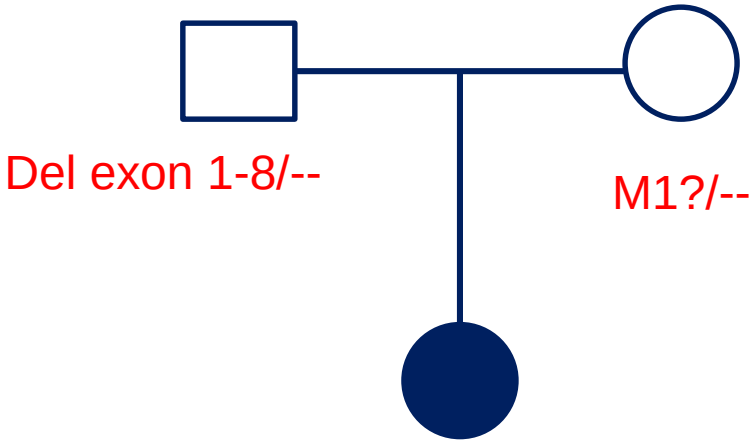
9KB deletion including GUSB exons 1-8 (268 markers)



Courtesy to Tatiana Tvrdik and Xinjie Xu

Case 4 Genotype

GUSB



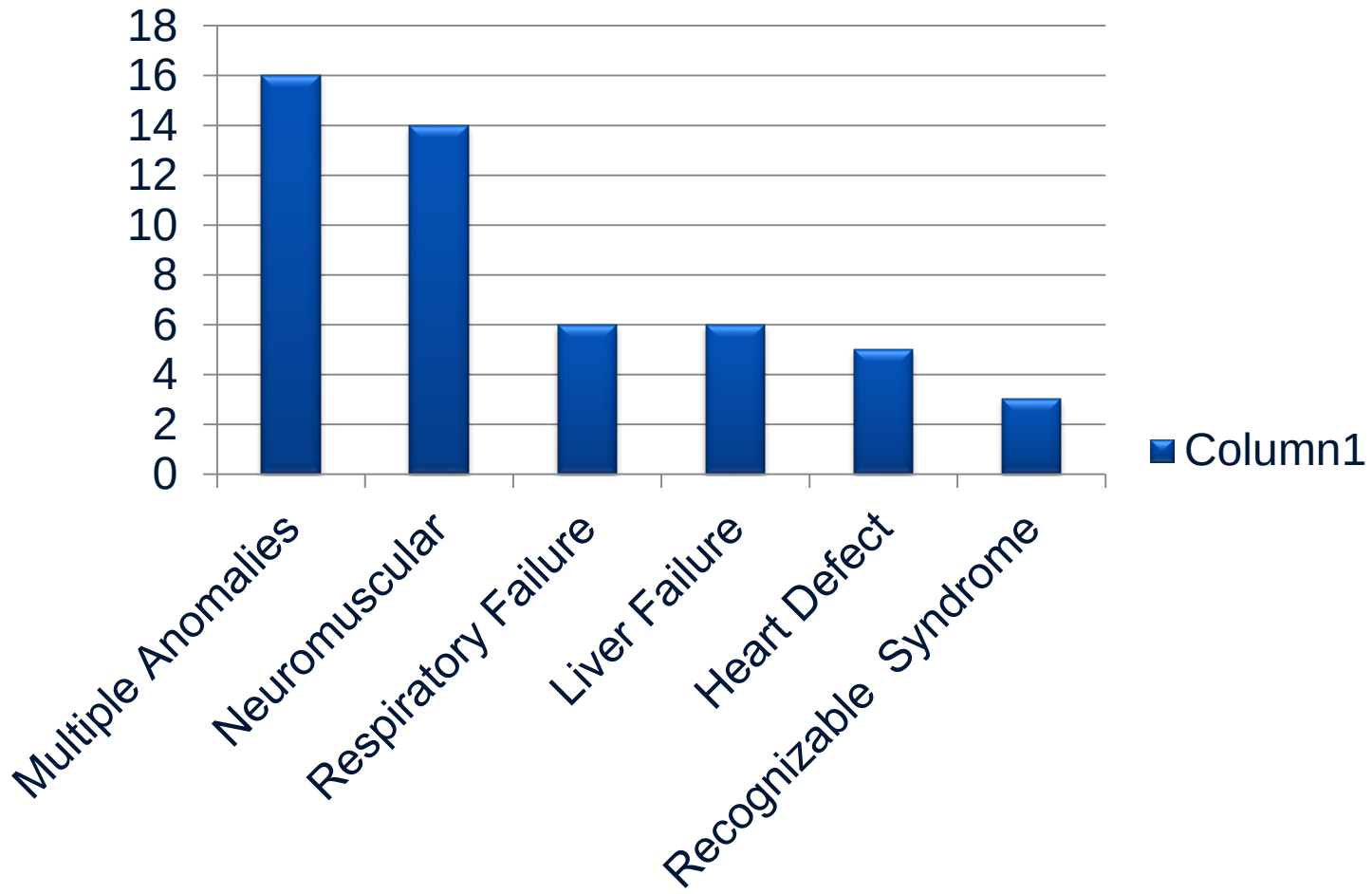
Mucopolysaccharidosis type VII

- Also known as Sly syndrome
- Caused by biallelic pathogenic variants in the *GUSB* gene
- Defect in the enzyme: beta glucuronidase
- Accumulation of GAGs (glycosaminoglycans) causes dysfunction of multiple organs
- Skeletal anomalies and short stature, pulmonary disease, cardiovascular complications, joint stiffness, hepatosplenomegaly, hernias, coarse features, corneal clouding, and varying degrees of intellectual disability, prenatal hydrops

Courtesy to Tatiana Tvrdik

Summary of first 50 Cases

Phenotypes


18
16
14
12
10
8
6
4
2
0
Multiple Anomalies
Neuromuscular
Respiratory Failure
Liver Failure
Heart Defect
Recognizable Syndrome
Column1

RapidSeq Diagnostic Yield and TAT

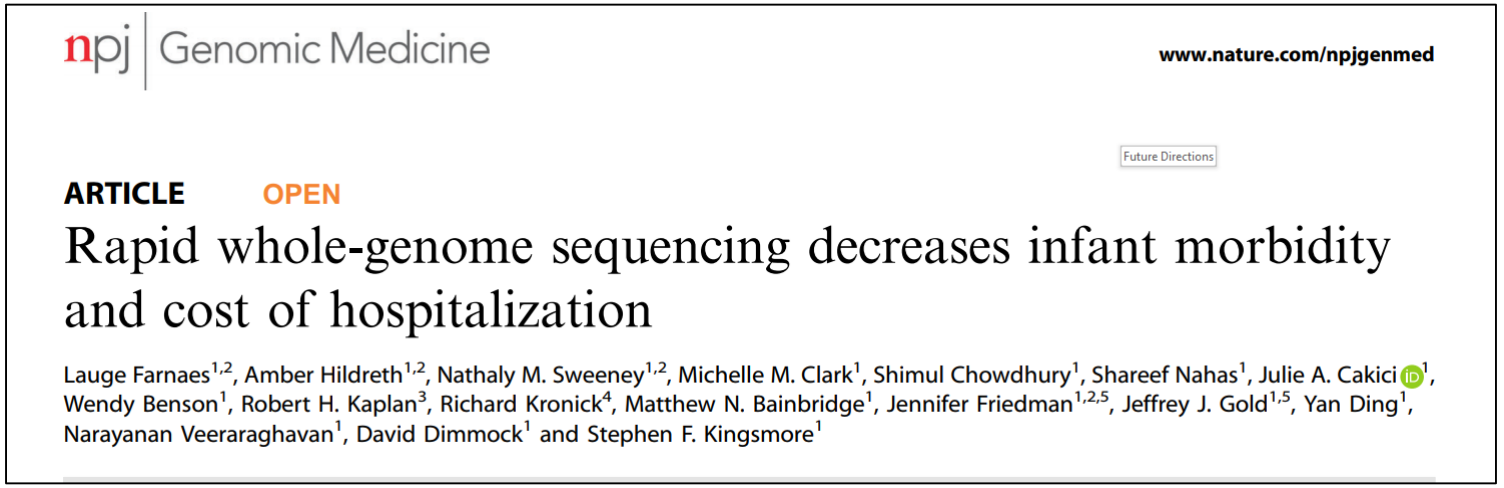
- Positive: 25/50 (50%)
- Twenty-five positive cases: 13 De Novo, 11 Recessive, 1 dominant (maternal inherited)
- Partially positive 1/50 (2%) – inherited
- Negative 20/50 (40%)
- Uncertain 4/50 (8%)
- All genes are OMIM/HGMD known disease-causing genes
- Average prelim report issued in 9 days, final reports in 16 days (8-37 days)

Other Rapid Tests on the Market

- Baylor: Critical Trio Whole Exome Sequencing, TAT 2 weeks
- GeneDx: XomeDxXpress (WES with a verbal Result in 7 Days), TAT 2 weeks
- Ambry: ExomeNext-Rapid, TAT 8-14 days
- PerkinElmer: WES – STAT– 7-10 days
- Fulgent: Clinical Exome (4681 genes)
- Rady: rapid Whole Genome Sequencing (rWGS) (currently research only)

Courtesy to Tatiana Tvrdik

Rapid Whole Genome Sequencing



npj | Genomic Medicine

www.nature.com/npjgenmed

Future Directions

ARTICLE OPEN

Rapid whole-genome sequencing decreases infant morbidity and cost of hospitalization

Lauge Farnaes[1,2], Amber Hildreth[1,2], Nathaly M. Sweeney[1,2], Michelle M. Clark[1], Shimul Chowdhury[1], Shareef Nahas[1], Julie A. Cakici[1], Wendy Benson[1], Robert H. Kaplan[3], Richard Kronick[4], Matthew N. Bainbridge[1], Jennifer Friedman[1,2,5], Jeffrey J. Gold[1,5], Yan Ding[1], Narayanan Veeraraghavan[1], David Dimmock[1] and Stephen F. Kingsmore[1]

Rady Children Hospital: rapid Whole Genome Sequencing (rWGS) in NICU, PICU and cardiovascular intensive care unite (currently research only)

npj Genomic Medicine (2018) 3:10

Rapid Whole Genome Sequencing

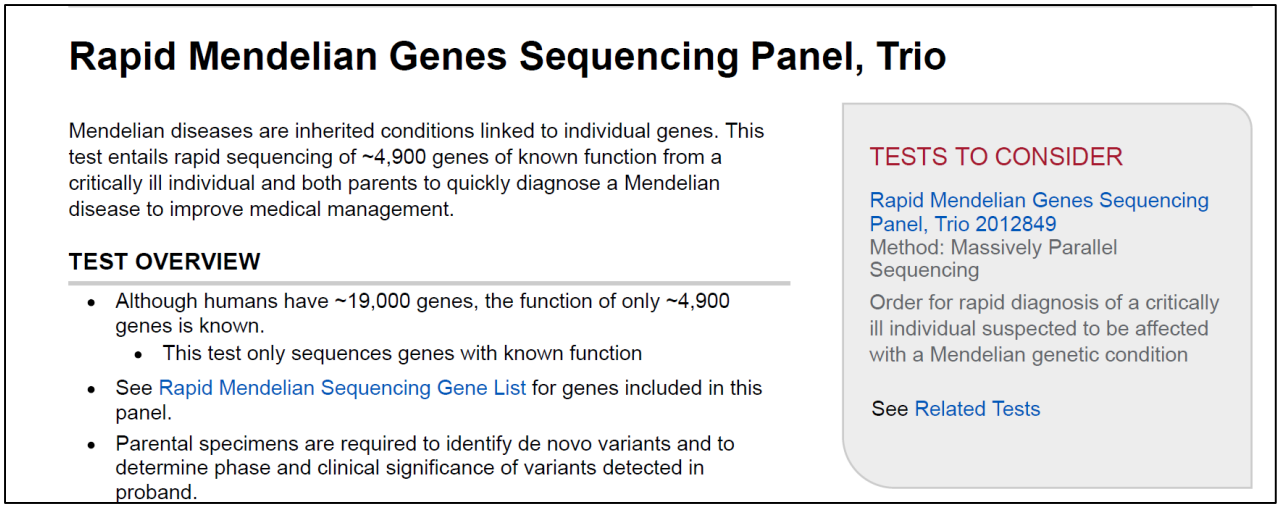
- rWGS performed in 42 cases: 29 trios, 1 quad, 9 mother-infant duos, 3 proband only
- Positive yield: 18 (43%) diagnosed by rWGS, 4 (10%) diagnosed by standard test
- TAT: two weeks
- 18 genes are included in the Rapid Seq 4500+ Gene Panel

Rapid Mendelian Genes Sequencing Panel, Trio

- The panel updated : ~4900 genes
- Specimens: peripheral blood
- TAT: 2-4 weeks
- Only final report (no preliminary report)
- Trio: require proband and parental specimens
- Consent required

Additional Technical Information

- Tests to consider
- Test overview
- Test Interpretation
  - Clinical sensitivity
  - Reporting and interpretation
  - Secondary findings
  - Limitations
  - Analytical sensitivity and specificity



Rapid Mendelian Genes Sequencing Panel, Trio

Mendelian diseases are inherited conditions linked to individual genes. This test entails rapid sequencing of ~4,900 genes of known function from a critically ill individual and both parents to quickly diagnose a Mendelian disease to improve medical management.

TEST OVERVIEW

- Although humans have ~19,000 genes, the function of only ~4,900 genes is known.
  - This test only sequences genes with known function
- See Rapid Mendelian Sequencing Gene List for genes included in this panel.
- Parental specimens are required to identify de novo variants and to determine phase and clinical significance of variants detected in proband.

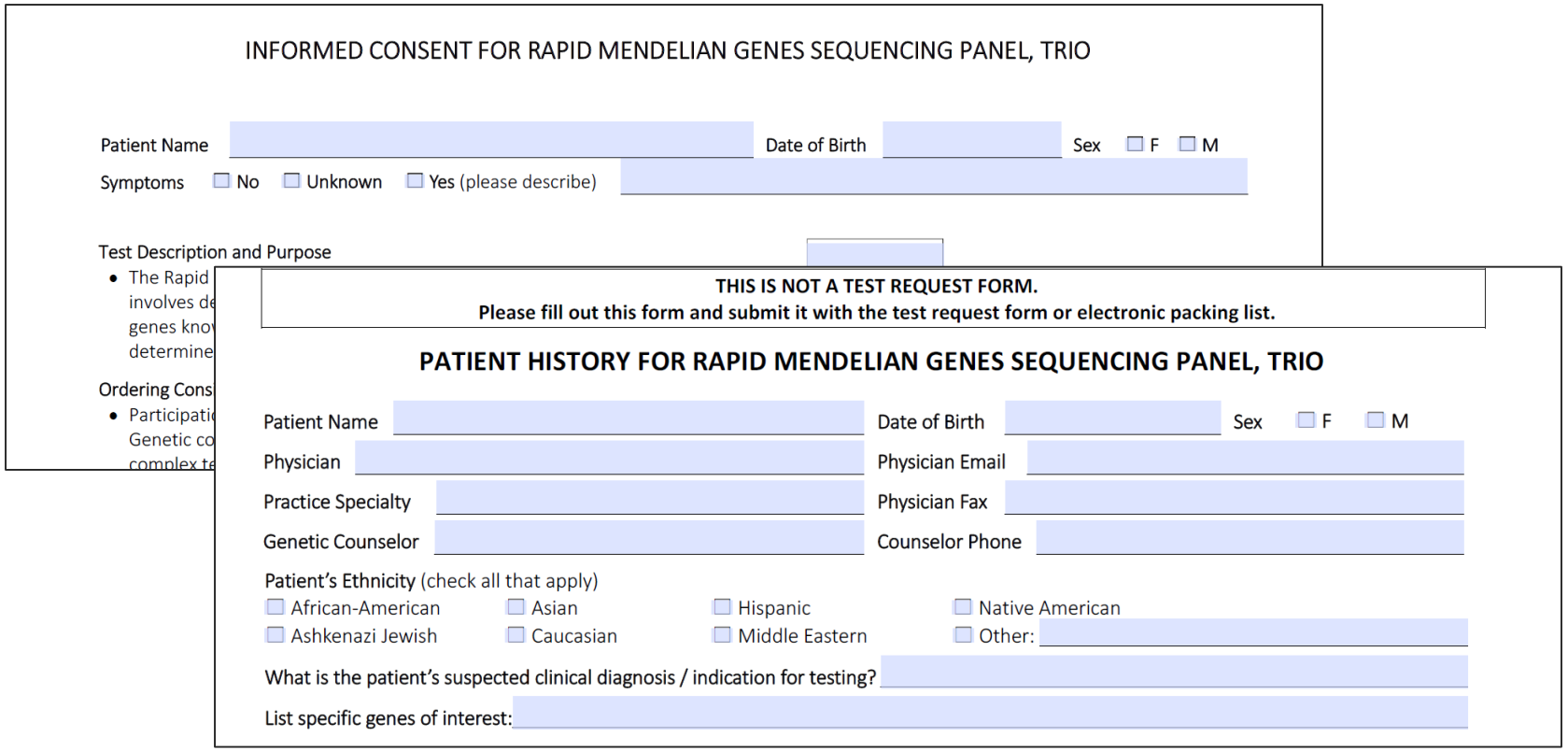
TESTS TO CONSIDER

Rapid Mendelian Genes Sequencing Panel, Trio 2012849
Method: Massively Parallel Sequencing

Order for rapid diagnosis of a critically ill individual suspected to be affected with a Mendelian genetic condition

See Related Tests

Informed Consent and Patient History



INFORMED CONSENT FOR RAPID MENDELIAN GENES SEQUENCING PANEL, TRIO

Patient Name _____ Date of Birth _____ Sex ☐ F ☐ M

Symptoms ☐ No ☐ Unknown ☐ Yes (please describe) _____

Test Description and Purpose

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THIS IS NOT A TEST REQUEST FORM.
Please fill out this form and submit it with the test request form or electronic packing list.

PATIENT HISTORY FOR RAPID MENDELIAN GENES SEQUENCING PANEL, TRIO

Patient Name _____ Date of Birth _____ Sex ☐ F ☐ M

Physician _____ Physician Email _____

Practice Specialty _____ Physician Fax _____

Genetic Counselor _____ Counselor Phone _____

Patient's Ethnicity (check all that apply)

☐ African-American ☐ Asian ☐ Hispanic ☐ Native American

☐ Ashkenazi Jewish ☐ Caucasian ☐ Middle Eastern ☐ Other: _____

What is the patient's suspected clinical diagnosis / indication for testing? _____

List specific genes of interest: _____

Rapid Mendelian Sequencing Gene List

Rapid Mendelian Sequencing Gene List

December 2018

Gene	Coding Exons Not Covered	OMIM Number	Gene Aliases
ADK		102750	AK
ADNP		611386	KIAA0784, ADNP1
ADORA1		102775	RDC7
ADORA2A		102776	ADORA2, RDC8
ADRA1A	*NM_001322503.1: 2* *NM_033303.4: 3*	104221	ADRA1C, ADRA1L1
ADRA2A		104210	ADRA2, ADRA2R, ADRAR
ADRA2B		104260	ADRA2L1, ADRA2RL1, ADRARL1
ADRA2C		104250	ADRA2L2, ADRA2RL2, ADRARL2
ADRB1		109630	ADRB1R
ADRB2		109690	ADRB2R, ADRBR, BAR, B2AR
ADRB3		109691	
ADSL		608222	
ADSSL1		612498	FLJ38602
AEBP1		602981	ACLP

Conclusion

- Rapid Mendelian Genes Sequencing Panel with 4900 known disease-causing genes will improve the precision diagnosis of disease in neonatal intensive care.
- Guide for precision treatment
- Reduce the mortality and cost in NICU
- Building the communication between clinicians and laboratorians is critical for the data interpretation and result delivery.

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