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Genetic disease testing

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Genetic diseases or disorders are caused due to abnormality or mutations in the genetic makeup. As of July 2024, Online Mendelian Inheritance in Man (OMIM) listed 7,528 single locus (Mendelian) diseases that are associated with 4913 genes,[1] and new genetic disorders are constantly being described in medical journals.[2] Around 80 percent of rare diseases are of genetic origin.[3] Currently, around 300 million people live with rare diseases.[3] Genetic disorders are broadly categorized into three types: single-gene defects, chromosomal abnormalities, and multifactorial conditions.[4] Genetic diseases can be caused due to mutations in a single gene or multiple genes.[5]

Single gene disorders

- **Autosomal disorders** are caused by a mutation in one of the 22 autosomal chromosomal pairs. These can be autosomal dominant or autosomal recessive. Autosomal dominant disorders happen when a gene mutation or abnormality from one of the parents passes on the disorder to the child. A few examples of autosomal dominant disorders are Huntington's disease, Marfan syndrome, and achondroplasia.[6] Autosomal recessive disorders happen when a gene mutation of abnormality is passed on to the child from both parents. A few examples of autosomal recessive disorders are cystic fibrosis, Tay-Sachs disease, sickle cell disease, and thalassemia.[7]
- **X-linked disorders** occur when a mutation in X-chromosome is passed on to the child. There are at least 533 disorders due to the involvement of the genes on the X chromosome. A few examples of the x-linked chromosomes are: Red-green color blindness, hemophilia, and Duchene muscular dystrophy.[8]
- **Y-linked disorders** occur when a mutation in the Y-chromosome is passed on by a father to his son. A few examples of Y-linked chromosomes are Y chromosome infertility and some cases of Swyer syndrome.[9]
- **Trinucleotide repeat disorders**, also called triplet repeat disorders, are a set of over 30 genetic disorders caused by a mutation in which repeats of three nucleotides (trinucleotide repeats) increase in copy numbers until they cross a threshold above which they cause developmental, neurological, or neuromuscular disorders. Depending on its location, the unstable trinucleotide repeat may cause defects in a protein encoded by a gene; change the regulation of gene expression; produce a toxic RNA; or lead to production of a toxic protein.[10,11,12] A few examples of trinucleotide repeat disorders are myotonic dystrophy (DM), Huntington disease, spinocerebellar ataxia, Friedreich ataxia, and fragile X syndrome.[13]
- **Mitochondrial mutation disorders** can affect one part of the body or many parts, including the brain, muscles, kidneys, heart, eyes, and ears, as mitochondria are the powerhouse of energy. Examples of mitochondria diseases are Barth syndrome, chronic progressive external ophthalmoplegia, Kearns-Sayre syndrome, Leigh syndrome, mitochondrial DNA depletion syndromes, mitochondrial encephalomyopathy, lactic acidosis, mitochondrial neurogastrointestinal encephalomyopathy, myoclonic epilepsy with ragged red fibers, neuropathy, ataxia, retinitis pigmentosa, and Pearson syndrome.[14]
- **Imprinting disorders** are congenital conditions that are due to disturbances of genomic imprinting. Genomic imprinting is the process by which only one copy of a gene in an individual (either from their mother or father) is expressed, while the other copy is suppressed. A few examples of imprinting disorders are Prader–Willi syndrome, Angelman syndrome, and Beckwith–Wiedemann syndrome.[15]

Chromosomal disorders

Chromosomal disorders are characterized by a morphological or numerical alteration in single or multiple chromosomes, affecting autosomes, sex chromosomes, or both.[4] Chromosome abnormalities occur due to an error in cell division (mitosis or meiosis), which may occur in the prenatal, postnatal, or preimplantation periods. Chromosomal disorders are classified as numerical or structural and constitutional or acquired. A few examples of chromosomal disorder are:

- **Down's syndrome** or trisomy 21
- **Edward's syndrome** or trisomy 18
- **Patau syndrome** or trisomy 13
- **Klinefelter's syndrome** or presence of additional X chromosome in males
- **Turner syndrome** or presence of only a single X chromosome in females

Multifactorial disorders

Multifactorial disorders, also called complex disorders, occur due to the influence of multiple genes acting in concert with environmental factors.[5] This class of disorders probably represents the single largest class of inherited disorders affecting the human population. Heart disease, diabetes, cancer, asthma, schizophrenia, osteoporosis are all examples of multifactorial disorders.

Testing for genetic diseases

Genetic testing involves analysis of DNA, chromosomes, or proteins from an individual's blood, skin, hair, or other tissue specimens for a change, or mutation, which is associated with a genetic condition. When a mutation occurs, it may affect all or part of a gene and can result in an abnormal function leading to disease.[16] Three major types of genetic testing are available in laboratories: cytogenetics examine structure and number of chromosomes under the microscope, biochemical tests measure proteins produced by genes, and molecular tests look for DNA mutations [16]

Cytogenetics: This testing involves the analysis of cells in a sample of blood, tissue, amniotic fluid, bone marrow, or cerebrovascular fluid to identify any changes in an individual's chromosomes. There are three major methods of cytogenetic testing:

Standard karyotyping: The first cytogenetics method used in the 1950s to visualize human chromosomes and their differences linked to conditions such as Down syndrome. In this method, chromosomes are paired and arranged in a standardized format known as karyotype or karyogram to provide a genome-wide snapshot of an individual's chromosomes. Karyotypes are prepared using standardized staining procedures to reveal characteristic structural features for each chromosome. The stain most commonly used is Giemsa stain and produces G-banding at the site of the condensed chromosomes.[17]

Karyotyping is one of the most preferred methods to detect structural and numerical abnormalities and diagnose specific birth defects, genetic disorders, and even cancers. Clinical cytogeneticists analyze human karyotypes to detect gross genetic changes—anomalies involving several megabases or more of DNA. Karyotypes can indicate changes in chromosome number associated with aneuploid conditions, such as trisomy 21 (Down syndrome), as well as more subtle structural changes, such as chromosomal deletions, duplications, translocations, or inversions.

Fluorescent in situ hybridization (FISH): A molecular cytogegenetic technique developed in the early 1980s that uses fluorescent probes to bind to particular parts of a nucleic acid sequence (DNA or RNA) with a high degree of sequence complementarity. In this technique, the full set of chromosomes from an individual is affixed to a glass slide and then exposed to a fluorescently labeled probe. The fluorescently labeled probe finds and then binds to its matching sequence within the set of chromosomes. With the use of

Disease category	Metabolic disease
Disorders of amino acid metabolism (amino acid disorders)	• Phenylketonuria • Maple syrup urine disease
Disorders of organic acid metabolism (organic acidemias and acidurias)	• Methylmalonic aciduria • Propionic aciduria • Glutaric acidemia type 1
Disorders of fatty acid oxidation	• Medium-chain acyl-CoA dehydrogenase deficiency
Disorders of urea cycle metabolism (urea cycle disorders	• Citrullinemia • Argininemia • Ornithine transcarbamylase deficiency
Disorders of cholesterol synthesis	• Smith-Lemli-Opitz syndrome
Lysosomal storage disorder	• Gaucher disease • Fabry disease • Hurler syndrome • Niemann-Pick disease
Disorders of mitochondrial function (mitochondrial diseases)	• Leber's hereditary optic neuropathy (LHON) • Mitochondrial myopathy, encephalopathy, lactic acidosis, • and stroke-like episodes (MELAS) • Myoclonic epilepsy with ragged-red fibers (MERRF)
Disorders of carbohydrate metabolism (carbohydrate metabolism disorders)	• Galactosemia • Fructose intolerance
Disorders of connective tissue (e.g., collagen and fibrillin)	• Marfan syndrome • Osteogenesis imperfecta

Table 1. Metabolic disorders for which biochemical genetic tests are performed.

a fluorescent microscope, the chromosome and sub-chromosomal location where the fluorescent probe bound can be seen.[18] It is utilized to diagnose genetic diseases, gene mapping, and identification of chromosomal abnormalities, and may also be used to study comparisons among the chromosomes' arrangements of genes of related species.

Comparative genomic hybridization (CGH) and array comparative genomic hybridization (aCGH): Comparative genomic hybridization (CGH) is a molecular cytogenetic method to analyze copy number variations (CNVs) without the need for culturing cells. This technique aims to quickly and efficiently compare two genomic DNA samples — a test and a reference arising from two sources — that are most often closely related, because it is suspected that they contain differences in terms of either gains or losses of either whole chromosomes or subchromosomal regions. This technique was originally developed to evaluate the differences between the chromosomal complements of solid tumor and normal tissue.[19]

CGH has an improved resolution of 5–10 megabases compared to giemsa banding karyotyping and fluorescence in situ hybridization (FISH) that are limited by the resolution of the microscope utilized.[20, 21]

Array comparative genomic hybridization (aCGH): Also called as microarray-based comparative genomic hybridization, matrix CGH, array CGH is a molecular cytogenetic technique for the detection of chromosomal copy number changes on a genome-wide and high-resolution scale.[22] Array CGH compares the patient's genome against a reference genome and identifies differences between the two genomes, and thereby locates regions of genomic imbalances in the patient, utilizing the same principles of competitive fluorescence in situ hybridization as traditional CGH.

With the introduction of array CGH, the main limitation of conventional CGH, a low resolution, is overcome. In array CGH, instead of using metaphase chromosomes as in traditional CGH, cloned DNA fragments (+100–200 kb) are used of which the exact chromosomal location is known. This allows the detection of aberrations in more detail - makes it possible to map the changes directly onto the genomic sequence with much higher resolution compared to traditional CGH.[23] Using this method, copy number changes at a level of 5–10 kilobases of DNA sequences can be detected. This method allows one to identify new recurrent chromosome changes such as microdeletions and duplications in human conditions such as cancer and birth defects due to chromosome aberrations.[24]

Biochemical genetic testing: Some individuals exhibit "inborn errors of metabolism," a genetic abnormality from birth that affects their body's metabolism. Biochemical genetic tests are highly complex tests that are used to evaluate enzyme activity; functional

Technology	Advantages	Limitations	Applications
Polymerase chain reaction (PCR)[27] and real-time PCR	Simple, rapid, high-sensitivity, high-specificity	Possibility of false-positive results due to contamination and amplification bias; prior sequence data is required for primer design	Genotyping, gene expression analysis, mutation detection
Multiplex ligation-dependent probe amplification (MLPA)[28]	Detects small rearrangements; up to 40 targets; high throughput; low cost	Cannot detect copy neutral loss of heterozygosity. May have problems with mosaicism, tumor heterogeneity, or contamination with normal cells.	Detects copy number variations in complete chromosomes or single exons. Detects DNA methylation changes.
DNA microarray[29]	High throughput, accurate, rapid, high sensitivity	Costly, complex data analysis	Genotyping, copy number variation, detection
Sanger sequencing[30]	High accuracy, relatively long-read lengths, widely available, single-nucleotide resolution	Low throughput, relatively expensive, labor-intensive, inherent sequencing bias, inability to detect structural variants	Genotyping, mutation detection, validating results from next-generation sequencing
Next-generation sequencing (NGS)[31]	High throughput, compared to Sanger sequencing, cost effective, high speed, better data resolution, accuracy	Sequencing errors can occur in every process. Can exhibit GC bias, result influenced by quality and purity of DNA or RNA used. Storage and management of huge data generated is challenging	Whole genome sequencing, whole exome sequencing, targeted gene sequencing, RNA sequencing, enables detection of rare genetic variants

Table 2. Molecular tests for genetic diseases.

status of proteins; and levels of metabolites such as amino acids, organic acids, and fatty acids from a wide variety of specimen types such as urine, whole blood, plasma, serum, cerebrospinal fluid, muscle biopsy, and other tissues. These tests are used to evaluate and diagnose, monitor treatment, and clinically manage such individuals.[25] **Table 1** provides a few examples of metabolic disorders for which biochemical genetic tests are performed.[25]

Molecular tests: These techniques have greatly advanced over the years and act as powerful tools for diagnosis, genetic consultation, and prevention of genetic diseases.[26] These are also used as follows:

- Determine presymptomatic individuals' illness risk
- Detect asymptomatic recessive trait carriers
- Diagnose prenatal conditions not yet evident in pregnancy

Several factors are considered when selecting the appropriate test, including suspected conditions and their possible genetic variations.[26] A broad genetic test — whole genome sequencing or whole exome sequencing — is employed when a diagnosis is uncertain. A targeted test is preferred for suspected specific conditions. A targeted test could be for a single gene or a panel of genes and could be to detect point mutations, deletions, insertions, or copy number variations. Gene expression tests are performed to detect whether genes are active (producing mRNA and protein) or inactive. Too much activity (overexpression) or too little activity (underexpression) of specific genes may suggest particular genetic disorders, including various cancer types. Some of the commonly used methods used in molecular testing and their specific applications are provided in **Table 2**.

Conclusion

To date there are 7,000 types of rare diseases and 300 million people live with rare diseases.[3] 80% of rare diseases are due to genetic causes, which means around 240 million people live with genetic disease. Hence genetic disease is a huge burden to the world. However, due to the complexity and variability, many genetic diseases are still not diagnosed and do not have proper treatment. That said, we can be hopeful that with the advancement of science and technology that is happening at such a rapid pace we would be successful in offering precise solutions to patients with genetic diseases in the near future.

The advancements are taking place in various areas; for example, CRISPR/Cas systems and small interfering ribonucleic acid (RNA) are helping to expand our understanding of genetics at the molecular level and various omics technology along with artificial intelligence are enabling better diagnosis and monitoring of genetic diseases and cell and gene therapy, which help to better manage patients having genetic diseases.

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