The Genetics of Monogenic Diseases: The Search Continues

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What are Monogenic Diseases?

> "Our own **genomes** carry the story of evolution, written in **DNA**, the language of **molecular genetics**, and the narrative is **unmistakable**".
> *Kenneth R. Miller*

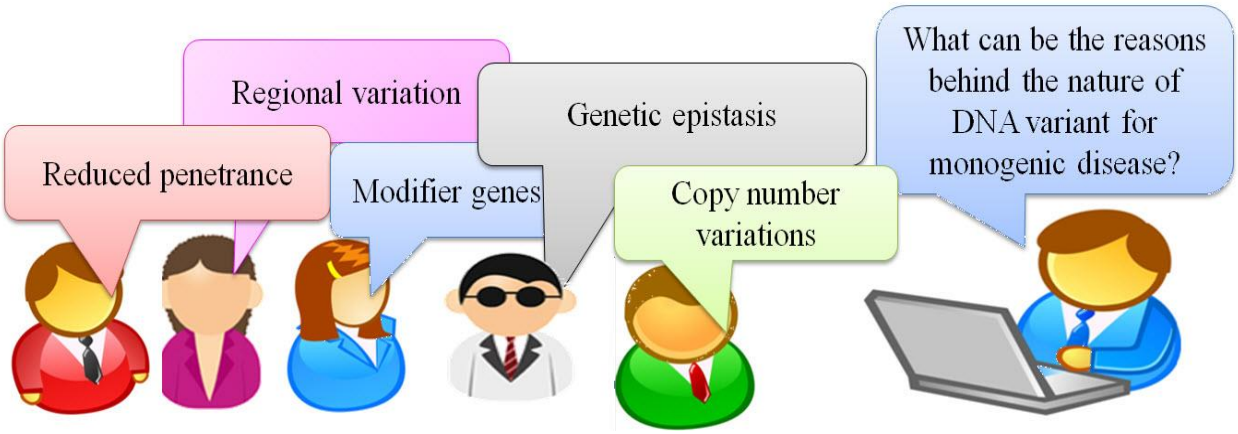
Clinical molecular studies have rapidly evolved as a promising technology, where recent advances have been made in the discovery of faulty genes which cause genetic disorders (1). Genetic disease is any condition in which there is an identifiable genetic component in causation of the disease (1, 2). The concept as well as the elucidation of genetic disease has taken up a pace parallel to the development in medical genetics (3). Sickle cell disease caused by a molecular defect in the gene of a hemoglobin chain was the first genetic disease to be characterized by Linus Pauling (4). Subsequently, a number of gene mutation(s) causing diseases have been identified (5).

> Monogenic diseases are inheritable as per the Mendel's laws of inheritance. Therefore, they are also known as **Mendelian diseases**.

Every genetic disease is associated with a genetic as well as an environmental component (6). A broad category of genetic diseases, *i.e.*, monogenic or single gene disease, is associated with major mutations or highly penetrant mutations in a single gene present in all cells of the body (7). The prevalence of known monogenic diseases is quite rare as an individual disease. They collectively affect less than 1% of the world's total population (8).

Monogenic diseases are majorly categorized into 4 inheritance patterns, *i.e.*, autosomal dominant, autosomal recessive, X-linked (dominant and recessive) or others. Autosomal dominant monogenic disorders involve the modification to only one gene copy, whereas, recessive disorders occur due to mutation in both alleles. X-linked monogenic diseases are linked to a defect in the genes present in the X (sex) chromosome, where alleles associated with the mutation can be either dominant or recessive (5, 8).

How to Detect the Responsible Gene?



Genes account to play an important as well as extremely diversified role in the etiology of human diseases, where a single rare mutation is fully responsible for causation of a monogenic Mendelian disease (9). The brainstorming efforts of scientists and researchers have resulted in the detection of genes responsible for almost 50% of the rare monogenic diseases (10). This has happened in the last three decades due to the rapid advancements and dramatic improvements in the DNA sequencing technologies involving exome sequencing, microarray technologies, including oligonucleotide array comparative genomic hybridization and single nucleotide-polymorphisms (SNP) genotyping arrays, as well as the next-generation sequencing (NGS) with "paired-end" methods. It enabled the scientists to understand and perform whole-genome analysis by the discovery of the submicroscopic copy-number variations (CNVs) present in the genomes (10, 11). It has resulted in a decrease in the time lag from months or years to days/ weeks and that too with accuracy (3). The advancement in technology has also helped scientists to plan and execute new strategies to analyze causative mutations responsible for the monogenic diseases which are not amenable to linkage-based positional cloning (12, 13).

The major drawbacks related to the analysis of the causative variants are **technical** as well as **genetic**.

However, the role and limitations of genetic testing is still a question of debate for many other monogenic diseases (14). The limited knowledge in the field of genetics, in terms of human genetic variation, is mainly due to heterochromatin polymorphisms. Though, various strategies are involved in the detection of gene mutation causing monogenic disease, the clinical molecular laboratories are enrolled in detection of the sequence variants in the specimens of suspected patients (1).

Technical issues are the detection of false positive and false negative variants which are of major concern. A variant arising from highly polymorphic genes, assembly misalignment and misleading reference genome information is considered as a false positive variant (15). The efforts of scientists have resulted in the detection of false positive gene variants in many monogenic diseases such as Marfan Syndrome where out of 23 variants of gene *FBN1*, 14 were detected as false positives (16).

The genetic hindrance lies in the locus heterogeneity, which refers to the presence of multiple as well as distinct genes responsible for a disease (for one clinical phenotype). Hereditary ataxia, hereditary spastic paraplegia and many other neurological disorders are good examples to explain the complexity associated with detection of variant where the major problem is to prove the causality of a mutation.

Is the DNA Variant a Benign Polymorphism or a Pathogenic Mutation?

A sequence variation is caused due to a mutation, where the variation might be functionally silent or could lead to a disease, depending upon the environmental or the genetic factors. The functionally silent variants are called benign polymorphism, whereas the disease causing variants

are called pathogenic mutations. Pathogenic mutations generally cause a disease with a typical pattern of the Mendelian inheritance (17).

The concept of human genetic variation has been revealed, however, the causation as well as elucidation of more than 3000 monogenic diseases is still under the cover (6). The reason might be the unanswered question related to the detection of monogenic disease which arises with an incomprehensive understanding of the clinical significance of any sequence variant (1). A substantial clinical variability influence many Mendelian diseases as the patients with same mutation(s) might develop a very severe/ mild form of a monogenic disease or show no symptoms (9). Though, the American College of Medical Genetics and Genomics (ACMG) recommendations have provided interpretative categories of sequence variants and an algorithm for interpretation, the recommendations did not provide defined terms or detailed variant classification guidance (1). A healthy individual harbors many potentially disadvantageous, asymptomatic variants (18, 19). As per the scientific community, the causality of a disease is confirmed if it fulfills the following criteria (Figure 1):

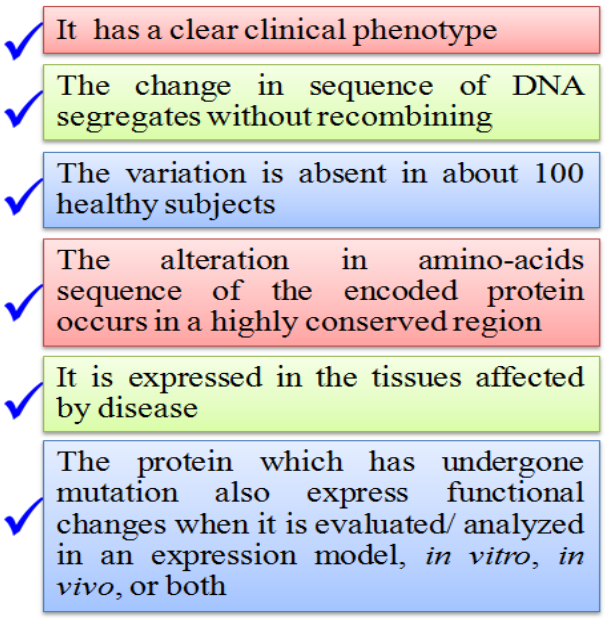


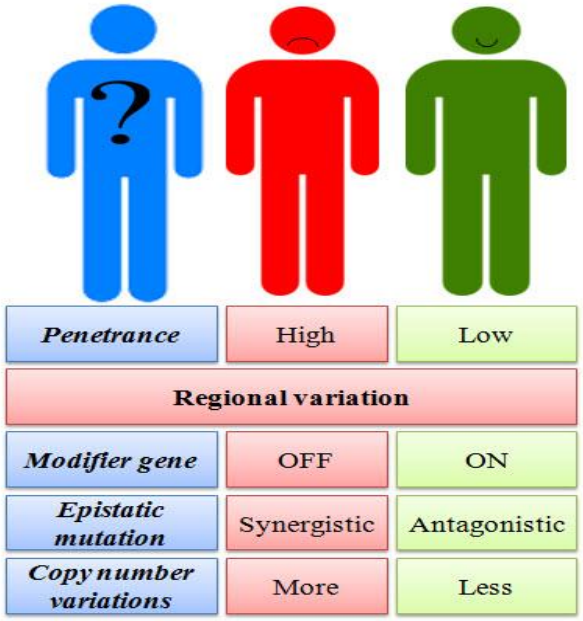
Figure 1: Criteria for the Causality of a Genetic Disease

Such criteria have to be fulfilled by the pathogenic mutation, where if even a single point is not fulfilled, the change in DNA sequence is considered as benign (20). A suitable illustration for the same can be given using a study conducted in 2001 which focused on the causative mutation leading to dyskalemic periodic paralysis (21).

A disease is considered as one with reduced penetrance where the individuals with a particular pathogenic mutation do not show clinical signs of the associated disorder. It is a common reason for complication in the detection of sequence variant in monogenic diseases as many mutations fail to cause disease in at least a proportion of individuals who carry them. It is observed as a characteristic of the underlying mutation in disorders with autosomal (dominant) inheritance such as congenital cataract with *GJA3* gene (22-25).

Regional variation might be another possible reason where a more severe phenotype, a pathogenic mutation, is observed to cause symptomatic monogenic disease. This can be exemplified with a study on Dent disease, an X-linked disorder characterized by low-molecular-weight (LMW) proteinuria, hypercalciuria, nephrocalcinosis, urolithiasis and renal dysfunction. Generally, it is caused by mutations in at least two genes, *i.e*. *CLCN5* and *OCRL1* with similar genetic background and phenotypes in European countries and USA. However, in Japan, the genes leading to Dent disease were *CLCN5* and/or *OCRL1* and/or some other genes, not detected previously. Though, the genetic background was almost similar as observed in Europe and the USA, the presence of dysfunctions also varied. Hypercalciuria is found to be more prominent and affects more than 90% patients (especially children) in other countries. However, it was observed only in 51% of Dent disease patients in Japan. Many Dent disease patients in Japan suffered from renal impairment which was unexpectedly observed in children. This proved that Japanese Dent disease has a wider clinical spectrum than Dent disease in Europe and the USA (26).

For more information on human genes and genetic disorders, visit http://www.ncbi.nlm.nih.gov/omim/, an **Online Mendelian Inheritance in Man** (OMIM) knowledgebase.



The difference in expression of disease depends on many factors, out of which, modifier genes play an important role. The concept of modifier genes was introduced in 1941. Since then, modifier genes have been defined by many scientists and that too in their own way. One of most easily understood definition was given by Gruneberg in 1963. He defined modifier gene as a gene which bears the capacity to modify the expression of a mutant gene without affecting the normal condition. The search of modifier genes and genes responsible for a disease is different in terms of phenotype under question (disease phenotype, which can be affected or unaffected *vs.* clinical phenotype which is obviously affected), and the study population (identification of a modifier gene involves the selection of associated clinical phenotype). They influence the phenotypic outcome of a given genotype *via* same/ related, or a parallel biological pathway as the disease gene (27, 28). Cystic fibrosis (CF), a monogenic, life-shortening, recessive disorder displays varied clinical presentation. An allelic variation in CF transmembrane conductance regulator gene (CFTR), *i.e.*, the responsible gene, alone is associated with multiple organ failure. However, in the presence of modifier genes, a good, CFTR independent genetic control is presented by many manifested diseases such as lung disease, neonatal intestinal obstruction, diabetes, and anthropometry (29).

Genetic epistasis, a phenomenon involving the effect of one gene being dependent on the presence of one or more 'modifier genes', can also be a possible explanation. It can also be

defined as the interaction/ combination of (modifying) genes at different loci to change the disease phenotype (30). Epistatic mutation responsible for a disorder involves multiple modifier genes to contribute to the phenotype by altering the penetrance, expressivity, pleiotropy, and severity of a disease. The alteration can be a synergistic (enhances the primary defect leading to a more severe phenotype) or antagonistic epistasis (reduces it to impart a protective effect) (31). In other words, a gene getting influenced by another/ modifier gene might be replaced with a shared effect of multiple genes. Though, this makes search for the causative variant more complex , but, search of such mutations might help clinicians in searching newer diagnostic, prognostic, and therapeutic strategies.

CNVs result from any rearrangement in genetic material due to loss or gain of specific genomic segments. They could individually, or together with the genes located in it, lead to phenotypic abnormalities which cause monogenic diseases (32). The phenotypes of a disease are also associated with genomic CNVs, the comprehensive mapping of which helps to study the correlation of variant with its disease phenotypes (33). A change in CNV associated with a single gene has the potential to revolutionize our knowledge about benign polymorphism and pathogenic variant. Mutations such as deletions and duplications occurring in the HLA class III genes encode the complement components C2 and C4 in the healthy individuals (34). However, such mutations are also associated with systemic lupus erythematosus since many years (35, 36).

VariantMaster, innovative software, developed by a research team from the Department of Genetic Medicine and Development of the Faculty of Medicine of the University of Geneva, analyzes genome sequencing data for identification of mutations causing monogenic diseases. The software can be downloaded freely from http://sourceforge.net/projects/variantmaster/.

Search of the Causative Variant: A Challenge for Clinicians

Identification of the disease-causing mutations requires brainstorming and straining through a massive number of sequence variants. To ascertain the relationship between genes/ variants and diseases/ symptoms, the accurate and comprehensive analysis using molecular genetic testing is important. However, this search for the needle in the haystack continues to be an ongoing task for molecular diagnostic practice. To unlock the causative variant is very challenging where the association of disease with genotype and/ or phenotype is difficult to establish. It is due to the contribution of both, genetic and environmental factors, which combine to influence the genetic characteristics of a disease. A single contributing factor might be small enough to be masked by other influencing factors, involving environment and ethnicity, as well as individual genetic

background. Concentrating beyond genes and DNA, and understanding a disease as an entity with respect to the individual, family, population and the environment is also warranted.

Thus, no single genetic approach is a golden strategy to identify the sequence variant responsible for monogenic diseases. There is a need to set guidelines which meet all the conditions required for comprehensive and successful identification of sequence variant in monogenic diseases, where a single or a combination of approaches used for genome-wide screenings might offer the best strategy. Therefore, there is a necessity to explore the increasing advancement opportunities and challenges to expand the 'atlas' of genetics, emphasizing monogenic diseases.

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