Editorial Article

Recent Advances in Genetic Tests for Rare Disease Diagnosis

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Introduction

Diagnosis is the most important step in any clinical process as it plays a vital role in therapeutic decision making. It is majorly categorized into hematologic, chemical, microbiological, immunological, and molecular diagnostics. With increasing insight into the pathology of diseases, molecular diagnostics has become a popular method of diagnosis. Apart from diseases that are commonly seen in a population, there are certain diseases called rare diseases that affect fewer members in a population. The World Health Organization categorizes a disease or disorder under rare diseases if the prevalence is in 1 or fewer individuals per 1000 people in a population. Gene is the most important motive, often linked to these uncommon diseases. Hence, "Genetic Testing", becomes an essential diagnostic procedure with rare diseases. From Karyotyping to Next Generation Sequencing (NGS), genetic tests have evolved to the modernity. This editorial highlights the recent advances in genetic testing and their clinical application for the detection of rare diseases.

The gold standard method of genetic testing

Genetic Testing refers to the group of tests performed to identify alterations in chromosomes, DNA, or proteins that may contribute to disease pathology. The term genetic testing is broad and includes many tests performed on different genetic material using various techniques.[1] Apart from diagnosis, genetic testing is also conducted for others such as Carrier Testing, Prenatal Testing, Presymptomatic Testing, Pharmacogenetics, Newborn Screening, and Forensic Testing. Some conventional methods used in Genetic Testing are Karyotyping, Polymerase Chain Reaction (PCR), Fluorescence in Situ Hybridization (FISH), Comparative Genomic Hybridization (CGH), Gene Expression Profiling, etc. The rising awareness in Genetics has paved the way for introducing of new, advanced, and efficient techniques for the diagnosis of Genetic disorders and rare diseases.

Karyotyping

Karyotyping is still the gold standard in the evaluation of chromosomal abnormalities, through which the chromosomes are arranged in a sequential order based on their shape and size to detect many chromosomal abnormal conditions. This technique is also provided for a genome-wide view of all the chromosomes in the metaphase cell with information on the numerical and structural rearrangements such as deletion, duplication, inversion, and translocation in the clonally abnormal cell. These methods allow identification of chromosomal variations and must be useful for human studies, including genetic diseases and cancer. This technique can also be automated for quick screening of human populations.[2]

Polymerase chain reaction

Polymerase chain reaction (PCR) is the in vitro multiplication of the DNA strands and it is used to increase the quantity of the DNA sample that is obtained. There are many types of PCR methods like the reverse transcriptase PCR, multiplex PCR, nested PCR which are changed versions of the generic Polymerase Chain Reaction.[3] With the initiation of PCR, molecular diagnostics crosses the threshold of proven laboratory for the provision of genetic services, such as screening and identification for known and unknown mutations.

Fluorescence in situ Hybridization

Fluorescence in situ Hybridization (FISH) is a technique where the DNA is hybridized with fluorescence probes to facilitate DNA analysis. It is based on the principle that a DNA strand binds to its complementary strand. FISH is highly specific, sensitive, and takes less time. These qualities make FISH as a readily acceptable diagnostic tool in clinical laboratories. This technique is mainly used to detect minuscule structural and numerical anomalies of the chromosome, deletions, and amplification of the genes, and identification of marker chromosomes.[4]

Comparative genomic hybridization

Comparative genomic hybridization (CGH) is a cytogenetic testing method that is applied for analyzing copy number variants (CNV) in the DNA test sample through a comparative analysis with the normal DNA sample that acts as the reference without culturing the cells. CGH in a single assay can detect genome-wide duplications and deletions with greater resolution when compared to karyotyping and FISH techniques. It is a popular diagnostic tool used in the detection of microdeletions and microduplication syndromes.[5]

Gene expression profiling

Gene expression profiling is the process through which the gene expression levels of many genes are measured simultaneously.[6] This gene expression measurement provides detailed insight into the cellular function. This molecular profiling technique is a powerful predictive and prognostic marker for clinical diagnosis and decision making[7]

Current trends in genetic testing

Next-generation sequencing of the whole genome

DNA sequencing is a method through which the order of the nucleotides is determined; this method helps to identify mutations in the genome. Sanger sequencing method is used for sequencing the DNA and sequencing of the whole genome, which needs many days. Next-generation sequencing (NGS) is a method that has superseded Sanger sequencing because of its efficiency and capability of analyzing many genes simultaneously at a faster rate. NGS has become the test method of choice for somatic mutations, germline mutations, and also oncology cases in many diagnostic laboratories. Some clinical applications of NGS that are in practice or being researched to bring Next Generation Sequencing into clinical diagnostics are HLA (Human Leukocyte Antigen) typing, RNA sequencing and expression, microbial analysis, and circulating tumor DNA (ctDNA) testing. NGS of ctDNA, otherwise known as the liquid biopsy is now available in clinical laboratories. This method is used to analyze the whole genome of a tumor which can help in cancer diagnosis and also monitoring for cancer relapse and progression in the future.[8]

Whole exome sequencing

Exome refers to the group of exons which are the protein-coding regions of the genome. Whole exome sequencing (WES) is a fairly new method through which only the exons of a genome are sequenced. The whole Genome analysis has shown that the exons make up only 1% of the genome. The information provided by WES is fully comparable with the amino acid sequence of the encoded protein. WES is primarily used to detect mutations in the coding regions of the genome. The remarkable size difference between the genome and exome makes WES cheaper and quicker compared to whole-genome sequencing. Exome sequencing has helped to detect tiny sequence differences among the exomes of many individuals. WES is helpful in detecting Single Nucleotide Polymorphisms (SNP) and the risk of individuals developing any disease.[9]

Clinical exome sequencing

Clinical exome sequencing (CES) is like Whole exome sequencing (WES), however, CES only screens for genes that are known to cause diseases. CES is a more comprehensive test that helps to diagnose rare Mendelian disorders that could not be diagnosed with biochemical, other genetic or radiological techniques. Trio based exome sequencing, also known as Trio-CES is a testing method that parallelly analyzes the whole exome of the proband along with the whole exome of the parents. This method facilitates the discovery of de novo mutations in the proband.[10]

Conclusion

The upcoming years will continue to bring-in new tests and improve the quality of the pre-existing techniques. Genetic Testing has enabled the monitoring of subtle alterations in the chromosomes and genes. This will help clinicians to make wise decisions regarding the selection of therapies and also to monitor the progression of a disease. Genetic Testing has also aided in the identification of high-risk individuals, which makes disease management easier by early intervention. Though we have reached this far in developing ideal diagnostic tests, certain milestones such as reproducibility, accuracy, and cost-effectiveness are yet to be a matter of concern.

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