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INVITED EDITORIAL
Genotype, Malleotype, Phenotype, and Randomness: Lessons from Neurofibromatosis-1 (NF-1)

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Neurofibromatosis-1 (NF-1) is a complex disorder. The total number and range of severity of features of the disorder require an entire book for their full consideration (Riccardi 1992). Moreover, many of the features have an important age factor: the timing of the onset and the degree of severity are a function of age. For example, most patients with NF-1 do not develop cutaneous neurofibromas until early adolescence, and the total number of neurofibromas thereafter is primarily a linear function of age. The older the patient, the larger the number of neurofibromas. Conversely, café au lait spots are present from early infancy, and their numbers (in contrast to the freckling that is also part of the disorder) are relatively constant until late middle age (late 40s and early 50s), when the total number of spots declines. Some features are congenital, and some develop later. Therefore, comparisons of patients must take into account whether the feature being considered is congenital in origin or whether its presence and severity are a function of the patient's age.

In addition, what first appears to be a quantitative trait for the purpose of judging the severity of the disease may prove to be less straightforward at the final interpretation. For example, I have proposed (Riccardi 1992) that short stature as a part of NF-1 is really a discontinuous trait, and the key issue is its presence or absence, not merely the average heights of persons with and without the disease. If you have NF-1, you either will or will not have an optic glioma. If you have NF-1, you either will or will not have short stature. If you have NF-1 and short stature, the severity will have some range, from mild to severe. To presume a meaningful spectrum of height measurements of all patients with NF-1 misses a key point: such a spectrum mixes those with and without the disorder's consequences yet attributes the average solely to the disorder.

In the context of the wide diversity of expression of NF-1, it certainly is realistic to consider whether there might be groupings or clusterings of features that would be helpful either for counseling patients about expectations or for getting a pathogenetic handle on the disorder. In this issue of the *Journal,* Easton et al. (1993) present their work, which attempts to identify whether certain features of NF-1, or levels of severity of selected features, are more likely to occur as a function of genetic relatedness within families. They conclude that additional genetic influences ("modifying genes") are necessary to explain the clustering that appears to be present.

The purpose of this editorial is to draw attention to this important work and, as well, to consider other genetic and nongenetic explanations of the data. There are two main questions that one has to consider. First, did the study design influence the outcome? Second, given the legitimacy of the outcome, is there an alternative explanation to the "modifying genes" posited in the article's title? The study puts a great deal of reliance on the concordance between members of MZ twin pairs. However, since these two individuals are matched *exactly* for age, one is left with the question of whether it is the precise age matching or the genetic identity that accounts for the concordance. And, even if one presumes a greater similarity of expression of NF-1 mutations among close relatives than among distant relatives, the question remains whether this requires epigenetic influences, i.e., modifier genes. My own prejudice, based on an experience with just over 1,100 patients with NF-1 (Riccardi 1992), is that, in general, there is as much variation observed within fami-

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lies as there is when one family is compared with another.

Genotype and Phenotype

In February 1972, as a genetics fellow in Boston, I was asked to give a pediatric grand-rounds presentation on the topic of my choice. A sage adviser suggested that I select a topic that might also reflect a career focus. In this context, I considered neurofibromatosis (NF), for many reasons, not the least of which was the tremendous variation that I had already appreciated in the disorder that was to be designated as "NF-1" 10 years later (Riccardi 1982).

That variation—from family to family, from person to person, and from one body part to another for a particular patient—was the key intellectual challenge, the basis for a lifelong commitment to the neurofibromatoses. That is, how does one account for multiple levels of variation for a singular clinical disorder? Among the possible explanations there are seven major categories that can be resolved into two groups.

The first group essentially entails genotype-phenotype correlations, including two with stable genotypes, a third reflecting a malleable (i.e., potentially changing) genotype, and a fourth reflecting mosaicism: (1) multiple loci, (2) multiple alleles for a single locus, (3) malleable loci, and (4) postzygotic mutations. The second group of explanations to account for the variable expression of NF-1 entails emphasis on factors extrinsic to the mutant gene: (5) epigenetic factors, (6) environmental factors, and (7) random—i.e., stochastic—factors.

Multiple Loci

Among the neurofibromatoses there are at least two clinically distinct forms, NF-1 and NF-2, each with its own gene locus. Other loci for NF as such have not been identified, although a distinct locus for familial meningioma is likely to exist near the NF-2 locus on the long arm of chromosome 22 (Rey et al. 1993).

To date, if a clinician versed in NF diagnoses a patient's condition to be NF-1, genetic linkage studies or direct mutation analyses, when informative, have, with no exceptions, confirmed an NF-1 locus mutation. This is true even if there are features above and beyond the accepted *inclusive* criteria for NF-1 (National Institutes of Health 1988) to suggest an additional or alternative diagnosis, such as the Noonan syndrome or the Watson syndrome, respectively. Rarely, however, there have been cases with atypical NF (i.e., not clearly NF-1 or NF-2) with germinal mutations at the NF-1 17q11.2 locus (Wallace et al. 1990).

The other universally acknowledged form of NF, specified as NF-2 in 1982 (Riccardi 1982) and previously known as "bilateral acoustic NF" is known to have a locus on chromosome 22 (Trofatter et al. 1993). Thus, the two clinically distinct types of NF also reflect distinct loci.

A somewhat different issue is whether mutations in pseudogenes for NF-1 might result in disorders that are the same as or resemble NF-1. Such pseudogenes are apparently present on chromosomes 2, 14, 15, and 20–22 and perhaps on chromosome 12 (D. Marchuk, personal communication 1993). Through May 1993, no data have emerged to purport or confirm that NF-1 pseudogene mutations lead to any disorder, let alone resemble one of the neurofibromatoses. Ultimately, neither clinical nor genetic heterogeneity in NF-1 is explained by multiple loci or pseudogenes.

Multiple Alleles for a Single Locus

On the one hand, many types of mutations at the NF-1 locus have been described. These include exon point mutations, insertions, deletions, and cytogenetically detectable translocations. However, a simple, straightforward genotype-phenotype correlation has not been possible with the limited data available. On the other hand, the existence of the Watson syndrome, an apparently allelic form of NF-1 (Allanson et al. 1991), suggests that certain NF-1 alleles may be distinctive in their clinical expression, and Stephens and her colleagues (Kayes et al. 1992) have shown that a large deletion, entirely removing one allele of the NF-1 gene, may lead to extraordinary clinical problems, including mental retardation and perhaps a distinctive facies. Moreover, the full expression of NF-1 caused by such a deletion argues against a significant modifying influence of the intact normal allele. In any event, allelism is not a frequent explanation for NF-1 variability from one family to another, and it is not relevant to variability within a family.

Malleable Locus

An allele may represent a normal variant (a polymorphism) or be considered an aberration (a mutation), the basis for disease. Among the recent lessons that geneticists have learned, however, is that a mutation—a change in the sequence of DNA base pairs—may not stay the same from one person to another, as has been shown, for example, for the fragile-X syndrome and for Steinert myotonic dystrophy (Caskey et al. 1992). More-

over, a mutation may even differ between one tissue or organ site and another within the same patient (Chao et al. 1993). It is as though a certain type of locus may encode a gene that is very plastic or malleable: it can be shaped and reshaped by adding or removing elements.

The malleable gene is shaped one way or another as a function of the individual from whom it was transmitted. Thus, for example, the molecular details of the fra(X) gene—and, in turn, the gene's expression—depend on whether it was received from a mother or a father (Smits et al. 1992) and, further, on the sex of the transmitting grandparent, and so on. The key is the predictable and high frequency of *both additions and losses* of the modifying elements of the fra(X) gene. It is easy to envision the process as something akin to molding clay or putty. The point is that, from the molecular standpoint, we now have a way potentially to account for variable gene expression (i.e., clinical phenotype), on the basis of individual-specific but alterable changes in the nature of the gene itself. Certain forms of such a malleable gene would define specific malleotypes, such that each malleotype would be associated with a particular phenotype. Genomic imprinting is another way to approach this same notion of gene plasticity (Barlow 1993).

The point here is not to pursue the notion of malleable genes and the malleotype concept to its logical extreme; rather, it is to highlight that variation in a mutant gene's expression within a family, as has been described by Easton et al. (1993), does not require the influence of *other* genes to explain an apparent genetic contribution to the variation.

Postzygotic Mutations

Postzygotic mutations leading to germinal mosaicism, somatic mosaicism, or both (Edwards 1989) might explain some instances of variation from one generation to the next, but not variation within generations. Specifically for NF-1, while predecessors of unequivocal cases may have atypical or incomplete forms of NF, once the NF-1 is clinically full-blown, it is consistently so among *all* affected descendants (Riccardi and Lewis 1988). Somatic mosaicism for a postzygotic mutation is thus an unlikely explanation of NF-1 variable expressivity.

Epigenetic Factors

If genes other than the allele of the mutant gene appear to influence the expression of the mutant allele, then we are actually considering epigenetic factors. The apparent clustering of some features of NF-1, as a function of genetic relatedness of the subjects—especially, MZ twins—has been interpreted by Easton et al. (1993) to demonstrate an epigenetic basis for at least selected aspects of NF-1 variability.

Environmental Factors

To date, no environmental factors have convincingly been shown to influence the presence or severity of any feature of NF-1, with the possible exception of mechanical trauma as it relates to the presence of neurofibromas (Riccardi 1990). Thus, it is unlikely that a shared common environment would explain any apparent clustering of NF-1 features among close relatives.

Stochastic Factors

For a disease as multifaceted and age dependent as is NF-1, the significance of randomness and chance cannot be overstated. On the basis of the presence of one or two features of the disorder, two patients may appear to be more similar to each other than to other patients with NF-1, but that similarity may merely be random, a matter of chance. Because the similarities among the sporadic (simplex) cases of NF-1 have been as consistent as the similarities among patients belonging to the same family (Riccardi 1992), I have been strongly influenced to believe that these similarities represent chance more than they reflect something about the nature of the NF-1 gene or about the relatedness of the similarly affected family members. Consider, for example, that, if any person with NF-1 has a 1-in-20 chance of manifesting a trait, the likelihood of any two people manifesting the same trait is 1 in 400. For 80,000 people with the disorder, we would expect 200 pairs to show concordance for a given trait. If we now consider 10 different traits, each with a 5% chance of being present, then we expect 2,000 pairs to show some concordance. That is, there is a baseline 2.5% chance that any pair of patients with NF-1 will be concordant for a feature that occurs with a frequency of 5%. In this context, I would consider that, within a given family with NF-1 (i.e., for which we are dealing with a single allele at the 17q11.2 locus), the key modifying factor is stochastic, with a secondary importance for environmental factors, especially trauma, and a third, least critical, epigenetic factor.

Conclusion

The study by Easton et al. (1993) is an important one. It documents elements of the care and thoughtfulness that are required to investigate a disorder as complex as NF-1. Studies such as this simply must be done as we

seek to link what we know about NF-1 mutations at the molecular level with what we know about their consequences for individual persons at the clinical level.

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