Genetic Ophthalmology and the Era of Clinical Care

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Genetics is rapidly entering the realm of clinical medicine. The January and February 2007 issues of the Archives of Ophthalmology, a member of the JAMA and Archives Journals family of publications, provide a view into the field of medical genetics through the remarkable recent advances in ophthalmic genetics that are beginning to drive medical vision care.1,2

Discovering Genetic Factors for Ocular Disease

Nearly 25 centuries ago Hippocrates recognized that the trait of blue eyes is inherited, and in 350 BC, Aristotle commented on the transmission of vision impairment across generations.1 Yet, as recently as 1980, only a few ocular disease genes had been identified.4 Now, little more than 2 decades later, and greatly facilitated by the databases and technologies produced by the Human Genome Project, more than 400 genes are cloned or mapped that cause or contribute to eye and vision diseases.3,5 This large number is nearly one fifth of the current set of human genes cloned for all mendelian disease traits.6

A majority of the known ocular disease genes, and most of those that manifest at an early age, are inherited as a mendelian autosomal-dominant, autosomal-recessive, or X-linked trait.1,6-10 The genetic elucidation of corneal and retinal dystrophies, hereditary congenital cataracts, and early onset open-angle and congenital forms of glaucoma provide insight into biological pathways and encoded protein function. Defects in developmental genes such as PITX2, FOXC1, PAX6, and VMD2 are responsible for multiple associated ocular anomalies such as microphthalmia, aniridia, or retinal degenerations, and some have associated systemic defects.10 In the January issue of the Archives of Ophthalmology, Ito et al11 showed that a novel missense mutation in FOXC1, which caused malformations in the anterior chamber and systemic defects, had significantly impaired protein function with decreased capacity to localize to the nucleus, bind DNA, and transactivate reporter genes. In another article in that issue, Beby et al12 describe the first case in which an Arg116Cys CRYAA gene mutation is associated with early onset autosomal-dominant nuclear cataract and iris coloboma. The mutant Arg116Cys protein exists as a highly oligomerized protein with conformational changes and reduced chaperone activity, a function that normally suppresses aggregation of other proteins.13

Finding the genetic factors responsible for non-mendelian diseases was predicted to be much more difficult because analysis of pedigrees had suggested that for most diseases of this type, many genes would be involved, with no single gene contributing a particularly strong effect.14 Until recently, the approach to investigate such conditions was limited to studies that examined variation in candidate genes, chosen on the basis of various hunches about their involvement in the particular condition. These hunches, however, were usually incorrect, and progress was slow.15 All that now is changing. The International HapMap Project16,17 produced a detailed map of human genetic variation across the genome and revealed that a smaller set of 300 000 to 500 000 variants can serve as a proxy for the roughly 10 million common genetic variants in the human genome. Coupled with a profound decrease in the cost of genotyping, this new information has ushered in the new era of genome-wide association studies, which has the power to uncover the basis of complex traits in any common disease, wherever they happen to be located in the genome.

Beyond the single-gene mendelian traits, genetics is beginning to uncover the basis of complex traits that cause more common diseases. Primary open-angle glaucoma is a common neurodegenerative eye disease that affects more than 2 million individuals living in the United States.18 Adult-onset primary open-angle glaucoma typically exhibits complex inheritance and is usually associated with elevated intraocular pressure.10 As reported in the January issue of the Archives of Ophthalmology, Duggal et al19 performed a genome-wide scan of intraocular pressure in a population-based cohort with a median age of 64 years to identify genes that control this quantitative trait. Seven loci of interest were

See also the January and February 2007 issues of the Archives of Ophthalmology.
identified, and 2 regions at chromosomes 19p and 2p colocalized with blood pressure loci. The results extend the scope of primary open-angle glaucoma susceptibility genes to quantitative trait loci that may control systemic pressure in the vascular system and the eye.

Seven million US adults have clinical signs of age-related macular degeneration (AMD), including the 1.8 million who are legally blind through progressive loss of central and reading vision. The genetic breakthrough for AMD occurred only 2 years ago when a common coding variant in the complement factor H (CFH) gene was identified and shown to account for nearly 40% of the genetic risk for AMD cases. HapMap resources had recently become available and were used for association studies that netted the CFH gene, illustrating the power of this new comprehensive genetic tool.

Studies by Schaumberg et al and by Schuler et al in the January issue of the Archives of Ophthalmology have again confirmed the role of CFH. A total of 4 cloned genes are now implicated in the risk for developing AMD. As anticipated, the AMD genetic risk paradigm is complicated, reflecting interactions among multiple risk alleles. Gene-environment interactions contribute additional complexity. Ophthalmic genomics thus may serve as a test bed for advancing understanding about such interactions and whether, for instance, known AMD risk factors of smoking and obesity may interact with genetic contributions and the interplay of gene-environment interactions for major systemic disease.

Therapeutic Strategies for Ophthalmic Genetic Disease

The next frontier that links gene discovery to medicine involves exploring therapies for the many genetically defined ocular diseases. In the February issue of the Archives of Ophthalmology, Qi et al describe a therapeutic strategy for Leber hereditary optic neuropathy, a disease in which mitochondrial DNA mutations cause acute vision loss in midlife. Leber hereditary optic neuropathy cells transfected in vitro with adenov-associated virus expressing superoxide dismutase 2 showed increased expression of mitochondrial superoxide dismutase and had an 89% increase in cell survival compared with Leber hereditary optic neuropathy controls. Bolstering antioxidant defenses locally through gene delivery could be a promising treatment option that protects patients against vision loss.

Some ocular diseases have crisp and even remarkable end points that provide a significant opportunity for conducting clinical trials, such as the potential to restore sight to the blind. This exciting possibility may soon occur from a human clinical trial (scheduled to begin in 2007) for RPE65 gene replacement in patients with Leber congenital amaurosis (LCA), an autosomal recessive form of childhood blindness.

One form of LCA is caused by mutations in the RPE65 gene. In less than 15 years, researchers and clinicians have moved rapidly from the discovery of the RPE65 gene to an upcoming human phase 1 clinical trial. The gene was cloned in 1993 and an RPE65 knockout mouse model was established, which determined that RPE65 plays an essential role in the metabolism of vitamin A retinoid in the eye. By 1998, RPE65 mutations were identified as the cause of congenital blindness in some children with LCA because the consequent absence of 11-cis-retinal in the retina precludes functional vision. By 1998, RPE65 mutations were identified as causing LCA in Briard dogs, thereby providing a large animal model of LCA disease. By 2000, a multi-institutional team of researchers had injected a single dose of a gene therapy treatment containing the RPE65 gene driven by an adeno-associated virus (AAV) vector into the eyes of 3 Briard dogs with LCA. Within a few weeks, the dogs recovered significant sight, which allowed them to navigate and interact visually with the trainers. Treated dogs have retained vision with no sign of complications for more than 5 years. This represents the first time that vision has been successfully restored in a large animal model of retinal degeneration. The extension to humans of ocular gene delivery preclinical trials has now been approved by the US Food and Drug Administration and the National Institutes of Health Recombinant DNA Advisory Committee for a phase 1 open-label, single dose escalation study to assess the safety of AAV-RPE65 subretinal delivery in patients with LCA. The first human data from this intervention may become available later this year.

A New Requirement for Widely Accessible Genetic Testing

The public is rapidly becoming informed about genetic medical discoveries, testing, and the potential for treatments through the Internet and media resources, and in one study, most US adults reported that they want information about genetic disorders in their families from their physicians. Genetic testing on a clinical basis, however, is currently available for only a limited number of inherited eye diseases. Information from GeneTests indicates that testing for many conditions is available from only 1 or a few laboratories in the United States and worldwide and primarily from laboratories conducting research studies. The complexity of classifying the types of ocular disease manifestations attributable to mutations in a given gene requires extensive analysis that only a few laboratories have achieved. In addition, the manner in which results are communicated to patients must convey medically useful prognostic information. Such information derives from comprehensive genotype-phenotype correlation studies.

Examples of these studies are reported in the February issue of the Archives of Ophthalmology. The study by Wong et al is a large-scale investigation of 890 patients with von Hippel-Lindau disease, an uncommon multisystem, dominantly inherited cancer syndrome with ocular manifestation. Von Hippel-Lindau disease may cause the formation of retinal capillary

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hemangioblastomas, which can result in vision loss and sometimes the loss of the eye. Genotyping clarified the relationship with ocular involvement, and aids in the counseling of patients about the risk of developing retinal angiomatosis, and in the resulting visual morbidity based on their genotype. The study by Wu et al involves the rare pediatric cases of vitreoretinopathies, which share a common pathology of aberrant retinal development and are associated with mutations occurring in the Norrie disease (NDP) gene. The genotyping of 109 cases implicated mutations in NDP that disrupt a cysteine-knot motif with severe retinal dysgenesis, while patients with noncysteine NDP mutations had abnormal vascular and retinal development and phenotypes consistent with a less severe phenotype. The retinal phenotype correlated well with the genotype findings. Wu et al concluded that patients who exhibit severe retinal dysgenesis should be suspected of carrying a mutation that disrupts the cysteine-knot motif in the NDP gene.

Molecular testing can aid in identifying mutations and can help in establishing and confirming a diagnosis. Molecular testing can identify carrier status or rule out the presence of familial mutations in nonsymptomatic at-risk relatives. The clinical need and clear benefit of relevant genetic testing for a number of blinding diseases is exemplified by the serious consequences of ocular cancer. Retinoblastoma is a malignant tumor that occurs in about 1 in 20,000 children and is almost always fatal if left untreated. Through early diagnosis and genotyping, however, modern treatment methods provide a survival rate of more than 96%.

The February issue of the Archives of Ophthalmology also contains several articles relevant to the importance of molecular diagnosis in clinical care. Daiger et al and Stone focus on genetic testing strategies for mendelian eye diseases, and, in particular for retinitis pigmentosa, which leads progressively to total blindness. Both articles suggest that within 5 to 10 years, molecular testing of newly diagnosed patients with inherited eye diseases will be a routine part of clinical practice and will uncover the underlying disease-causing mutation in 90% of cases. Mutations in approximately 75 genes are currently known to cause retinitis pigmentosa. It is now possible to identify the disease-causing mutations for 56% of patients with autosomal dominant retinitis pigmentosa, 30% with recessive retinitis pigmentosa, and nearly 90% with X-linked retinitis pigmentosa. Downs et al show the utility, scope, and current limitations of genetic testing in a mutational analysis of 8 retinal genes performed on samples obtained from referred patients. The genetic basis of disease was determined in 133 of 266 diagnostic tests. Additional tests identified carriers and mutations in nonsymptomatic patients who had known familial mutations. As improved diagnostic technologies become more widely available, reliable, and inexpensive, molecular diagnostic testing for all diseases will become routine in predicting risk and prognosis and a core component in clinical care.

While several hundred genes have been identified as causing inherited ocular disease, diagnostic molecular genetic testing remains a cottage industry and is fragmented across many laboratories that provide access primarily on a research basis. Progress has been made but public availability is still limited. As described by Daiger et al and by Blain and Brooks, the National Eye Institute (National Institutes of Health) created a partnership of laboratories across the vision research community and established the National Ophthalmic Disease Genotyping Network to broaden accessibility of diagnostic genetic testing. The National Ophthalmic Disease Genotyping Network resource can provide patients with medically useful information while simultaneously laying the foundation for the next generation of research through a secure database of phenotype parameters, facilitating genotype-phenotype analysis. Providing accessible clinical genotyping to patients with hereditary eye diseases might engage them to participate in clinical therapy trials and simultaneously provide a DNA repository coupled with anonymous phenotypic information for researchers. A front-end coordinating center will provide ready access to the National Ophthalmic Disease Genotyping Network for clinicians beyond academic centers. This initiative should stimulate grassroots patient interest and involvement in genetic-based clinical care as the entire field of medicine moves quickly into this new arena. However, the ethical, legal, and social issues attendant to medical genetic programs must be addressed. In particular, the need for effective federal legislation to prevent genetic discrimination in health insurance and the workplace grows more compelling by the day, as more individuals become eligible for genetic analysis and expect that the results of their genetic tests will be used to help and not harm them.

Internet-accessible databases will be invaluable in elucidating the pathophysiology of common diseases by serving as repositories for the evolving knowledge of disease-causing mutations, nondisease-causing polymorphisms, and genotype-phenotype correlations. The new Genotype and Phenotype Database is an Internet repository initiated in 2006 and managed by the National Center for Biotechnology Information. The database is designed to collect and distribute data from genome-wide studies that explore the association between specific genes (genotype information) and observable traits, such as blood pressure and weight, or the presence or absence of a disease or condition (phenotype information). The first release of the Genotype and Phenotype Database contains data from 2 studies: the Age-Related Eye Diseases Study, a 3640-patient, multicenter, prospective 7-year study of the clinical course of AMD and age-related cataracts, and the Parkinsonism Study, which gathered DNA, cell line samples, and detailed phenotypic data on 2573 study participants. The data from the Genotype and Phenotype Database will enable interested researchers an unprecedented level of detail and information on the association between study participants’ genetic makeup and clinical variables.

Conclusion
From those first observations many centuries ago of the inheritance of eye diseases, ophthalmic genetics has now pro-
gressed to identify several hundred genes that cause ocular disease and blindness, and remarkable advances are being made toward human gene–based ocular therapy. As illustrated by the January and February 2007 issues of the Archives of Ophthalmology on ophthalmic genetics, this is an exciting time when advances in genomic technologies and translational research are beginning to provide for better eye care.

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