Glaucoma Genetics in 2009

Patients' risk of developing disease depends on the genetic mutation.

BY JOHN H. FINGERT, MD, PHD

Genes play an important role in glaucoma. Epidemiological studies, twins studies, and reports of families in which glaucoma is transmitted as a Mendelian trait show that genes are involved in the pathogenesis of this disease. Only during the past decade, however, have researchers identified specific glaucoma genes. This article reviews two important genes that confer risk for glaucoma in vastly different ways.

Mutations in one gene, myocilin (*MYOC*), confer a high likelihood for the development of glaucoma and almost never occur in subjects with healthy eyes. Conversely, mutations in the lysyl oxidase-like 1 gene (*LOXL1*) are common among both patients with exfoliation syndrome and normal subjects. Even so, *LOXL1* mutations, also called *risk alleles*, are detected at a statistically higher frequency in patients with exfoliation syndrome than in normal subjects. Individuals who carry *LOXL1* risk alleles are at a higher risk for developing exfoliation syndrome and secondary glaucoma than those who do not carry these alleles. Given the high frequency of *LOXL1* risk alleles in the general population, however, most carriers never develop disease.

The previous examples illustrate the different ways that genes may contribute risk for disease. In some cases, mutations in a single gene, such as *MYOC*, may be the principal risk factor for disease. In other cases, genes such as *LOXL1* confer risk that only leads to disease when combined with the action of other genetic and environmental factors.

MYOCILIN-ASSOCIATED GLAUCOMA

Clinical Features

Patients with glaucoma that is caused by defects in the myocilin gene generally have one of two distinct clinical presentations. One set of myocilin mutations is associated with juvenile open-angle glaucoma (JOAG) that is characterized by the early onset of disease, particularly high IOP, and a strong family history. Myocilin mutations have been associated with the autosomal dominant inheritance of JOAG in many large families and are responsible for 8% to 63% of the cases of JOAG overall.[1-4]

A second set of *MYOC* mutations is associated with more typical adult-onset primary open-angle glaucoma (POAG). In fact, the most commonly detected myocilin mutation in the United States, GLN368STOP, is associated with late-onset glaucoma that is clinically indistinguishable from POAG cases without myocilin mutations.[5] Many mutations in myocilin have been discovered in populations of POAG patients from around the world. Overall, mutations in myocilin are associated with 3% to 4% of the cases of POAG.[6]

Elevated IOP appears to be a common feature of all myocilin-associated glaucoma, although the IOP is more markedly elevated in cases of JOAG.

Mechanism of Disease

The mechanism by which mutations in the myocilin gene lead to glaucoma is unclear. In fact, very little is known about the basic biology of myocilin, except that it is produced in the ocular tissues that are vital to the regulation of IOP (the trabecular meshwork[7] and ciliary body[8]) and that, under normal circumstances, myocilin is secreted into the aqueous humor.

The normal function of myocilin is unknown, and studies of experimental strains of mice have shown that neither an excess nor an absence of myocilin activity leads to glaucoma.[9-11] Instead, mutations in myocilin cause disease by altering the normal behavior of the encoded protein. Whereas normal myocilin protein is secreted from the trabecular meshwork cells, mutant myocilin protein is retained and accumulates intracellularly.[12] Recent investigations have suggested that mutations may alter the protein structure of myocilin so that it binds to proteins that divert it from the secretory pathway to intracellular compartments, the peroxisomes.[13] These studies suggest that the abnormal intracellular accumulation of myocilin protein may damage the tissues of the iridocorneal angle (especially the trabecular meshwork cells) and lead to reduced aqueous outflow, elevated IOP, and eventually damage to the optic nerve and glaucoma.

Discovering the Genetic Etiology of Primary Open-Angle Glaucoma

You can never have too many patients.

BY LOUIS R. PASQUALE, MD

An old adage in baseball is *you can never have too much pitching*. In the field of glaucoma genetics, one can never have too many cases and controls when looking for the genes responsible for genetically complex diseases such as primary open-angle glaucoma (POAG).

Having the human genome sequence now makes it possible to find genes for POAG using a whole genome approach. Genome-wide studies use a collection of unrelated cases and controls without preconceived hypotheses about what the protein products of the genes do or where the genes for POAG might be located in the human genome. This agnostic approach can work as long as the sample size is large enough to overcome the multiple-comparison challenge encountered when one genotypes cases and controls at more than 650,000 locations throughout the genome simultaneously. Sample size calculations for the number of cases and controls actually needed to find POAG genes are preliminary, because the underlying genetic architecture of POAG is currently unknown.

At Harvard Medical School, Janey Wiggs, MD, PhD, and I are funded to use a genome-wide approach to find the genes for POAG in 1,200 cases and 1,200 controls through an initiative called *GLAUGEN* (the *Glau*coma *Gen*e *En*vironment Initiative). Collaborators at Brigham Women's Hospital (Jae Hee Kang, ScD) and the Harvard School of Public Health (David Hunter MBBS, ScD) among others are assisting us in this effort.

To further advance this research, the National Eye Institute is supporting another genome-wide scan on a second set of 2,000 cases and 2,000 controls. Dr. Wiggs and Michael Hauser, PhD, at Duke University are the coprincipal investigators. Termed *NEIGHBOR* (*NEI* *G*laucoma *H*uman Genetics Colla*bor*ation), this initiative involves a consortium of investigators from the following institutions:

- Bascom Palmer Eye Institute, University of Miami School of Medicine
- Duke Eye Center, Duke University School of Medicine
- Hamilton Glaucoma Center, University of California, San Diego
- Kellogg Eye Center, University of Michigan School of Medicine
- Pittsburgh Eye and Ear Institute, University of Pittsburgh School of Medicine
- Stanford University School of Medicine
- West Virginia University Eye Institute, West Virginia School of Medicine
- Wilmer Eye Institute, Johns Hopkins School of Medicine

The results of these studies will provide important information about the genetic factors that predispose people to developing POAG. We anticipate that POAG will consist of several distinct disease subgroups and that there may be unique genes that operate within specific ancestral groups.

Because over 100 genes with modest effect sizes may be operating in POAG, we may need several thousand more DNA samples to achieve a better understanding of the disease. Ultimately, the information gained from the genetic dissection of POAG may lead to earlier diagnosis and better treatments. We can purify DNA from mouthwash samples as well as blood samples, and ophthalmologists around the country can contribute to this effort. Those interested in learning more about the studies described herein or who have patients that they would like to enroll may contact the study coordinator, Elizabeth Delbono, MPH, at (800) 368-8143.

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Study Supports Polygenic Nature of Glaucoma

BY JULIA T. LEWANDOWSKI, SENIOR ASSOCIATE EDITOR

An experimental model in Baker's yeast that investigated the effect of variations in the *UTP21* gene on protein synthesis has clarified the role of the *WDR36* gene in the pathogenesis of human primary open-angle glaucoma (POAG).[1] Investigators previously thought that several alterations in the sequence of the *WDR36* were exclusive to individuals with adult-onset POAG. The discovery that the variations occurred with similar frequency in populations without glaucoma, however, suggested that the *WDR36* gene only causes glaucoma in the presence of additional factors.

The investigators tested their hypothesis by modifying the *UTP21* gene in yeast to mimic the POAG-related variations identified in *WDR36*. Because both genes process precursors to ribosomal RNA (rRNA), variations in their sequences can adversely affect the assembly of ribosomes and thus impair the organelles' ability to produce normally functioning proteins.

A comparison of cultures grown from the modified yeast cells showed that several variants of *UTP21* and their corresponding homologues in *WDR36* (R495Q [D685G], 1567V [1604V], D621G [D685G], and F634V [M671V]) significantly affected the processing of rRNA only in the presence of a mutated *STI1* gene. This cochaperone gene, which is also known as the *heat shock protein-organizing protein* in humans, not only packages the proteins produced by ribosomes, but it also provides neuroprotection for retinal cells and modulates responses to stress.

Based on these observations, the investigators concluded that the variants listed earlier "are likely to encode subtle defects in *WDR36* that, in certain environmental or genetic contexts, such as a mutation of *STI1*, predispose [an individual] to progression of glaucoma."[1]

A better understanding of the polygenic nature of glaucoma, they added, "may lead to identification of other co-modifying glaucoma genes" and spur the development of new therapeutic targets.[1]

"If we can understand who gets glaucoma, then we're in a much better place to prevent it," stated Michael A. Walter, PhD, an associate professor of ocular genetics at the University of Alberta in Edmonton, Alberta, Canada, and one of the study's investigators, in a news release. "If we can understand why they get glaucoma, then we have some important clues to use in developing second-generation medications that treat the disease itself."

1. Footz TK, Johnson JL, Dubois S, et al. Glaucoma-associated WDR36 variants encode functional defects in a yeast model system. Hum Mol Gen. 2009;18(7):1276-1287.

Testing for Myocilin Mutations

For many diseases, genetic testing has the potential to provide valuable information to patients and their physicians. Test results may be complex, however, and may affect other family members. Consequently, experienced physicians and genetic counselors should be involved in decisions about genetic testing and the interpretation of results.

Nonetheless, some general principles suggest which patients may benefit most from genetic testing for myocilin mutations, which account for approximately one in 25 cases of POAG. Large-scale population-based testing for myocilin mutations is not currently feasible given the relatively low prevalence of disease-causing mutations. Testing may be warranted, however, for a select subset of patients who have high-risk features of disease, such as individuals with a strong family history of glaucoma, an early onset of disease, and markedly elevated IOP. A high proportion of patients with these characteristics have a mutation in the myocilin gene. Genetic testing frequently may provide useful information to these individuals and their physicians. In particular, it would be helpful to screen young, unaffected at-risk myocilin family members to appropriately utilize resources and to follow closely those harboring disease-causing mutations.

EXFOLIATON SYNDROME AND THE LYSYL OXIDASE-LIKE 1 GENE

Recently, a genome-wide scan for risk alleles identified a pair of variations in the *LOXL1* gene, Arg141Leu and Gly153Asp. Each was observed in patients with exfoliation syndrome at a significantly higher frequency than in normal control subjects.[14] Initial studies with Icelandic and Swedish subjects[14] were confirmed with studies of populations from the United States,[15-18] Australia,[19] Japan,[20-24] India,[25] and Germany and Italy.[26] As mentioned earlier, although these "high-risk" *LOXL1* alleles are more common among patients with exfoliation syndrome, they also occur in a majority of normal subjects. As a result, most carriers of high-risk alleles never develop disease. The high frequency of *LOXL1* risk alleles in the general population suggests that other genes and possibly environmental factors determine which carriers of *LOXL1* risk alleles go on to develop exfoliation syndrome.

Mechanisms of Disease

LOXL1 encodes an enzyme that has an important role in elastin synthesis[27] and is expressed in many tissues of the eye, including the cornea, iris, ciliary body, lens capsule, and optic nerve.[19] The risk alleles (Arg141Leu and Gly153Asp) are located within a segment of the encoded LOXL1 propeptide that is cleaved to form an active enzyme after binding to its substrates tropoelastin and fibulin-5.[27-29] Although the mechanism by which these risk alleles contribute to the development of exfoliation syndrome is still under investigation, one hypothesis suggests that the risk alleles may alter cleavage of the LOXL1 propeptide, binding to substrates, or enzymatic activity.[14] Such altered function may lead to the accumulation of fibrillar material in the anterior segment of the eye and the pathologic features of exfoliation syndrome.

Testing for High-Risk LOXL1 Alleles

The high prevalence of *LOXL1* risk alleles limits the current utility of genetic testing. At present, the vast majority of test results would be false positives (carriers of *LOXL1* risk alleles who do not have exfoliation syndrome) and would not provide useful information to patients and their physicians. When additional risk factors are identified, more comprehensive testing will be possible and will likely provide useful diagnostic and prognostic data.

SUMMARY

Genes contribute to the development of glaucoma in multiple ways. In some cases, rare mutations in single genes can cause disease that is inherited as a Mendelian trait (eg, myocilin and autosomal dominant JOAG). In such instances, a mutation in a single gene contributes risk for disease that overwhelms other factors, and the vast majority of mutation carriers develop disease. Other cases of glaucoma are likely caused by the combined action of several common genetic risk alleles. In these instances, only the combined risk from multiple factors is sufficient to cause disease. Although individuals carrying any one risk allele (like a *LOXL1* risk allele) are at higher risk for glaucoma, most do not have the disease. ❑

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