INVITED EDITORIAL
Phenotypes of Patients with "Simple" Mendelian Disorders Are Complex Traits: Thresholds, Modifiers, and Systems Dynamics

Katrina M. Dipple[1] and Edward R. B. McCabe[1,2,3,4]

[1]Department of Pediatrics, UCLA School of Medicine, and Mattel Children's Hospital at UCLA, [2]Mental Retardation Research Center, [3]Brain Research Institute, and [4]Molecular Biology Institute, UCLA, Los Angeles

One of the firmly held concepts in human molecular genetics has been that, if we can understand the details of specific genetic mutations and their effects on protein products, we will be better able to correlate genotype with phenotype. One of the promises of this concept is that such a knowledge base will move clinical genetics into a predictive mode: knowledge of the mutant alleles responsible for a disease would permit an accurate prediction of the prognosis and a better-informed selection among therapeutic strategies for any individual patient. As the mutations have been identified for a series of diseases, it has become clear that the correlation between genotype and phenotype is often incomplete. What has emerged is the recognition that, for many diseases, only a subset of all mutations reliably predicts phenotype.

We propose that there are two thresholds relating mutant protein function to phenotype: a level below which the severe phenotype will always be observed and another level above which the phenotype will be uniformly mild (fig. 1). Between these two thresholds is an indeterminate range, in which mutations would not correlate with phenotype and additional unlinked genes and/or environmental factors would influence the final phenotype. For many diseases, both thresholds may be observed; but, for others, one or both thresholds may not be seen. For example, there may not be any mutation with sufficiently low protein function to result uniformly in the severe phenotype and/or with sufficiently adequate function to be associated consistently with the mildest phenotype.

This conceptual construct would suggest at least five models for a prototypical autosomal recessive biphenotypic disorder (fig. 1). The simplest, but extremely rare (perhaps nonexistent), example would involve a discrete threshold for protein function, with mutations consistently leading to predictable functional consequences above or below the threshold (discrete-threshold model). The remaining models would be nondiscrete, and each would have mutations that would be indeterminate and that would not reliably predict phenotype. In the two-threshold nondiscrete model, specific alleles might be associated at all times with either the mild or the severe phenotype, and other mutant alleles would be indeterminate. For the indeterminate mutations, protein function would be in an intermediate range, in which other nonallelic genetic variations and/or environmental effects would influence the in vivo function and therefore the clinical phenotype. In the single-threshold, severe/indeterminate nondiscrete model, specific mutations would be observed that would be associated uniformly with the severe phenotype, but none would be observed to correlate consistently with the mild phenotype. The single-threshold, mild/indeterminate nondiscrete model would have a group of mutations associated reliably with protein function resulting in the mild phenotype and another group that would show no correlation with phenotype. Finally, for some diseases, no threshold would be observed, because there would be no correlation between any genotypes and the clinical phenotypes (no-threshold model). For most "single-gene" disorders, there will be a considerable range of protein function and a significant number of alleles that will not correlate absolutely with clinical phenotype, because of the effects of additional independently inherited genetic variations and/or environmental influences. For these "simple" Mendelian disorders, the phenotypes are, in fact, complex traits.

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Address for correspondence and reprints: Dr. E. R. B. McCabe, Department of Pediatrics, UCLA School of Medicine, Los Angeles, CA 90095-1752. E-mail: emccabe@mednet.ucla.edu

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Gaucher Disease

In this issue, Koprivica et al. (2000) report the genotyping of DNA from 128 individuals with type 1 (nonneuronopathic) and 24 individuals with type 3 (chronic neuronopathic) Gaucher disease, as well as the identification of >97% of the mutant alleles. They examined the frequency of mutant alleles among Ashkenazi Jewish and non-Ashkenazi patients, compared the results with those of other reports, and attempted to correlate genotype with phenotype. They concluded that certain mutations

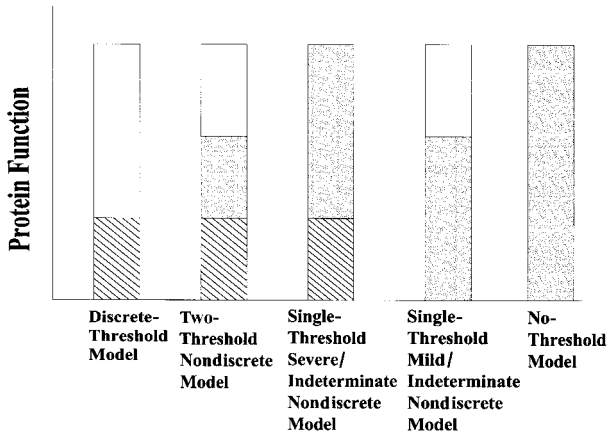

Figure 1 Threshold models relating mutant-protein function to phenotype in a biphenotypic disorder. The hatched areas indicate the severe mutations, the stippled areas indicate the indeterminate mutations, and the white areas indicate the mild mutations. In the discrete-threshold model, mutations would consistently result in functional consequences above or below an absolute threshold; in the two-threshold nondiscrete model, specific alleles might be associated consistently with either the mild or the severe phenotype, whereas others would be indeterminate with intermediate function influenced by independent genetic and environmental factors; in the single-threshold nondiscrete models (severe/indeterminate and mild/indeterminate), mutations would be associated only with the severe or mild phenotype, respectively, and all other mutations would be indeterminate; and, in the no-threshold model, no mutations would correlate absolutely with the phenotype. The levels of the thresholds shown in the figure are arbitrary and will vary dramatically between different proteins. A thorough examination of genotype-phenotype correlations is required before a disorder can be assigned to a particular model, since limited information may lead to misassignment.

appear to predict specific Gaucher disease types. For example, N370S as a heterozygous or homozygous allele has been observed only in individuals with type 1 Gaucher disease, which suggests that sufficient glucocerebrosidase activity is expressed by N370S to "protect" the individual from neuronopathic disease. Other mutations, however, did not appear to have reliable genotype-phenotype correlations. For example, the N188S mutation, elsewhere associated with relatively mild Gaucher disease and thought to protect against the neuronopathic phenotype (Kim et al. 1996; Choy et al. 1999), was observed in a patient with the more-severe type 3 disease in the study by Koprivica et al. (2000). The lack of consistency between the R463C allele and phenotype was particularly remarkable. R463C homozygosity has been reported in patients with type 1 (Hatton et al. 1997) and type 3 (Gurakan et al. 1999) disease. In the study by Koprivica et al. (2000), severe or null alleles observed in patients with type 2 disease were found in compound heterozygosity with R463C among patients with type 1 and type 3 disease. The authors of that report noted that siblings with identical genotypes may differ clinically and considered that limitations in the prediction of phenotype from genotype might be due to the contribution of additional genes and environmental factors to the individual's phenotype.

In the discussion that follows, we will consider a small group of diseases from among many possible examples in which influences beyond the primary genotype modify the individual's phenotype.

Other Biochemical Genetic Disorders

In addition to Gaucher disease, there are many other inborn errors of metabolism that are inherited in a simple Mendelian fashion, for which the phenotypes appear to be inherited as complex traits. One of the first to be recognized in this category was phenylketonuria (PKU) (Scriver and Waters 1999). This biochemical disorder is inherited in an autosomal recessive pattern, and the classic phenotype includes high blood phenylalanine levels and mental retardation in the untreated individual. The patients detected by newborn screening who have classic PKU require treatment with a phenylalanine-restricted, tyrosine-supplemented diet, whereas patients with milder hyperphenylalaninemia may not require dietary intervention. The isolation of the phenylalanine hydroxylase (*PAH*) gene brought hope of predicting the phenotype and, therefore, of determining the need for dietary therapy on the basis of genotype. Although some studies have found a correlation between genotype and in vitro or in vivo phenotype, not all have confirmed this relationship (reviewed in Enns et al. 1999). A meta-analysis of 365 patients, who represented 73 different *PAH* mutations and 161 genotypes, found that 11 of the mutations were inconsistent in their in vivo phenotypes (Kayaalp et al. 1997). Seven *PAH* mutations exhibited discordance between in vitro and in vivo phenotypes. A study of patients from seven European centers evaluated a model for prediction of four phenotypic categories on the basis of *PAH* genotype (Guldberg et al. 1998). The phenotype predicted by the model was inconsistent with the observed phenotype in 88 (13.5%) of 650 patients. A study of 133 ethnically diverse patients showed no correlation between the mutation severity and phenylalanine levels or between genotype and either pretreatment circulating-phenylalanine concentrations or intelligence (Enns et al. 1999). The authors of that report cautioned that "prognosis may not be predicted with precision based on mutation analysis" in an individual patient (Enns et al. 1999, p. 594). Expression of *PAH* missense mutations in mammalian cells showed that the mutations could affect the enzyme activity, the levels of phenylalanine hydroxylase protein, both, or neither (Scriver and Waters 1999). PAH proteins with missense mutations may have increased susceptibility to proteolytic degradation, and the levels of phenylalanine

are dependent on the in vivo steady-state level of the PAH protein (the balance between the formation and the degradation of the proteins) and/or the residual activity in the mutant protein.

A detailed study of two *PAH* mutations (one causing mild disease and one causing a severe phenotype) showed in vivo activity to be what one would expect—the mild phenotype had an activity level of 26% of normal, whereas the severe phenotype had much lower activity (4.6% of normal) (Waters et al. 1998). The disparity in activity levels was not due to changes in the catalytic activity of the phenylalanine hydroxylase enzyme but to differences in the amount of PAH protein present. These observations support the hypothesis that, at least for some mutations, it is the degradation of the mutant protein in vivo that determines the PAH activity (Waters et al. 1998). Scriver and Waters (1999) argue that interindividual differences in the gene products involved in protein stability, such as the chaperones and proteolytic enzymes, may be responsible for phenotypic variability among individuals with the identical genotype. This concept of selective degradation of mutant protein has been seen with other enzymes, such as hypoxanthine-guanine phosphoribosyltransferase (Capecchi et al. 1974).

There are lysosomal storage disorders in addition to Gaucher disease—namely, α- and β-mannosidase, and fucosidase deficiencies—that lack a consistent genotype-phenotype relationship (Michalski and Klein 1999). These disorders are caused by accumulation of glycoproteins that are unable to be properly degraded in the patients' lysosomes. Despite the fact that all the patients with these autosomal recessive disorders have low or undetectable activity of the respective enzyme, the clinical phenotypes are seen as a continuum, from the severe, infantile type I to the milder, juvenile type II forms. Other biochemical and environmental factors are thought to influence the clinical phenotype (Michalski and Klein 1999). In α-mannosidosis, the patients have mental retardation, hypotonia, impaired hearing, lens opacification, macroglossia, and immunodeficiency, from splice-site, missense, and nonsense mutations. The most common mutation (R750W) is seen in 21% of the patients of European descent and is associated with a range of clinical phenotypes. With fucosidosis, the patients present with a progressive neurological decline and with mental retardation. They have growth retardation, coarse faces, infections, angiokeratoma, and dysostosis multiplex, due to nonsense, frameshift, splice-site, and missense mutations in the *FUCA1* gene. Although the missense mutations occurring in the conserved areas of the protein have a more severe clinical phenotype, there is a range of phenotypic severity within the same family (Michalski and Klein 1999). These findings suggest that other genes or exogenous factors are involved in determining the severity of the clinical phenotype.

Phenotypic Variability within Families

In addition to the metabolic disorders mentioned above, there are numerous other examples of genetic disorders in which the genotype does not correlate with the clinical phenotype. Among the cardiac diseases, for example, mutations in the genes *KVLQT1, HERQ, SCN5A,* and *KCNE1,* which encode the voltage-gated ion channels that regulate the contraction of the heart, cause the hereditary form of long-QT syndrome (Ackerman 1998). The clinical phenotype varies from a prolonged QT interval, seen only on electrocardiogram, to syncope, seizures, or sudden death. Even within the same family, the identical mutation is associated with a phenotype that varies by features such as the age at onset of symptoms (e.g., 9 vs. 57 years) and the severity of symptoms (Ackerman 1998).

Intrafamilial phenotypic variability may also be seen with mutations in *PAX2,* a paired-box gene involved in kidney and eye development. These mutations cause dominantly inherited optic-nerve colobomas, hearing loss, vesicoureteral reflux, and renal anomalies (Sanyanusin et al. 1995). Within one family we can see the clinical variability imposed on the same frameshift mutation (c.1104delC) (Sanyanusin et al. 1995; Schimmenti et al. 1995). All affected individuals (the father and three sons) had optic-nerve colobomas but had varying degrees of renal problems and hearing loss. Two of the boys had renal failure requiring transplantation (at ages 5 and 14 years), but the other brother had milder renal disease, and the father's renal disease was so mild that it was undetected until the renal failure was diagnosed in his children. Only the father and one son had hearing loss. This variability within one family, all of whose members had the same mutation, suggests that additional environmental factors or genes that are inherited independently of *PAX2* are influencing the clinical phenotype.

This variability may also be observed in the neurogenetic disorders, one example being Charcot-Marie-Tooth disease (CMT). The dominant, type 1 CMT has been subclassified on the basis of the gene involved: "CMT1A" for mutations in the peripheral myelin protein 22 gene (*PMP22*), "CMT1B" for mutations in the myelin protein 0 gene (*MPZ*), and "CMT1X" for mutations in the connexin 32 gene (*Cx32*) (Haites et al. 1998). However, the genotype does not predict the clinical phenotype, which can be quite variable. Mutations in the early-growth response 2 gene (*ERG2*) have been observed in individuals with the clinical diagnosis of CMT1, as well as in others with a related but clinically distinct congenital hypomyelination (CH). Point mu-

tations in the *MPZ* gene may cause CMT1, CMT2, CH, and Dejerine-Sottas syndrome (Haites et al. 1998). In one study of 116 patients with CMTX and *Cx32* mutations, the disease severity was variable. This clinical variability was noted within families of defined genotypes, and clinical differences and severity were more prominent in males than in females (Hahn et al. 1999), which suggests that other factors are involved in determining the clinical phenotype. A study of 30 patients from 19 families with an autosomal recessive neurodegenerative disorder—Wolfram syndrome (optic atrophy and juvenile-onset diabetes mellitus)—showed no correlation between the severity of the clinical phenotype and the mutation in the Wolfram syndrome gene (*WFS1*) (Hardy et al. 1999). In addition, there was variability in the clinical phenotype within some families with *WFS1* mutations.

There are also endocrine disorders for which genotype may not predict phenotype. In some cases of congenital adrenal hyperplasia (CAH) due to 21-hydroxylase deficiency encoded by *CYP21,* there is a lack of genotype-phenotype correlation (Carlson et al. 1999). Fourteen of 26 mutations showed inconsistency in the relationship between the phenotype predicted from the genotype and the observed phenotype (Wilson et al. 1995). Some of the patients with the same mutation had either the classic or the nonclassic form. Of the 13 patients with the V281L/Del mutation, 11 had nonclassic CAH, 1 had salt wasting, and 1 had simple virilization (Wilson et al. 1995; Carlson et al. 1999). Mutations in *SRY,* the gene encoding the testis-determining factor, are associated with disorders of sexual differentiation (Vilain and McCabe 1998). However, the clinical outcome depends on other factors, as evidenced by a family with a single-base-pair substitution (588G→C; V196L) that presents with two distinct clinical phenotypes: three XY sex-reversed females and two XY males (Vilain et al. 1992). Another striking example is in a family with an R311H mutation in the *c-erbAß* gene that encodes the thyroid-hormone receptor (Geffner et al. 1993). The proband presented with a severe form of selective pituitary resistance to thyroid hormone and was heterozygous for the R311H mutation. Her unaffected father and unaffected half-sister were also heterozygous for the same mutation. These examples reinforce the concept that other environmental factors or genes inherited independently are affecting the clinical phenotype in these endocrine disorders.

Identification of Phenotypic Modifiers

Factors modifying phenotype have been identified in some cases. One example is a polymorphism that affects expression of the D178N mutation in the prion protein gene (*PRNP*), which causes familial Creutzfeldt-Jakob (fCJD) disease in some individuals and fatal familial insomnia (FFI) in others (Harder et al. 1999). The phenotypic differences among individuals with the D178N mutation appear to segregate with the 129M/V polymorphism: 129M is seen with FFI, and 129V is found with fCJD. The molecular basis for this phenotypic modification in the patients with either FFI or fCJD is thought to involve different proteinase-resistant prion-protein isoforms associated with the polymorphism.

Phenotypic modifiers have been identified in other types of disorders, including the hemoglobinopathies. Sickle-cell disease is the prototypical hemoglobinopathy, with hemoglobin S the initial hemoglobin variant to be described (Pauling et al. 1949) and to have its amino acid mutation reported (Ingram 1956). The phenotype of homozygous SS sickle-cell disease is known to be modified by concomitant expression of hemoglobin F in hereditary persistence of fetal hemoglobin (HPFH) (Stamatoyannopoulos et al. 1975). HPFH may be linked to the β-globin gene cluster on chromosome 11p in some families but not in all (Donald et al. 1988; Thein et al. 1994). The 20-fold variation in hemoglobin F production among normal individuals and those with sickle-cell disease is influenced by age, sex, α-globin gene number, β-globin haplotype, and the F-cell production (FCP) locus that maps to Xp22.2 (Dover et al. 1992; Chang et al. 1995, 1997). In studies of SS subjects from Jamaica and France, the FCP locus accounted for 40% of the variation in hemoglobin F (Chang et al. 1995, 1997). Another locus that appears to be important in controlling the level of fetal hemoglobin expression maps to 6q (Craig et al. 1996).

For many of the thalassemias, it is also difficult to predict phenotypes from genotypes (Ho et al. 1998*a*, 1998*b;* Weatherall 1998). Among those with hemoglobin E/β-thalassemia, the variability in phenotype remains largely unexplained, although there is a correlation between the level of F expression and the total hemoglobin concentration (Rees et al. 1998). In one family with hemoglobin E segregating with pyrimidine 5′ nucleotidase (P5N) deficiency, homozygous EE disease, which is normally relatively mild, results in severe anemia, with P5N deficiency causing hemoglobin instability (Rees et al. 1998). This example suggests yet another possible genetic-modifier mechanism that might influence the phenotypes among the hemoglobinopathies.

Modifier Genes in Model Organisms

To better understand how modifier genes influence phenotype, it will be important to study the numerous examples of modifier genes that exist in model organisms. One prototypical gene that is influenced by a variety of modifiers is the *Drosophila white* eye locus involved in pigment deposition. *Modifier of white* (*MOW*) (Bhadra and Birchler 1996), *lightener of white* (*LOW*) (Bhadra

et al. 1997*a*), *ultra female overexpression* (*Ufo*) (Bhadra et al. 1997*b*), and *sugarless* (*sgl*) (Benevolenskaya et al. 1998) are recently identified genes that regulate expression of *white*. The *sgl* gene encodes uridine diphosphate glucose dehydrogenase and is thought to modulate growth-factor signaling by effects on cell-surface glycosaminoglycans (Benevolenskaya et al. 1998).

Vulval induction in *Caenorhabditis elegans* provides examples of modifier genes influencing specific signal-transduction cascades and modulating large chromosomal regions. The *ksr-1* (kinase suppressor of ras) gene encodes a member of the Raf family of serine/threonine protein kinases that modifies the RAS-mediated signal-transduction pathway involved in vulval induction (Sundaram and Han 1995). The *tam-1* (tandem-array modifier) gene appears to influence context-dependent gene silencing and belongs to the synMuv (synthetic multivulva phenotype) group of genes, which functions in the regulation of the RAS pathway (Hsieh et al. 1999).

In yeast there are also many examples of modifier genes, including those which influence the abundance of cell-surface enzymes (Na et al. 1995) and complex subcellular compartmented protein degradation (Ohsumi 1999).

Model organisms demonstrate the wide variety of modifier genes that influence cellular systems and present candidate mechanisms for considerations in mammals. Numerous examples of transgenic and knockout mice are known in which the phenotypic differences between strains are obvious. As the catalogue of murine and human genes becomes complete, candidate mechanisms from other model organisms may be extrapolated more efficiently to these mammals.

Thresholds for Protein Function

With the accumulation of detailed information about the mutations in "single-gene" disorders, geneticists have observed that the correlation between genotype and phenotype is inconsistent. The frequent lack of predictability in this relationship acknowledges that the primary mutant gene product is embedded within a highly complex system in which a multiplex of genetic polymorphisms, additional nonallelic mutations, and environmental influences represent the differences between individuals. Perhaps what is more impressive than the inconsistency between genotypes and phenotypes, given the complexity of the systems, is the observation of the occasional mutation that does show a reliable correlation with phenotype. When such a predictable relationship does exist, it is usually because the function of the mutant gene product exceeds a threshold, above which systemic influences cannot compromise the collective operational integration or, alternatively, below another threshold, beneath which the function of the mutant protein cannot be raised by other variables within the system. Between these two thresholds is an indeterminate range in which the mutant products have a level of residual function that may be influenced by additional systemic perturbations, to result in either of the dichotomous phenotypes.

One example of a disease with an apparent threshold is the autosomal dominant disorder Marfan syndrome, in which mutations in the fibrillin 1 gene cause a highly variable clinical course ranging from isolated ectopia lentis to aortic-root dilatation or neonatal lethality (Ramirez et al. 1999). All types of mutations are seen in the fibrillin 1 gene, including missense and nonsense mutations, deletions, mRNA instability, splice-site changes, and skipped exons. For most patients, there is no clear relationship between the mutation and the clinical phenotype, except that mutations in the middle of the gene tend to be found with the more severe clinical phenotypes. Recent studies of fibrillin 1–gene targeting in mice generated two types of mutations—one that had a large in-frame deletion and decreased expression and another with decreased expression of normal fibrillin 1. Selective breeding of the two murine lines provided a spectrum of phenotypes among the mice that was similar to that seen in humans. On the basis of observations in the mice and humans, the authors of that report speculated that there is a "critical threshold" of functional microfibrils below which symptoms are observed (Ramirez et al. 1999, p. 206).

Mutations: Embedded within Complex Systems

The recognition that simple Mendelian traits are, in fact, complex traits represents a logical extension of concepts developed by metabolic-control analysis (MCA) (Krauss and Quant 1996; Brand 1997; Schilling et al. 1999). MCA has shown that metabolic pathways are not controlled by single rate-limiting steps and that control is shared among all steps, with more than one step having significant influence on pathway flux (Krauss and Quant 1996). Regulation occurs at the systems level and is mediated by effectors internal or external to the regulated system (Krauss and Quant 1996; Brand 1997). Because the activity of the particular steps in the pathway may be influenced by nonallelic polymorphisms and additional independent mutations, individuals within the population will differ in flux through the various steps in the pathway, imposing an additional magnitude of genetic complexity on systems that would already be incredibly intricate, even if individuals were genetically identical.

The rarity of the true single-gene disorder affirms the observations from MCA. No single gene product is rate limiting in any system, and more than one gene product will influence the activity and regulation of the system. The organism represents a collection of systems that is

highly complex because of unique genetic and environmental contributions. Thus, there is considerable opportunity for perturbation of the primary mutation's influence on the systems composite, which frequently results in phenotypic differences among individual patients, even within the same family.

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