

## Chapter 2

# Molecular Diagnostics as Basis of Personalized Medicine

### Introduction

Molecular diagnostics, the use of diagnostic testing to understand the molecular mechanisms of an individual patient's disease, will be pivotal in the delivery of safe and effective therapy for many diseases in the future. Role of molecular diagnostics in personalized medicine covers the following aspects:

- Early detection and selection of appropriate treatment determined to be safe and effective on the basis of molecular diagnostics
- Integration of molecular diagnostics with therapeutics
- Monitoring therapy as well as determining prognosis

In parallel with two important components of personalized medicine – pharmacogenetics and pharmacogenomics (compared in Table 4.1)– there are two types of tests relevant to personalized medicine.

1. A pharmacogenomic test is an assay intended to study interindividual variations in whole genome single nucleotide polymorphism (SNP) maps and haplotype markers, alterations in gene expression, or inactivation that may be correlated with pharmacological function and therapeutic response. In some cases the pattern or profile of the change rather than the individual biomarker is relevant to diagnosis.
2. A pharmacogenetic test is an assay intended to study interindividual variations in DNA sequence related to drug absorption and disposition (pharmacokinetics), including polymorphic variations in genes that encode the functions of transporters, metabolizing enzymes, receptors, and other proteins.

### Molecular Diagnostic Technologies

Molecular diagnostic technologies have been reviewed in a detailed report on this topic (Jain 2009a). Molecular diagnostics are used for genetic testing and have the potential to be applied for genetic screening of large populations. They can also be

used as adjuncts to clinical trials. A classification of molecular diagnostic technologies relevant to personalized medicine is shown in Table 2.1. Some of these technologies, which are used for mutation detection, overlap with technologies for detection of SNPs described later in this chapter. The two most important technologies relevant to personalized medicine are SNP genotyping and microarray /biochip.

## DNA Sequencing

DNA sequencing was initially used only for research purposes but has now become a routine tool in molecular diagnostics. The technologies are described in a special report on this topic (Jain 2009b). An important characteristic of a diagnostic assay is the specificity of the nucleic acid sequence that is detected. Several research and clinical laboratories are now using DNA/RNA sequencing technology for the following applications that are relevant to personalized medicine:

- HIV resistance sequence analysis
- HCV genotyping
- Genetic diseases

Most new sequencing techniques simulate aspects of natural DNA synthesis to identify the bases on a DNA strand of interest either by “base extension” or “ligation.” Both approaches depend on repeated cycles of chemical reactions. However, cost can be lowered and speed is increased by miniaturization to reduce the amount of chemicals used and to read millions of DNA sequences simultaneously. Several technologies are available for sequencing.

## Biochips and Microarrays

### *DNA Biochip Technology for Developing Personalized Medicine*

Biochip is a broad term indicating the use of microchip technology in molecular biology and can be defined as arrays of selected biomolecules immobilized on a surface. This technology has been described in more detail elsewhere (Jain 2009c). DNA microarray is a rapid method of sequencing and analyzing genes. An array is an orderly arrangement of samples. The sample spot sizes in microarray are usually less than 200  $\mu\text{m}$  in diameter. It is comprised of DNA probes formatted on a microscale (biochips) plus the instruments needed to handle samples (automated robotics), read the reporter molecules (scanners), and analyze the data (bioinformatic tools). Selected applications of biochip technology relevant to personalized medicine are listed in Table 2.2.

**Table 2.1** Examples of molecular diagnostic technologies used for personalized medicine

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Polymerase chain reaction (PCR)-based methods
Cold-PCR
Digital PCR
DirectLinear™ analysis
Quantitative fluorescent PCR
Real-time PCR
Reverse transcriptase (RT) PCR
Restriction fragment length polymorphism
Scorpions™ (DxS Ltd): closed-tube platform for the efficient homogeneous detection of PCR amplicons
Single-strand conformational polymorphism
Non-PCR methods
Arrayed primer extension
Enzyme mutation detection
Fluorescence resonance energy transfer (FRET) based assays: Invader assay
Locked nucleic acid (LNA) technology
Peptide nucleic acid (PNA) technology
Transcription-mediated amplification
Gene chip and microfluidic microarrays
Nanodiagnosics
Nanoparticle-based integration of diagnostics with therapeutics
Nanotechnology-based refinement of diagnostics for pharmacogenetics
Toxicogenomics
Single nucleotide polymorphism genotyping
DNA methylation studies
Gene expression based tests
DNA sequencing
Multiplex DNA sequencing
Sequencing in microfabricated high-density picoliter reactors
Whole genome sequencing
Cytogenetics
Comparative genomic hybridization (CGH)
Fluorescent in situ hybridization
Proteomic-based methods
Fluorescent in situ protein detection
Protein/peptide arrays for identification of multiple biomarkers in blood and tissue samples
Protein biochip technology
Toxicoproteomics
MicroRNA-based diagnostics
Molecular imaging
Functional MRI with nanoparticle contrast
FDG-PET
Optical imaging
Point-of-care diagnostics

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**Table 2.2** Applications of biochip technology relevant to personalized medicine

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Rapid DNA sequencing
Drug discovery and development
High-throughput drug screening
Design and stratification of clinical trials
Drug safety: applications in pharmacogenetics
Toxicogenomics
Clinical drug safety
Molecular diagnostics
Genetic screening
Detection of mutations
Inherited disorders
Identification of pathogens and resistance in infections
Molecular oncology
Cancer prognosis
Cancer diagnosis
Pharmacogenomics
Gene identification
Genetic mapping
Gene expression profiling
Detection of single nucleotide polymorphisms
For storage of the patient's genomic information
Integration of diagnosis and therapeutics

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Microarrays allow scientists to look at very subtle changes in many genes simultaneously. They provide a snapshot of what genes are expressed or active, in normal and diseased cells. When normal cells or tissues are compared to those known to be diseased, patterns of gene expression can emerge, enabling scientists to classify the severity of the disease and to identify the genes that can be targeted for therapy. This is how microarrays can potentially be used to develop personalized medical treatments. Figure 2.1 shows how the applications of biochips for pharmacogenetics and SNP genotyping form the basis for development of personalized medicine.

Microarray technology not only helps to make sense of the vast amount of genomic information but also enables its application to the patient by early detection of disease and prediction of drugs response. Although some problems of standardization and integration with electronic records remain, microarrays are promising for efficient, cost-effective, and personalized approaches to human health care. Microarray results can be comparable across multiple laboratories, especially when a common platform and set of procedures are used. Improving and standardizing microarray experiments will also enable early detection of diseases like cancer. This study may bring us one step closer to personalized medical treatment.

Numerous biochip technologies are available for clinical applications. The best known are the GeneChip (Affymetrix) and the AmpliChip CYP450 (Roche), which was cleared by the regulatory authorities for marketing in the US and the EU as an in vitro laboratory diagnostic test in 2004. The test is performed using DNA that is

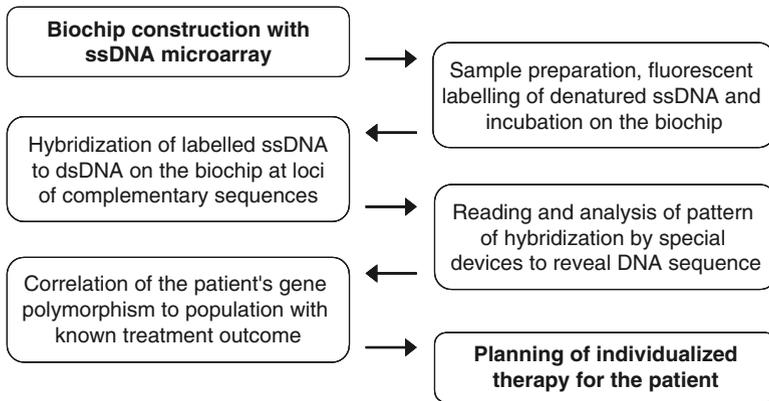


Fig. 2.1 Role of biochips/microarrays in personalized medicine ©Jain PharmaBiotech

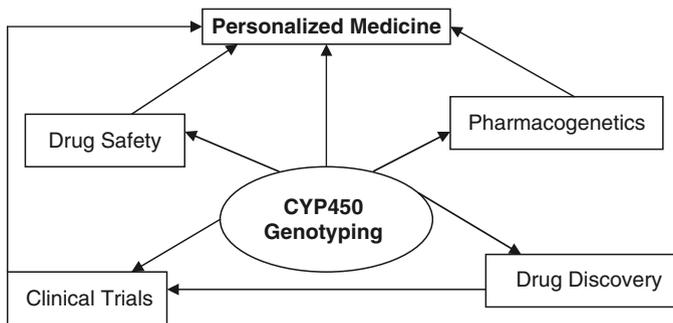


Fig. 2.2 Role of CYP450 genotyping in development of personalized medicine ©Jain Pharma-Biotech

extracted from a patient’s blood. DNA sequence is determined on the basis of the sequence of the probe molecule to which the DNA is most similar. AmpliChip CYP450 contains more than 15,000 different oligonucleotide probes to analyze both the sense and the antisense strands of an amplified target DNA sample (Jain 2005a). Virtually all known polymorphisms and alleles of CYP2D6, and the two most frequent for CYP2C19, can be detected simultaneously. AmpliChip CYP450 provides comprehensive coverage of gene variations, which play a role in the metabolism of approximately 25% of all prescription drugs. AmpliChip CYP450 test is intended to be an aid for physicians in individualizing treatment doses for patients on therapeutics metabolized through these genes. The role of CYP450 genotyping in development of personalized medicine is shown in Fig. 2.2.

## ***Role of Protein Biochips in Personalized Medicine***

Most of the biochips use nucleic acids as information molecules but protein chips are also proving to be useful. Profiling proteins will be invaluable, for example, in distinguishing the proteins of normal cells from early-stage cancer cells, and from malignant, metastatic cancer cells that are the real killers. In comparison with the DNA microarrays, the protein arrays, or protein chips, offer the distinct possibility of developing a rapid global analysis of the entire proteome leading to protein-based diagnostics and therapeutics.

Of all the applications of protein microarrays, molecular diagnostics is most clinically relevant and would fit in with the coming trend in individualized treatment. These technologies have an advantage in diagnosis of some conditions. For example, different proteins such as antibodies, antigens, and enzymes can be immobilized within protein microchips. Miniaturized and highly parallel immunoassays will greatly improve efficiency by increasing the amount of information acquired with single examination and reduce cost by decreasing reagent consumption.

ProteinChip (Vermillion, Inc.) has a role in proteomics comparable to that of GeneChip in genomics. It is based on SELDI (surface-enhanced laser desorption/ionization) process, which has four parts as applied to patient samples:

1. Patient sample of proteins is processed on the ProteinChip Array.
2. Enhance the “signal-to-noise” ratio by reducing chemical and biomolecular “noise” (i.e., achieve selective retention of target on the chip by washing away undesired materials).
3. Read one or more of the target protein(s) retained by a rapid, sensitive, laser-induced process (SELDI) that provides direct information about the target (molecular weight).
4. Process (characterize) the target protein(s) at any one or more locations within the addressable array directly in situ by engaging in one or more on-the-chip binding or modification reactions to characterize protein structure and function. Software produces map of proteins, revealing expression of marker protein with color change in the patient sample as compared to the control sample.

Proteomic pattern analysis might ultimately be applied as a screening tool for cancer in high-risk and general populations. This also applies to autoimmune diseases, by screening sera of patients or high-risk individuals for the presence of specific autoantibodies, using arrays of large numbers of recombinant proteins of known identity. Such arrays overcome the problems associated with variation of protein levels in conventional tissue extracts and hence improve reproducibility as a prerequisite for diagnostic use. High-throughput protein arrays have the potential to become diagnostic tools, eventually arriving at the doctor’s office and as over-the-counter devices. However, techniques to enable efficient and highly parallel identification, measurement, and analysis of proteins remain a bottleneck. A platform technology that makes collection and analysis of proteomic data as accessible as genomic data is yet to be developed. Sensitive and highly parallel technologies analogous to the nucleic acid biochip, for example, do not exist for protein analysis.

Protein chips will be particularly useful for clinical implementation of personalized medicine. Profiling proteins on biochips will be useful for distinguishing the proteins of normal cells from early-stage cancer cells, and from malignant metastatic cancer cells. In comparison with the DNA microarrays, the protein microarrays/chips, offer the possibility of developing a rapid global analysis of the entire proteome leading to protein-based diagnostics and therapeutics. Of all the applications of protein microarrays, molecular diagnostics is most clinically relevant and would fit in with the coming trend in individualized treatment. These technologies have an advantage in diagnosis of some conditions. For example, different proteins such as antibodies, antigens, and enzymes can be immobilized within protein biochips.

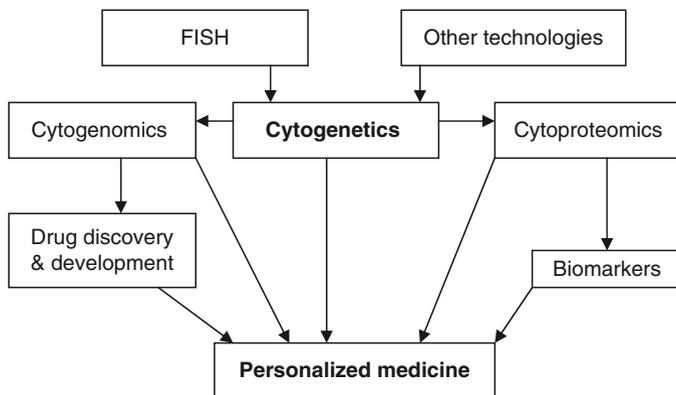
## Cytogenetics

The term “cytogenetics” has been classically used for studies of the cellular aspects of heredity. It has been used mainly to describe the chromosome structure and identify abnormalities related to disease. Besides clinical diagnostics, cytogenetics has been used for basic genomic research as well. It is better to include cytogenetics under the term “cytomics,” which means that the structural and functional information is obtained by molecular cell phenotype analysis of tissues, organs, and organisms at the single cell level by image or flow cytometry in combination with bioinformatic knowledge extraction concerning nucleic acids, proteins, and metabolites (cellular genomics, proteomics, and metabolomics), as well as cell function parameters like intracellular pH, transmembrane potentials, or ion gradients. The broader scope of biology at cell level can be covered by terms such as cytogenomics, cytometabolomics, and cytoproteomics. Because of its important role in diagnosing disease at molecular level, cytogenetics is an important part of molecular diagnostics and can be referred to as molecular cytogenetics. Cytogenetic technologies are described in detail in a special report on this topic (Jain 2009n).

### *Molecular Cytogenetics as Basis for Personalized Medicine*

Exciting advances in fluorescent in situ hybridization (FISH) and array-based techniques are changing the nature of cytogenetics, in both basic research and molecular diagnostics. Cytogenetic analysis now extends beyond the simple description of the chromosomal status of a genome and allows the study of fundamental biological questions, such as the nature of inherited syndromes, the genomic changes that are involved in carcinogenesis, and the 3D organization of the human genome. The high resolution that is achieved by these techniques, particularly by microarray technologies such as array comparative genomic hybridization, is blurring the traditional distinction between cytogenetics and molecular biology.

Classic cytogenetics has evolved from black and white to technicolor images of chromosomes as a result of advances in FISH techniques, and is now called molecular



**Fig. 2.3** Relation of cytogenetics to personalized medicine ©Jain PharmaBiotech

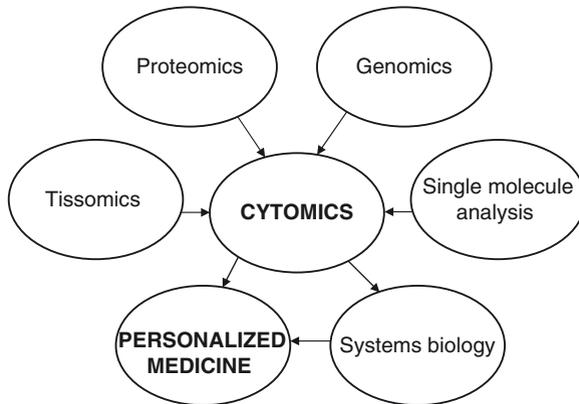
cytogenetics. Improvements in the quality and diversity of probes suitable for FISH, coupled with advances in computerized image analysis, now permit the genome or tissue of interest to be analyzed in detail on a glass slide. It is evident that the growing list of options for cytogenetic analysis has improved the understanding of chromosomal changes in disease initiation, progression, and response to treatment.

The architecture of the human genome as revealed by the human genome sequencing project explains the recurrence of microdeletions and microduplications caused by a non-allelic homologous recombination involving segmental duplications created during the evolution of primates. The new data have greatly contributed to our understanding of human chromosomal diseases. Molecular cytogenetics will enable the further assessment of molecular basis of structural chromosome anomalies.

Cytogenetics is related to other technologies in the same way as genetics and hence to personalized medicine with the difference that everything is at cell level (Fig. 2.3).

### *Cytomics as a Basis for Personalized Medicine*

In addition, differential molecular cell phenotypes between diseased and healthy cells provide molecular data patterns for (a) predictive medicine by cytomics or for (b) drug discovery purposes using reverse engineering of the data patterns by biomedical cell systems biology. Molecular pathways can be explored in this way including the detection of suitable target molecules, without detailed a priori knowledge of specific disease mechanisms. This is useful during the analysis of complex diseases such as infections, allergies, rheumatoid diseases, diabetes, or malignancies. The top-down approach reaching from single cell heterogeneity in cell systems and tissues down to the molecular level seems suitable for a human



**Fig. 2.4** Relation of cytomics to personalized medicine ©Jain PharmaBiotech

cytome project to systematically explore the molecular biocomplexity of human organisms. The analysis of already existing data from scientific studies or routine diagnostic procedures will be of immediate value in clinical medicine, for example as personalized therapy by cytomics (Valet 2005). Relation of cytomics to personalized medicine and other related technologies is shown in Fig. 2.4.

## SNP Genotyping

### *Technologies for SNP Analysis*

Technologies used for detection and analysis of SNPs are shown in Table 2.3. These are described in more detail elsewhere (Jain 2009a) but some are described briefly in the text following the table. Desirable characteristics of a genotyping technology are the following: (1) robust performance and accuracy across a variety of circumstances; (2) high-throughput performance; and (3) low cost. Sequencing offers the highest degree of specificity and selectivity. Restriction fragment length polymorphism, TaqMan assays and DNA microarrays are also frequently used genotyping methods.

### *Applications of SNPs Relevant to Personalized Medicine*

High-resolution genome-wide association studies using panels of 300,000 to 1 million SNPs aim to define genetic risk profiles of common diseases. These studies provide an opportunity to explore pathomechanism of human diseases and are unbiased by previous hypotheses or assumptions about the nature of genes that influence

**Table 2.3** Technologies for SNP analysis

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Digital Genetic Analysis
DNA chips and microarrays
DNA sequencing
Electrochemical DNA detection
Solution-borne ferrocene-modified DNAs
Redox-active intercalators
Surface-bound molecular beacon-like DNA
Fluorescence-detected 5'-exonuclease assays
Hybridization assays
Allele-specific oligomer hybridization
Array hybridization assays, e.g., MASDA (multiplexed allele-specific diagnostic assay)
Hybridization with PNA probes
Invader assay
Mass spectrometry (MS)
Matrix Assisted Laser Desorption Ionization Time of Flight MS (MALDI-TOF MS)
Competitive Oligonucleotide Single Base Extension
Nanoparticle probes
Oligomer-specific ligation assays
PCR-based methods
PCR-CTPP (confronting two-pair primers)
Degenerate oligonucleotide primed (DOP)-PCR
TaqMan real-time PCR
Smart amplification process version 2
Peptide nucleic acid (PNA) probes
Primer extension
Pyrosequencing
Single base extension-tag array on glass slides (SBE-TAGS)
Single molecular fluorescence technology
Triplex Assay (Genetic Technologies, Inc.)
WAVE System's Temperature Modulated Heteroduplex Analysis method
Zinc finger proteins

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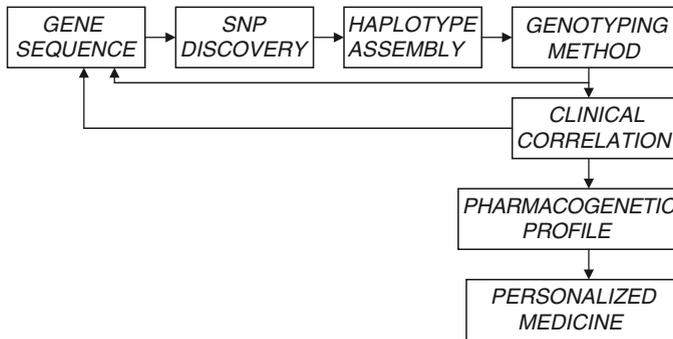
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complex diseases. Many genetic variants identified as risk factors for diseases by such studies have been localized to previously unsuspected pathways, to genes without a known function.

In the absence of functional information about which polymorphisms are biologically significant, it is desirable to test the potential effect of all polymorphisms on drug response. Potential uses of SNP markers include drug discovery and prediction of adverse effects of drugs. Role of SNPs in personalized medicine is shown in Fig. 2.5.

SNPs have the following relation to an individual's disease and drug response:

- SNPs are linked to disease susceptibility.
- SNPs are linked to drug response, e.g. insertions/ deletions of ACE gene determine the response to beta blockers.



**Fig. 2.5** Role of SNPs in personalized medicine. © Jain PharmaBiotech

- SNPs can be used as markers to segregate individuals with different levels of response to treatment (beneficial or adverse) in clinical settings.
- SNPs have a role in clinical trials as genotyping is important in design and interpretation of clinical studies.

Advantages of molecular genetic profiling in clinical studies are the following:

- It is a contribution to molecular definition of the disease.
- Correlation of drug response to the genetic background of the patient.
- Prediction of dose-response and adverse effects.
- SNP mapping data can be used to pinpoint a common set of variant nucleotides shared by people who do not respond to a drug.

### ***Concluding Remarks on SNP Genotyping***

Several methods are available for SNP genotyping. For ten or fewer SNPs and sample numbers in the thousands, the current gold standard is TaqMan real-time PCR (Life Technologies). MassARRAY system (SEQUENOM), is a mass spectrometry-based platform suitable for high throughput and up to 1,000 SNPs. Pyrosequencing (Biotage AB), a sequencing-by-synthesis method can be used for up to 100 SNPs. Affymetrix provides the densest coverage at the whole-genome level with its GeneChip Human Mapping 500K Array Set and Affymetrix GeneChip® Scanner 3000 MegAllele, based on Molecular Inversion Probe Technology, and enables the highest level of multiplexing that is commercially available, as well as increase throughput with low capital investment. Illumina is supplementing its current 100K chip with a 250K chip. Restriction fragment length polymorphism analysis is laborious and hit-and-miss as success depends on whether the restriction enzyme recognizes particular SNPs. It is relatively inexpensive, which makes it appropriate for a small number of SNPs and a small number of samples. New methods

for SNP genotyping are being investigated. The presence of a single base pair mismatch can be identified by the conductance of the molecule and can cause a change in the conductance of dsDNA by as much as an order of magnitude, depending on the specific details of the double helix and the SNP.

Pharmacogenetic capabilities have changed remarkably since the first SNP map from the SNP Consortium became freely available in 2001. It is now possible to use SNP-mapping technologies to create a genetic profile of each individual that can be used to identify patterns of susceptibility genes for common diseases, as well as genetic risk/efficacy factors that are related to the effects of drugs. Interindividual variability in drug response, ranging from no therapeutic benefit to life-threatening adverse reactions, is influenced by variation in genes that control the absorption, distribution, metabolism, and excretion of drugs.

An example of how SNP genotyping may be applied in medicine is the evidence of association between an SNP in the TNFR (tumor necrosis factor receptor) II gene and rheumatoid arthritis. TNF is a powerful mediator of inflammation in rheumatoid arthritis. In vivo, its acute effects are limited by binding to soluble receptors (TNFR), suggesting that TNFR genes could be important candidate risk factors, the strongest association being observed in patients with a family history of this disease. The TNFR2 polymorphism or other genetic variations in the TNF or related genes may be useful markers for susceptibility to familial rheumatoid arthritis treatment response to TNF inhibitors.

## Haplotyping

An alternative approach to SNP genotyping is haplotyping. Haplotyping information makes it possible to highlight the structure of the genome, notably through haploblocks which correspond to segments of chromosomes unlikely to undergo a crossing-over event. Haplotyping is a way of characterizing combinations of SNPs that might influence response and is considered to be a more accurate measure of phenotypic variation. However, SNP-based tests have greater power when the number of causative SNPs (a subset of the total set of SNPs) is smaller than the total number of haplotypes. One limitation of haplotyping is that haplotypes need to be determined for each individual, as SNPs detected from a pool of DNA from a number of individuals cannot yield haplotypes.

Until whole-genome sequencing of individual patients becomes feasible clinically, the identification of SNPs and haplotypes will prove instrumental in efforts to use genomic medicine to individualize health care. When an extensive inventory of genome-wide SNP scans has been assembled across diverse population samples, maps using SNP and/or haplotypes will dictate that it will not be necessary to identify the precise genes involved in determining therapeutic efficacy or an adverse reaction. Linkage disequilibrium (LD) methods can provide robust statistical correlations between a patient's response/risk index for a given drug class and a specific LD-SNP/haplotype profile.

Candidate gene-based haplotype approach has been applied to the pharmacogenetics of drug response and adverse events. Clinical trials using haplotyped individuals were the first genetically personalized medical treatments.

## *HapMap Project*

Compared to the map of the human genome, which provides a route finder in genetics, a haplotype map will show the sites along the way. HapMap, a public resource created by the International HapMap Project ([www.hapmap.org](http://www.hapmap.org)), is a catalog of genetic variants (SNPs) that are common in human populations. It will enable efficient and large scale studies in genetics and show common variants that cause disease. The HapMap project is the first major post-genomic initiative and is built on the experience gained from sequencing the human genome. The results will provide the physicians with basics of pharmacogenomics to enable them to give personalized treatments to their patients.

HapMap will accelerate the discovery of genes related to common diseases, such as asthma, cancer, diabetes, and heart disease. This information will aid researchers searching for the genetic factors that affect health, disease, and responses to drugs and the environment. HapMap is a shortcut to scanning through millions of SNPs. One need only to find blocks into which the genome is organized, each of which may contain several SNPs. SNPs in a haplotype block are inherited together and the pattern of SNPs in a haplotype block is unique for an individual. Currently this information is being used for the development of genetic panels to be used in pharmacogenomic and disease risk assessment studies. HapMap would be useful in the US where little is known of the genealogy of the population. Some population groups, however, share haplotype patterns from their common ancestors. HapMap program would be superfluous in Iceland, where it is possible to isolate disease genes in the highly structured genealogy of Iceland for any disease with a prevalence of more than 0.2%.

The consortium's new goal is to build an improved version of the HapMap that is about five times denser than the original plan. This "Phase II" HapMap will take advantage of the rapid, high-throughput genotyping capacity of Perlegen Sciences to test another 4.6 million SNPs from publicly available databases, and add that information to the map. Perlegen received a \$6.1 million award from the NIH's National Human Genome Research Institute (NHGRI) to add data on 2.25 million additional SNPs to HapMap. The new development, enabled by a partnership among multiple funding sources, will expand that effort and test virtually the entire known catalog of human variation on the HapMap samples. This will increase the density of SNP "signposts" across the genome from the current average of 1 every 3,000 bases to about 1 every 600 bases.

Successful genome-wide association studies are the most visible and exciting outcome of HapMap to date, with the large number of robust and highly replicated genetic associations with common diseases providing novel and unexpected

insights into the pathophysiology of disease (Manolio et al. 2008). The HapMap has also been invaluable in developing genotyping and analytic methods, and providing samples for validation of variation detection methods and standardization of laboratory processes. Application of these association findings is expected to produce new advances in the prevention and treatment of common diseases.

### ***Predicting Drug Response with HapMap***

A pharmacogenetic study in cardiovascular disease using a model based on HapMap revealed that haplotype constituted by allele Gly16 (G) at codon 16 and allele Glu27 (G) at codon 27 genotyped within the beta2AR candidate gene exhibits a different effect on heart rate curve than the rest of haplotypes (Lin et al. 2005). Parents with the diplotype consisting of two copies of haplotype GG are more sensitive in heart rate to increasing dosages of dobutamine than those with other haplotypes. This model provides a powerful tool for elucidating the genetic variants of drug response and ultimately designing personalized medications on the basis of each patient's genetic constitution.

### **Nanodiagnostics for Personalized Medicine**

Nanotechnology is the creation and utilization of materials, devices, and systems through the control of matter on the nanometer-length scale, i.e., at the level of atoms, molecules, and supramolecular structures. It is the popular term for the construction and utilization of functional structures with at least one characteristic dimension measured in nanometers (a nanometer is one billionth of a meter ( $10^{-9}$  m)). Nanobiotechnology is the application of nanotechnology in life sciences and is the subject of a special report (Jain 2009d). Application of nanobiotechnology in molecular diagnostics is called nanodiagnostics and is described in a book on Nanomedicine (Jain 2008). Because DNA, RNA, protein, and their functional subcellular scaffolds and compartments, are in the nanometer scale, the potential of single molecule analysis approach would not be fully realized without the help of nanobiotechnology. Advances in nanotechnology are providing nanofabricated devices that are small, sensitive and inexpensive enough to facilitate direct observation, manipulation, and analysis of a single biological molecule from a single cell. This opens new opportunities and provides powerful tools in the fields such as genomics, proteomics, molecular diagnostics, and high throughput screening.

Various nanodiagnostics that have been developed will improve the sensitivity and extend the present limits of molecular diagnostics (Jain 2007). Numerous nanodevices and nanosystems for sequencing single molecules of DNA are feasible. It seems quite likely that there will be numerous applications of inorganic nanostructures in biology and medicine as markers. Given the inherent nanoscale of receptors,

pores, and other functional components of living cells, the detailed monitoring and analysis of these components will be made possible by the development of a new class of nanoscale probes. Biological tests measuring the presence or activity of selected substances become quicker, more sensitive, and more flexible when certain nanoscale particles are put to work as tags or labels. Nanoparticles are the most versatile material for developing diagnostics.

Nanomaterials can be assembled into massively parallel arrays at much higher densities than is achievable with current sensor array platforms and in a format compatible with current microfluidic systems. Currently, quantum dot technology is the most widely employed nanotechnology for diagnostic developments. Among the recently emerging technologies, the one using cantilevers is the most promising. This technology complements and extends current DNA and protein microarray methods, because nanomechanical detection requires no labels, optical excitation, or external probes and is rapid, highly specific, sensitive, and portable. This will have applications in genomic analysis, proteomics, and molecular diagnostics. Nanotechnology has potential advantages in applications in point-of-care (POC) diagnosis: on patient's bedside, self-diagnostics for use in the home, integration of diagnostics with therapeutics, and for the development of personalized medicines.

### *Cantilevers for Personalized Medical Diagnostics*

An innovative method based on cantilevers has been developed for the rapid and sensitive detection of disease- and treatment-relevant genes (Zhang et al. 2006). This method detects active genes directly by measuring their transcripts (messenger RNA (mRNA)), which represent the intermediate step and link to protein synthesis. Short complementary nucleic acid segments (sensors) are attached to silicon cantilevers which are 450 nm thick and therefore react with extraordinary sensitivity. Binding of the targeted gene transcript to its matching counterpart on one of the cantilevers results in optically measurable mechanical bending. Differential gene expression of the gene 1-8U, a potential marker for cancer progression or viral infections, could be observed in a complex background. The measurements provide results within minutes at the picomolar level without target amplification, and are sensitive to base mismatches. An array of different gene transcripts can even be measured in parallel by aligning appropriately coated cantilevers alongside each other like the teeth of a comb. The new method complements current molecular diagnostic techniques such as the gene chip and real-time polymerase chain reaction (PCR). It could be used as a real-time sensor for continuously monitoring various clinical parameters or for detecting rapidly replicating pathogens that require prompt diagnosis. These findings qualify the technology as a rapid method to validate biomarkers that reveal disease risk, disease progression, or therapy response. Cantilever arrays have potential as a tool to evaluate treatment response efficacy for personalized medical diagnostics.

## ***Nanopore-Based Technology for Single Molecule Identification***

As single molecules are driven through a nanopore by a voltage differential, the 3D charge profile of a molecule is measured by the field-effect transistors (FETs), enabling each molecule in the sample to be uniquely identified and precisely quantified. This method does not require fluorescent or other labels, thermal cycling, or optics. This technology offers the prospect to eventually correlate DNA and its expressed proteins with specific disease states using an inexpensive, disposable, and portable device. For example, the device has the potential to enable development of exquisitely targeted treatments using sequencing data both from a patient and from the disease-causing pathogen. Compared to other nanopore-based technologies for measuring molecules using electronic signals, the Eagle approach achieves a 1,000-fold higher sensitivity as a result of the FETs embedded in the nanopores. This technology could potentially be the first to enable the identification and measurement of both DNA and proteins in a single sample at the same time. The technology could have significant implications for advancing personalized medicine on the basis of its potential for faster, more efficient, and less expensive protein and nucleic acid identification.

## **Application of Proteomics in Molecular Diagnosis**

Discovery of the genetic sequence encoding a protein by nucleic acid technologies is not sufficient to predict the size or biological nature of a protein. Studies at the messenger RNA level can assess the expression profiles of transcripts but these analyses measure only the relative amount of an mRNA encoding a protein and not the actual amount of protein in a tissue. To address this area, several protein-based analysis technologies have been developed. Proteomic technologies are described in detail in a special report on this topic (Jain 2009e). Proteomics-based assays are considered to be a distinct group within molecular diagnostics and should not be confused with immunoassays although some proteomic technologies are antibody-based.

Technologies with the greatest potential are 2D PAGE, antibody-based screening, protein-binding assays, and protein biochips. 2D PAGE is combined with mass spectroscopy-based sequencing techniques, which identify both the amino acid sequences of proteins and their posttranslational appendages. This approach is combined with database search algorithms to sequence and characterize individual proteins. Role of proteomics in the discovery of biomarkers will be described in [Chapter 3](#).

## ***Comparison of Proteomic and Genomic Approaches in Personalized Medicine***

Although proteomic and genomic approaches can be complementary, there are some similarities and differences that are shown in [Table 2.4](#).

**Table 2.4** Comparison of proteomic and genomic approaches in personalized medicine

Genotype/haplotype	Gene/protein expression	Protein function studies	Metabonomics
Polymorphisms related to a specific level of enzyme activity	Protein function is inferred from expression levels of mRNA or protein	Direct measurement of protein function	Infers level of protein function from metabolic profile
Genotype does not always correlate with protein function	Gene/Protein expression does not always correlate with protein expression/protein function	Direct measurement of protein function under conditions which mimic drug exposure	Levels of endogenous metabolites rather than exogenous levels; under static conditions
Does not account for polypharmacy, inducers, and inhibitors	Does not account for polypharmacy, inducers, and inhibitors	Accounts for polypharmacy, inducers, and inhibitors	Accounts for polypharmacy, inducers, and inhibitors
Qualitative Identifies polymorphism found to correlate to fast or slow phenotype	Quantitative Identifies increased or decreased expression of mRNA or protein	Quantitative Identifies responders, non- responders, and those that will experience toxicity at standard doses	Qualitative Identifies responders, non- responders, or those that will experience toxicity
Allows semi categorical individualization	Lack of correlation, makes individualization inaccurate	Allows accurate individualization of therapy to treat many of those originally identified as non-responders or at risk for toxicity	Non-responders or those who will experience toxicity are not treated with specific agent

## Gene Expression Profiling

The activity of a gene, so called gene “expression” means that its DNA is used as a blueprint to produce a specific protein. The first step of gene expression is transcription, the process by which the sequence of DNA bases within a gene is used as a template to synthesize mRNA. Following transcription, the nascent mRNA is processed and transported out of the nucleus and into the cytoplasm of the cell. Once in the cytoplasm, the mature mRNA is engaged in the last step in gene expression, translation – the process by which proteins are synthesized. Finally there is posttranslational modification of proteins into mature forms. Each of these steps in gene expression is subject to precise cellular controls that collectively allow the cell to respond to changing needs.

Less than half of all genes are expressed in a typical human cell, but the expressed genes vary from one cell to another and from one individual to another.

**Table 2.5** Selected methods for gene expression profiling

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Genome-wide methods
Microarrays: whole genome expression array
Serial analysis of gene expression (SAGE)
Expressed sequence tags (ESTs) analysis
Gene expression profiling based on alternative RNA splicing
Tangerine expression profiling
Individual sequences
Real time RT-PCR
Competitive RT-PCR
RNase protection assay
T cell receptor expression analysis
Analysis of single-cell gene expression
RNA amplification
Monitoring in vivo gene expression
Magnetic resonance imaging

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Gene expression is used for studying gene function. Gene expression profiling, therefore, is relevant to personalized medicine. The temporal, developmental, topographical, histological, and physiological patterns in which a gene is expressed provide clues to its biological role. All functions of cells, tissues, and organs are controlled by differential gene expression. Malfunctioning of genes is involved in most diseases, not only inherited ones. Knowledge of which genes are expressed in healthy and diseased tissues would allow us to identify both the protein required for normal function and the abnormalities causing disease. This information will help in the development of new diagnostic tests for various illnesses, as well as new drugs to alter the activity of the affected genes or proteins. Gene expression profiling is relevant to development of personalized medicine and some of the technologies used will be described briefly. Various techniques for detection of gene expression are shown in Table 2.5.

### ***DNA Microarrays for Gene Expression Studies***

DNA microarrays have become the main technological workhorse for gene expression studies. To date, detection platforms for most microarrays have relied on short (25 base) oligonucleotides synthesized in situ, or longer, highly variable length DNAs from PCR amplification of cDNA libraries. Long (50–80 base) oligonucleotide arrays are now available and might eventually eliminate the use of cDNA arrays. The technology has advanced to such a point that researchers now demand microarrays that are cost-effective and have flexibility and quality assurance. Although there are other, non-array methods for analyzing gene expression, such as SAGE, the simplicity of the oligonucleotide approach makes it the most attractive option for the gene expression profiling. Important applications are in drug discovery,

a file that is now flooded with potential targets. Microarrays will play an essential role in overcoming this obstacle in both target identification and in the long road of drug discovery and development. Two important therapeutic areas for gene expression profiling using microarrays are cancer and neurological disorders.

### ***Analysis of Single-Cell Gene Expression***

Analysis of single-cell gene expression promises a more precise understanding of human disease pathogenesis and has important diagnostic applications. Single cell isolation methods include flow cytometry cell sorting and laser capture microdissection. Besides the gene expression analysis, the following nucleic acid amplification methods are suitable for single-cell analysis:

- Single cell phenotyping
- Homomeric tailed PCR, which allows unbiased amplification of RNA
- RNA amplification

Gene expression analysis of single cells is providing new insights into disease pathogenesis, and has applications in clinical diagnosis. Molecular signatures of some diseases can best be discerned by analysis of cell subpopulations. Studies in disease-relevant cell populations that identify important mRNA (and protein) differences between health and disease should allow earlier diagnosis, better therapeutic intervention, and more sensitive monitoring of treatment efficacy. This will facilitate the development of personalized medicine on the basis of the molecular signatures of the diseased cell population.

Current assays for gene expression destroy the structural context. By combining advances in computational fluorescence microscopy with multiplex probe design, it is possible that expression of many genes can be visualized simultaneously inside single cells with high spatial and temporal resolution. Use of the nucleus as the substrate for parallel gene analysis can provide a platform for the fusion of genomics and cell biology and it is termed “cellular genomics.” This technique takes a snapshot of genes that are switched on in a single cell. Used on a breast biopsy or suspect skin mole, it could pick out the first one or two cells that have harmful genes and become malignant.

### ***Gene Expression Profiling Based on Alternative RNA Splicing***

RNA splicing is an essential, precisely regulated process that occurs after gene transcription and before mRNA translation. A gene is first transcribed into a pre-mRNA, which is a copy of the genomic DNA containing intronic regions destined to be removed during pre-mRNA processing (RNA splicing), as well as exonic sequences that are retained within the mature mRNA. During splicing, exons can

either be retained in the mature message or targeted for removal in different combinations to create a diverse array of mRNAs from a single pre-mRNA, a process referred to as alternative RNA splicing. Splicing is the crucial and tightly regulated step between gene transcription and protein translation. Alternative splicing could be responsible for generating up to three times as many proteins as the 20,000–25,000 genes encoded by the human genome. The ability to analyze RNA splicing events gives a unique understanding of the sequences that are critical for normal cellular function. The control of alternative RNA splicing can be deregulated in human disease as a consequence of alterations within signaling cascades, within the spliceosome machinery, or within the genes that are spliced. This allows the identification of novel splice variants that cannot be detected using oligonucleotide microarray technology. Comparisons of alternative RNA splicing repertoires will not only provide such expression markers but will also aid candidate gene selection for SNP analyses, defining the location of the relevant SNPs within the genes. An increased understanding of the mechanism of alternative splicing and the further characterizations of splice variants will have a significant impact on pharmacogenomics and development of personalized medicine. Alterations in RNA splicing have a significant impact on drug action and can be exploited to generate pharmacogenomics tools in several ways.

- Alteration of alternative RNA splicing events triggered by drug or chemicals action constitutes a route through which relevant candidate genes can be selected for further genotyping because these genes are likely to lie within crucial pathways of drug action.
- Analyses of RNA splicing might provide a rapid method for detection of polymorphisms across the whole gene.
- RNA splicing alteration libraries between responders and non-responders would constitute a discovery tool for SNPs that are relevant to pharmacogenomics.

## **Molecular Imaging and Personalized Medicine**

Positron emission tomography (PET) is the most sensitive and specific technique for imaging molecular pathways *in vivo* in humans. PET uses positron emitting radionuclides to label molecules, which can then be imaged *in vivo*. The inherent sensitivity and specificity of PET is the major strength of this technique. Indeed, PET can image molecular interactions and pathways, providing quantitative kinetic information down to sub-picomolar levels. Generally, the isotopes used are short-lived. Once the molecule is labeled, it is injected into the patient. The positrons that are emitted from the isotopes then interact locally with negatively charged electrons and emit what is called annihilating radiation. This radiation is detected by an external ring of detectors. It is the timing and position of the detection that indicates the position of the molecule in time and space. Images can then be constructed tomographically, and regional time activities can be derived. The kinetic data produced provide information about the biological activity of the molecule. Molecular imaging

provides *in vivo* information in contrast to the *in vitro* diagnostics. Moreover, it provides a direct method for the study of the effect of a drug in the human body. Molecular imaging plays a key role in the discovery and treatment process for neurological diseases such as Alzheimer's disease and cancer. The ability to image biological and pathological processes at a molecular level using PET imaging offers an unparalleled opportunity to radically reform the manner in which a disease is diagnosed and managed. Its translation into clinical practice will impact upon personalized medicine.

### ***Monitoring In Vivo Gene Expression by Molecular Imaging***

Molecular imaging is an emerging field of study that deals with imaging of disease on a cellular and molecular level. It can be considered as an extension of molecular diagnostics. Technologies encompassed within molecular imaging include optical, magnetic resonance imaging (MRI), and nuclear medicine techniques. In contradistinction to "classical" diagnostic imaging, it sets forth to probe the molecular abnormalities that are the basis of disease rather than to image the end effects of these molecular alterations. Radionuclide imaging, MRI, and positron emission tomography (PET) can be used to visualize gene expression. Several current *in vitro* assays for protein and gene expression have been translated into the radiologic sciences. Endeavors are under way to image targets ranging from DNA to entire phenotypes *in vivo*. The merging fields of molecular biology, molecular medicine, and imaging modalities may provide the means to screen active drugs *in vivo*, image molecular processes, and diagnose disease at a presymptomatic stage.

### **Glycomics-Based Diagnostics**

Glycomics is the study of glycans, which are information-rich molecules, composed of complex carbohydrates (sugars or polysaccharides) that are often attached to proteins, lipids, and cells and it focuses on inflammatory therapies. Interactions between carbohydrates and proteins mediate intracellular traffic, cell adhesion, cell recognition, and immune system function. Glycans are downstream in the biological information flow and are therefore closer to the actual state of affairs. They can generate information, which is more relevant to the pharmacological aspects of drug behavior than either DNA or proteins by themselves.

Glycominds, Ltd.'s personalized medicine approach to inflammatory disorders is on the basis of glycan molecules. This approach has significant advantage over SNPs and other DNA-based pharmacogenomics assays because the patient's inflammation level is correlated to his history of infection and physiological state, not just to his DNA. Autoimmune research based on protein-glycan interactions generates superior analysis. Using GlycoChip® arrays, Glycominds measures binding

at the antibody level, including sub-types (i.e. IgG, IgM, and IgA), T-cell glycan adhesion, and glyco-related serum proteins. By combining its proprietary knowledge of protein-glycan interactions with its superior approach to inflammation biomarker research, Glycominds' strategy is to discover exceptional biomarkers that will serve as personalized medicine tests. These novel biomarkers open up an unexplored angle for drug pharmacodynamics. An example of the application of this technology is Glycominds' gMS™ assay for multiple sclerosis (MS) that will enable to stage the predicted disease activity and identify the most appropriate treatment strategy in patients presenting with a first demyelinating event. Patients who may benefit from disease modifying therapy could commence it earlier and more aggressively if needed. Conversely, patients who are not at immediate risk and might not benefit from therapy could transiently avoid the effects of inconvenient and costly treatments. Glycominds is sponsoring two studies, PRACTIMS and DECISION, to validate its MS marker.

## Combination of Diagnostics and Therapeutics

Combination of diagnosis with therapeutics, wrongly referred to as “theranostic” is an important component of personalized medicine. A more appropriate term is “pharmacodiagnostic.” The diagnostics is linked to the therapeutic substance to select patients who would be suitable for treatment by a particular drug. The drug and the diagnostic test are marketed together. There are several such combinations in the market particularly for the treatment of cancer.

## Point-of-Care Diagnosis

Point of care or near patient testing means that diagnosis is performed in the doctor's office or at the bed side in case of hospitalized patients or in the field for several other indications including screening of populations for genetic disorders and cancer. POC involves analytical patient testing activities provided within the healthcare system, but performed outside the physical facilities of the clinical laboratories. It does not require permanent dedicated space, but instead includes kits and instruments, which are either hand carried or transported to the vicinity of the patient for immediate testing at that site. The patients may even conduct the tests themselves at home. After the laboratory and the emergency room, the most important application of molecular diagnostics is estimated to be at the point-of-care. There are many reasons for the substantial growth of POC testing, but perhaps the most significant is that the accuracy and reliability of POC tests now approach that of high-volume analyzers used in clinical laboratories. POC diagnosis is important for the development of personalized medicine and various applications are listed in Table 2.6.

For physicians, the benefit of being able to obtain test results quickly at the bedside or in a critical care setting often outweighs the somewhat higher cost per

**Table 2.6** Applications of point-of-care diagnosis

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In the hospital
Emergency room testing for various pathogens in 'untested' blood donations
Rapid tests in emergency departments for microorganisms in severe diarrhea, meningitis, etc.
Intensive care
Operating room
In the physician's office
Testing for viruses causing coughs and colds
Detection of bacterial infections to select appropriate antibiotic
Screening for cancer
In field studies
Screening of populations for genetic disorders
Testing of patients in clinical trials
Detection of microorganisms that are associated with bioterrorism
Identification of patients with communicable diseases at the point of immigration.
Food testing
In the home
Self testing by the patient
Testing at home by visiting healthcare personnel

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test associated with POC testing. This is particularly true in the coronary care units of hospital emergency departments, where new cardiac marker tests can provide rapid results that physicians can use to make critical patient management decisions. The demand for POC tests has also stimulated an increase in their diversity. A small variety of home tests such as ovulation predictors, pregnancy tests, fecal occult blood assays, and blood glucose monitors have been available for years. More recently, FDA has approved home-use tests for monitoring bladder cancer, anticoagulation therapy, urinary tract infections, HIV status, drugs of abuse, and even risk assessment for preterm labor and delivery.

Point-of-care diagnosis is well known with simple biochemical tests such as blood glucose monitoring. Role of biochips for this purpose is still in development. Protein chips, particularly microfluidic immunoassays, appear to be likely to get to point-of-care first as several technical problems associated with use of nucleic acid chips outside the laboratory are being worked out. Biochip and microfluidic technologies are also used for miniaturizing other laboratory tests such as cell count and automated immunoassays. Continued improvements in biosensor technology and miniaturization will increase the ability to test for many analytes at or near the patient. Hand-held diagnostic devices, biochips and electrochemical devices for the detection of DNA are particularly suited for point-of-care diagnostics. Nanotechnology would be another means of integrating diagnostics with therapeutics. Nanotechnology-based diagnostics provides the means to monitor drugs administered by nanoparticle carriers. Nanodiagnostic sensors might be incorporated in nanorobotic devices in the future for navigating the body to detect and destroy viruses or cancer cells.

## ***Point-of-Care Diagnosis of Infections***

In medicine, quantitative measurement of specific strains of infectious organisms is very important in emergency situations because the physician must start therapy immediately if the patient is in critical condition. An effective test must be precise, rapid, and also able to measure the infectious burden. At the same time, better testing will quickly identify the organism's strain and drug susceptibility, reducing the delay in finding the right antibiotic.

Traditional diagnostic testing often requires several days to isolate and grow the infectious organism, and to test its sensitivity to specific antibiotics. Until then, the physician must use powerful broad-spectrum antibiotics. Widespread use of these antibiotics leads to the emergence of drug resistance, which then narrows the number of drugs available to treat serious infections. Infectio Diagnostic, Inc. is developing PCR-based tests for the under-1-h detection and identification of infectious agents, thus revolutionizing the decision-making process of health care professionals.

Detection, identification, and characterization of pathogens are being revolutionized by the combination of the seemingly disparate fields of nucleic acid analysis, bioinformatics, data storage and retrieval, nanotechnology, physics, microelectronics, and polymer, solid state, and combinatorial chemistry. The first application of DNA chips in POC testing will probably be for identifying pathogens and their antimicrobial resistance potential. These developments, particularly with regard to POC testing, have important implications for the delivery of health care. It will be possible to miniaturize test kits, which can be swallowed or added to body fluids and coupled with data transmitters so that results can be sent to remote site for analysis.

## ***Advantages vs. Disadvantages of Point-of-Care Diagnosis***

Advantages of POC diagnosis are

- Appropriate immediate prescribing according to diagnosis
- Rapid implementation of measures for control of infections
- Decreased dependency of remote areas on distant diagnostic facilities
- Rapid diagnosis, alleviating unnecessary anxiety associated with waiting for results
- Contributing to decreased overall cost of health care by reducing inappropriate treatments while waiting for traditional laboratory diagnosis
- No need for transport of specimens

Disadvantages of POC diagnosis are

- Misuse or misinterpretation of test result, particularly if used at home
- Overutilization of services leading to rise of cost of health care

- Potential loss of epidemiological data
- Less opportunity for large scale automation
- Inadequate discussion or patient counseling
- Reduced opportunity for internal and external quality assurance, with associated risk of misdiagnosis
- Medicolegal implications

### ***Future Prospects of Point-of-Care Diagnosis***

POC-testing is destined to become a major force in the development of healthcare delivery. Advances will be on four fronts:

1. Scope: Expanding the POC format into new categories of in vitro diagnostic testing.
2. Connectivity: Communicating test results externally with ease and flexibility.
3. Non-invasiveness: Improving the way test samples are obtained from the body.
4. Miniaturization: Reducing the size of the devices to enable novel uses.

The major technological requirements to reduce complications of POC have been identified by both the manufacturers and the regulators. These focus on reduction of dependence on the operator and seamless automation of quality control.

## **Genetic Testing for Disease Predisposition**

Genetic testing is a broad term, which covers several techniques, including those used to determine paternity and in forensic medicine. However, most genetic tests are used to confirm a suspected diagnosis, to predict susceptibility to an illness, to identify individuals who carry a specific genetic mutation but remain unaffected themselves, or to predict how an individual is likely to respond to a certain therapy. Genetic tests are also used to screen fetuses, newborns, and embryos used in in vitro fertilization for genetic defects. Over 1,500 genetic tests are available including those that indicate susceptibility to cancer, neurological disorders, and heart disease.

Testing for gene mutations that confer susceptibility to adult-onset disorders has potential benefits, but these must be balanced against the psychological harms, if any. The published findings on the psychological effects of such testing focus on Huntington's disease, which has the most available data, and the hereditary cancer syndromes. Most of the evidence suggests that non-carriers and carriers differ significantly in terms of short-term, but not long-term, psychological adjustment to test results. The psychological impact of genetic testing depends more on pretest psychological distress than the test result itself.

## *Personal Genetic Service*

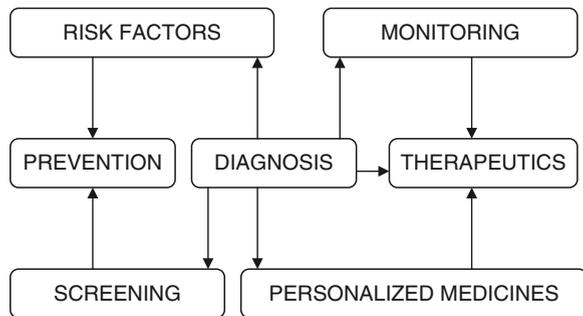
A large number of companies offer tests to screen for diseases with a genetic component or to identify those at risk of developing a certain disease. Some of the companies developing genetic tests are mentioned in other categories such as those involved in prenatal and cancer diagnostics.

Commercialization of genetic technologies is expanding the horizons for the marketing and sales of direct-to-consumer (DTC) genetic tests. Several companies are involved in this activity. A selection of companies offers genetic screening tests directly to consumer, usually via Internet. This list does not include companies offering genetic testing only for paternity, athletic ability, etc. At least three companies – 23andMe, DeCode Genetics, and Navigenics/Affymetrix – have made available DTC “personal genome services” that rely on the same arrays of 500,000 to 1 million SNPs used in genome-wide association studies. The best organized program is that of Navigenics/Affymetrix, which also provides genetic counseling.

## **Role of Diagnostics in Integrated Healthcare**

### *Concept of Integrated Healthcare*

Advances in medical genetics, molecular diagnostics, and genome-based medicines will enable integrated healthcare systems incorporating genetic screening, prevention, diagnosis, therapy, and monitoring. Diagnosis and therapy would be central in such a system as shown in Fig. 2.6. A suitable term to describe such a system has not been coined as yet. The term “integrative medicine” is applied to indicate integration of complimentary medicine in traditional. The first example of the combination of diagnostics and therapeutics was in the management of AIDS. HIV genotyping tests were used to detect resistance to antiviral drugs and molecular diagnostics tests were conducted for viral quantification to monitor therapy. The



**Fig. 2.6** A scheme of integrated healthcare and personalized medicine

initiative for development of such systems has come from the pharmaceutical industry as no academic or government organization has taken interest in this approach. Although the industry has a vested interest in the development of combined systems, there are advantages for the practicing physicians as well.

A combined system for diagnosis and therapeutics will have other components. The term diagnosis will broadly include screening for identification of risk factors, whereas therapeutics would also include monitoring of therapy. Prevention is added to this system because detection of predisposing factors can enable disease prevention by correction of risk factors or pre-emptive treatment. A key factor that will drive the integration of diagnostics and therapeutics is the availability of improved and more precise diagnostic methods, which are easy to perform and are not expensive. As discovery of disease genes progresses, the genes may form the link between diagnosis and gene-based medicines.

## *Components of Integrated Healthcare*

### **Screening**

It would be ideal to detect predisposition and risk factors before the development of a disease. The classical risk factors for major diseases are known but screening for genetic risk factors would be helpful in detecting specific risk factors for certain diseases. This would form the basis of preventive strategies. Search for disease targets is revealing a variety of molecular markers that can be used for molecular diagnosis, staging, and stratification of patient. Molecular diagnostics can be used for detection of disease predisposition. With increasing emphasis on preventive medicine, there will be an increasing emphasis on automated genotyping and individual risk profiling. Proactive identification of risk would enable prevention and management in a logical manner.

### **Disease Prediction**

Predictive genetic testing is the use of a genetic test in an asymptomatic person to predict future risk of disease. These tests represent a new and growing class of medical tests, differing in fundamental ways from conventional medical diagnostic tests. The hope underlying such testing is that early identification of individuals at risk of a specific condition will lead to reduced morbidity and mortality through targeted screening, surveillance, and prevention.

### **Early Diagnosis**

Early diagnosis of a disease before the symptoms appear is desirable but it is not possible for most of the diseases. Currently, early detection and treatment of disease

is on the basis of clinical chemistry methods combined with family history, lifestyle risk factors, and diagnostic imaging. Rapid advances, however, are being made in this direction.

### **Prevention**

This could imply early detection and prevention of progression of a degenerative disease. Correction of risk factors may prevent either the development of a disease or its complications. Pre-emptive treatment may be on the basis of a correctable gene abnormality. In the conventional practice of neurology, it can be compared to repair of an intact asymptomatic intracranial aneurysm to prevent subarachnoid hemorrhage.

### **Therapy Based on Molecular Diagnosis**

While the companion tests for therapeutic products themselves will be technically simple and most likely test for SNP variants, issues surrounding their development, regulatory approval, marketing, and reimbursement remain to be established. Therapy based on diagnosis is applicable to early, acute, or chronic stages of a disease. The patient may be treated by a medication determined to be safe and effective on the basis of molecular diagnostics. Not only would the cause of the illness be better defined by the molecular diagnosis, but also the most effective specific medication for the disease in a particular patient could be selected.

### **Monitoring of Therapy**

Appropriate diagnostic tests can facilitate the frequent monitoring of the effects of therapy to verify the success by objective measurements and to detect the failure of therapy as early as possible so that appropriate changes in treatment can be instituted. Molecular diagnostic methods are an important part of monitoring of therapy.

### ***Advantages and Limitations of Integrated Healthcare***

Main advantages of the combined approach are as follows:

- A physician can provide comprehensive care for the patient without fragmentation of the components to several other physicians.
- Less wastage of ineffective costly therapies with financial savings and reduction of undesirable adverse effects for the patients. Expensive treatments may not be authorized without a definite diagnosis. Selection of drugs will be guided by unique genetic profile of the patient in order to optimize safety and efficacy.

- The patients themselves can conduct some of the tests under development.
- Genetic screening is linked to the treatment and if there is no treatment available for the genetic disorder, the patient may opt for foregoing the diagnostic test.

The interest of the biopharmaceutical industry is in packaging diagnostic and therapeutic materials to facilitate marketing. However, there are some limitations as follows:

- This approach cannot be universally applicable to all disorders.
- Not all the tests and treatments can be packaged together.
- The concepts of integration of various components in improving care of patients and reducing healthcare costs will need to be proven by further studies.

Nevertheless, the concept of integrating diagnosis and therapy, as well as monitoring, is a useful one for improving the general quality of healthcare in this age of super-specialization and fragmentation of care among numerous specialists who may not communicate well with one another.

## **Future of Molecular Diagnostics in Personalized Medicine**

It is widely anticipated that the molecular diagnostic industry will continue to grow at double-digit pace to meet increasing demand for personalized medicine from 2008 to 2013. A wide variety of drugs in late preclinical and early clinical development are being targeted to disease-specific gene and protein defects that will require co-approval of diagnostic and therapeutic products by regulatory agencies. An increasingly educated public will demand more information about their predisposition for serious diseases and how these potential illnesses can be detected at an early stage when they can be arrested or cured with new therapies custom-designed for their individual clinical status. To respond to this demand, major pharmaceutical companies will partner with diagnostics companies or develop their own in-house capabilities that will permit efficient production of more effective and less toxic integrated personalized drug and test products. For clinical laboratories and pathologists, this integration of diagnostics and therapeutics represents a major new opportunity to emerge as leaders of the new medicine, guiding the selection, dosage, route of administration, and multidrug combinations and producing increased efficacy and reduced toxicity of pharmaceutical products.

## **Summary**

Molecular diagnostics includes some of the most important technologies for the development of personalized medicine. These are introduced briefly. Diagnosis at molecular level includes molecular imaging as well. These technologies will be

important for integration of diagnostics with therapeutics, which is an important component of personalized medicine. Apart from diagnosing disease, molecular diagnostics is used for determining the pathogenesis of disease, as well monitoring the effect of treatment.



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