The data, devices, and molecular techniques that have become part of the practice of medicine as a result of the Human Genome Project are for most clinicians both inspiring and intimidating. While the potential for tests that can help make very accurate diagnoses of rare genetic diseases and that can identify genetic predispositions to more common ones is exciting, the translation of this concept to practice has been slower and more complicated than most people would have imagined.

The reasons for the slow translation of genomic discoveries from the laboratory to the clinic range from the vagaries of intellectual property law to the different strategies that various countries have chosen to pay for the medical care of their citizens. However, our understanding of the genes involved in inherited eye disease is now sufficiently advanced and molecular technology is sufficiently powerful that we can reasonably expect the deployment of useful tests for nearly all inherited eye diseases during the next 5 to 10 years (Table). The success of this ambitious undertaking will depend on (1) the active participation of experienced clinicians, (2) the development and use of mutation detection probability distributions (MDPDs),1 (3) the use of a standardized method for estimating the pathogenic probability (EPP)1 of individual sequence variations, (4) the thoughtful use of a variety of mutation detection strategies that are carefully matched to specific diagnostic situations, (5) the use of hypothesis-driven sequential testing instead of “shotgun testing,” and (6) the use of up-to-date Internet-accessible databases as repositories for evolving knowledge about disease-causing mutations, nondisease-causing polymorphisms, and genotype-phenotype correlations.

Genetic eye diseases range in prevalence from common disorders like macular degeneration, which affects as many as 1 in 3 people older than 75 years,2 to specific subtypes of rare conditions that each occur in only a few people in the United States per year. In the future, genetic testing will play an important role in the management of disorders at both ends of this spectrum. However, the strategies that will be employed for the development and deployment of genetic tests for very common disorders will be somewhat different than for the long list of mendelian eye diseases that are collectively common but individually fairly rare (affecting fewer than 1 in 1000 people). This article will focus on genetic testing strategies for the latter conditions.

INDICATIONS FOR GENETIC TESTING

For discussion purposes, the indications for genetic testing can be divided into 5 broad categories: treatment, diagnosis, prognosis, counseling, and research. However, even the most superficial consideration of these categories quickly reveals that there is significant overlap among them. For example, accurate genetic testing is an absolute requirement for any type of gene replacement therapy. Thus, a genetic test that detects 2 inactivating mutations in the RPE65 gene3 can simultaneously confirm the clinical diagnosis,
Moreover, the act of performing a genetic test is a tangible sign to patients at risk of losing their vision. However, after conveying this message, a physician imagines that in some way they may be part of a more optimistic future. If a test result is positive, that result also serves to connect the patient and their family to a very specific part of the research world and allows them to focus their questions and reading on the part of this world that is most relevant to them.

A physician's ability to give a patient an accurate prognosis is based on the accuracy and precision of the genetic test is a tangible sign to patients and their families that their physician imagines that in some way they may be part of a more optimistic future.
The ability to connect clinical findings to a specific address in the genomic domain has already been mentioned. Performing tests for variations in known genes can also dramatically speed the discovery of new disease genes. Many clinical entities (eg, LCA and Bardet-Biedl syndrome) are genetically heterogeneous (caused by mutations in different genes in different people). In both of these 2 diseases, the genes responsible for the majority of cases have been identified (Figure 1), but the genes responsible for a significant subset (20%-30%) remain to be discovered. As a result, any effort to discover the remaining genes will be 3 to 5 times more likely to succeed if laboratories have access to samples from patients with these diseases who have already been screened for mutations in known genes with negative results.

WHAT IS A GENETIC TEST?

In the broadest sense, a genetic test is any clinical or laboratory maneuver that has the potential to increase or decrease the likelihood that a patient has an inherited disease. Thus, an abnormal electoretinogram in an asymptomatic 10-year-old child of a parent with autosomal-dominant retinitis pigmentosa is as much a genetic test as a molecular investigation of the rhodopsin, RDS, and RPE genes of the same family. The concept illustrated in this example deserves great emphasis: a knowledgeable clinician is arguably the single most important component of the genetic testing process. Laypeople, regulatory agencies, and indeed many physicians often have a much narrower view of a genetic test. They tend to see it as the performance of 1 or more laboratory techniques that result in a black-and-white answer about the presence or absence of a disease-causing mutation. They tend to believe that as the technology gets better and better, the tests will get better and better. The implication of this view is that the physician’s role will eventually be reduced to little more than phlebotomist. In 1990, it may have been true that the rate-limiting steps of genetic testing for rare eye diseases were mostly in the laboratory. However, in 2006, the most rate-limiting steps in the translation of genomic information from the laboratory to the clinic are to inform clinicians about the availability of these tests (Table) and to educate them about their proper use and interpretation.
CLINICALLY USEFUL TESTS

There are at least 4 features of a genetic test that are of interest to a clinician and his or her patient: the cost, the turnaround time, the report, and the likelihood that the test will assist in the management of the patient. Some of these features are logistically in opposition to one another. That is, if a laboratory takes steps to make the turnaround time of a test very short or the report very detailed and customized, the cost of the test will go up. Similarly, if a laboratory tries to make a test very global so that it evaluates even very unlikely possibilities, the likelihood of a positive result will go up, but the turnaround time and cost of the test will also go up. If any of these parameters gets too out of balance, it will render the test sufficiently impractical that it will not be widely available, if at all.

Experience has shown that none of these 4 test parameters poses an absolute barrier to the use of a test. Many physicians have sent samples to research laboratories even when their turnaround times were measured in years and their written reports were nonexistent. Similarly, some highly motivated families are willing to pay thousands of dollars out of their own pockets for a test, even when the likelihood of a positive result is quite low. However, the demand for tests performed under these extreme circumstances is very low and will never be sufficient to drive genetic testing to a point where it will be considered standard of care. With this in mind, we sampled the opinions of many clinicians and patients across the country and used these opinions to empirically choose a blend of parameters that would make a genetic test maximally attractive. We refer to a test that falls within these empirically determined bounds as a clinically useful test.

In this context, we herein define a clinically useful test as one that has an average cost of less than $500, a turnaround time of 8 weeks or less, an easily understood report written in a standard format with a defined nomenclature, and a greater than 50% chance of a clinically meaningful result (when properly ordered by a knowledgeable clinician). These threshold values of the 4 parameters are debatable and will undoubtedly change as technology improves, as more genes are discovered, and as clinicians become more experienced in using these tests to their best advantage. Here, it is sufficient to make the point that clinical skill plays a critical role in the utility of any genetic test and that the favorable balancing of these 4 parameters will be necessary to elevate genetic testing from a futuristic curiosity to the standard of care.

NORMAL GENETIC VARIATION

The human genome contains a lot of information. Each person inherits its 3 billion nucleotides from each parent, many of which could, if altered, give rise to disease. If one built a model of the human genome out of pennies, with each one representing a single nucleotide, this model would consist of 2 rows of pennies that would circle the globe 1.42 times at the equator. If all of these pennies had 1 of 4 dates (representing the 4 possible nucleotides in DNA), one’s task in finding the cause of an autosomal-dominant retinal disease like malattia leventinesi or rhodopsin-associated retinitis pigmentosa would be to circle the globe 1.42 times looking for 1 penny with an altered date. This task would be complicated by the fact that many variations in the genome do not cause disease and simply represent the normal genetic variation among individuals. In fact, any 2 unrelated human beings will vary from one another at about 1 nucleotide position out of every 1000. Thus there are millions of nondisease-causing polymorphisms, which would complicate the search for the true disease-causing mutation in this example.

THE MUTATION DETECTION PROBABILITY DISTRIBUTION

Fortunately, disease-causing sequence variations are not distributed evenly throughout this large genomic space. Most of them are found in or very near the coding sequences of a gene, thereby limiting the space in which one has to look by at least 10-fold. Of course, once a specific gene or series of genes has been associated with a given clinical entity, the coding sequences of these genes become much likelier locations for disease-causing sequence changes in people with that clinical disease than other places in the genome. Moreover, even within genes, disease-causing mutations are not evenly distributed because certain segments of genes encode protein domains that subserve specific functions. In many genes, new mutations are more likely to be found near the location of previously identified mutations than they are elsewhere in that gene.

Using one’s past experience with mutation discovery as a guide for future mutation discovery in the same population is the central idea behind the MDPD (Figure 2). With this method, one simply keeps track of all plausible disease-causing sequence variations that are associated with a given clinical entity in a given population and uses this ever-growing experience to fine tune the mutation-hunting strategy. For example, with a DNA sequence-based mutation detection strategy, one would divide the genes known to cause a specific disease into segments that could be assessed by individual DNA sequencing reactions and then sequence these segments in order of decreasing likelihood of detecting a mutation based on one’s past experience in that population. For genetically heterogeneous diseases (those caused by different genes in different patients), following the MDPD frequently results in switching back and forth between genes during the assay (Figure 2). For recessive diseases in which 2 different disease alleles are expected, the discovery of the first allele causes one to refocus the investigation on that one gene (whose segments are also screened in MDPD order), because the discovery of the first allele makes it much more likely that the second allele will lie in that gene than in any other gene in the genome. The MDPD method can dramatically reduce the cost of genetic testing because the most common alleles are detected early in the testing process. In addition, many segments of genes are ex-
tremely unlikely to cause disease and in most situations should not be screened at all. Sequencing the entire coding region of such a gene would increase the cost and the time required for a test with a near zero increase in yield.

THE VALUE OF CLINICAL HYPOTHESES

The primary assumption underlying all clinical genetic testing is that there is a predictable relationship between the presence or absence of certain sequence variations (referred to as a patient’s genotype) and a patient’s clinical appearance or disease outcome (referred to as the phenotype). A strong genotype-phenotype correlation has value in both directions. In the genotype-to-phenotype direction, a correlation might allow a clinician to predict the severity of an outcome or the response to a given intervention based on the presence of a specific mutation in a specific gene. However, in the phenotype-to-genotype direction, a correlation might allow a clinician to predict that a mutation would be found in a specific gene or group of genes based on a specific set of clinical observations. In fact, all MDPDs require the specification of a phenotype for them to have any meaning. That is, the MDPD simply shows the probability of finding variations that cause a specific phenotype in specific portions of specific genes.

Sometimes the association between the genotype and phenotype is so strong and so specific that the MDPD consists of a single point. For example, the rare mendelian maculopathy known as malattia leventinese, or Doyne honeycomb retinal dystrophy (Figure 3), is caused by a single variation (Arg345Trp) in the fibulin 3 gene. If one clinically suspects this disease, there is only 1 nucleotide in the entire human genome that needs to be evaluated and if that nucleotide is not abnormal, the diagnosis cannot be malattia leventinese. This extreme example shows the potential value of a very specific clinical hypothesis in narrowing the search for a disease-causing variation.

One might imagine that as one’s ability to molecularly interrogate the genome gets faster and less expensive that the value of specific pretest clinical hypotheses would diminish. In fact, there are certain allele-specific testing strategies that simultaneously evaluate hundreds or thousands of potential disease-causing variations for very little cost per allele. Large-scale parallel testing may be useful in certain parts of a testing algorithm, there are at least 2 serious weaknesses of such an approach that usually preclude its use as a laboratory’s sole means of genetic testing. First, although the cost per assessed allele is very small, if the clinical hypothesis is very strong, the vast
century, many clinical laboratories have performed large-scale parallel testing of disease alleles. A single laboratory can process more than 100 samples per day, whereas parallel testing of 400 samples would take at least 3 times longer. This advantage is significant:

- ...allel approach would as a result be quite a bit more expensive than a sequential approach. Second, and more importantly, every individual carries multiple disease-causing variations in the heterozygous state. For example, 1 in 25 Caucasian individuals is heterozygous for a cystic fibrosis allele that would cause the disease in an offspring if a spouse contributed a second similar allele. Similarly, there are so many different genes that cause autosomal-recessive retinitis pigmentosa that 1 in 5 people is heterozygous for a true retinitis pigmentosa–causing mutation, even though fewer than 1 in 5000 people inherit 2 such alleles of the same gene. Given these facts, parallel testing of thousands of disease alleles will frequently detect at least 1 true disease allele that has nothing to do with a patient’s disease, just as one would expect to have many false-positive tests if one ordered hundreds of conventional clinic laboratory tests on a single patient without regard to his or her clinical diagnosis. Thus, if one does perform large-scale parallel testing of any type as part of a genetic testing algorithm, one has to be significantly more skeptical of any positive result and apply very stringent criteria for accepting such findings as the true explanation for a patient’s disease.

### THE VALUE OF A MULTIPLATFORM APPROACH

There are a number of different methods one can use to evaluate the DNA of a patient in search of disease-causing variations. Most of the approaches in common use today begin by first amplifying 1 or more segments of genomic DNA using the polymerase chain reaction. After amplification, the polymerase chain reaction products are then evaluated by some combination of enzyme digestion, electrophoresis, hybridization, silver staining, fluorescent labeling, scanning, and/or liquid chromatography. As suggested previously, each method has certain strengths and weaknesses and no single method is optimal in all circumstances. Automated DNA sequencing consists of polymerase chain reaction amplification, sequence-specific fluorescent labeling, and liquid chromatography, and is perhaps the most robust single method for mutation detection today. It can evaluate more than 600 contiguous nucleotides in the genome in both directions for about $12. However, the following example illustrates how the thoughtful use of a much less sophisticated method (single-strand conformational polymorphism analysis [SSCP]) can be combined with automated DNA sequencing to dramatically decrease the cost and increase the speed of a genetic test in a specific clinical situation.

**Figure 4.** Mutation analysis of intron 26 of the CEP290 gene. A, Single-strand conformational polymorphism analysis of 10 individuals with Leber congenital amaurosis. Asterisk indicates 3 of these individuals who harbor an A to G missense variation at nucleotide 1655 of intron 26 that results in an aberrant migration pattern using this electrophoretic technique. Automated DNA sequencing of 1 of these individuals (B) reveals a homozygous G at position 1655. C, DNA sequence chromatogram of a healthy individual who is homozygous for the wild-type sequence.
When sequence variations are so rare that family information is absent or scant, one can also use predictions of the severity of a mutation's effect on protein structure.

Thus, if one wishes to estimate the disease-causing potential (pathogenicity) of a given mutation as accurately as possible, one needs to have access to mutation-screening data from hundreds of healthy and affected individuals as well as an up-to-date summary of the published literature about this gene. Armed with this information, one can make a reproducible estimate of the pathogenicity of a sequence variation using a defined set of rules, which vary depending on the inheritance pattern of the disease in question. A more detailed discussion of these rules has been published elsewhere, and in the present context it is sufficient to make the point that all sequence variations are not equally likely to cause disease and that methods exist for estimating this likelihood.

Although practicing clinicians may have a general knowledge of the principles behind the estimates of pathogenic probability, it is a wholly unreasonable expectation that they will have access to all the necessary information to perform such a calculation themselves each time they interpret a test for one of their patients. Instead, they need to be able to rely on the laboratory that performs the test to provide them with this estimate just as any other clinical laboratory would be expected to provide a range of normal results for each of the evaluations they perform.

The system that we advocate divides the pathogenic potential of all observed sequence variations into 4 categories ranging from 0 to 3. Zero denotes variations that are extremely unlikely to cause disease, while 3 is used to refer to variations whose pathogenicity is strongly supported by all available data. This simple system allows physicians to translate their clinical experience from one disease to another.

CONCLUSION

For many inherited eye diseases, there is no longer any need for a physician to say, “I’m sorry, there is nothing we can do.” Genetic tests are becoming faster, more affordable, more likely to yield a positive result, and more likely to be covered by insurance. In fact, it is now a practical possibility to offer testing to every person in the United States affected with some rare disorders like LCA and Bardet-Biedl syndrome. Although new gene discoveries and technical improvements in the laboratory will continue to be important, the greatest opportunities for moving this field forward lie in the engagement of practicing clinicians in every aspect of the genetic testing process.

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REFERENCES


21. Chiang AP, Nishimura D, Searby C, et al. Comparative genomic analysis identifies an ADP-ribosylation factor-like gene as the cause of Bardet-


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**Archives Web Quiz Winner**

Congratulations to the winner of our September quiz, Navneet Kumar, MBBS, Dr Rajendra Prasad Center for Ophthalmic Sciences, All India Institute of Medical Sciences, New Delhi, India. The correct answer to our September challenge was congenital iris ectropion. For a complete discussion of this case, see the Clinicopathologic Reports, Case Reports, and Small Case Series section in the October ARCHIVES (Grieshaber M, Orgul S, Bruder E, Hadziselimovic F, Flammer J. Congenital iris ectropion and glaucoma associated with intestinal neuronal dysplasia: a manifestation of a neural crest syndrome. *Arch Ophthalmol*. 2006;124:1495-1497).

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