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Gene-based approach to human gene-phenotype correlations

THADDEUS P. DRYJA*

Ocular Molecular Genetics Institute, Harvard Medical School and Massachusetts Eye and Ear Infirmary, 243 Charles Street, Boston, MA 02114

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ABSTRACT Elucidating the genetic basis of human phenotypes is a major goal of contemporary geneticists. Logically, two fundamental and contrasting approaches are available, one that begins with a phenotype and concludes with the identification of a responsible gene or genes; the other that begins with a gene and works toward identifying one or more phenotypes resulting from allelic variation of it. This paper provides a conceptual overview of phenotype-based vs. gene-based procedures with emphasis on gene-based methods. A key feature of a gene-based approach is that laboratory effort first is devoted to developing an assay for mutations in the gene under regard; the assay then is applied to the evaluation of large numbers of unrelated individuals with a variety of phenotypes that are deemed potentially resulting from alleles at the gene. No effort is directed toward chromosomally mapping the loci responsible for the phenotypes scanned. Example is made of my laboratory's successful use of a gene-based approach to identify genes causing hereditary diseases of the retina such as retinitis pigmentosa. Reductions in the cost and improvements in the speed of scanning individuals for DNA sequence anomalies may make a gene-based approach an efficient alternative to phenotype-based approaches to correlating genes with phenotypes.

A major goal of the current generation of geneticists is to construct a table correlating genes with human phenotypes. The table will be large. One column will be composed of the approximately 5,000 phenotypes that are currently known (1) (perhaps many more), and the second column will list approximately 60,000 to 70,000 transcriptional units now estimated to be in the human genome (2). Gene-phenotype associations may be indicated by lines connecting entries in the "phenotype" column with loci in the "gene" column. At the moment, the table is only partially completed; a few hundred genes have been identified as causes for specific diseases or other phenotypes. Encyclopedic versions of the current state of this table exist, such as McKusick's *Mendelian Inheritance in Man* (1).

Contemplation of this table suggests two starting points for research aimed at identifying the connections between specific genes and phenotypes. One method begins with the phenotype column. Patients having a particular phenotype are gathered and analyzed. Clues pointing to the responsible genes are obtained from clinical findings and from biochemical and genetic analyses. Genes are evaluated until one is found with sequence abnormalities specific for the phenotype. Depending on the overall method, this approach may be referred to as "reverse genetics;" in this article it is referred to as the "phenotype-oriented approach." It is the method responsible for most of the human gene-disease correlations discovered thus far.

The second approach, less commonly used, is to start from the gene column. A gene is isolated and its protein product is partially characterized. Work then is directed toward finding what human phenotype would result from mutations in it. This technique will be referred to as the "gene-oriented approach." It may remind one of what sometimes is referred to as the "candidate gene" approach, but it is conceptually distinct. When a molecular geneticist has a phenotype-oriented mindset, the candidate genes are those genes deemed to have a high likelihood of being the cause for a particular phenotype in question. With a gene-oriented mindset, in contrast, the goal is to investigate the role of a particular gene in human disease. With that locus as a reference, one actually searches for "candidate phenotypes" that might be caused by alleles of it.

Note that with either the phenotype- or gene-oriented approach, one does not actually scan the entire opposing column to discover a gene-phenotype association. This has been too time consuming and therefore impractical to do. Rather, one narrows the search using various methods. For the phenotype-oriented approach, the currently customary intermediate step is to determine the approximate chromosomal location for the gene in question. This usually requires large families with many living affected individuals, or, alternatively, with a few, but distantly related, affected individuals. With such families, linkage analyses permit the mapping of a gene to an approximate location in the human genome. The chromosome location also can be presumptively mapped if some affected individuals have deletions or translocations detectable cytogenetically: If a particular chromosomal region is commonly affected, a responsible gene is often in the same area. Once the approximate chromosomal location is known, one can confine the evaluation of the "gene column" to only those genes in that region, which at this point are called "candidate genes." Because the success of this method depends on figuring out the chromosomal position of the locus, the method is referred to as "positional cloning."

Other methods besides positional cloning are used in the phenotype-oriented approach. If individuals with a particular phenotype first are discovered to have a specific biochemical abnormality, then that information can be used to narrow the scope of the gene scan to those genes expressing proteins in the defective biochemical pathway. Sometimes the biochemical abnormality pinpoints the responsible gene. For example, biochemical analyses showed that many albinos lacked tyrosinase activity, a key enzyme in the synthetic pathway for melanin. This knowledge made it logical, in the context of a phenotype-based approach, to begin the search for genes causing albinism with the tyrosinase gene. Similarly, genes causing sickle cell anemia, gyrate atrophy, and phenylketonuria were found to be due to β -globin, ornithine aminotransferase, and phenylalanine hydroxylase, respectively, in each

case through the analysis of only one member of the gene column.

The contrasting gene-oriented approach has not been widely used probably because it was not deemed attractive for a number of reasons. One reason is that the approach seemed unwieldy because of the apparently large number of phenotypes that must be scanned before having a reasonable chance of finding a gene-phenotype correlation. However, just as for phenotype-based approaches, shortcuts exist that considerably reduce the amount of work involved. The pattern of expression of a gene can provide an important insight: Genes expressed only in the inner ear, for example, are most likely to be associated with hereditary forms of deafness or imbalance, a set of entities that comprise a very small subset of the phenotype column. Although the determination of chromosomal map positions is not an essential part of this approach, if a chosen gene happens to be from a region known to be implicated in a plausibly corresponding phenotype, it is wise to screen individuals with that phenotype early on. A more direct gene-oriented method is to study the phenotype of animals with transgenic or naturally arising mutations in the gene of interest; one then focuses the evaluation of humans on those with phenotypes similar to those seen in the homologously mutant animals.

Another argument against a gene-based approach to gene-phenotype correlations is based on the smaller size of the phenotype column compared with the genotype column. Assuming a one-to-one mapping, this discrepancy in size indicates that most genes would have no companion in the phenotype column. It could be that the relatively low number of known human phenotypes (about one-tenth the number of genes) is because many genes are essential for life, so that mutations in them are embryonic lethals. Or, some sets of genes might have overlapping or redundant function, so that defects in any one member of a set produce no phenotype or one that is too subtle to be recognized. If most human genes are essential to life or are redundant, studies of genes selected at random for evaluation in large sets of humans frequently would turn up nothing of value. However, the small size of the phenotype column could be primarily the result of our inability or failure to recognize many human phenotypes. A multitude of human personality traits that are genetic probably exist but are not yet individually listed in the phenotype column. Another reason for the small size of the phenotype column is our inability to distinguish similar phenotypes that are caused by different genes. Numerous examples exist where a clinically defined disease is found to have underlying nonallelic heterogeneity in which defects in any of a number of genes can be the cause. As extreme examples, at least 30 genes now are known to be responsible for congenital deafness (3, 4), and, as will be discussed below, perhaps 50 or more genes can cause hereditary forms of blindness such as congenital amaurosis and retinitis pigmentosa (5–7). The actual number of genes responsible for just one of these phenotypes could be much higher, perhaps well over 100. So, the assumption of one-to-one genotype-phenotype mapping is false. Even for genes where mutations are embryonic lethals, precedent exists for viable individuals with corresponding phenotypes. For example, patients with McCune–Albright syndrome or paroxysmal nocturnal hemoglobinuria are mosaic for defects in the essential proteins $Gs\alpha$ or phosphatidylinositol glycan class A, respectively (8, 9). Some oncogenes provide special examples of phenotypes revealed only in mosaic individuals. In summary, it may be that a sizable proportion and perhaps most human genes correspond to clinically evident phenotypes. There is a reasonable likelihood that, for any individual gene of interest, one will find some phenotype associated with alleles of it if one can evaluate a sufficiently large number of individuals.

For either a gene-based or phenotype-based approach, the allele-phenotype associations that are encountered initially

will provide only tentative evidence of an etiological relationship. Additional evidence supporting a causal relationship could come from the documentation of a statistically significant association of alleles with a phenotype, the detection of new germ-line or somatic mutations appearing together with the phenotype, the observation of a corresponding phenotype in lower animals with homologous alleles, or the demonstration of biochemical properties of the allelic variants of the protein product that reasonably explain the phenotype.

Identifying Phenotypes Due to Genes Expressed in the Retina

In the last decade my laboratory's research has been organized according to a gene-based approach to identifying gene-phenotype correlations. The genes selected for study are expressed by specialized cells of the retina, such as the photoreceptor cells, and most of the genes code for proteins in the phototransduction cascade. Many of these human genes were made available by other investigators or were directly isolated using already cloned homologues from other species. Because most of the selected genes are expressed almost exclusively by photoreceptors (the pineal gland is the only extra-retinal site that also expresses many members of this pathway), it was reasonable to predict that individuals with defects in these genes would exist (i.e., the mutants would not be lethal). Because the protein products of these loci were known to play key roles in the physiology of rod or cone photoreceptors (10–13), it was expected that alleles would produce phenotypes affecting the retina, so these sorts of phenotypes were targeted for evaluation. Effort was devoted to collecting a large number of individuals with a variety of hereditary and sporadic diseases of the retina such as retinitis pigmentosa (progressive degeneration of rod and cone photoreceptors with symptomatic visual loss usually in early adulthood), congenital amaurosis (blindness before 1 year of age due to retinal degeneration or dysfunction), cone-photoreceptor degeneration (degeneration of cone photoreceptors with relative sparing of rods), stationary night blindness (defective function of the rod photoreceptor mechanism with normal function and no degeneration of cones), and macular degeneration (degeneration of the posterior region of the retina leading to loss of the central visual field). Many of these diseases were known to be genetically heterogeneous because affected families could exhibit dominant, recessive, X-linked, or maternal inheritance patterns. And some, such as sector retinitis pigmentosa or birdshot chorioretinopathy, always are found as simplex cases (i.e., they are sporadic). Most of the patients were ascertained through a close collaborator, Eliot Berson, whose clinic specializes in the diagnosis, care, and study of patients with retinitis pigmentosa and allied diseases. Currently the laboratory has leukocyte samples from more than 6,000 individuals.

Initial experiments, mostly done before 1990, involved scanning sets of a few hundred individuals for gene deletions or rearrangements using Southern blot methods. The low sensitivity of the Southern blot technique in detecting point mutations explained the early failure to make any gene-phenotype correlations (14–16). With the advent of PCR-mediated direct DNA sequencing and mutation scanning methods such as single-strand conformation analysis (17), it became feasible to evaluate in a reasonable amount of time hundreds of individuals with a technique that detects about 90% of all DNA sequence anomalies (18–21). The merit of the approach then became clear. Between 1990 and 1997, 14 genes were analyzed in cohorts of about 100 to 1,000 unrelated individuals. Of these, defects in seven so far have been found to be the cause of photoreceptor phenotypes (Table 1).

A few of these gene-phenotype correlations were made contemporaneously with other groups using the customary

positional cloning approach (e.g., identification of the *RDS* gene as a cause of dominant retinitis pigmentosa; refs. 22 and 23). Others ultimately may have been made that way. For some of the correlations, however, it is very difficult to envision how positional cloning could have ever been successful. Not only was previous linkage data not available, but it is unlikely that linkage to some of the responsible regions could have ever been collected, due to the low number of families accounted for by some of them. For example, the genes encoding the α - and β - subunits of rod cGMP-phosphodiesterase and the α -subunit of the rod cGMP-gated cation channel each account for 4% or less of families with autosomal recessive retinitis pigmentosa (24–26). No previous linkage data indicated responsible genes in the chromosomal regions with these genes (5q31.2-q34, 4p16.3, and 4p14-q13, respectively; refs. 27–29). No families with retinitis pigmentosa due to these gene defects and suitably sized for linkage studies are known to exist even now. In fact, at least one of the gene-phenotype correlations was made in spite of previous mapping information: the *ROM1* gene had been mapped to chromosome 11q13 (30, 31), and a very specific phenotype of the retina (autosomal dominant vitelliform macular degeneration) had been mapped to the same chromosome interval (32), yet no mutations in *ROM1* have been found in patients with this disease (31). Our group found *ROM1* mutations instead to be a cause of digenic retinitis pigmentosa, a disease not previously linked to this region with an inheritance pattern previously unrecognized (33).

No phenotypes were found by our laboratory for seven of the genes. Three of these are still actively under investigation, and it is possible that corresponding phenotypes will be uncovered. Many conceivable explanations could account for the negative results for the remainder. The single-strand conformation analysis method used for detecting mutations misses about 10% of DNA sequence anomalies. In addition, the introns, the promoter region, and the 5' and 3' untranslated regions of most genes were not evaluated. Another possible explanation is that the retina-specific expression pattern may be incorrect for some of the genes. Those genes may have an essential role in some extraocular tissue, causing mutations in them to be embryonic lethals. The explanation intuitively most likely in my mind, however, is that an insufficient number and diversity

of individuals have been evaluated. Mutation rates and carrier frequencies for most genes are very low, and it may be that individuals with phenotypes corresponding to some of the genes were not included in the analysis. This is evidently the case for the arrestin gene. As the same time as our evaluation of this gene, another group found a mutation associated with Oguchi disease (34), a rare condition in which patients require 2–3 hr to adapt to the dark but otherwise have excellent vision. Only three unrelated individuals with Oguchi disease were available for evaluation in my laboratory; none had mutations in the arrestin gene. Instead, all had pathogenic mutations in the gene encoding rhodopsin kinase (35), an enzyme that prepares photoactivated rhodopsin for deactivation by arrestin.

Other groups appear to have used a gene-oriented approach to finding correlating phenotypes. A multiyear effort to understand the genes encoding the red, green, and blue opsins that enable color vision first identified the responsible genes and then scanned individuals with defective color vision to discover mutations (36, 37). A gene-based approach is embodied in the analysis of phenotypes manifest in transgenic mice with null mutations in genes expressed in neurons of the central nervous system (38, 39), although apparently little successful work has been devoted to identifying the corresponding human phenotypes.

One final observation from these findings is that in many instances different alleles in a single gene cause different phenotypes or different inheritance patterns. One example is provided by the genes encoding red and green opsin. Defects in these can cause protan and deutan color blindness (37), blue cone monochromacy (40), or a form of macular degeneration (41). Another example is the rhodopsin gene, where some alleles cause dominant retinitis pigmentosa, others recessive retinitis pigmentosa, and still others dominant stationary night blindness (see Table 1). This phenomenon of “gene sharing” by phenotypes has been noted for other phenotypes not specific to the retina (for example, the *RET* gene where different alleles can cause Hirschprung disease or multiple endocrine neoplasia type 2A or type 2B; ref. 42). The implication is that evaluation of a gene cannot be considered complete just because one correlating phenotype is found.

Table 1. Status of gene evaluations in the author’s laboratory

Evaluations successfully identifying a correlating phenotype		Evaluations not successful in identifying a correlating phenotype	
Gene	Correlating Phenotype(s)	Gene	Number of patients evaluated
Rhodopsin	Dominant RP (45), recessive RP (46), dominant stationary night blindness (47)	Arrestin	272 unrelated patients evaluated [Oguchi disease by another group (34)]
Rod transducin, α subunit	Dominant stationary night blindness (48)	Rod cGMP-phosphodiesterase, γ subunit	Over 1,000 unrelated patients evaluated, of whom 704 have been reported (52)
Rod cGMP-phosphodiesterase, α subunit	Recessive RP (24)	Recoverin	Evaluation of 596 unrelated patients (53)
Rod cGMP-phosphodiesterase, β subunit	Recessive RP (49)	11-cis-retinal dehydrogenase	Evaluation of 180 patients
Rhodopsin kinase	Oguchi disease (35)	Guanylate cyclase activating protein	Still actively being evaluated in the author’s laboratory
Peripherin/rds	Dominant RP (23), digenic RP (33), retinitis punctata albescens (50) [dominant retinitis pigmentosa and dominant macular dystrophies and cone/rod degenerations by other groups (22,51)]	<i>IRBP</i> (interphotoreceptor retinoid binding protein)	Still actively being evaluated in the author’s laboratory
<i>ROM1</i>	Digenic RP (33)	<i>TULP1</i> (tubby-like protein-1) (54)	Still actively being evaluated in the author’s laboratory

Future Role for a Gene-Based Approach to Identifying the Genetic Basis of Human Phenotypes

Despite the successes and clear logic of positional cloning, we are beginning to face the law of diminishing returns with it (43, 44). Many of the monogenic, clinically distinctive phenotypes that are amenable to gene identification through straightforward positional cloning already have been discovered or currently are being tackled by positional cloning efforts in progress. Most of the phenotypes that remain are found in relatively small pedigrees that do not permit chromosomal mapping with satisfactory precision for a linkage-based approach. A large number of human phenotypes are defined by simplex cases that do not permit clear categorization into a Mendelian inheritance pattern; many conceivably could represent phenotypes caused by mosaicism for gene defects that otherwise would be lethal, or they could be the result of combinations of redundant genes that, because they are unlinked, would only rarely be transmitted together to produce the same phenotype in a second generation. Also remaining are multifactorial phenotypes, caused by alleles at many genes together with environmental factors, and monogenic phenotypes with a great deal of underlying nonallelic heterogeneity. Phenotype-oriented approaches to deal with these sorts of phenotypes frequently require hundreds or thousands of participating individuals, numbers comparable to what is required for a fairly high success rate with a pure gene-based approach.

The arguments for a gene-based approach to identifying gene-phenotype correlations are not meant to discredit or devalue positional cloning or other phenotype-based approaches. The point is rather that a gene-based mind-set cultivates alternative, and in some ways complementary, approaches that can have a superb track record in some situations. It particularly is amenable for the analysis of large collections of individuals with related phenotypes who are well categorized phenotypically. For example, large sets of patients with psychiatric diseases, autism, personality disorders, or other phenotypes specific to the central nervous system might be particularly valuable resources for the evaluation of the numerous genes that are expressed specifically in the brain. Another possible gene-based approach begins with the existing collection of reference pedigrees (such as the CEPH pedigrees) used to develop the existing human linkage maps. Now that the positions of thousands of genes on the map are known, it might be very rewarding to document interesting phenotypes present in the members of those reference families so that one might search for gene-phenotype correlations.

It may be feasible someday to determine the entire nucleotide sequence of both parental copies of the human genome in any patient under study. This would be the ultimate in phenotype-based technology: individuals with a phenotype in question would have their genomes sequenced and compared with an established reference sequence. Any genetic cause for the phenotype would be embodied in the set of peculiar sequences shared by those individuals. Before we arrive at that level of genomic sequencing technology, we will come to a stage, which is now just beginning, where individual genes or sets of genes can be evaluated for mutations quickly and at a low per-assay cost. Mutation-screening technologies are becoming increasingly streamlined and automated, and soon thousands of individuals will be evaluated efficiently in a modest-sized laboratory, or tens of thousands of individuals in a large laboratory. Much of the effort behind the analysis of individuals for DNA sequence anomalies in a specific gene is in the development of the assay. Once an assay is working, the additional effort required to analyze a few extra individuals is small. Microchips that can quickly provide the complete DNA sequence of chosen genes are an illustrative example of this. Once developed, a chip is cheap to reproduce. As inexpensive chips proliferate through human genetics research laborato-

ries, they will be used to evaluate large numbers of individuals for defects in the included genes, sometimes to test rationally based predictions of genotype-phenotype associations, sometimes for no good reason but simply because the incremental cost for each analysis, once a chip and the leukocyte DNA sample are on hand, will be so low. Occasionally a novel allele will be found to correlate with a phenotype, or particular variants will be found to modulate or modify phenotypes that are caused primarily by alleles at a different locus. In short, a global gene-based approach to identifying the genetic basis for human phenotypes might occur unintentionally as a byproduct of advances in mutation screening technology.

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