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Advancements of Genetic testing in clinical laboratories: A detailed review

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Abstract

The development in the genetic research has increased demand of genetic testing for number of inherited disorders. As the clinical applicability of the genetic tests has increased enormously, the awareness for genetic disorders and their diagnosis has also set an amplified trend in public. Genetic tests help in assessing the carrier testing in patient and their family, pre-natal and new born screenings which further can be used for personal decision making. Overall, genetics testing is classified into three major types: cytogenetic (karyotyping, Fluorescent in-situ hybridization, comparative genomic hybridization), biochemical (high-performance liquid chromatography, gas chromatography, mass spectrometry and tandem mass spectrometry) and molecular (polymerase chain reaction, RT-PCR, DNA microarray, Sanger sequencing and next generation sequencing). Genetic tests are important because genetic information not only provide helpful information about patient but their blood relatives as well. However, it should be considered that the genetic information should be only accessed by few people and under what conditions it should be used. Some of the challenges related to the adoption of genetic testing for diagnosis and treatment of patients are the lack of demonstrated clinical validity and the existence of alternative approaches that have lower costs.

Keywords: Cytogenetics, Genetic testing, Molecular genetics, Next generation sequencing, Polymerase chain reaction.

INTRODUCTION

The understanding of the genetics and the related diseases were revolutionized by the progress of the human genome project [1]. The development in the genetic research has increased demand of genetic testing for number of inherited disorders [2]. As the clinical applicability of the genetic tests has increased enormously, the awareness for genetic disorders and their diagnosis has also set an amplified trend in public [3].

The genetic tests can be done either by investigating the DNA or RNA (direct testing), looking at markers co-inherited with a disease-related gene (linkage testing), assaying metabolites (biochemical testing), or analysing the chromosomes (cytogenetic testing). Genetics has important role in diagnosis and progression of hematologic tumors. The utilization of genetic tests in deciding specific treatment of various hematologic malignancies as well as for evaluation of depth of treatment response is rapidly advancing. Cancer cells have complex genetic alterations and small genomic changes which remain undiagnosed can be detected by comparative genomic hybridization (CGH) and single nucleotide polymorphisms (SNP). Genetic tests help in assessing the patient and the patient's family disease risks and these can be used for personal decision making other than medical care [4,5].

Applications and types of genetic testing

1. Uses of Genetic Testing

Genetic testing serves a wide range of purposes, including:

- **Newborn Screening**: This is the most widely used form of genetic testing. Early identification of genetic disorders in newborns enables timely interventions to either prevent the onset of symptoms or reduce the severity of the disease.
- **Carrier Testing**: This test helps individuals or couples determine if they carry a mutated allele that could be passed on to their offspring, potentially leading to recessive conditions such as cystic fibrosis or sickle cell anemia.

Carrier testing is often recommended for those with a family history of genetic disorders or for individuals from ethnic groups with a higher prevalence of certain genetic conditions. Ethical guidelines and legal requirements must be adhered to during carrier testing, including obtaining consent before sharing information. Healthcare professionals involved in family screening are obligated to disclose relevant findings to the family.

- **Prenatal Diagnostic Testing**: Conducted during pregnancy, these tests identify genetic or chromosomal abnormalities in a fetus. Couples at high risk of genetic disorders often opt for prenatal testing, which has also become standard practice in in-vitro fertilization procedures to screen for potential genetic risks. Types of prenatal tests include:
  a. **Non-invasive Prenatal Testing (NIPT)**: This blood test examines fetal DNA in the mother's bloodstream as early as 10 weeks into pregnancy to screen for conditions such as Down syndrome (trisomy 21), trisomy 18, trisomy 13, and sex chromosome abnormalities.
  b. **Chorionic Villus Sampling (CVS)**: Performed between 10-13 weeks of pregnancy, this procedure involves collecting placental tissue to test for chromosomal abnormalities and genetic conditions.
  c. **Amniocentesis**: Typically carried out between 15-20 weeks of pregnancy, this test analyzes amniotic fluid to detect chromosomal abnormalities and neural tube defects.
- **Confirmatory Testing**: Genetic testing can confirm a diagnosis in individuals presenting symptoms or be used to monitor disease progression or treatment response.
- **Predictive or Pre-dispositional Testing**: These tests identify individuals at risk of developing a genetic condition later in life, even if they currently show no symptoms. Predictive testing is particularly beneficial for those with a family history of genetic disorders, allowing for early risk assessment and potential preventive measures.
- **Minimal Residual Disease (MRD) Detection**: Following procedures like stem cell or bone marrow transplants, genetic tests evaluate chimerism status by amplifying short tandem repeats (STR) and variable nucleotide tandem repeats (VNTR) markers using PCR and gel electrophoresis. These methods help predict graft failure and disease relapse. Advanced techniques, such as real-time PCR for detecting SNPs or insertion/deletion polymorphisms, offer greater sensitivity in assessing chimerism.

This comprehensive approach to genetic testing facilitates early detection, diagnosis, and management of various genetic conditions.

2. Types of Genetic Testing

Genetic testing is categorized into three main types based on the nature of the abnormality being analyzed: cytogenetic, biochemical, and molecular testing. Each type is tailored to identify specific genetic irregularities.

2.1. Cytogenetic Testing

Cytogenetic testing involves analyzing entire chromosomes to identify structural or numerical abnormalities. Chromosomes from dividing human cells can be visualized under a microscope. T lymphocytes from white blood cells are commonly used for cytogenetic testing because they are easily collected and can rapidly divide in culture.

2.1.1. Karyotyping

Karyotyping involves culturing cells obtained from blood, amniotic fluid, or tissue biopsies. After 72 hours of culture, chromosomes are harvested, fixed onto slides, and stained to produce distinct bands that enable detailed structural analysis. This method is used to detect chromosomal anomalies such as Down syndrome (Trisomy 21), Edwards syndrome (Trisomy 18), Patau syndrome (Trisomy 13), Turner syndrome (Monosomy X), Cri-du-chat syndrome (5p deletion), Klinefelter syndrome (47,XXY), and Philadelphia chromosome, among others.
Karyotyping is also used in diagnosing conditions like pancytopenia, where red blood cells, white blood cells, and platelets are reduced. In such cases, a bone marrow aspiration test combined with karyotyping of bone marrow samples can help identify the genetic cause.

2.1.2. Fluorescent In Situ Hybridization (FISH)

Fluorescent in situ hybridization (FISH) was developed in the 1960s by Joseph Gall and Mary Lou Pardue and further refined by John et al. in 1969 [6]. It is a powerful tool for identifying chromosomal abnormalities such as insertions, deletions, translocations, and specific DNA sequences. The process involves hybridizing nuclear DNA (from interphase or metaphase chromosomes) fixed on a microscopic slide with a fluorescently labelled probe [7]. The probe, labeled with a fluorophore or hapten, binds to complementary sequences on the target DNA. After denaturation and annealing, the hybridized DNA is visualized under a fluorescence microscope. The resulting fluorescent signals indicate the hybridization sites.
FISH has been instrumental in diagnosing genetic disorders such as Prader-Willi syndrome, Angelman syndrome, 22q13 deletion syndrome, Cri-du-chat syndrome, chronic myelogenous leukemia, acute lymphoblastic leukemia, and Down syndrome.

2.1.3. Comparative Genomic Hybridization (CGH) or Chromosomal Microarray Analysis (CMA)

Comparative genomic hybridization (CGH) is a cytogenetic technique used to detect gains or losses of DNA that are not observable through standard chromosome analysis. It can identify small deletions and duplications but is unable to detect structural changes like balanced translocations or inversions.

The CGH process begins with isolating DNA from a test sample and a reference sample. Each sample is labeled with different fluorescent dyes, denatured, and mixed in equal proportions. The labeled DNA fragments are hybridized onto a normal metaphase chromosome spread. Competition between green and red-labeled DNA fragments for hybridization at their chromosomal origin allows the detection of genetic imbalances. The fluorescence ratio indicates genetic gains or losses at specific loci.

While CGH provides a global view of chromosomal imbalances, it has limitations. It cannot identify structural chromosomal abnormalities without copy number changes, such as balanced translocations or inversions. Moreover, it does not provide insights into tissue architecture and is a time-intensive method [8].

These cytogenetic techniques collectively enable the detection and analysis of genetic anomalies, playing a critical role in diagnosing genetic conditions and guiding treatment strategies.

Table 1: Comparison among different methods of cytogenetics

Characteristics	Karyotyping	FISH (Fluorescence In Situ Hybridization)	CGH (Comparative Genomic Hybridization)
Definition	This technique is used for visualizing the complete set of chromosomes to detect large chromosomal abnormalities.	A molecular cytogenetic technique used to detect specific DNA sequences on chromosomes.	Technique to detect copy number variations (CNVs) across the genome without needing specific DNA sequence knowledge.
Resolution	Low resolution (can detect changes > 5 Mb)	Medium resolution (can detect specific sequences, down to ~100 kb)	High resolution (detects CNVs at the kilobase level)
Abnormalities Detected	chromosomal abnormalities such as aneuploidy, deletions, translocations	Specific gene loci or structural abnormalities like microdeletions or duplications	Copy number variations (gains or losses of chromosomal regions)
Visualization	Chromosome bands under a light microscope	Fluorescent probes that bind to target sequences	A ratio of fluorescence intensity between sample and reference DNA
Required Sample	Requires fresh samples (blood, bone marrow)	Fresh or fixed cells/tissues (interphase or metaphase)	DNA extracted from any tissue source
Processing Time	Longer (usually one-two weeks, due to need for cell culture, harvesting and analyzing)	Faster (one-three days)	Moderate (depends on array type, ~one week)
Advantages	Detects large-scale chromosomal abnormalities (whole chromosome gains/losses)	Detects specific, targeted DNA changes; can be done on non-dividing cells	Genome-wide analysis of CNVs; higher resolution for identifying smaller abnormalities
Disadvantages	Low resolution, cannot detect smaller abnormalities	Limited to the regions only where probes are designed, requires prior knowledge of the mutation	Cannot detect balanced rearrangements (translocations, inversions); detects only CNVs

1.1. Biochemical genetic testing

The enormous numbers of biochemical reactions that routinely occur in cells require different types of proteins. An alteration or variation in any type of protein can result in altered function of the cells and therefore causing disease/syndrome. Testing for biochemical diseases is done by different techniques that examine the altered protein. With

help of different biochemical tests activity of protein, enzymes, metabolites levels in a sample, structural protein analysis can be done. Due to the less stability of proteins, the samples required for test should be collected, transported and stored according to the laboratory specifications.
A range of different technologies such as high-performance liquid chromatography (HPLC), tandem mass spectrometry (MS/MS) and gas chromatography/mass spectrometry (GC/MS) permit the qualitative as well as quantitative detection of metabolites.
Next generation sequencing can detect the cause of disease at the genetic level. It can contribute to detect variations of diseases-caused genes conveniently and accurately, so as to help the diagnosis of in born errors of metabolism.

1.2. Molecular genetic testing

DNA testing can be done to check the small variations as well as number of mutations present in a single case. There are examples of genetic conditions which are caused by many different mutations making molecular genetic testing a challenging task. For example, more than 1,000 mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene can cause cystic fibrosis (CF). However, with the advancement and implementation of NGS testing, various genes related to cystic fibrosis can be tested at once, which can cut down the time consumed in detecting the genes and related variants.

Methods in Molecular Testing

Direct DNA analysis is done when the target genetic sequence is already known. Several molecular technologies, including direct sequencing, polymerase chain reaction-based assays (PCR), restriction fragment length polymorphism (RFLP) and hybridization, can be used to perform testing.

PCR and Nucleic Acid Amplification

PCR is a laboratory technique designed to amplify specific segments of DNA or RNA using the DNA polymerase enzyme, particularly Taq DNA polymerase, derived from *Thermus aquaticus*[9,10]. Introduced in 1985 by Kary Mullis and colleagues, a discovery for which they were later awarded the Nobel Prize, PCR revolutionized molecular biology with its ability to amplify and analyze DNA fragments efficiently [11].
PCR operates through three primary steps:

1. Denaturation: The double-stranded DNA is separated by heating.
2. Annealing: Short primers bind to complementary sequences on the template DNA.
3. Elongation: DNA polymerase synthesizes a new strand by extending the primers.

This technique is a cornerstone in diagnosing bacterial and viral infections, as well as for screening genetic disorders due to its sensitivity and ability to process multiple samples simultaneously [12].

Real-Time PCR

Real-time PCR, an advanced variation of conventional PCR, allows rapid detection of DNA segments by incorporating fluorescent dyes. Unlike traditional PCR, it eliminates the need for post-amplification steps, such as gel electrophoresis. Real-time PCR detects amplicons in real-time, making it highly efficient and faster [13]. Applications of real-time PCR include detecting viral pathogens such as enteroviruses, West Nile virus, Dengue virus, Hantavirus, and SARS. It is widely used for HIV and HCV viral load testing and for identifying microorganisms like bacteria, fungi, and parasites by targeting their RNA. Since RNA presence often correlates with viable organisms, real-time PCR is particularly effective in studying gene expression [13]. Despite its advantages, real-time PCR is costlier than conventional PCR.

Reverse Transcriptase PCR (RT-PCR)

RT-PCR converts RNA into complementary DNA (cDNA) using reverse transcriptase. This technique is particularly useful for studying gene expression and is often combined with conventional PCR to analyze specific genes [10]. RT-PCR gained prominence during the COVID-19 pandemic for its high sensitivity, specificity, and rapidity in detecting the SARS-CoV-2 virus.

DNA Microarray Analysis

Also known as DNA chip analysis, this technique measures gene expression by hybridizing mRNA to complementary DNA templates. DNA microarrays allow simultaneous analysis of thousands of genes, with fluorescence signals indicating the level of gene expression. Computational tools analyze the hybridization data to provide insights into gene activity [14].

DNA Sequencing Techniques

DNA sequencing methods were pioneered in the 1970s by Walter Gilbert's group at Harvard and Frederick Sanger's team at Cambridge. Though initially expensive and time-consuming, advancements in technology have made DNA sequencing

faster, safer, and more affordable. The Sanger sequencing method was instrumental in completing the Human Genome Project in 2003 [15-17].

Sanger Sequencing

Sanger sequencing, also called the dideoxy chain termination method, uses chain-terminating dideoxynucleotides (ddNTPs). Unlike deoxynucleotides, ddNTPs lack a free 3′ hydroxyl group, which prevents the addition of further nucleotides, halting DNA strand elongation [15].

A typical Sanger sequencing reaction requires the following:

- Template DNA to be sequenced.
- DNA polymerase to catalyze the reaction.
- Primers that anneal to the target sequence.
- A mixture of dNTPs and fluorescently labeled ddNTPs.

During the reaction, random incorporation of ddNTPs terminates the DNA strands at various points. The fragments are then separated by size using capillary electrophoresis. A laser excites the fluorescent tags on the terminal nucleotides, and the emitted fluorescence is detected by a computer, which determines the DNA sequence based on the order of the fragments.

The Sanger method remains a gold standard for accuracy, especially for sequencing long DNA fragments up to 900 nucleotides. It has applications in diagnostics, research, and large-scale genomic projects like the Human Genome Project.

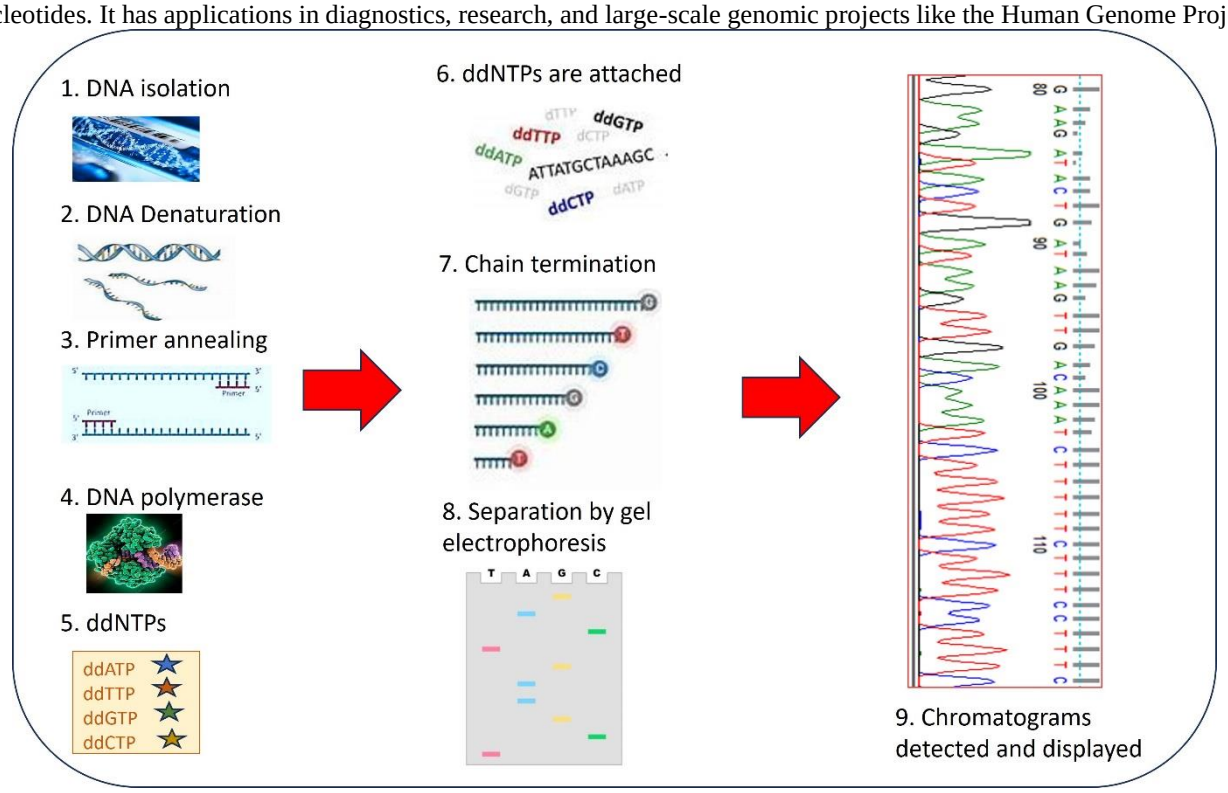

Figure 1: Figure illustrating Sanger Sequencing workflow.

Next Generation Sequencing

Next Generation Sequencing, also referred to as parallel sequencing, enables the simultaneous sequencing of multiple DNA or RNA fragments, or even entire genomes, within a relatively short time frame. This technique integrates advanced sequencing chemistries, sequencing platforms, and bioinformatics tools to achieve its high efficiency. NGS workflows include several steps: DNA fragmentation, library preparation, massive parallel sequencing, bioinformatics analysis, and the annotation and interpretation of variants or mutations. Bioinformatics plays a critical role in aligning the fragmented DNA reads to a reference genome, allowing accurate reconstruction. The human genome, comprising approximately three billion bases, is sequenced multiple times to ensure data reliability and depth [18].

NGS is versatile and can sequence DNA or RNA at various scales:

1. **Whole-Genome Sequencing (WGS)**: WGS involves sequencing nearly all nucleotides in an organism's genome. While it has broad applications in research, its clinical use is more limited, primarily in diagnosing constitutional

genetic diseases rather than somatic mutations in cancers. WGS is particularly beneficial for identifying rare genetic disorders.

2. **Whole-Exome Sequencing (WES)**: WES focuses on sequencing the entire coding region of an individual's genome, which accounts for about 1% of the human genome. It is commonly employed for identifying mutations within protein-coding genes.
3. **RNA Sequencing (Transcriptome Analysis)**: RNA sequencing, also known as transcriptome analysis, involves sequencing all RNA transcripts, including mRNA, rRNA, tRNA, microRNAs, and non-coding RNAs. It is especially useful for detecting fusion genes and studying gene expression, with a specific emphasis on mRNA sequencing.
4. **Targeted Resequencing**: This method focuses on sequencing specific genes of interest, offering higher sequencing depth compared to WGS. It is widely used in oncology for analyzing mutations in targeted genes associated with specific cancers. For instance, NGS panels can be customized for myeloid leukemia, breast cancer, or respiratory diseases. The increased depth of sequencing in targeted panels enables the detection of mutations with varying allelic frequencies, which is critical for clinical diagnostics [19-21].

NGS has revolutionized genomics, offering unparalleled precision and scalability for research and clinical applications.

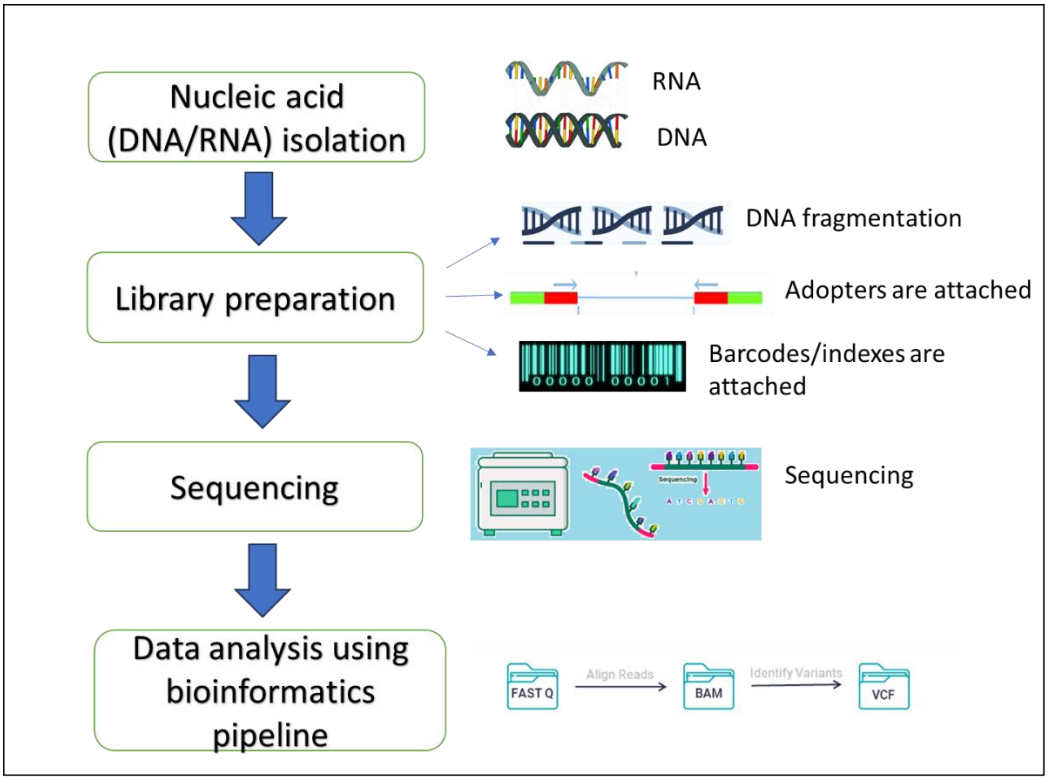

Figure2: Flowchart showing the overview of NGS workflow.

NGS clinical application is not limited to diagnosis. It is also widely used in identifying mutation targets for targeting therapy and in identifying a high-risk population for certain hereditary cancers. Over the past few years, numerous molecular targeting drugs have been developed [22]. Now a days different platforms are available to carry NGS which use different chemistries to carry out sequencing.

Roche 454 Technology: The Roche 454 system was the first commercially successful NGS platform, utilizing pyrosequencing technology. This approach detects pyrophosphate, a byproduct of nucleotide incorporation, to determine whether a specific base was added to a growing DNA chain [23]. Known for its long-read lengths (600–800 nucleotides) and relatively high throughput (25 million bases with 99% accuracy or better), 454 sequencing was widely used for genome sequencing and metagenomic studies, facilitating efficient genome assembly.

Ion Torrent™ Technology: Ion Torrent technology directly translates nucleotide sequences into digital data using a semiconductor chip [24]. During DNA synthesis, the incorporation of a nucleotide into the growing DNA chain releases a hydrogen ion, altering the pH of the solution. This pH change is recorded as a voltage shift by an ion sensor, similar to a pH meter. If no nucleotide is incorporated, no voltage spike is observed. Ion Torrent supports automated library and template preparation through the Ion Chef™ system, offering applications such as targeted and de novo DNA/RNA

sequencing, transcriptomics, microbial sequencing, copy number variation detection, small RNA/miRNA sequencing, and CHIP-seq [25].

Illumina Technology: Illumina has become a dominant player in the second-generation sequencing market, leveraging technology initially developed by Solexa and Lynx Therapeutics. Illumina sequencing employs "bridge amplification," where DNA molecules (approximately 500 base pairs) with ligated adapters undergo amplification on a glass slide coated with complementary oligonucleotides. This method supports various protocols, including whole-genome sequencing, exome and targeted sequencing, RNA sequencing, metagenomics, CHIP-seq, and methylome analysis [26].

Pharmacogenetics

Role of Genes in Drug Response: Genes significantly influence an individual's response to medications, and genetic testing can guide treatment strategies by reducing adverse effects and preventing complications. Pharmacogenetic testing identifies individuals at risk of severe drug reactions, helping tailor therapeutic approaches and avoid ineffective or harmful treatments. Despite its potential, the integration of pharmacogenetics into clinical practice remains limited due to gaps in actionable variant knowledge and the availability of genetic testing for only a small number of variants and drugs [27].

Genetic Testing for Drug Dosing: Genetic variants affecting drug metabolism (pharmacokinetics) are instrumental in determining appropriate drug doses to prevent under-treatment or adverse effects due to high drug exposure [28]. For example, dosing of the oral anticoagulant warfarin, used to treat and prevent thromboembolic disorders, shows significant interindividual variability influenced by dietary and genetic factors.

Warfarin has a narrow therapeutic range, and deviations in the international normalized ratio (INR) can be harmful. Genetic testing for variants in the *CYP2C9* gene (involved in warfarin metabolism), the *VKORC1* gene (the target of warfarin action), and the *CYP4F2* gene (related to vitamin K recycling) is recommended to optimize dosing. These tests enable precise adjustments to ensure therapeutic efficacy while minimizing risks of over- or under-coagulation.

Ethical and Societal Challenges in Genetic Testing

The widespread use of genetic testing in workplaces or within families may lead to concerns about unfavorable interactions between genetics and the environment, potentially fostering discrimination. Employers might prefer to hire individuals with a "healthy" genetic profile to reduce healthcare and insurance costs, which could otherwise become a financial burden for the company. In recent years, instances of genetic testing in the workplace have raised significant concerns regarding individual autonomy, privacy, and dignity [29].

To address the risk of workplace discrimination and unfair treatment by insurance companies, the United States has implemented various anti-discrimination laws. One notable example is the Genetic Information Non-Discrimination Act (GINA). This legislation prohibits employers and insurers from using genetic information to make decisions regarding health coverage or employment, thereby protecting individuals from genetic discrimination.

Challenges and limitations of genetic testing

Some of the challenges related to the adoption of genetic testing for diagnosis and treatment of patients are the lack of demonstrated clinical validity and utility of some genetic variants (such as most of the genetic variants identified in genome-wide association studies) and the existence of alternative approaches that have lower costs (e.g., dosing blood levels of the drug instead of adjusting based on genetic testing).

CONCLUSION

Genetic tests are important because genetic information not only provide helpful information about patient but their blood relatives as well. However, it should be considered that the genetic information should be only accessed by few people and under what conditions it should be used [30].

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