

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

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### Abstract

Glaucoma is a complex group of diseases causing progressive degeneration of the optic nerve. Despite strong evidence of hereditary contributions to glaucoma, relatively few genetic mutations associated with glaucoma risk have thus far been identified. Glaucoma likely represents a complex genetic trait influenced by multiple loci and many confounding factors, increasing the challenge in identifying glaucoma's hereditary basis. In this article, a brief history of the search for glaucoma-causing mutations is presented, highlighting recent breakthroughs that have identified lysyl oxidase-like 1 (LOXL1) as a major genetic risk factor contributing to exfoliative glaucoma. In considering challenges that remain ahead, 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.

### Keywords

Glaucoma, genetics, exfoliation, pseudoexfoliation, animal models

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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.

In recent years, 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 for developing glaucoma compared with the general population.<sup>1,2</sup> Also, different forms of glaucoma exhibit ethnic biases. For example, African-Americans are four to five times more likely to have primary open-angle glaucoma than white Americans.<sup>3</sup> Likewise, primary angle-closure glaucoma is much more common in East Asians than Europeans.<sup>4</sup> These observations, and many others, collectively indicate that heredity plays an important role in glaucoma.

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 used to overcome this matter 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.<sup>5</sup> 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.<sup>6,7</sup> 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.<sup>7</sup> A series of population-based screens has indeed indicated that approximately 3–5% of primary open-angle glaucoma cases worldwide are attributable to defects in the myocilin gene.<sup>8,9</sup> More recently, several groups have generated myocilin mouse models.<sup>10–13</sup> 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 after 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,<sup>14</sup> 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 Lysyl Oxidase-like Protein 1 Gene

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,<sup>15,16</sup> but until recently no causative mutations had been identified. In a large genome-wide association study among Scandinavian glaucoma patients (14,672 controls and 274 patients with exfoliation syndrome), the lysyl oxidase-like protein 1 (LOXL1) gene (*Loxl1*) has been identified as the first known genetic risk factor contributing to exfoliation syndrome.<sup>17</sup> A strong association was detected between exfoliation syndrome and two single nucleotide polymorphism (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.<sup>17</sup>

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.

## Toward a Genetic Test for Exfoliation Syndrome

Since the initial discovery of the role of LOXL1, there has been a flurry of research findings influencing exfoliation syndrome. Several studies have subsequently replicated the LOXL1 association.<sup>18,19</sup> Some interesting matters have also been raised; perhaps the most interesting comes from a careful consideration of 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.<sup>17</sup> 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.<sup>18</sup> Therefore, the majority of people with high-risk LOXL1 alleles likely are, and will remain, unaffected. This indicates that although LOXL1 is an important component of risk for exfoliation syndrome, additional factors must 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 would solve. When the identities of the additional risk factors for exfoliation syndrome 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 multifactorial disease at the genetic level.<sup>20</sup> 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 use in our laboratory, is genetic studies 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 indicate that many exciting opportunities are now available.

Multiple advances are being made with animal models relevant to pathways of exfoliation syndrome. Mice with a genetic defect in the lysosomal trafficking regulator (*Lyst*) gene recapitulate multiple aspects of human exfoliation syndrome.<sup>21,22</sup> 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.<sup>23</sup> 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, and the LYST protein is not 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 under way.

Another significant opportunity using mouse genetics pertains to mouse models of *Lox11*. Interestingly, mice containing a targeted knockout of

*Lox11* have existed for several years,<sup>24</sup> but, curiously, there has been no mention of exfoliation syndrome-like phenotypes. This may indicate that *Lox11* knockout mice have a different phenotype from what is caused by the non-synonymous mutations found in humans. Simply stated, mutations resulting in proteins with defective function often behave differently from mutations that result in no protein at all. Alternatively, *Lox11*-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 *Lox11* mutant mice also exhibit iris transillumination defects. It will also be important to determine whether *Lox11*-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.

### Conclusion

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 technologic 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 use experimental approaches that reduce complexity, including the synergistic use of genetic approaches in mice to identify additional genes of importance. ■

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