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# Toward a Genetic Understanding of Glaucoma—Breakthroughs and Challenges from Studies of Exfoliation Glaucoma

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## Why Study Glaucoma Genetics?

The promise of glaucoma genetic studies is simple. Defining genetic risk-factors predisposing people toward glaucoma empowers the design of new therapeutic strategies. All current glaucoma medications and surgeries target the same risk-factor, intraocular pressure. This strategy is often adequate, but for some patients alternative approaches could be of substantial benefit. By promoting new opportunities for early detection and additional options for medical intervention, the potential long-term benefits of studying glaucoma genetics are clear.

The actual work to discover the genetic causes of glaucoma is far from simple. Given the biological intricacies of the anatomy and physiology involved in glaucoma, most geneticists have long suspected that the glaucoma phenotype would involve equally intricate genetic pathways. Over the past year, a significant breakthrough has occurred in the identification of a gene with major importance to exfoliation syndrome and its sequela, exfoliation glaucoma. Here, we present an abbreviated version of the molecular search for glaucoma genes that provides a context for contemplating the broader significance of this recent progress. Our central tenet is that the search for glaucoma's genetic elements will continue to require experimental ingenuity and synergistic approaches involving animal models that render the complexity of glaucoma more tractable.

## An Optimistic Beginning: Glaucoma Genes Exist

Glaucoma tends to run in families. First-degree family members of individuals with primary open-angle glaucoma have an approximately eight to ten-fold increased risk of developing glaucoma compared to the general population.[1, 2] Also, different forms of glaucoma exhibit ethnic biases. For example, African-Americans are 4 to 5 times more likely to have primary open-angle glaucoma than white Americans.[3] Likewise, primary angle-closure glaucoma is much more common in East Asians than Europeans.[4] These observations, and many others, collectively indicate that heredity plays an important role in glaucoma.

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In recent years several groups have become actively engaged in studies to identify genetic variations associated with glaucoma susceptibility. Each of these studies has had several inherent hurdles to contend with. For instance, one major problem facing glaucoma genetics pertains to patient classifications. Because the glaucomas consist of many individual diseases with overlapping phenotypic similarities, a genetic analysis that included individuals with different forms of glaucoma would likely lead to confounding results. A strategy that has been repeatedly utilized to overcome this issue is to initially focus on analysis of forms of glaucoma with strikingly unique features, either based on age of onset (such as juvenile and congenital forms of glaucoma) or based on clinical presentation (such as secondary glaucomas associated with pigment dispersion or exfoliation syndrome). An interesting example of glaucoma genetics that benefited from this strategy comes from studies of the myocilin gene and its role in glaucoma.

The discovery of myocilin as a glaucoma-causing gene was a culmination of multiple experimental approaches. In one line of work, the myocilin protein was identified by cellular studies of the trabecular meshwork.[5] Independently, a positional cloning approach with an unusually large family pedigree affected by autosomal dominant juvenile open-angle glaucoma led to the identification of disease causing mutations within the myocilin gene on chromosome 1q.[6, 7] Having initially identified myocilin's contribution to this early-onset form of glaucoma, subsequent studies then went on to test whether myocilin mutations are also associated with the much more common and later onset primary open-angle form of glaucoma.[7] A series of population based screens have indeed indicated that approximately 3% to 5% of primary open angle glaucoma cases worldwide are attributable to defects in the myocilin gene.[8, 9] More recently, several groups have generated myocilin mouse models.[10–13] Combined, these efforts are collectively contributing to a better understanding of myocilin function and bringing potential therapies derived from this knowledge ever closer.

## A Pessimistic Reprise: Where Are the Other 95% of Mutations?

A full decade past the initial identification of myocilin's role in glaucoma, it is useful to broadly compare the initial successes with myocilin to what other gains have been made in glaucoma genetics. Although tremendous progress has been made,[14] much more remains to be accomplished. Known mutations still only account for a limited percentage of glaucoma cases. Where are all the other mutations? In considering this question, many geneticists had begun to suspect that perhaps the myocilin gene might be atypical. If most genes associated with glaucoma influence only a small percentage of cases, perhaps myocilin was found early on because of its unusually large significance. Accordingly, perhaps most of what remains to be discovered would be mutations in a large number of genes, each accounting for only a small fraction of all glaucoma. In late 2007, this assumption was challenged by a breakthrough finding related to the genetics of exfoliation syndrome.

## Success in Exfoliation Syndrome: Identification of *LOXL1*

Significant advances in glaucoma genetics have been precipitated by studies unraveling the genetic factors of exfoliation syndrome. Exfoliation syndrome has long been appreciated to

have strong hereditary contributions,[15, 16] but until this past year no causative mutations had been identified. In a very large genome-wide association study among Scandinavian glaucoma patients (14,672 controls and 274 patients with exfoliation syndrome), the lysyl oxidase-like protein 1 gene (*LOXL1*) has been identified as the first known genetic risk factor contributing to exfoliation syndrome.[17] A strong association was detected between exfoliation syndrome and two SNP genetic markers in *LOXL1*. The high-risk alleles defined by these markers occur within the vast majority of patients with exfoliation syndrome. Remarkably, individuals homozygous for both markers have about a 700 fold increased risk for exfoliation syndrome. If the risk associated with these alleles could be avoided, it would be predicted to eliminate over 99% of exfoliation syndrome cases.[17]

In addition to the importance to exfoliation syndrome, these findings also have an important broader implication. As with *LOXL1* in exfoliation syndrome, there could also be additional glaucoma-associated genes in other forms of glaucoma with very large influences that remain to be identified. Perhaps with recent technological advancements, such as the ability to readily perform high-density genome-wide genetic association studies, the years to come will bring many more similar advances.

## Necessary, But Not Sufficient, Contributions of *LOXL1* to Exfoliation Syndrome

Since the initial discovery of the role of *LOXL1*, there has been a flurry of research findings impacting exfoliation syndrome. Several studies have subsequently replicated the *LOXL1* association.[18, 19] Some interesting issues have also been raised. Perhaps the most interesting comes from a careful consideration of the *LOXL1* allele frequencies. In addition to being found in the vast majority of patients with exfoliation syndrome, there is also a high occurrence of high-risk *LOXL1* alleles among the general population. Within the original Scandinavian populations studied, the high-risk alleles were observed at a frequency of approximately 50% in the general population.[17] All of the follow-up studies confirming a role for *LOXL1* in disease have also observed this high incidence of high-risk alleles among the general population. Approximately 25% of the general population is even homozygous for the highest risk allele.[18] Thus, the majority of people with high-risk *LOXL1* alleles likely is, and will remain, unaffected. This indicates that although *LOXL1* is an important component of risk for exfoliation syndrome, additional factors must also play a role. Thus, *LOXL1* mutations appear necessary, but not sufficient to cause exfoliation syndrome in most people. Identification of the additional factors determining risk remains an important future goal in studies of exfoliation syndrome.

These findings also have an important clinical ramification; a genetic test for exfoliation syndrome is still impractical. Because the high-risk alleles are so common, a genetic test based on current knowledge would create far more problems than it solved. When the identities of the additional risk-factors for exfoliation are known, this type of genetic test may eventually have clinical utility.

## Reducing Genetic Complexity with Mice

As with many common diseases, glaucoma likely represents a complex multi-factorial disease at the genetic level.[20] There are multiple strategies for experimentally dealing with this complexity. As in the case of *LOXL1*, one strategy for dealing with complexity is to perform studies with greater statistical power by involving very large numbers of subjects. However, this approach is expensive and may not be practical for all forms of glaucoma, especially rare forms of glaucoma where large numbers of patients are not available. Another approach for dealing with genetic complexity, and one that we particularly utilize in our laboratory, is to use genetic approaches with inbred mice.

Mice have an anatomy, physiology, and genome very similar to humans. Mice also exhibit susceptibility to many of the same diseases, including glaucoma. Because potentially confounding factors such as genetic background and environment can be controlled in mice, studies with mice allow the opportunity to identify genetic and environmental factors with even modest phenotypic contributions. Once defined in mice, research gains can then be brought back into focused human studies where there is direct relevance to patient care. However, to apply these advantages to the study of exfoliation syndrome a challenge arises. Few, if any, mouse models of exfoliation syndrome have previously been described. However, recent reports presented at Annual Meetings of The Association for Research in Vision and Ophthalmology (ARVO) indicate that many exciting opportunities are on the horizon.

Multiple advances are being made with animal models relevant to pathways of exfoliation syndrome. At an ARVO Special Interest Group session entitled “Genetics and Molecular Pathophysiology of Exfoliation Syndrome”, we recently presented data that mice with a genetic defect in the *Lyst* gene recapitulate multiple aspects of human exfoliation syndrome. Our consideration of *Lyst* as a candidate impacting exfoliation began from the observation that *Lyst* mutation results in an unusual pattern of iris transillumination defects. The iris transillumination defects occurring in *Lyst* mutant mice are a known, but often overlooked, aspect of exfoliation syndrome.[21] Building on this initial finding, we have also observed the presence of an exfoliative-like material and pronounced iris pigment dispersion in eyes of *Lyst* mutant mice. The *Lyst* gene has not previously been considered a candidate for exfoliation syndrome, nor is the LYST protein known to be a member of a LOXL1 pathway. Thus, this phenotype-driven approach in mice has led us to a novel hypothesis that would otherwise not have been considered; the *LYST* gene is a potential contributor to exfoliation syndrome in humans. Experiments to test this hypothesis directly are currently underway.

Another significant opportunity utilizing mouse genetics pertains to mouse models of *Loxl1*. Interestingly, mice containing a targeted knockout of *Loxl1* have existed for several years,[22] but curiously, there has been no mention of exfoliation syndrome-like phenotypes. This may indicate that *Loxl1* knockout mice have a different phenotype than what is caused by the non-synonymous mutations found in humans. Simply stated, mutations resulting in proteins with defective function often behave differently than mutations that result in no protein at all. Alternatively, *Loxl1* dependent phenotypes may be subtle and will require detailed examination to detect. In considering the findings with *Lyst* mutant mice, it will be

particularly interesting to determine whether *Loxl1* mutant mice also exhibit iris transillumination defects. It will also be important to determine whether *Loxl1*-dependent phenotypes in mice are genetic background dependent, perhaps differing when the same mutant allele is studied in different inbred strains of mice. Such a finding could serve as an important first step toward identification of additional genetic factors interacting with *LOXL1*.

## Concluding Remarks

The genetic pathways involved in susceptibility to many common diseases are intricate and often difficult to dissect. Glaucoma is no exception. The recent discovery of the *LOXL1* gene as a genetic risk factor for exfoliation syndrome has suggested that some glaucoma genes with very large influence remain to be identified. Given the power of recent technological advances, the overall future appears bright for glaucoma genetics. However, the example of *LOXL1* also emphasizes what most geneticists have long suspected; glaucoma is a multifactorial disease. Thus, in moving forward, it will be important to utilize experimental approaches that reduce complexity, including the synergistic use of genetic approaches in mice to identify additional genes of importance.

## Biographies

Colleen Trantow is a graduate student at The University of Iowa. Her thesis work involves studies of *Lyst*-dependent phenotypes and their relevance to secondary forms of glaucoma.

Michael G. Anderson, Ph.D., is an Assistant Professor of Molecular Physiology and Biophysics at The University of Iowa. Having performed graduate work in *Drosophila* genetics and postdoctoral studies in mouse genetics at The Jackson Laboratory in Bar Harbor, Maine, he is currently applying his knowledge of genetic approaches with model organisms to study glaucoma.

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