The genetic kaleidoscope of vision

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Abstract:
Site-specific phenotypic effects of the 73 known alleles in the rhodopsin gene that cause retinal degeneration are difficult to interpret because most alleles are documented in only one case or one family, which means variation in effects could actually arise from interactions with other loci. However, sample sizes necessary to detect epistatk. interaction may place an answer to this question beyond our grasp.

Article:
[DAIGER ET AL.] The comprehensive review of **DAIGER ET AL.** documents genetic heterogeneity, allelic heterogeneity, and clinical heterogeneity in a diverse collection of mutations causing degeneration of the human retina. Listed here are 42 mapped loci, one of which (rhodopsin) has at least 73 alleles causing a wide array of phenotypes in terms of the quality, severity, and consistency of effects. While proposing a classification scheme to bring some order to a bewildering complexity in a single gene, the authors point out that many vital aspects of the gene's role in development remain unexplored. A great deal has been accomplished in the biochemical genetics of retinal degeneration, but continuing study suggests a limitless diversity and provokes questions about the goals of this research and the likelihood of achieving them.

1. The chemically complete retina. Understanding a system of 42 genes is challenging enough, yet there must be thousands of genes expressed in the retina, many of which are fixed in the population and do not produce disease. It makes good sense to concentrate on genes associated with disorders of vision if one hopes to someday prevent blindness. At the same time, the connectedness of the metabolic system in the retina dictates that the effects of a mutation at one locus will not be well understood until its associated protein can be placed in a biochemical context that includes the protein products of fixed loci. Thus, it would be helpful to know how many genes are expressed in the retina and how many are unique to the retina. Is it important for science to identify and characterize all of these, and will this knowledge benefit those who suffer from retinal dystrophy? The target article has set forth much that is already known but, has given little more than a hint at the goals of this enterprise. In particular, will the knowledge of the DNA sequence of various alleles causing some form of retinal degeneration be used primarily to screen for genetic defects and possibly abort embryos destined to suffer reduced vision in later life, or is there hope for ameliorating these hereditary conditions when the specific allele is known? A simple catalog of alleles and phenotypes is sufficient for eugenic purposes, whereas practical intervention to prevent retinal degeneration will necessitate a much more comprehensive understanding of the relations between rhodopsin and its environment.

2. Site-specific phenotypic effects? The answers to these questions will depend critically on the general properties of biochemical gene action. In particular, should we expect that changing a specific amino acid in a protein should result in a specific array of phenotypic effects at the level of retinal function in anyone possessing that allele? If yes, then there is no need to understand much about associated proteins derived from fixed loci or polymorphic loci not strongly tied to disease. The familiar catalog of loci related to specific disease syndromes can be expanded into a *catalog of alleles* or groupings of alleles, each with a characteristic syndrome. This seems to be the answer provided by the authors when they state "the clinical phenotype is a consequence of where and when the mutation affects the function of rhodopsin" (sect. 4.2, para. 1).

There is no doubt that an amino acid substitution in a critical protein *can* alter neural function. Rigorously controlled studies of oisogenic mice differing at a single amino acid in only one protein find that the mutants deviate phenotypically from their normal siblings, at least when group averages are compared. However, it does not follow that the average phenotypic difference is attributable solely to the amino acid difference. Coisogenic littermates share thousands of other genes and a multifaceted laboratory environment in common, and the genetic difference occurs in a context that may be critically important for the phenotypic outcome. For example, epistatic interaction with the genetic background can markedly alter the consequences of mutations such as *diabetes* and *reeler* in mice (Cavincss et al. 1972; Coleman 1981) and interaction with the rearing environment can sometimes be quite dramatic (e.g., Lee & Bressler 1981).

The appropriate research designs for assessing epistasis and gene-environment interaction always require individuals with a specific genotype at a designated locus to be combined with different genetic backgrounds or reared in different circumstances. To achieve this, the investigator must have access to a fairly large number of individuals carrying the identical mutation. In this regard, the yellow flag for caution begins to wave when the authors inform us in section 4.1.1 that "The majority of mutations in Table 2 are unique, occurring in one patient or one family only." Is it not possible or even likely that certain mutations with apparently variable effects are working in concert with other retinal genes that, while not causing disease on their own account, are interactants that strongly influence the consequences of a rare rhodopsin allele? And might not some of the apparently consistent effects of other mutations simply reflect the lack of genetic variation in interacting loci within certain families? The great phenotypic diversity attributed in this article to allelic heterogeneity may perhaps result from epistatic interaction or even gene-environment interaction, as **DAIGER ET AL.** acknowledge briefly in section 3.2.2.

Of course, the argument can work both ways. When a mutation in a gene results in substantially different disease in different people, epistasis might be invoked to explain this, but the effect could just as well arise from allelic diversity. In this respect, reliable knowledge about the DNA sequence in the rhodopsin gene should help to discriminate between these two kinds of causes. We begin with the observations that there are in fact many alleles of the rhodopsin gene and there are also many phenotypes. But are the two variations closely associated and, if so, why?

3. *interaction, the night blindness of statistical analysis.* It is instructive to explore the kinds of data that will be needed to address two important questions about allelic heterogeneity. Both of these involve the question of sample size required to render a statistical procedure sufficiently sensitive to more subtle genetic effects.

One question is simply whether two alleles do indeed differ significantly in the frequency of associated phenotypes. Suppose most cases can be dichotomized into early and later onset of retinal degeneration, and let the probability of the early form be p_i and p, for two alleles. The null hypothesis is that $p_1 = p_2 = p$, whereas the alternative hypothesis is that $p_1 = p + c$ and $p_2 = p - c$, where c is the deviation from an average of the two alleles. If one plans to test the significance of a difference in sample proportions of the early onset form using a Z test with a Type I error probability of α, two-tailed, and wants the test to have Type II error probability β, equivalent to statistical power of $1 - \beta$, then the appropriate sample size is given by a formula based on a normal approximation to the binomial distribution.

$$N = \frac{\left(Z_{\alpha/2} \sqrt{\frac{pq}{8}} + Z_{\beta} \sqrt{\frac{pq - c^2}{8}} \right)^2}{c^2}$$

Suppose *p* = .5. To achieve power of 90% when α = .05, the necessary sample sizes per group are indicated in Table 1. It could be quite difficult to find enough humans with each of the rare alleles. In this regard, it appears the authors are on the right track by seeking commonalities in terms of domain of action of various alleles, where samples could reasonably be pooled across similar alleles.

In order to test the classification scheme more rigorously, it would help not only to work with adequate samples but also to obtain continuous measures of the phenotypes associated with retinal degeneration, such as age at

onset of symptoms, rate of degeneration, and percent of the retina impaired. These dependent variables could then be assessed with multivariate statistics to determine the significance and strength of relationships with region occupied by a mutation (as in sects. 4.2.2 to 4.2.5). This would allow for exceptions, such as the 1289de117 and 1312de124 mutations, to weaken an interesting relationship without negating it altogether. The other important question about phenotypic consequences of allelic heterogeneity concerns the possible role of epistasis. Two alleles might have quite different effects on one genetic background but appear to differ less radically on another background. Consider the example in Table 2 where hypothetical group means are given in arbitrary units. The allelic difference is twice as large on background B compared to A. A simple formula that provides a normal approximation to the noncentral t distribution is convenient for estimating the necessary sample sizes (Wahlsten 1991). Suppose the standard deviation within a group is $\sigma = 7.5$ units and a planned contrast is used to test the difference between the effects of the two alleles without regard to genetic background. To achieve power of 80% when a two-tailed test with $\alpha = .05$ is used, the investigators must study about 10 patients in each of the four groups. However, to test the hypothesis of interaction between allele and genetic background with the same level of power, the sample size must be 73 *patients* per group! As a general rule, the necessary sample size is inversely proportional to the square of the size of the effect, Thus, the sample sizes appropriate for studying many kinds of interactions in a serious way are substantially larger than those that investigators commonly employ when looking at the average effects of an allelic difference [see Wahlsten "Insensitivity of the Analysis of Variance to Heredity-Environment Interaction" *BBS* 13(1) 1990].

Table 1 (Wahlsten). *Sample size to yield power of 90%*

c	p_1	p_2	N
.05	.55	.45	128
.10	.6	.4	32
.15	.65	.35	14
.2	.7	.3	8

Table 2 (Wahlsten). *Model of epistatic interaction*

	Allele 1	Allele 2
Background A	10	15
Background B	11	21

The tyranny of numbers appears to render unanswerable the question of epistatic interaction when there are so few families with rare alleles. If we cannot address this question in a serious way, I believe investigators should inform readers that they cannot argue strongly for the reductionistic thesis that each allele or molecular domain codes for a specific clinical syndrome.

Given the statistical difficulties of human genetic research, perhaps it might be helpful to *assess* allelic interactions in animal models of retinal degeneration using transgenic methods to create animals with different rare alleles. Once accomplished, large numbers of retinal degenerate mice could be produced.