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Editorial Office

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Seth GS Medical College and KEM Hospital, Parel, Mumbai 400012, India.
E-mail: jpgm@jpgmonline.com

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One gene, many phenotypes

Prasun P, Pradhan M, Agarwal S

Sanjay Gandhi Post
Graduate Institute of
Medical Sciences,
Lucknow, India

Correspondence:

Mandakini Pradhan
E-mail: mandakini@sgpgi.ac.in

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ABSTRACT

“Phenotype” is the visible or quantifiable effect of the expression of a gene, whereas the specific genetic constitution responsible for a phenotype is called “genotype”. It was hoped that phenotype could be accurately predicted if the genotype could be characterized. But, the relationship between the genotype and phenotype is not straightforward. Similar genetic lesions can have entirely different phenotypes. In recent years, there has been tremendous progress in the understanding of the genetic basis of diseases. The extent to which it will be possible to relate findings at the DNA level to the clinical phenotype is difficult to delineate on many occasions. The elucidation of mechanisms underlying genotype-phenotype discrepancies is important as it will influence the use of DNA-based tests in the diagnosis, therapy and counseling of individuals affected with genetic disorders. This issue is pertinent to almost every aspect of medical practice and research in this post-genome era. In this article, we have tried to summarize those factors which are responsible for varied manifestations of lesion(s) in a single gene.

KEY WORDS: Genotype, genotype-phenotype correlation, phenotype

Mutations in different genes can lead to similar phenotype e.g., hereditary spherocytosis can be due to mutations in the genes encoding for spectrin, ankyrin, protein 4.2 or band 3.^[1-4] In contrast, defects in a single gene have been implicated in different phenotypes. For example, cystic fibrosis is caused by homozygous/compound heterozygous mutations in the *CFTR* gene. Mutations in the same gene can lead to isolated congenital bilateral absence of vas deferens (CBAVD).^[5-7] There may be considerable phenotypic heterogeneity even among individuals who have identical mutations at the disease-causing locus. This is best exemplified by sickle cell anemia, in which all patients are homozygous for a similar genetic lesion in the beta globin gene, but the phenotypic diversity ranges from ‘life-threatening’ to ‘symptom-free’.^[8-14]

With rapid advancement in the field of genetics, thousands of genes involved in human diseases have been cloned. It was expected that knowledge of mutations would lead to consistent genotype-phenotype correlations, clarifying why a given genetic change results in a particular phenotype. However, genotype-phenotype correlation is often incomplete. Monogenic diseases provide the simplest models for studying genotype-phenotype relationships. The understanding of mechanisms underlying genotype-phenotype discrepancies is important, as it will move clinical genetics towards predictive medicine, allowing better selection of therapeutic strategies and individualized counseling of persons affected with genetic disorders.

From ‘Genotype’ to ‘Phenotype’

There are several steps involved in the expression of a gene.

These are:

1. Transcription: Process whereby genetic information is transmitted from DNA to mRNA.
2. mRNA processing: The mRNA leaves the nucleus and undergoes a number of modifications such as, 5' capping, polyadenylation and splicing. The intervening sequences (non-coding regions) are excised and the exons (coding regions) are joined to form the mature RNA during the process of splicing.
3. Translation: Process whereby the mRNA is decoded on ribosomes to direct synthesis of specific proteins.
4. Posttranslational modification: Many proteins undergo modification before they attain functional activity. These modifications are of various types. The most common are the specific cleavage of precursor proteins; formation of disulfide bonds; or covalent addition or removal of groups leading to modifications such as acetylation, formylation, glycosylation, hydroxylation, methylation, oxidation or phosphorylation.

Each of these steps in the gene expression is subject to complex regulations and multiple interactions, which can result in variable and unexpected expressions of the same gene. For example, alternative mRNA splicing can produce several species of mRNA from a single gene.^[15] The polypeptide chains produced after translation may be modified in numerous ways leading to many versions of the final protein. Thus, a single gene can generate hundreds and possibly thousands of different protein molecules by the processes of alternative splicing and posttranslational modifications.

At present, only a fraction of gene function can be inferred

from the primary gene sequence. The need of developing strategies to define gene function and better understanding of the biological systems has led to the emergence of many revolutionary disciplines like proteomics, functional genomics, chemical genetics and systems biology.

‘Proteomics’ focuses on gene products i.e., proteins, which are the active agents in cells. It attempts to characterize proteins, compare variations in their expression levels in normal and disease states, study protein-protein interactions and identify their functional roles.^[16-18] ‘Systems biology’ (also called ‘integrated biology’) uses an integrated approach to understand the biological systems. The organism or the biological system is analyzed in its entirety rather than by just studying limited number of components at a time. Information regarding all the mRNA levels (transcriptome) and protein levels (proteome) in a biological system during health and disease are collected and analyzed.^[19]

It is hoped that the processes underlying health and disease will be better understood in the near future with these novel and integrative approaches.

One Gene, Many Phenotypes

I. One gene, many mutations, many phenotypes

It is very interesting to know that mutations at a single locus can lead to diseases with entirely different clinical features. For example, mutations in the *RET* gene have been implicated in the etiology of Hirschprung disease as well as multiple endocrine neoplasia (MEN) Type 2. This phenomenon, whereby different mutations at the same locus result in different phenotypes is known as allelic heterogeneity. The underlying mechanism is either quantitative or qualitative change in the gene product. Some of the examples of allelic heterogeneity have been listed in Table 1. A few illustrative examples are discussed here.

Nonfunctional vs. partially functional/truncated gene product

Duchenne and Becker muscular dystrophies are caused by mutations in the dystrophin gene. Mutations that partially inactivate the gene product cause Becker muscular dystrophy (BMD), while mutations which completely inactivate the gene product produce Duchenne muscular dystrophy (DMD).

Loss of function vs. gain of function

The *RET* gene codes for a tyrosine kinase receptor. Loss of function mutations in *RET* that lead to nonfunctional product

or lower expression of *RET* give rise to Hirschprung disease. Gain of function mutations at the same locus that produce constitutively activated receptors lead to MEN Type 2. Similarly, loss of function mutations at *FGFR1* locus cause an autosomal dominant form of Kallman syndrome characterized by anosmia and hypogonadotropic hypogonadism, while gain of function mutations at the same site lead to a form of craniosynostosis (Pfeiffer syndrome).^[20,21]

II. One gene, one mutation, many phenotypes

The phenomenon of allelic heterogeneity is not unexpected, as the gene product may get differentially changed by the different mutations and so the phenotypes. More surprising is the fact that individuals with similar genetic lesions can have significantly different clinical manifestations. This is well observed in autosomal dominant disorders, where ‘pleiotropy’, ‘variable expressivity’ and ‘reduced penetrance’ have been classically described. Pleiotropy is the condition whereby a single gene mutation has multiple consequences in numerous tissues. Even in the same family, two individuals carrying the same mutant genes may have different disease manifestations. Expressivity is defined as the severity of the phenotype. When the severity of disease differs in people with same genotype, the phenotype is said to have variable expressivity. Penetrance is the proportion of persons with a particular genotype who manifest the disease. The reduced penetrance leads to ‘skipping of generation’. Neurofibromatosis Type 1 (NF1) is characterized by extreme clinical variability, not only between unrelated individuals and among affected individuals within a single family but even within a single individual with NF1 at different times in life. The mutation in the *NF1* gene can produce different lesions in different tissues such as café-au-lait spots, neurofibroma, iris hamartoma, skeletal abnormalities or mental retardation (pleiotropy). Each of these pleiotropic effects can have varying severity among the affected family members (variable expressivity). The mechanisms underlying such clinical variations are often unclear. It is supposed to be the result of the modifying effects of other genes, as well as due to interaction with environmental factors. Some of the known mechanisms responsible for variable manifestations of a single gene lesion have been discussed briefly in the subsequent sections.

Mosaicism (gene dosage effect)

Mosaicism is the existence of two cell lines with different genetic constitution that have been derived from a single zygote. It arises as a result of occurrence of new mutation during development. The stage at which the mutation occurs

Table 1: Selected examples of allelic heterogeneity

Disease	Gene	Disease
Hurler syndrome	<i>IDUA</i>	Scheie syndrome
Charcot-marie-tooth neuropathy	<i>PMP22</i>	Hereditary neuropathy with pressure palsy
Hyperkalemic periodic paralysis	<i>SCN4A</i>	Paramyotonia congenita
Creutzfeldt- Jacob disease	<i>PRNP</i>	Familial fatal insomnia
Pseudohypoparathyroidism IA	<i>GNAS1</i>	Albright hereditary osteodystrophy
Kennedy disease	<i>AR</i>	Androgen insensitivity
Cystic fibrosis	<i>CFTR</i>	Congenital bilateral absence of vas deferens
Duchenne muscular dystrophy	<i>DMD</i>	Becker muscular dystrophy
Hirschprung disease	<i>RET</i>	Multiple endocrine neoplasia Type 2

determines the proportion of cells bearing the lesion. It is an important cause of phenotypic heterogeneity among individuals who carry the same genetic lesion.^[22] The phenotypic severity is determined by the proportion of cells carrying the mutation. This is best exemplified in mitochondrial disorders. There are thousands of mitochondrial DNA (mtDNA) molecules in a cell. When a mutation occurs in the mtDNA, it is at first present in only one of the mtDNA molecules. At cell division, the mtDNA molecules replicate and sort randomly among the daughter cells. Each daughter cell may receive very different proportions of mitochondria carrying normal and mutant mtDNA. The phenotype will depend upon three factors: the relative abundance of mutant mtDNA (heteroplasmy), the tissue distribution of the mutant mtDNAs and the vulnerability of each tissue to impaired oxidative metabolism (threshold effect). Thus, reduced penetrance, variable expression and pleiotropy are typical features of kindred with mitochondrial disorders. For example, a deletion of 4977 bp of mtDNA is commonly encountered in Kearns-Sayre syndrome (characterized by the triad of pigmentary retinopathy, external ophthalmoplegia and onset before the age of 20 years). The same deletion has also been identified in cases of Pearson syndrome (sideroblastic anemia, exocrine pancreatic dysfunction) and progressive external ophthalmoplegia. The different phenotypes from the same deletion are due to tissue distribution of the defect. If the defect is present in mitochondria of all tissues, the phenotype is Kearns-Sayre syndrome. In Pearson syndrome, the defect is localized mainly to the hematopoietic tissue, while the defect is confined to the skeletal tissues in progressive external ophthalmoplegia.^[23,24]

Another striking example of phenotypic diversity arising from mosaicism is the androgen insensitivity syndrome (AIS). Androgen insensitivity syndrome is the major cause of male pseudohermaphroditism. It is an X-linked disorder caused by mutations in androgen receptor (AR) gene. Androgen insensitivity syndrome can be subdivided into three highly variable phenotypes: complete AIS, when the affected persons have female external genitalia; partial AIS, when the genitalia are ambiguous; and mild AIS, when the affected individuals have normal male external genitalia. In a number of cases, identical mutations have resulted in significantly different phenotypes. This is due to somatic mosaicism.^[25-28] The co-expression of wild allele shifts the AIS subtype to a higher degree of virilization than expected from the mutant allele alone.

Modifier genes

A modifier gene is defined as an inherited genetic variation that affects the phenotypic expression of another gene. It can affect the pleiotropy, penetrance or expressivity of the disease. Depending upon the nature of modifying effect, modifier genes might cause more severe phenotypes, less severe phenotypes, novel phenotypes or wild-type (normal) phenotypes.^[29]

Modifiers causing less severe (reduced) phenotype

Beta thalassemia

The severity of anemia in beta thalassemia reflects the degree of globin chain imbalance. The excess of alpha globin chain

precipitates in red cell precursors leading to ineffective erythropoiesis. This imbalance can be genetically modified by two factors—variation in amount of gamma globin response and alpha globin chain production.^[30,31] The beta thalassemia patients who co-inherit alpha globin gene deletions will have less redundant alpha globin chains and tend to have less severe phenotype. Similarly, increased synthesis of gamma globin chain will reduce the disease severity by increasing HbF level. The gamma globin response is also genetically determined. The C→T polymorphism at position -158 of the gamma globin gene is associated with enhanced HbF response. There are many other loci that are not linked to the beta globin gene but modify HbF response. Linkage studies have mapped these loci to three regions of the genome—chromosome 6q23, 8q11 and Xp22.^[32-34]

Sickle cell anemia

HbF is the best understood genetic modifier of sickle cell anemia. HbF inhibits polymerization of HbS. Increasing levels of HbF progressively ameliorate the disease. The HbF response is genetically determined as discussed under the 'beta thalassemia' section. Similarly, the coinheritance of deletion of alpha globin genes modifies the phenotype of sickle cell disease. Alpha globin gene deletions cause reduced intracellular concentration of HbS leading to reduction in rigidity of red cells and consequently longer erythrocyte lifespan, raised hematocrit and blood viscosity. Clinically, these changes produce beneficial and harmful effects. They seem to protect against stroke but predispose to more frequent painful episodes and osteonecrosis.^[8,35-38]

Spinal muscular atrophy (SMA)

Spinal muscular atrophy is a recessive neuromuscular disorder caused by homologous loss of *SMN1* gene function. There are four types of SMA according to the age of onset and disease severity. Type 1 patients show onset within six months after birth and usually die before two years of age. Type 4 is characterized by age of onset > 30 years and only very mild signs of muscle weakness.

SMN2 is a nearly identical copy of *SMN1*. *SMN2* produces transcripts lacking exon 7. About 10% of *SMN2* transcripts are correctly spliced and encode a protein identical to *SMN1*. The severity of SMA has been found to be influenced by the number of *SMN2* copies.^[39] Increasing number of *SMN2* copy reduces severity of disease. About 10% of Type 1 patients carry two copies, 82% of Type 2 SMA patients have three *SMN2* copies, whereas Type 3 patients have minimum three to four *SMN2* copies.

Modifiers causing more severe (enhanced) phenotype

The severity of anemia in beta thalassemia depends on the degree of globin chain imbalance. It is an autosomal recessive condition. The heterozygotes for beta thalassemia mutations are clinically asymptomatic as the degree of imbalance is insignificant. But, the coinheritance of extra alpha globin genes (alpha triplication) increases the imbalance. This leads to symptomatic disease in heterozygotes, sometimes manifesting as 'intermedia' phenotype.^[40-42]

Alternative splicing

Alternative mRNA splicing is another mechanism responsible for different expression of similar genotype. Two illustrative examples are given below.

(A) Duchenne muscular dystrophy and BMD are caused by mutations in the dystrophin gene. Duchenne muscular dystrophy is a severe muscle-wasting disease arising from defects in the dystrophin gene, typically nonsense or frameshift mutations that preclude the synthesis of a functional protein. Becker muscular dystrophy generally arises from in-frame deletions that allow synthesis of a shorter but still semifunctional protein. But, nonsense mutations which should cause DMD have been reported in BMD. This is due to alternative mRNA splicing— skipping of the affected exon leads to removal of the nonsense mutation from the dystrophin mRNA.^[43-45] This results in production of partially functional dystrophin and BMD phenotype.

(B) Cystic fibrosis is an autosomal recessive disorder. The genotype delta F508/R117H can lead to either severe phenotype of cystic fibrosis leading to respiratory failure or the milder phenotype, in which the only manifestation is congenital bilateral absence of vas deferens (CBAVD). The *CFTR* gene has two intron 8 variants. One is associated with efficient mRNA splicing, while the other causes inefficient splicing. The R117H allele is capable of producing partially functional protein. The R117H allele associated with efficient splicing leads to production of some amount of partially functional protein and hence milder phenotype (CBAVD). On the other hand, severe phenotype results if the intron 8 variant causes inefficient splicing and production of nonfunctional protein.^[46-50]

Epigenetic mechanisms

Epigenetics is the study of stable alterations in gene expression that arise during development and cell proliferation. Epigenetic phenomena modulate when and at what level genes are expressed. Thus, the expression of a mutation also depends upon the activity state of the locus carrying it; the mere presence of a genetic defect may not be enough for clinical expression. Genomic imprinting and X-inactivation are examples of epigenetic phenomena.

Genomic imprinting

The expression of a gene depends upon the parent who passed on the gene. For example, two different diseases – Prader-Willi syndrome and Angelman syndrome – are due to deletion of the same part of chromosome 15. When the deletion involves the chromosome 15 inherited from the father, the child has Prader-Willi syndrome, but when the deletion involves the chromosome 15 inherited from the mother, the child has Angelman syndrome. This is a striking example of how the parental origin of a genetic defect influences the clinical phenotype. *UBE3A* is the gene implicated in Angelman syndrome. It is subject to imprinting, being expressed only from the maternal allele in the brain.^[51-55] A *UBE3A* mutation inherited from the mother will lead to Angelman syndrome, while paternal *UBE3A* mutation will be silent.

X-inactivation

X inactivation in females is a random process. Female carriers of X-linked recessive conditions (e.g. hemophilia, DMD) are asymptomatic. But, occasionally they may show mild or even full expression of the disease which may approach that of a hemizygous male. This is due to nonrandom inactivation of X chromosome. By chance, most of the X chromosomes carrying the normal allele get inactivated resulting in clinical expression of the disease.

Gene and environment

Virtually all human diseases result from the complex interplay of genetic susceptibility factors and modifiable environmental factors. This is most obvious in the context of common illnesses such as diabetes, coronary artery disease or cancer. But, environmental factors play a significant role in the expression of monogenic disorders too. For example, inherited metabolic disorders manifest when there is introduction of the substrate for which the metabolism is defective. Similar genetic defects may have different phenotypes if the environmental factors are not similar.

Conclusions

Knowing a gene mutation is only a step in predicting its consequences. The effect of a mutation is determined by other genetic and environmental modifiers. Recently, a large number of candidate genes have been discovered for common disorders like hypertension, diabetes, cancer etc. The genotype-phenotype correlation^[56] is much more complex in these disorders where genetic modifiers and environmental factors are complexly interwoven. However, the same principles outlined for monogenic disorders apply. The prediction of phenotype based upon the results of DNA-based tests may be fallacious at times. But the judicious use of these results will definitely serve in anticipating a complication, selecting an appropriate therapeutic regimen and better counseling.

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