Chapter 1

Molecular Diagnostics: Past, Present, and Future

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1.1 INTRODUCTION

Molecular or nucleic acid-based diagnosis of human disorders is referred to as the detection of the various pathogenic mutations in DNA and/or RNA samples in order to facilitate detection, diagnosis, subclassification, prognosis, and monitoring response to therapy. Molecular diagnostics combines laboratory medicine with the knowledge and technology of molecular genetics and has been enormously revolutionized over the last decades, benefiting from the discoveries in the field of molecular biology (see Table 1.1). The identification and fine characterization of the genetic basis of the disease in question is vital for accurate provision of diagnosis. Gene discovery provides invaluable insights into the mechanisms of disease, and gene-based markers allow physicians not only to assess disease predisposition but also to design and implement improved diagnostic methods. The latter is of great importance, as the plethora and variety of molecular defects demands the use of multiple rather than a single mutation detection platform. Molecular diagnostics is currently a clinical reality with its roots deep into the basic study of gene expression and function.

1.2 HISTORY OF MOLECULAR DIAGNOSTICS: INVENTING THE WHEEL

In 1949, Pauling and his coworkers introduced the term *molecular disease* into the medical vocabulary, based on their discovery that a single amino acid change at the β-globin chain leads to sickle cell anemia, characterized mainly by recurrent episodes of acute pain due to vessel occlusion. In principle, their findings have set the foundations of molecular diagnostics, although the big revolution occurred many years later. At that time, when molecular biology was only hectically expanding, the provision of molecular diagnostic services was inconceivable and technically not feasible. The first seeds of molecular diagnostics were provided in the early days of recombinant DNA technology, with many scientists from various disciplines working in concert. cDNA cloning and sequencing were at that time invaluable tools for providing the basic knowledge on the primary sequence of various genes. The latter provided a number of DNA probes, allowing the analysis via Southern blotting of genomic regions, leading to the concept and application of restriction fragment length polymorphism (RFLP) to track a mutant allele from heterozygous parents to a high-risk pregnancy. In 1976, Kan and coworkers carried out, for the first time, prenatal diagnosis of α-thalassemia, using hybridization on DNA isolated from fetal fibroblasts. Also, Kan and Dozy, in 1978, implemented RFLP analysis to pinpoint sickle cell alleles of African descent. This breakthrough provided the means of establishing similar diagnostic approaches for the characterization of other genetic diseases, such as phenylketonurea (Woo *et al.*, 1983), cystic fibrosis (Farrall *et al.*, 1986), and so on.

At that time, however, a significant technical bottleneck had to be overcome. The identification of the disease causing mutation was possible only through the construction of a genomic DNA library from the affected individual, in order first to clone the mutated allele and then determine its nucleotide sequence. Again, many human globin gene mutations were among the first to be identified through such approaches (Busslinger *et al.*, 1981; Treisman *et al.*, 1983). In 1982, Orkin and his coworkers showed that a number of sequence variations were linked to specific β-globin gene mutations. These groups of RFLPs, termed *haplotypes* (both intergenic and intragenic), have provided a

TABLE 1.1 The timeline of the principal discoveries in the field of molecular biology, which influenced the development of molecular diagnostics.

Date	Discovery
1949	Characterization of sickle cell anemia as a molecular disease
1953	Discovery of the DNA double helix
1958	Isolation of DNA polymerases
1960	First hybridization techniques
1969	*In situ* hybridization
1970	Discovery of restriction enzymes and reverse transcriptase
1975	Southern blotting
1977	DNA sequencing
1983	First synthesis of oligonucleotides
1985	Restriction fragment length polymorphism analysis
1985	Invention of PCR
1986	Development of fluorescent *in situ* hybridization (FISH)
1988	Discovery of the thermostable DNA polymerase – Optimization of PCR
1992	Conception of real-time PCR
1993	Discovery of structure-specific endonucleases for cleavage assays
1996	First application of DNA microarrays
2001	First draft versions of the human genome sequence
2001	Application of protein profiling in human diseases
2005	Introduction of the high-throughput next-generation sequencing technology

first-screening approach in order to detect a disease-causing mutation. Although this approach enabled researchers to predict which β-globin gene would contain a mutation, significantly facilitating mutation screening, no one was in the position to determine the exact nature of the disease-causing mutation, as many different β-globin gene mutations were linked to a specific haplotype in different populations (further information is available at http://globin.bx.psu.edu/hbvar; Hardison *et al.*, 2002; Patrinos *et al.*, 2004; Giardine *et al.*, 2007).

At the same time, in order to provide a shortcut to DNA sequencing, a number of exploratory methods for pinpointing mutations in patients' DNA were developed. The first methods involved mismatch detection in DNA/DNA or RNA/DNA heteroduplexes (Myers *et al.*, 1985a, b) or differentiation of mismatched DNA heteroduplexes using gel electrophoresis, according to their melting profile (Myers *et al.*, 1987). Using this laborious and time-consuming approach, a number of mutations or polymorphic sequence variations have been identified, which made possible the design of short synthetic oligonucleotides that were used as allele-specific probes onto genomic Southern blots. This experimental design was quickly implemented for the detection of β-thalassemia mutations (Orkin *et al.*, 1983; Pirastu *et al.*, 1983).

Despite the intense efforts from different laboratories worldwide, diagnosis of inherited diseases on the DNA level was still underdeveloped and therefore still not ready to be implemented in clinical laboratories for routine analysis of patients due to the complexities, costs, and time requirements of the technology available. It was only after a few years that molecular diagnosis entered its golden era with the discovery of the most powerful molecular biology tool since cloning and sequencing, the polymerase chain reaction (PCR).

1.3 THE PCR REVOLUTION: GETTING MORE OUT OF LESS

The discovery of PCR (Saiki *et al.*, 1985; Mullis and Faloona, 1987) and its quick optimization, using a thermostable *Taq* DNA polymerase from *Thermus aquaticus* (Saiki *et al.*, 1988) has greatly facilitated and in principle revolutionized molecular diagnostics. The most powerful feature of PCR is the large amount of copies of the target sequence generated by its exponential amplification (see Fig. 1.1), which allows the identification of a known mutation within a single day, rather than months. Also, PCR has markedly decreased or even diminished the need for radioactivity for routine molecular diagnosis. This has allowed molecular diagnostics to enter the clinical laboratory for the provision of genetic services, such as carrier or population screening for known mutations, prenatal diagnosis of inherited diseases, or in recent years, identification of unknown mutations, in close collaboration with research laboratories. Therefore, being moved to their proper environment, the clinical laboratory, molecular diagnostics could provide the services for which they have been initially conceived.

The discovery of PCR has also provided the foundations for the design and development of many mutation detection schemes, based on amplified DNA. In general, PCR either is used for the generation of the DNA fragments to be analyzed, or is part of the detection method. The first attempt was the use of restriction enzymes (Saiki *et al.*, 1985) or oligonucleotide probes, immobilized onto membranes or in solution (Saiki *et al.*, 1986) in order to detect the existing genetic variation, in particular the sickle cell disease-causing

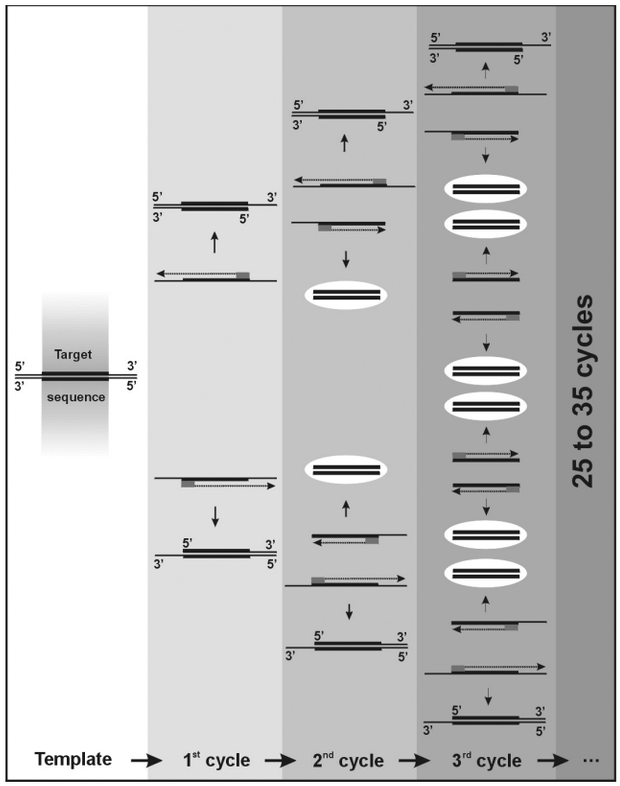

FIGURE 1.1 The PCR principle. Thick and thin black lines correspond to the target sequence and genomic DNA, respectively; gray boxes correspond to the oligonucleotide primers, and the correct size PCR products are included in the white ellipses. Dashed lined arrows depict the elongation of the template strand.

mutation. In the following years, an even larger number of mutation detection approaches have been developed and implemented. These techniques can be divided roughly into three categories, depending on the basis for discriminating the allelic variants:

1. *Enzymatic-based methods*. RFLP analysis was historically the first widely used approach, exploiting the alterations in restriction enzyme sites, leading to the gain or loss of restriction events (Saiki *et al.*, 1985). Subsequently, a number of enzymatic approaches for mutation detection have been conceived, based on the dependence of a secondary structure on the primary DNA sequence. These methods exploit the activity of resolvase enzymes T4 endonuclease VII, and, more recently, T7 endonuclease I to digest heteroduplex DNA formed by annealing wild-type and mutant DNA (Mashal *et al.*, 1995). Digestion fragments indicate the presence and the position of any mutations. A variation of the theme involves the use of chemical agents for the same purpose (Saleeba *et al.*, 1992; see also Chapter 3). Another enzymatic approach for mutation detection is the oligonucleotide ligation assay (Landegren *et al.*, 1988). In this technique, two oligonucleotides are hybridized to complementary DNA stretches at sites of possible mutations. The oligonucleotides' primers are designed such that the 3′ end of the first primer is immediately adjacent to the 5′ end of the second primer. Therefore, if the first primer matches completely with the target DNA, then the primers can be ligated by DNA ligase. On the other hand, if a mismatch occurs at the 3′ end of the first primer, then no ligation products will be obtained.
2. *Electrophoretic-based techniques*. This category is characterized by a plethora of different approaches designed for screening of known or unknown mutations, based on the different electrophoretic mobility of the mutant alleles, under denaturing or non-denaturing conditions. Single strand conformation polymorphism (SSCP) and heteroduplex (HDA) analyses (Orita *et al.*, 1989; see Chapter 4) were among the first methods designed to detect molecular defects in genomic loci. In combination with capillary electrophoresis (see Chapter 5), SSCP and HDA analysis now provide an excellent, simple, and rapid mutation detection platform with low operation costs and, most interestingly, the potential of easily being automated, thus allowing for high-throughput analysis of patients' DNA. Similarly, denaturing and temperature gradient gel electrophoresis (DGGE and TGGE, respectively) can be used equally well for mutation detection (see Chapter 6). In this case, electrophoretic mobility differences between a wild-type and mutant allele can be ''visualized'' in a gradient of denaturing agents, such as urea and formamide, or of increasing temperature. Finally, an increasingly used mutation detection technique is the two-dimensional gene scanning, based on two-dimensional electrophoretic separation of amplified DNA fragments, according to their size and base pair sequence. The latter involves DGGE, following the size separation step.
3. *Solid phase-based techniques*. This set of techniques consists of the basis for most of the present-day mutation detection technologies, since they have the extra advantage of being easily automated and hence are highly recommended for high-throughput mutation detection or screening. A fast, accurate, and convenient method for the detection of known mutations is reverse dot-blot, initially developed by Saiki and coworkers (1989) and implemented for the detection of β-thalassemia mutations. The essence of this method is the utilization of oligonucleotides, bound to a membrane, as hybridization targets for amplified DNA. Some of this technique's advantages is that one membrane strip can be used to detect many different known mutations in a single individual (a one strip/one patient type of assay), the potential of automation, and the ease of interpretation of the results, using a classical avidin-biotin system. However, this technique cannot be used

for the detection of unknown mutations. Continuous development has given rise to allele-specific hybridization of amplified DNA (PCR-ASO, Chapter 2) on filters and recently extended on DNA oligonucleotide microarrays (see Chapters 16 and 17) for high throughput mutation analysis (Gemignani *et al.*, 2002; Chan *et al.*, 2004). In particular, oligonucleotides of known sequence are immobilized onto appropriate surfaces and hybridization of the targets to the microarray is detected, mostly using fluorescent dyes.

The choice of the mutation detection method is dependent upon a number of variables, including the mutation spectrum of a given inherited disorder, the available infrastructure, and the number of tests performed in the diagnostic laboratory, and recently with issues of intellectual properties (see also section 1.5.1 and Chapter 36). Most of the clinical diagnostic laboratories have not invested in an expensive high technology infrastructure, since the test volumes, that is, the number of tests expected to be performed, have not been large enough to justify the capital outlay. Therefore, simple screening tests such as SSCP and HDA were and still are the methods of choice for many clinical laboratories, as they allow for rapid and simultaneous detection of different sequence variations at a detection rate of close to 100%. Although PCR has significantly facilitated the expansion of molecular diagnostics, it nonetheless has a number of limitations. First of all, amplification of CG repeat-rich regions can be problematic for *Taq* polymerase, which sometimes leads to the classic alternative of Southern blot analysis. Also, *Taq* polymerase is error prone at a range of 10^{-4} to 10^{-5} per nucleotide, which is strongly influenced by the conditions of the amplification reaction, such as magnesium or deoxyribonucleotide concentration, pH, temperature, and so on. Polymerase errors can contribute to unspecific background, depending on the detection method, resulting in limiting the detection level. To overcome these technical problems, positive results should be confirmed by alternative methods or by using high fidelity thermostable polymerases.

Finally, it needs to be stressed that despite the wealth of mutation detection methodologies, DNA sequencing is still considered the gold standard and the definitive experimental procedure for mutation detection. However, the costs for the initial investment and the difficulties for standardization and interpretation of ambiguous results have restricted its use only to basic research laboratories.

1.4 MOLECULAR DIAGNOSTICS IN THE POST-GENOMIC ERA

In February 2001, with the announcement of the first draft sequence of the human genome (International Human Genome Sequencing Consortium, 2001; Venter *et al.*, 2001) and subsequently with the genomic sequence of other organisms, molecular biology has entered into a new era with unprecedented opportunities and challenges. These tremendous developments put pressure on a variety of disciplines to intensify their research efforts to improve by orders of magnitude the existing methods for mutation detection, to make available data sets with genomic variation and analyze these sets using specialized software, to standardize and commercialize genetic tests for routine diagnosis, and to improve the existing technology in order to provide state-of-the-art automated devices for high-throughput genetic analysis.

The biggest challenge, following the publication of the human genome draft sequence, was to improve the existing mutation detection technologies to achieve robust cost-effective, rapid, and high-throughput analysis of genomic variation. In the last couple of years, technology has improved rapidly and new mutation-detection techniques have become available, whereas old methodologies have evolved to fit into the increasing demand for automated and high-throughput screening. The chromatographic detection of polymorphic changes of disease-causing mutations using denaturing high-performance liquid chromatography (DHPLC; for review, see Xiao and Oefner, 2001) is one of the new technologies that emerged. DHPLC reveals the presence of a genetic variation by the differential retention of homo- and heteroduplex DNA on reversed phase chromatography under partial denaturation.

Single-base substitutions, deletions, and insertions can be detected successfully by UV or fluorescence monitoring within two to three minutes in unpurified PCR products as large as 1.5 kilo bases. These features, together with its low cost, make DHPLC one of the most powerful tools for mutational analysis. Also, pyrosequencing, a non-gel-based genotyping technology, provides a very reliable method and an attractive alternative to DHPLC (Chapter 8). Pyrosequencing detects *de novo* incorporation of nucleotides based on the specific template. The incorporation process releases a pyrophosphate, which is converted to ATP and followed by luciferase stimulation. The light produced, detected by a charge coupled device camera, is ''translated'' to a pyrogram, from which the nucleotide sequence can be deducted (Ronaghi *et al.*, 1998).

The use of the PCR in molecular diagnostics is considered the gold standard for detecting nucleic acids and it has become an essential tool in the research laboratory. Real-time PCR (Holland *et al.*, 1991) has engendered wider acceptance of the PCR due to its improved rapidity, sensitivity, and reproducibility (see Chapter 7). The method allows for the direct detection of the PCR product during the exponential phase of the reaction, therefore combining amplification and detection in one single step. The increased speed of real-time PCR is due largely to reduced cycles, removal of post-PCR detection procedures, and the use of fluorogenic labels and sensitive methods of detecting

their emissions. Therefore, real-time PCR is a very accurate and sensitive methodology with a variety of applications in molecular diagnostics, allows a high throughput, and can easily be automated and performed on very small volumes, which makes it the method of choice for many modern diagnostic laboratories.

Above all, the DNA microarray-based genotyping approach offers simultaneous analysis of many polymorphisms and sequence alterations (see Chapters 16 and 17). Microarrays consist of hundreds of thousands of oligonucleotides attached on a solid surface in an ordered array. The DNA sample of interest is PCR amplified and then hybridized to the microarray. Each oligonucleotide in the high-density array acts as an allele-specific probe and therefore perfectly matched sequences hybridize more efficiently to their corresponding oligonucleotides on the array. The hybridization signals, obtained from allele-specific arrayed primer extension (AS-APEX) (Pastinen *et al.*, 2000), are quantified by high-resolution fluorescent scanning and analyzed by computer software, resulting in the identification of DNA sequence alterations. Therefore, using a high-density microarray makes possible the simultaneous detection of a great number of DNA alterations, hence facilitating genome-wide screening. Several arrays have been generated to detect variants in the HIV genome (Kozal *et al.*, 1996; Wen *et al.*, 2000), human mitochondria mutations (Erdogan *et al.*, 2001), β-thalassemia (Chan *et al.*, 2004; Cremonesi *et al.*, 2007), and glycose-6-phosphate dehydrogenase (G-6-PD) deficiency mutations (Gemignani *et al.*, 2002), and so on.

In recent years, there has been a significant development of proteomics, which has the potential to become an indispensable tool for molecular diagnostics. A useful repertoire of proteomic technologies is available, with the potential to undergo significant technological improvements, which would be beneficial for increased sensitivity and throughput while reducing sample requirement (see Chapters 18 and 21). The improvement of these technologies is a significant advance toward the need for better disease diagnostics. The detection of disease-specific protein profiles goes back to the use of two-dimensional protein gels (Hanash, 2000), when it was demonstrated that leukemias could be classified into different subtypes based on the different protein profile (Hanash *et al.*, 2002). Nowadays, mass spectrometers are able to resolve many protein and peptide species in body fluids, being virtually set to revolutionize protein-based disease diagnostics (see Chapter 21). The robust and high-throughput nature of the mass spectrometric instrumentation is unparalleled and imminently suited for future clinical applications, as elegantly demonstrated by many retrospective studies in cancer patients (reviewed in Petricoin *et al.*, 2002). Also, high-throughput protein microarrays, constructed from recombinant, purified, and yet functional proteins, allow the miniaturized and parallel analysis of large numbers of diagnostic markers in complex samples. The first pilot studies on disease tissues are already starting to emerge, such as assessing protein expression profiles in tissue derived from squamous cell carcinomas of the oral cavity (Knezevic *et al.*, 2001), or the identification of proteins that induce an acute antibody response in autoimmune disorders, using auto-antigen arrays (Robinson *et al.*, 2002). These findings indicate that proteomic pattern analysis ultimately might be applied as a screening tool for cancer in high-risk and general populations.

The development of state-of-the-art mutation detection techniques has not only a positive impact on molecular genetic testing of inherited disorders, but also provides the technical means to other disciplines. Mutation detection schemes are applicable for the identification of genetically modified (GM) products, which may contaminate non-GM seeds, or food ingredients containing additives and flavorings that have been genetically modified or have been produced from GM organisms (see Chapter 29). The same techniques can ascertain the genotype of an animal strain (see Chapter 31). Another research area that benefits from the continuous development of mutation detection strategies is pharmacogenetics and pharmacogenomics (see Chapter 22), referred to as the effort to define the inter-individual variations that are expected to become integral for treatment planning, in terms of efficacy and adverse effects of drugs. This approach uses the technological expertise from high-throughput mutation detection techniques, genomics, and functional genomics to define and predict the nature of the response of an individual to a drug treatment, and to rationally design newer drugs or improve existing ones. Ultimately, the identified genomic sequence variation is organized and stored into specialized mutation databases, enabling a physician or researcher to query upon and retrieve information relevant to diagnostic issues (see Chapter 25).

Finally, and for the last 20 years, DNA analysis and testing has also significantly revolutionized the forensic sciences. The technical advances in molecular biology and the increasing knowledge of the human genome have had a major impact on forensic medicine (see Chapter 26). Genetic characterization of individuals at the DNA level enables identity testing from a minimal amount of biological specimen, such as hair, blood, semen, bone, and so forth, in cases of sexual assault, homicide, and unknown human remains, and paternity testing is also changing from the level of gene products to the genomic level. DNA testing is by far more advantageous over the conventional forensic serology, and over the years has contributed to the acquittal of falsely accused people (saving most of them even from death row) and the identification of the individual who had committed criminal acts (Cohen, 1995), and even helped to specify identities of unknown human remains, such as those from the victims at Ground Zero in New York, or from the skeletons of the Romanov family members (Gill *et al.*, 1994).

1.5 FUTURE PERSPECTIVES: WHAT LIES BEYOND

As an intrinsic part of DNA technology, molecular diagnostics are rooted in the April 1953 discovery of the DNA double helix. Today, it is clear that they embody a set of notable technological advances allowing for thousands of diagnostic reactions to be performed at once and for a range of mutations to be simultaneously detected. The reasons for this dramatic increase are two-fold. First of all, the elucidation of the human genomic sequence, as well as that of other species such as bacterial or viral pathogens, has led to an increased number of diagnostically relevant targets. Second, the molecular diagnostic testing volume is rapidly increasing. This is the consequence of a better understanding of the basis of inherited diseases, therefore allowing molecular diagnostics to play a key role in patient or disease management.

Presently, a great number of blood, hair, semen, and tissue samples are analyzed annually worldwide in both public and private laboratories, and the number of genetic tests available is steadily increased year by year. Taking these premises into account, we can presume that it is only a matter of time before molecular diagnostic laboratories become indispensable in laboratory medicine. In the post-genomic era, genetic information will have to be examined in multiple health care situations throughout people's lives. Currently, newborns can be screened for phenylketonurea and other treatable genetic diseases (Yang *et al.*, 2001). It is also possible that in the not-so-distant future, children at high risk from coronary artery disease will be identified and treated to prevent changes in their vascular walls during adulthood. Similarly, parents will have the option of being informed about their carrier status for many recessive diseases before they decide to start a family. Although not widely accepted, this initiative has already started to be implemented in Cyprus, where a couple at risk for thalassemia syndrome have been advised to undergo a genetic test for thalassemia mutations before their marriage (see also Chapter 37). Also, for middle-aged and older populations, scientists will be able to determine risk profiles for various late-onset diseases, preferably before the appearance of symptoms, which at least could be partly prevented through dietary or pharmaceutical interventions. In the near future, the monitoring of individual drug response profiles throughout life, using genetic testing for the identification of their individual DNA signature, will be part of the standard medical practice. Soon, genetic testing will comprise a wide spectrum of different analyses with a host of consequences for individuals and their families, which is worth emphasizing when explaining molecular diagnostics to the public (see also Chapter 38). All these issues are discussed in detail next. However, and in order to be more realistic, many of these expectations still are based on promises, though quite optimistic ones. Thus, some of the new perspectives of the field could be a decade away, and several challenges remain to be realized.

1.5.1 Commercializing Molecular Diagnostics

Currently, clinical molecular genetics is part of mainstream health care worldwide. Almost all clinical laboratories have a molecular diagnostic unit or department. Although in recent years the notion of molecular diagnostics has increasingly gained interest, genetic tests are still not generally used for population screening, but rather for diagnosis, carrier screening, and prenatal diagnosis, and only on a limited basis. Therefore, and in order to make molecular diagnostics widely available, several obstacles and issues need to be taken into consideration and resolved in the coming years.

The first important issue is the choice of the mutation detection platform. Despite the fact that there are over 50 different mutation detection and screening methods, there is no single platform or methodology that prevails for genetic testing. Genotyping can be done using different approaches, such as filters, gels, microarrays, microtiter plates; different amplification-based technologies; different separation techniques, such as blotting, capillary electrophoresis, microarrays, mass spectroscopy; and finally different means for labeling, such as radioactive, fluorescent, chemiluminescent, or enzymatic substances. The variety of detection approaches makes it not only difficult but also challenging to determine which one is better suited for a laboratory setting. Generally speaking, DNA sequencing is the gold standard for the identification of causative or non-DNA sequence variations, particularly with the advent of the next-generation sequencing technologies (see also Chapter 24). The initial investment costs and the expected test volume are some of the factors that need to be taken into consideration prior to choosing the detection technique. Related issues are also the costs of the hardware and software, testing reagents, and kits. The latter is of great importance, since the fact that most of the diagnostic laboratories today are running ''home-brew'' assays – for example, not using well-standardized genetic testing kits due to cost barriers, which brings to surface the issue of quality control of the reagents (see Chapter 40) and of safety (see Chapter 39). Currently, there are several clinical and technical recommendations for genetic testing for monogenic disorders that have been issued by several organizations (see Table 1.2).

Another very important issue is training the personnel of a molecular diagnostic laboratory, reflecting in the quality and the correct interpretation of the results. Continuous education of the personnel of the diagnostic laboratory is crucial for the accuracy of the results provided (see also Chapter 40). Many times, such as in the case of prenatal or pre-implantation

TABLE 1.2 Indicative clinical and technical recommendations for genetic testing for monogenic disorders. ACMG: American College of Medical Genetics, ASHG: American Society of Human Genetics.

Disease/syndrome	Gene	References
Alzheimer's disease	*ApoE*	ACMG (1995)
Canavan disease	*ASPA*	ACMG (1998)
Cystic fibrosis	*CFTR*	Dequeker *et al.* (2000), Grody *et al.* (2001a)
Thrombophilia	Factor V Leiden	Grody *et al.* (2001b)
Fragile X syndrome	*FMR1*	Maddalena *et al.* (2001)
Prader–Willi/Angelman syndrome	15q11-q13	ASHG/ACMG (1996)
Multiple endocrine neoplasia	*MEN1/MEN2*	Brandi *et al.* (2001)
Tuberous sclerosis	*TSC1/TSC2*	Roach *et al.* (1999)
Breast cancer	*BRCA1*	Sorscher and Levonian (1997)

diagnosis, irrevocable decisions need to be made, most of the time based on a simple test result. In the past decade, there has been a significant reduction in the number of incorrect genotypes diagnosed, as a result of continuous training and proficiency testing schemes (http://www.eurogentest.org). In the USA, there is a voluntary biannual proficiency testing for molecular diagnostic laboratories, while in Europe, the EuroGenTest European Network of Excellence (http://www.eurogentest.org) has been founded to promote quality in molecular genetic testing through the provision of external quality assessment (proficiency testing schemes) and the organization of best practice meetings and publication of guidelines. It is generally true that many geneticists and non-geneticist physicians would benefit from continuous education regarding the appropriate use of molecular diagnostic tests, which is necessary to evaluate the method pre-analytically and to interpret results.

The legal considerations and the ethical concerns are also hurdles that need to be overcome in the coming years. One issue is reimbursing of the diagnosis costs. At present, there are no insurance companies that reimburse the costs for molecular testing to the people insured; the necessary regulatory and legal framework remains to be established. ''Legalizing'' molecular testing, by the adoption of the relevant regulations, would probably result in an increase of the test volume and at the same time it can pose an immense barrier to uncontrolled genetic testing. Similarly, the need to obtain an informed consent from the patient to be analyzed is also of great importance and should be encouraged and facilitated by the diagnostic laboratory.

On the other hand, the issue of intellectual properties hampers the wide commercialization of molecular diagnostics. Almost all the clinically relevant genes have been now patented and the terms that the patent holders offer vary considerably (see Chapter 36). Among the difficulties that this issue imposes is the limiting choice of mutation detection platforms, the large loyalties for reagent use, and the exclusive sublicenses that many companies grant to clinical laboratories, leading eventually to monopolies. Since one of the biggest challenges that the clinical laboratory is facing is patent and regulatory compliance, partnerships and collaborations may be envisaged in order to take the technology licenses to the diagnostic laboratory that will subsequently develop, standardize, and distribute the assays. These will partly alleviate some of the intellectual properties issues. Finally, the issue of the medical genetics specialty is more urgent than ever. In the USA, medical genetics has been formally recognized as a medical specialty only within the past 15 years, and in Europe, medical genetics only recently has been formally recognized as a specialty (http://www.eshg.org). The implementation of this decision is still facing substantial difficulties (http://www.eshg.org/geneticseurope.htm), which will probably take years to bypass. With the completion of the Human Genome Project, genetics has become the driving force in medical research and is now poised for integration into medical practice. An increase in the medical genetics workforce, including geneticists and genetic counselors, will be necessary in the coming years. After all, the Human Genome Project has made information of inestimable diagnostic and therapeutic importance available and therefore the medical profession now has the obligation to rise to both the opportunities and challenges that this wealth of genetic information presents.

1.5.2 Personalized Medicine

The term ''personalized medicine'' refers to the practice of medicine where patients receive the most appropriate medical treatment, fitting dosage, and combination of drugs based on their genetic background. Some of the reasons for many types of adverse drug reactions are already known and often related to polymorphic gene alleles of drug metabolizing enzymes (Nebert and Menon, 2001; Risch *et al.*, 2002). The application of high-throughput genotyping tools for the identification and screening of single nucleotide polymorphisms (SNPs) eventually can lead to the determination of the unique molecular signature of an individual in a relatively short period of time. This way, individual drug responses can be predicted from predetermined genetic variances correlated with a drug effect. In other words, this will allow the physician to provide the patient with a selective drug prescription (see Chapter 22). A handful of pharmaceutical companies are developing a precise haplotyping scheme to identify individuals/patients who will derive optimal benefit from drugs currently under development. Clinicians will facilitate this effort by importing clinical data into this haplotyping system for a complete patient analysis and drug evaluation. In addition to these efforts, there is a growing need to incorporate this increasingly complex body of knowledge to standard medical practice. Incorporating pharmacogenomics-related courses in the standard curriculum of medical schools potentially can ensure that the forthcoming generation of clinicians and researchers will be familiar with the latest developments in that field and will be capable of providing patients with the expected benefits of personalized medicine.

Similarly, nutrigenomics (or nutritional genomics) investigates the interactions between nutrition and an individual's genome, and the consequent downstream effects on their phenotype with the aim of providing tailored nutritional advice or developing specialist food products (see Chapter 23). In other words, nutrigenomics recognizes that specific dietary advice that can be beneficial for one individual may be inappropriate, or actually harmful, to another. Although comparable to pharmacogenomics, nutrigenomics is still considered as an emerging science contrary to pharmacogenomics, which is considered to have ''come of age'' (Allison, 2008).

However, there are growing concerns regarding the ethical aspects of personalized medicine. First of all, equality in medical care needs to be ensured, when genetics foretell clinicians which patients would be less likely to benefit from a particular drug treatment. Second, it will become increasingly vital to devise operational tools for the prevention of stigmatization and discrimination of different populations, in particular on ethnic grounds (van Ommen, 2002), and therefore every precaution should be taken to eliminate all lingering prejudice and bias associated with the study of human genetic variation. Other dilemmas include the right to deny an available treatment from specific patient populations according to genetic-derived indications, as currently is the case with prenatal diagnosis (see also Chapter 37). Appropriate guidelines will be crucially needed for the successful implementation of pharmacogenomics into clinical practice.

1.5.3 Personal Genomics

The ultimate goal in health care over the next decades will be the efficient integration of molecular diagnostics with therapeutics. With the advent of next generation sequencing in 2005 (Margulies *et al.*, 2005) and the avalanche of developments in this field since then (see Chapter 24), experts believe that reasonably soon, people will be able to have their own genomes sequenced for under $1,000. This is going to involve sequencing technology that is much cheaper and faster than today's machines and several efforts are currently under way, often encouraged by major funding bodies (e.g. the European Commission-funded READNA consortium; http://www.cng.fr/READNA). When that point is reached, this can ultimately be translated in a patient being able to carry a smart card, like an ordinary credit card, providing secure access to his or her genetic information. So far, the entire DNA sequence of a handful of individuals has been sequenced, such as Craig Venter (Levy *et al.*, 2007), Jim Watson (Wheeler *et al.*, 2008), and so on, while ''the 1000 genomes'' project is an ambitious venture that involves sequencing the genomes of approximately 1,200 people from around the world, with the overall goal ''…to create the most detailed and medically useful picture to date of human genetic variation'' (http://www.1000genomes.org). In the future, a person may appear at the clinic for treatment, ''carrying'' its entire genome at hand or, alternatively, nanotechnology could eventually enable DNA analysis with a portable DNA sequencing device.

Even though the expectations are high and companies are currently using these new technologies to provide information to individuals to predict health and disease outcome, even behavioral traits, it is generally premature to make promises for clinically useful information from genomic analyses. Next to that, there is an inherent danger of overestimating the usefulness of the various personalized genomic tests that can be ordered directly by consumers. Unlike other genetic analyses, these tests provide sheer amounts of genetic information, but their diagnostic or prognostic value remains uncertain because of (a) the lack of information about the influence of environmental and other factors, and (b) the weak association for the vast majority of genetic loci with disease. In a carefully conducted study by Janssens and coworkers (2008), the scientific evidence supporting gene–disease associations for genes included in genomic tests offered commercially

to consumers online was assessed. These authors concluded that the synthetic odds ratios for the ''predictive genomic'' tests offered by seven of these companies were ranging from 0.54 to 0.88 for protective variants and from 1.04 to 3.2 for risk variants. Furthermore, genes in genomic profiles assessing the risk for developing cardiovascular disease were more frequently associated with non-cardiovascular diseases, and the same was true for associations of genomic profiles with bone diseases. These findings clearly demonstrate that, currently, there is insufficient scientific evidence to conclude that genomic profiles are useful in measuring genetic risk for common diseases or in developing personalized diet and lifestyle recommendations for disease prevention.

It is puzzling how these companies use their clients' genetic profiles to tailor individualized diets and lifestyle recommendations. Also, it is noteworthy that some of the companies that provide personal genomic tests have no physicians involved in ordering these tests, with the argument that ''...patients deserve direct access to their health information without a physician intermediary'' (http://www.nytimes.com/2008/06/26/business/26gene.html). Overall, although these tests could provide value to customers by offering tools for social networking or genealogy, there are questions whether and how to regulate these tests, about the extent to which they provide (useful) medical information and the risks from misinterpreting them (Magnus *et al.*, 2009).

1.6 CONCLUSIONS

In the coming years, molecular diagnostics will continue to be of critical importance to public health worldwide. Molecular genetic testing will facilitate the detection and characterization of disease, as well as monitoring of the drug response, and will assist in the identification of genetic modifiers and disease susceptibility. A wide range of molecular-based tests is available to assess DNA variation and changes in gene expression. However, there are major hurdles to overcome before the implementation of these tests in clinical laboratories, such as which test to employ, the choice of technology and equipment, and issues such as cost effectiveness, accuracy, reproducibility, personnel training, reimbursement by third-party payers, and intellectual property. At present, PCR-based testing predominates; however, alternative technologies aimed at exploring genome complexity without PCR are anticipated to gain momentum in the coming years. Furthermore, development of integrated chip devices (''lab-on-a-chip'') should facilitate genetic readouts from single cells and molecules. Together with proteomic-based testing, these advances will improve molecular diagnostics and will present additional challenges for implementing such technology in public or private research units, hospitals, clinics, and pharmaceutical industries.

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