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Ingenious way to find a needle in a haystack a genetic testing for rare eye diseases

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Finding disease-causing gene mutations is seen as the shortest cut to preventing or curing heritable diseases. However, each such procedure is resource-consuming and costly, and it becomes especially cost-ineffective for rare hereditary diseases. In this story, **Edwin M. Stone, M.D., Ph.D.**, director of the Carver Laboratory for Molecular Diagnosis, tells how his group came up with a cost-effective solution to genetic testing procedures for hereditary eye diseases.

History: Inherited diseases are caused by variations in DNA. Therefore, it is theoretically possible to diagnose any genetic disease by examining a patient's DNA for such variations. However, until very recently, a number of technical, financial, and ethical reasons have kept such testing out of the medical mainstream. Before the advent of DNA cloning in the 1970s, the only way to detect any sequence variation in the DNA of higher organisms was to observe variations in the proteins they encoded. This method allowed researchers to deduce the molecular nature of some genetic diseases, such as sickle-cell anemia. However, the great majority of disease-causing variations remained buried in the vast featureless and colorless polymer that is the human genome.

In the 1960s and 1970s, researchers discovered restriction enzymes and learned to use them to insert small bits of exogenous DNA into plasmids and viruses for replication. This process allowed individual genes to be isolated for the first time. It also made it possible to identify sequence variations within the human genome even when such variations did not cause a detectable change in an accessible protein. However, it was the development of the polymerase chain reaction (PCR) in the 1980s that made it possible to perform the types of genetic testing that are available today.

PCR has made it possible to perform tests on DNA samples consisting of only nanograms of DNA, while previous methods required more than a thousand times this amount. PCR also lends itself to automation and speed so that a single technician can perform an experiment to evaluate a single genetic locus in 400 or 500 people in a single day. In the past decade, the combination of cloning, PCR, automated DNA sequencing, and computer analysis of the resulting sequences has made it possible to embark upon

the sequence analysis of entire genomes (e.g., the Human Genome Project); the genomic sequences of several model organisms, including humans, have now been completed.

When specific human genes are screened for variations in a large number of different individuals, a large amount of variations is commonly found that does not appear to alter the structure and function of an individual. These silent variations are so common that any two human beings will differ from one another at least once every thousand nucleotides. This fact, coupled with the knowledge that the haploid human genome consists of more than three billion nucleotides, means that any two normal individuals will differ from one another at at least three million positions. Thus, finding a single nucleotide variation that is responsible for human disease is like trying to find a single steel needle in a haystack full of three million silver needles.

New facts: True disease-causing mutations can be distinguished from nondisease-causing polymorphisms in a number of ways, but the most common is to correlate the inheritance of a disease within a large family with the inheritance of a specific molecular variation. The second most common way is to use a similar correlative approach to evaluate a large number of unrelated individuals affected with a given disease and to demonstrate that a variation within a candidate gene is found more often among individuals with the disease than in ethnically similar unaffected individuals. Once such a correlation has been established in a statistically rigorous way, this information becomes the basis of a clinical molecular test. One might imagine that at this point, a for-profit testing firm could use such information to develop a commercially viable test and that in a fairly short period of time this test would be widely available to the medical community.

Although this has happened in some cases, many inherited disorders are so rare and the number of different disease-causing variations so numerous that there is simply insufficient demand to make such a test commercially viable. This is complicated by the fact that many diagnostically useful genomic sequences have been patented and that the legal costs involved in obtaining the rights to use the necessary intellectual property have made marginally viable genetic tests impossible to sustain.

For all of these reasons, most genetic testing for rare human diseases in the past decade has been performed on a research basis in the same laboratories that actually discovered the disease-causing genes. Although this was a reasonable approach for the first few years of human gene discovery, the past decade's explosive increase in the knowledge of disease-causing variations quickly exhausted the ability of research laboratories to keep pace.

In response to this challenge, University of Iowa Health Care researchers have explored a new strategy for providing practical genetic testing for rare eye diseases for the entire U.S. This strategy involves a partnership between the University of Iowa Roy J. and Lucille A. Carver College of Medicine, UI Hospitals and Clinics, the Department of Pathology, the research laboratories of the Center for Macular Degeneration, and the owners of the intellectual property of the relevant genes. In this approach, academic physicians use their expertise to design, perform, and interpret the tests and charge the patient only for the costs that are actually incurred in performing them (e.g., reagents and technician time).

In most cases to date, gene sequence patent holders have allowed their intellectual property to be used at no cost for this nonprofit endeavor, while in a few cases, they have asked for only a modest fee per test. The creation of the non-profit genetic testing laboratory at UI Hospitals and Clinics was greatly aided by a multi-million dollar endowment that was jointly provided by the Roy J. Carver Charitable Trust and the Department of Ophthalmology and Visual Sciences. For this reason, the laboratory is now known as the Carver Laboratory for Molecular Diagnosis.

Practice: The goal of the Carver Laboratory is to provide a wide variety of clinically useful tests, a rapid turnaround time, and an easily interpreted written report--all at a modest cost. Most tests offered by the laboratory cost well under $500. The most expensive test offered, involving the analysis of six different genes, costs a little over $1,000 (Table 1). In addition, the Center for Macular Degeneration offers expert genetic counseling regarding the tests' results for patients who do not have access to such counseling in their home communities.

The Carver Laboratory maintains a web page (http://www.carverlab.org/) with a wide array of information for physicians and patients about inherited eye diseases and eye disease genes. The web page can be used to learn more about the clinical features of these diseases, their mode of inheritance, and the ways in which patients can benefit from genetic testing. Visitors to the web page also have access to a variety of data about these genes, which have been collected by research laboratories at the UI Carver College of Medicine over the past 15 years.

The Molecular Ophthalmology Laboratory has screened thousands of patients with inherited eye diseases for variations in a large number of disease-associated genes. The knowledge gained from this experience is essential to the development of genetic tests that will benefit the patient. For example, some genes are very large while others are quite small. For large genes, it is not very efficient to screen the entire gene for variations, because most large genes have regions that harbor few if any disease-causing changes.

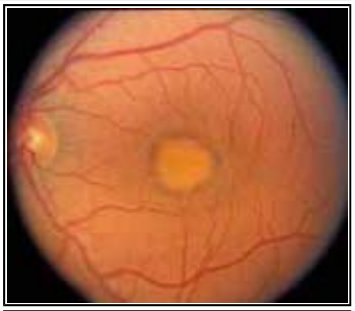
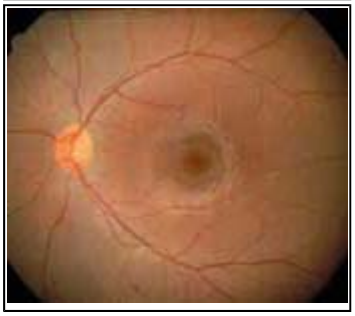
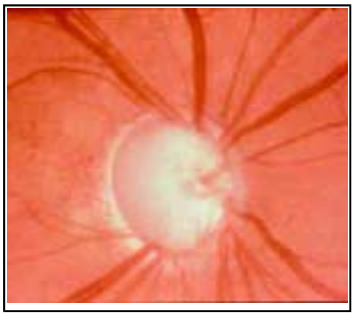
It is much more efficient to stratify the screening so that the regions of a gene that are known to harbor the most variations are screened first, with less promising regions screened later or not at all. For diseases that are caused by several different genes, such as Leber Congenital Amaurosis (LCA), the genes are not screened individually. Instead, the subparts of all six LCA genes are ranked according to the likelihood of harboring a disease-causing change, and these regions are screened in the order of decreasing likelihood of a significant finding.

LCA is a recessive disease, and affected patients are expected to carry two mutations--one inherited from each parent. Thus, once the first disease-causing variation is identified with the probability stratification approach described above, attention can then be shifted to finding the second mutation in the same gene. This sequential screening strategy, which takes full advantage of the large body of research data collected at the College in the past, can detect meaningful disease-causing mutations much faster and at a much lower cost than more conventional approaches.

The Carver Laboratory website features a table that summarizes the genomic structures of 34 genes that contribute to 19 different human eye diseases, as well as the laboratory's cumulative screening experience with these genes. As new variations are

detected by the nonprofit testing service, these changes will also be added to the web-based database.

Table 1: Tests Currently Offered

Disorder		Mode of Inheritance	Gene(s)
Best Disease		Autosomal Dominant	VMD2
Leber Congenital Amaurosis		Autosomal Recessive	AIPL1 CRB1 CRX GUCY2D RPE65 RPGRIP1
Leber Hereditary Optic Neuropathy		Mitochondrial	MTND1 MTND4 MTND6
Malattia Leventinese		Autosomal Dominant	EFEMP1
Pattern Dystrophy		Autosomal Dominant	RDS
Primary Open Angle Glaucoma / Juvenile Open Angle Glaucoma		Autosomal Dominant	GLC1A
Retinitis Pigmentosa		Autosomal Dominant	RHO RDS RP1
Dominant Stargardt Disease		Autosomal Dominant	ELOVL4

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