REVIEW

Genetics and genetic testing for glaucoma

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Purpose of review
In recent decades, investigators have identified numerous genes and genetic factors that cause or contribute risk for glaucoma. These findings have increased our understanding of disease mechanisms, provided us with new diagnostic tools, and may allow for development of improved therapies for glaucoma. However, genetic testing is most useful when it is reserved for appropriate patients. The purpose of this article is to review key points and recent developments regarding the genetics and genetic testing for glaucoma and to provide recommendations for when genetic testing may be warranted.

Recent findings
Large genome-wide association studies have identified multiple new susceptibility loci associated with primary open angle glaucoma and primary angle closure glaucoma.

Summary
Several glaucoma-causing genes and genetic risk factors for glaucoma have been discovered. As a result, there are specific clinical scenarios in which genetic testing is warranted. In select cases (i.e., familial juvenile open angle glaucoma), genetic testing can serve as a powerful tool to improve diagnostic accuracy, efficiency of disease surveillance, and selection of treatment, enabling physicians to better optimize care for their patients.

Keywords
genetic testing, genetics, glaucoma

INTRODUCTION

Glaucoma is a complex disease characterized by degeneration of the optic nerve and is the most common cause of irreversible blindness worldwide [1]. It is estimated that glaucoma will affect 79.6 million people by the year 2020 [2]. A genetic basis for glaucoma has been established through epidemiological studies [3], twin studies [4], reports of large families affected by glaucoma [5], genome-wide association studies (GWAS) [6,7▪▪], and animal models of glaucoma [8]. Early-onset glaucoma (before age 40) is more likely to be inherited in a Mendelian fashion involving single genes, whereas adult-onset glaucoma tends to follow more complex inheritance patterns involving multiple genetic factors [9].

In recent decades, researchers have identified multiple disease-causing mutations as well as numerous susceptibility loci associated with various forms of glaucoma. These findings have increased our understanding of disease mechanisms, provided new diagnostic tools, and facilitate development of improved therapies for glaucoma. However, the role of genetic testing in glaucoma is an evolving discussion, as the existence of such testing does not mean that it is appropriate for all patients.

Glaucoma can be categorized into cases with simple or with complex genetic basis. Some cases of glaucoma are caused primarily by a defect in a single gene. These cases of glaucoma have a simple genetic basis and are inherited in Mendelian patterns (i.e., as an autosomal dominant trait). Three genes, myocilin (*MYOC*), optineurin (*OPTN*), and TANK binding kinase 1 (*TBK1*) are each capable of causing open angle glaucoma with little influence from other genes or environmental factors. Other complex genetic cases of glaucoma are caused by the combined action of many genetic and environmental risk factors. Such genetic factors increase the risk for developing glaucoma, but each is incapable of causing disease in isolation. More than 20 genetic risk factors have been discovered for primary open angle glaucoma (POAG) and primary angle-closure

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KEY POINTS

- Simple genetic causes of open angle glaucoma include mutations in *MYOC*, *OPTN*, and *TBK1*, and are inherited as autosomal dominant traits with little to no influence from other risk factors.
- Complex genetic cases of glaucoma are caused by the combined action of many genetic and environmental risk factors, which cannot cause disease on their own.
- Recent large GWAS studies revealed new susceptibility loci for various forms of glaucoma, including one locus associated with visual field progression in POAG.
- Genetic testing for open angle glaucoma is currently recommended only in select cases for *MYOC* and *OPTN* mutations, but in these scenarios it is a powerful diagnostic and prognostic tool that can improve management and potentially help prevent vision loss.

glaucoma (PACG) and many more remain to be identified [10▪▪,11].

In this article, we review the most notable findings in the recent literature on the genetics of glaucoma, discuss examples of glaucoma with simple genetic bases, and describe key considerations associated with genetic testing for glaucoma. The genetics of primary congenital glaucoma and secondary forms of glaucoma are reviewed elsewhere [10▪▪,12,13].

RECENT DEVELOPMENTS IN GENETICS OF GLAUCOMA

Recent complex primary open angle glaucoma genetic study

In 2016, Bailey *et al.* [7▪▪] reported a large GWAS of POAG patients ($n = 3853$) and matched control subjects ($n = 33\,480$) from the United States and confirmatory cohorts from Europe, Australia, and Singapore. Strong associations were detected for three novel susceptibility loci for POAG: *TXNRD2* ($P = 4.05 \times 10^{-11}$), *ATXN2* ($P = 8.73 \times 10^{-10}$), and *FOXC1* ($P = 1.76 \times 10^{-10}$). *FOXC1* encodes a transcription factor that has important roles in the development of anterior segment structures. Mutations in the *FOXC1* gene had previously been associated with Axenfeld-Rieger syndrome [14], but this was the first report demonstrating its association with POAG. *ATXN2* had previously been implicated in other degenerative neurologic disorders including spinocerebellar ataxia type 2 [15] and amyotrophic lateral sclerosis (ALS) [16], which is notable because mutations in two glaucoma-causing genes (*OPTN* and *TBK1* described below) have also been linked with ALS [17,18,19–21]. *TXNRD2* is a nuclear gene that encodes a protein involved in mitochondrial function. Both *ATXN2* and *TXNRD2* were shown to be active in tissues that are central in the pathophysiology of glaucoma (i.e., trabecular meshwork, ciliary body, retina, and optic nerve). This large GWAS discovered novel genetic risk factors for POAG and has provided new insights into the pathogenesis of glaucoma. The functions of these risk factors suggest that studies of ocular development (*FOXC1*), neurodegeneration (*ATXN2*), and mitochondrial dysfunction (*TXNRD2*) may help define the biological pathways that contribute to POAG.

Recent complex primary angle-closure glaucoma genetic study

PACG is common in Asia and is a major cause of blindness [2,22,23]. In 2016, a large GWAS involving greater than 10 000 cases of PACG and nearly 30 000 controls from 24 countries identified multiple new susceptibility loci. In this report, Khor *et al.* [24▪▪] described five new genetic loci associated with PACG – *EPDR1*, *CHAT*, *FLIS3*, *FERMT2*, and *DPM2-FAM102A*. Each of these glaucoma risk factor genes is expressed in key anterior segment structures that are involved in angle closure as well as in the retina and optic nerve. Evaluation of the proteins encoded by these PACG risk factor genes suggests a potential role for abnormalities in cell-cell adhesion, collagen metabolism, and other molecular pathways in the development of PACG.

Primary open angle glaucoma risk factor associated with visual field progression

GWAS approaches have also been used to identify factors that are associated with progression of glaucoma severity. In 2015, Trikha *et al.* [25▪▪] reported an association between *TGFBR3-CDC7* and visual field progression in POAG patients from Singapore. The 1334 patients in this study were followed for a mean of 9 years and were genotyped at 10 previously reported glaucoma risk loci. One of these loci, *TGFBR3-CDC7*, was associated with visual field changes. POAG patients with one *TFGBR3-CDC7* risk allele had an odds ratio of 6.71 ($P = 0.003$) for visual field progression. This report is the first to identify glaucoma susceptibility loci that are associated with visual field progression.

SIMPLE GENETIC (SINGLE GENE) CAUSES OF OPEN ANGLE GLAUCOMA

Myocilin (*MYOC*) and glaucoma that most frequently occurs with higher intraocular pressure

Mutations in *MYOC* are the most common molecularly defined cause of open angle glaucoma [26▪].

Patients with *MYOC* typically have moderately to markedly high intraocular pressure (IOP) and dominantly inherited disease [27]. One set of *MYOC* mutations cause 4–60% of juvenile open angle glaucoma (JOAG) cases, while a different set of *MYOC* mutations cause 3–4% of POAG cases [27–29]. Patients with JOAG caused by *MYOC* mutations have an early onset of disease and markedly high IOP that frequently does not respond to medical therapies. Early surgical intervention is often required [30]. Conversely, patients with POAG caused by the Gln368Stop mutation in *MYOC* have later onset of disease and moderately high IOP. These patients have the same response to medical and surgical interventions as patients without an identified genetic mutation [31].

Although the normal function of the *MYOC* gene remains a mystery, much has been learned about how mutations in this gene lead to glaucoma. *MYOC* encodes a protein that is secreted by trabecular meshwork cells into the aqueous humor. Glaucoma-causing mutations prevent its secretion and lead to accumulation of abnormal MYOC protein in trabecular meshwork cells, which may compromise their function and ultimately lead to elevated IOP and glaucoma [32,33]. More recently, the pathogenesis of glaucoma caused by mutations in *MYOC* has been studied using laboratory mice engineered to carry the same glaucoma-causing mutations as human patients [34]. These transgenic mice, which carry a Tyr437His *MYOC* mutation, developed high IOP and optic nerve damage that mirrors the glaucoma seen in humans [35]. Moreover, abnormal MYOC protein is also retained within the trabecular meshwork cells of these mice, where its accumulation causes a stress response [36]. On the basis of these observations, Zode and colleagues hypothesized that *MYOC* mutations lead to production of abnormal, misfolded MYOC protein that accumulates in the endoplasmic reticulum of trabecular meshwork cells and has a toxic effect, which may be a key step in the development of myocilin-related glaucoma. This hypothesis was tested by treating the transgenic mice with a drug, 4-phenylbutyrate, which is a chemical chaperone that helps proteins fold into their correct conformations. Transgenic mice carrying a Tyr437His *MYOC* mutation treated with phenylbutyrate (either orally or as a medicated eyedrop) no longer had elevated IOP and did not develop glaucoma [36,37]. As such, phenylbutyrate appeared to cure myocilin-related glaucoma in mice. Phenylbutyrate has not yet been tested in humans, but could represent a novel treatment modality for patients with glaucoma caused by certain *MYOC* mutations.

Optineurin, tank binding kinase 1 and glaucoma that occurs with lower intraocular pressure

POAG that occurs at IOP $\leq$ 21 mm Hg has frequently been termed normal tension glaucoma (NTG). Mutations in two genes, *OPTN* and *TBK1*, have each been associated with about 1–2% of NTG cases and both exhibit autosomal dominant inheritance [17,18,38–43]. One *OPTN* mutation, Glu50Lys, has been detected in NTG patients in numerous independent studies and has a confirmed role in the pathogenesis of glaucoma. The data linking other *OPTN* mutations with glaucoma are conflicting [38,44,45]. Large duplications or triplications of a segment of chromosome 12q spanning the *TBK1* gene have been detected in NTG patients. Such *TBK1* gene duplications or triplications are responsible for about 1 in 100 NTG cases worldwide [18,40–43].

As previously noted, mutations in *OPTN*, *TBK1*, and the glaucoma risk factor *ATXN2* have also been discovered in patients with ALS, another neurodegenerative disease. Inactivating mutations in *OPTN* or *TBK1* may cause ALS, while a triplet repeat expansion in the *ATXN2* gene is associated with this disease [16,19–21]. Different mutations in these same genes cause NTG, solidifying their role in a family of degenerative diseases of the central nervous system and retina.

The two known NTG genes (*OPTN* and *TBK1*) encode proteins that directly interact with each other to activate autophagy, a catabolic cellular process in which intracellular proteins, organelles, and other cellular debris are captured, delivered to the lysosome, and degraded [46–48]. *TBK1* encodes a kinase that phosphorylates OPTN, which is an autophagy receptor. Mutations in *TBK1* or *OPTN* are thought to cause abnormal activation of autophagy which may damage retinal ganglion cells beyond repair as a mechanism for glaucoma. This hypothesis has been tested with studies of cells cultured from skin biopsies taken from NTG patients. Skin cells were reprogrammed to become induced pluripotent stem cells (iPSC) and then differentiated into neurons with features of retinal ganglion cells. Such iPSC-derived neurons produced from NTG patients with *TBK1* mutations showed abnormal activation of autophagy when compared to cells produced from control subjects [49]. These data support the hypothesis that *TBK1* mutations cause glaucoma via activation of autophagy in retinal ganglion cells. The pathogenesis of glaucoma caused by *OPTN* mutations has also been explored with transgenic mice and patient-derived iPSCs and these investigations suggest that molecules that

interfere with TBK1 and/or OPTN function may have therapeutic utility for these forms of glaucoma [50–52].

GENETIC TESTING FOR GLAUCOMA

Genetic testing may provide valuable data to patients and their physicians by enhancing early diagnosis, improving the accuracy of prognosis, and by identifying the most efficacious treatment regimen. By identifying high-risk patients who carry known disease-causing mutations, physicians know when to recommend closer monitoring and earlier treatment to prevent or minimize vision loss. If such a variant is identified, genetic testing also allows for screening in relatives to determine if they also warrant close surveillance. If this screening is negative, these family members can be reassured that their risk of developing glaucoma is likely no higher than the general population. Consequently, genetic testing may help direct scarce clinical resources to those that need them most.

However, careful selection of patients for genetic testing is vital to realize these potential benefits. Guidelines for genetic testing in ophthalmology have been provided by the American Academy of Ophthalmology, which suggest that testing is only recommended if the results will impact treatment or disease surveillance [53]. Moreover, testing should be sought from reputable laboratories that are Clinical Labs Information Act (CLIA) certified. Finally, genetic testing should be conducted by experienced physicians with the availability of genetic counseling to ensure that test results are appropriately explained to patients and their families.

GeneTests.Org is an excellent resource for identifying genetic tests that are available from both CLIA-certified diagnostic laboratories and from research laboratories. Genetic testing is widely available for *MYOC* mutations (JOAG and POAG) and for *OPTN* mutations (NTG). Testing for *TBK1* mutations is not currently available for clinical use.

Testing unselected patients with POAG for *MYOC* mutations has relatively low yield. Only 3–4% of POAG patients will have a positive result. Conversely, testing of high-risk populations may have a much higher utility. Specifically, patients that are relatives of those known to have *MYOC*-associated glaucoma may have up to a 50% risk of carrying a *MYOC* mutation. Other patients with JOAG or POAG that have early onset of disease, markedly high IOP, and strong family history of disease (dominant inheritance) also have a higher likelihood of having *MYOC*-associated glaucoma. It is most cost-effective to limit testing for *MYOC* to these higher-risk cases of JOAG or POAG. Unselected testing for *MYOC* should be reserved for research studies [54].

Similar recommendations can be made for testing NTG patients for *OPTN* mutations. *OPTN* testing should generally be reserved for select patients with NTG that have family members with known *OPTN*-associated glaucoma or for NTG patients with early onset of disease and strong family history [54].

Genetic testing for POAG that is caused by the interaction of many genetic risk factors is not recommend in 2016. Many POAG risk factors have been discovered, but none are capable of causing glaucoma on their own. Consequently, a testing algorithm will be necessary in which the risk from many factors is summed. Currently, it is unclear if enough genetic risk factors for glaucoma have been identified for this approach to be effective, nor has an algorithm for summing the risk of these factors been developed. In the future, however, it is likely that this could be a successful approach to evaluating risk for developing glaucoma for a larger segment of the population than is currently possible.

CONCLUSION

The genetic basis for glaucoma is well established, and numerous disease-causing genes and genetic risk factors have been identified. Recently reported large GWAS studies continue to reveal additional susceptibility loci for various forms of glaucoma, including one locus associated with visual field progression in POAG. Although genetic testing is currently recommended only in select cases for *MYOC* and *OPTN* mutations, in the appropriate clinical scenario it can be a powerful diagnostic and prognostic tool that may better guide surveillance, facilitate earlier treatment, and help prevent vision loss in these high-risk patients.

Additional important genetic risk factors for glaucoma will continue to be discovered. These findings have the potential to further improve our understanding of disease mechanisms and enhance our ability to diagnose and treat glaucoma.

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Conflicts of interest

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