

## **Clinical implications of recent advances in primary open-angle glaucoma genetics**

Hélène Choquet<sup>1</sup>, Janey L. Wiggs<sup>2</sup>, and Anthony P. Khawaja<sup>3</sup>

<sup>1</sup>Kaiser Permanente Northern California (KPNC), Division of Research, Oakland, CA 94612, USA.

<sup>2</sup>Department of Ophthalmology, Harvard Medical School, Massachusetts Eye and Ear Infirmary, Boston, MA, USA.

<sup>3</sup>NIHR Biomedical Research Centre, Moorfields Eye Hospital NHS Foundation Trust and UCL Institute of Ophthalmology, London, UK.

Corresponding Author: Hélène Choquet

## Abstract

Over the last decade, genetic studies, including genome-wide association studies, have accelerated the discovery of genes and genomic regions contributing to primary open-angle glaucoma (POAG), a leading cause of irreversible vision loss. Here, we review the findings of genetic studies of POAG published in English prior to September 2019. In total, 74 genomic regions have been associated at a genome-wide level of significance with POAG susceptibility. Recent POAG GWAS provide not only insight into global and ethnic-specific genetic risk factors for POAG susceptibility across populations of diverse ancestry, but also important functional insights underlying biological mechanisms of glaucoma pathogenesis. In this review, we also summarize the genetic overlap between POAG, glaucoma endophenotypes, such as intraocular pressure and vertical cup-disc-ratio, and other eye disorders. We also discuss approaches recently developed to increase power for POAG locus discovery and to predict POAG risk. Finally, we discuss the recent development of POAG gene-based therapies and future strategies to treat glaucoma effectively. Understanding the genetic architecture of POAG is essential for an earlier diagnosis of this common eye disorder, predictive testing of at-risk patients, and design of gene-based targeted medical therapies of which there are currently none available.

## 1. POAG genetics advances

### A. Recent GWAS of POAG led to the discovery of numerous genetic loci

In the last decade, genome-wide association studies (GWAS) have rapidly accelerated the discovery of genetic determinants of numerous diseases and complex traits, with more than 10,000 significant genome-wide associations reported to date(1-3). Unsurprisingly, this GWAS success in identifying risk loci has also applied to primary open-glaucoma (POAG). As of 2017, sixteen genomic regions associated with POAG at a genome-wide level of significance ( $P < 5 \times 10^{-8}$ ) had been reported(4), with most studies conducted in European and Asian populations(5-13). In the last 3 years, many other loci that contribute to POAG susceptibility have been discovered(14-17), bringing the total number of POAG loci to 74 (**Table 1**). This recent, rapid increase in the discovery of POAG loci is likely due to several factors: 1) the emergence of large and multiethnic biobank-based cohorts, such as the UK Biobank(18, 19) and the Kaiser Permanente GERA(20, 21) cohorts; 2) the availability of summary statistics of published GWAS to the scientific community (e.g. GWAS catalog(2)), which has enabled more rapid confirmation of association loci; 3) the combination of the results from different GWAS in large multiethnic meta-analyses(14, 17); and 4) the application of recently developed approaches (e.g. gene-based analysis) using GWAS summary statistics(22, 23).

### B. Shared and ethnic-specific genetic associations

Recent GWAS provide insight into global and ethnic-specific genetic risk factors for POAG susceptibility across populations of diverse ancestry. As of 2017, five genomic regions had been reported to be associated with POAG in populations of Asian ancestry, including *ABCA1*, *PMM2*, and *CDKN2B-AS1*(8, 10, 24). Associations at all of these loci were either previously reported or have been largely confirmed in populations of European ancestry(9, 13, 14, 17). A recent Japanese GWAS of POAG confirmed genome-wide associations at known POAG-loci, and identified seven novel loci, including *FNDC3B*, *ANKRD55-MAP3K1*, *LMX1B*, *LHPP*, *HMG2*, *MEIS2* and *LOXL1*(16). Among these newly identified POAG loci, 3 loci, including *LHPP*, *HMG2*, and *MEIS2*, replicated neither in European population nor in African population from this study. This suggests the possible existence of genetic-specificities at those *LHPP*, *HMG2*, and *MEIS2* loci for POAG for Japanese individuals, and future studies in multiethnic populations may confirm those Asian-specific POAG genetic associations.

Recently, progress has been made in the identification of genetic variants associated with POAG in populations of African ancestry(25-27). Because individuals of African ancestry have 3-5 times increased POAG risk, and have worse visual field damage and disease progression compared to other populations(28, 29), it is important to elucidate the genetic contribution to POAG pathogenesis to aid identification of high-risk groups. Recent GWAS of glaucoma/POAG in African ancestry populations enabled the identification of genome-wide significant associations at *DNAJC24-ELP4*, *TRIM9-TMX1*, *FAM86A-RBFOX1*, *EXOC4*, and *ENO4* loci, and suggestive associations at *COL21A1-DST* and *MNS1-ZNF280D*(25-27). However, these associations still await validation in external cohorts, with the exception of *TRIM9-TMX1* and *FAM86A-RBFOX1*, which reached nominal significance in African-Americans from GERA(14) and in African-Americans from BioMe(27), respectively. In

addition, recent genetic studies conducted in populations of African ancestry attempted to replicate the associations at loci previously identified in GWAS predominantly conducted in populations of European or Asian ancestry. While some studies could not replicate any of these loci(25, 30), others provided evidence of association at *TXNRD2*, *FNDC3B*, 8q22, *AFAP1*, *TMC01*, or *CDKN2B-AS1* loci(26, 27, 31, 32). Using a “local” replication strategy that considered different linkage disequilibrium (LD) patterns across study populations, Bonnemaier *et al.*(27) replicated in their African cohorts, the POAG-associations at *TXNRD2*, *TMC01*, and *CDKN2B-AS1*, originally identified in European populations(5, 13). This suggests that POAG-susceptibility loci identified in cohorts of European or Asian ancestry may be relevant to populations of African ancestry. Genetic risk scores based on the previously reported and newly discovered POAG-genetic variants have been shown to predict POAG and explain up to 4% of the overall POAG risk in populations of African ancestry(14, 25-27). These findings provide new insight into the genetic architecture of POAG in populations of African ancestry, and future studies investigating the genetic complexity of POAG in these populations would request studies of extremely large size.

In contrast to the recent progress made in populations of African ancestry, the genetics of POAG in Hispanic/Latino populations remain largely unexplored, and to date, no genome-wide significant signal has been detected in Hispanic/Latino populations. Choquet *et al.*(14) examined the associations of the loci previously identified in GWAS of European or Asian populations in their Hispanic/Latino sample, consisting in 411 POAG cases and 4,778 controls. Three loci replicated after correction for multiple testing, including *TMC01*, near *CDKN1A*, and *CDKN2B-AS1*; and two others: *AFAP1* and *ABCA1* were nominally significant. When investigating the associations of the novel POAG-loci identified in this study, a suggestive association between *PDE7B* and POAG risk in Hispanic/Latinos was observed(14). Further, previously known and newly discovered genetic variants from this study explained 3.3% of POAG variance in Hispanic/Latinos from GERA. Future efforts to identify the genetic factors for POAG risk in this ethnicity may aid in understanding of the genetic architecture of POAG.

### **C. Importance of genetic ancestry**

Beyond genomic regions identification, recent genetic studies of POAG have enabled the determination of ancestry and population substructure(14, 33). Because variation in POAG prevalence has been observed across populations of diverse ancestry, especially in African-Americans who have a higher risk for developing POAG, it is important to investigate whether differences in POAG prevalence are due to genetic ancestry. Choquet *et al.*(14) investigated the association between genetic ancestry, as represented by genetic principal components, and the risk of POAG within each of the 4 GERA ethnic groups. A higher risk of POAG was associated with: greater northern (versus southern) East Asian ancestry in the East Asian group; greater Native American (versus European) ancestry in the Hispanic/Latino group; and greater African (versus European) ancestry in the African-American group. These findings suggest that this within-group variation could be due to genetic risk factors that correlate with genetic ancestry. Consistently, a recent study(33) conducted in an African-American cohort, assessed the local genetic ancestry at *CDKN2B-AS1*, an important POAG-associated locus established in

populations of European and Asian ancestry. Interestingly, a significant association was observed between POAG risk and local African genetic ancestry at *CDKN2B-AS1*, and on average, POAG cases were of 90% African descent compared with 58% for controls(33). It is noteworthy that in this study(33), no significant single SNP-POAG associations at this locus was detected after correcting for multiple testing. These findings highlight the importance of considering the variability in linkage disequilibrium patterns across populations and genetic heterogeneity when conducting genetic studies.

#### **D. Genetic loci discovery led to biological pathways discovery**

Although GWAS-identified associations do not directly highlight a specific gene or mechanism, several genetic studies conducted follow-up experiments of candidate genes within identified POAG-genomic regions using animal models and human cell lines. Before 2017, only a few POAG-candidate genes (i.e., *SIX6*, *CDKN2B-AS*, and *CAV1/2*) have been investigated in functional studies(34-37). *SIX6* encodes a homeobox protein and plays an important role in the development of the eye, especially the morphology of the optic nerve and the formation of the retina(38-40). *In vivo* (zebrafish) and *in vitro* assays demonstrated that *SIX6* risk variants attenuated protein function, leading to a reduction in the number of retinal ganglion cells, which are the primary cell type affected in glaucoma, thereby increasing POAG risk(34). Consistently, a *SIX6* risk variant (rs33912345) increased the expression of *CDKN2A* (another well-established POAG- and normal tension glaucoma-locus), resulting in the senescence of retinal ganglion cells in cell line, animal models, and human glaucoma retinas(36). Further, mice homozygous for a deletion in *CDKN2B-AS* are more vulnerable to retinal ganglion cell loss in response to elevated intraocular pressure, compared to wild-type and heterozygous animals(35). Vulnerability to retinal ganglion cell loss manifests by microglial reactivity signs both in the retina and the optic nerve of mutated mice(35), which are early indicators of glaucoma onset or progression(41, 42).

*CAV1*, which is located within the glaucoma susceptibility locus *CAV1/CAV2*, encodes the caveolin 1. *CAV1* has been shown to maintain normal intraocular pressure (IOP) levels by participating to the caveolae formation in the Schlemm's canal and trabecular meshwork, thereby facilitating aqueous humor flow through the eye(37). Similarly, *FMNL2*, a gene in one of the recently discovered POAG-loci, that is also associated with intraocular pressure(43), supports trabecular meshwork function relevant to aqueous humor outflow regulation(14). Indeed, suppression of *FMNL2* expression using small interfering RNAs (siRNAs) caused trabecular meshwork cell morphological modifications, thereby decreasing contractile activity and the assembly of actin stress fibers(14). Investigation of expression profiles of genes associated with both IOP and glaucoma also revealed a high enrichment in the trabecular meshwork compared to other human ocular tissues(17). Functional follow-up experiments have been also conducted for another POAG candidate gene, *LMX1B*(14, 16), previously reported to cause nail-patella syndrome, a rare developmental disorder, with some patients presenting a similar “glaucoma” phenotype associated with structural defects of the eye(44-46). In mouse models of different genetic backgrounds, *Lmx1b* mutations can result in high IOP and glaucomatous nerve damage in eyes without developmental defects(14). This suggests that *LMX1B* acts via elevated IOP to affect glaucoma susceptibility, with some mutations causing a condition in mice that resembles POAG. These findings provide important

functional insights linking genetic susceptibility POAG-loci to the underlying mechanisms of glaucoma pathogenesis.

In addition to *in vivo/vitro* follow-up experiments, *in silico* analyses turned out to be effective to prioritize the causal gene within the identified locus and to discover biological mechanisms underlying POAG disease. These include publicly available tools and datasets providing genomic annotations, epigenetic marks, drug targets, gene expression, and expression quantitative trait locus (eQTL) information(47-53). The interpretation of the non-coding variants that account for the majority of GWAS-identified risk alleles is crucial to understand the biological mechanisms through which these risk variants act. Recent GWAS studies(15-17) performed pathway analyses and identified relevant biological pathways that might be involved in POAG pathogenesis; these include “epidermal growth factor receptor signaling”, “response to fluid shear stress”, “abnormal retina morphology”, and “vascular development”. Functional follow-up experiments in cell lines or animal models may confirm the involvement of these biological pathways and provide underlying mechanisms of glaucoma pathogenesis.

#### **E. Pleiotropy and genetic correlations between POAG, glaucoma endophenotypes, ocular and systematic diseases**

Many of the POAG-associated loci, recently identified by GWAS, are also associated with glaucoma endophenotypes and other ocular conditions. While common variants in POAG-loci(14, 16) *FNDCB3* and *FMNL2* were known to be associated with intraocular pressure(43, 54), common variants in *CDKN2B-AS* and *SIX6*(17) were known to be associated with vertical cup-disc ratio(55). Similarly, while common variation at *LOXL1* POAG-locus(16) has been reported to be associated with exfoliation syndrome/exfoliation glaucoma(56-58), common variation at *MYOF* POAG-locus(15) has been reported to be associated with refractive error(59). In addition, common and rare variants in *C9*, a gene identified to be associated with POAG(15) using a gene-based approach, have been associated with age-related macular degeneration(60-62). These results are consistent with results from a recent study showing significant genetic correlation between POAG and age-related macular degeneration(63).

Genetic correlation analyses, using a technique-cross-trait LD score regression(64), also revealed the relationships between POAG and systemic diseases, including type 2 diabetes and cardiovascular diseases, such as myocardial infarction and ischemic stroke(16). These findings support previous reports, showing that pleiotropy, a term that refers to individual genetic loci that influence the risk of multiple diseases or affect variation in multiple complex traits, is pervasive(1, 3, 64-66).

#### **F. Heritability and variance explained**

Beyond the identification of genomic regions, recent GWAS of POAG have facilitated the quantification of how much of the total additive genetic variation due to segregating variants in the population is tagged by genotyped SNPs(14, 67). This quantification of “array” or “SNP” heritability is informative with respect to the unknown genetic architecture of the disease. A recent phenome-wide heritability study conducted in the UK Biobank(19), based on self-reported disease information, reported an array heritability estimate of 26.0% (s.e.=6.0%) for glaucoma(67). Consistently, Choquet *et al.* estimated an overall heritability of 26.0% (s.e.=1.0%)

for POAG in the GERA non-Hispanic white ethnic group(14). However, our current knowledge of genome-wide significant POAG/glaucoma SNPs explains only ~3% of the genetic contribution to glaucoma susceptibility, suggesting that additional variants remain yet to be discovered(14).

To explain the remaining “missing” heritability of POAG, innovative approaches have been employed. Gharahkhani *et al.*(15) conducted a meta-analysis of genetic data from OAG and its correlated traits (e.g. intraocular pressure, optic disc parameters) to identify new loci. Using this innovative and integrative approach, they found additional loci associated with OAG (i.e. near *MYOF*, *LINC02052*, and *LMO7*) at genome-wide level of significance. They also identified an association with a previously unreported gene, complement factor 9 (C9)(15), using a fast and flexible set-Based Association Test (fastBAT) method(68). This gene would not have been identified in a standard single-variant analysis due to the limited statistical power to detect individual genetic variants with small effect sizes. Similarly, MacGregor *et al.*(17) conducted gene-based association analyses and identified 4 genes that were associated with POAG after multiple testing correction, including *BICC1*, *SLC38A3*, *KALRN*, and, *RELN*. Next-generation sequencing has been recently largely used to identify novel associations between low frequency (or rare) coding variants and disease susceptibility(69). Zhou *et al.*(70) conducted a whole exome sequencing (WGS) analysis on 187 patients with early onset advanced POAG and 103 controls without glaucoma and found enrichment of rare variants in camera-type eye development genes (i.e. *CRYBA4*; *GAS1*; *GJA8*; *HES5*; *MAB21L2*; *NEUROD4*; *NR2E1*; *PAX6*; *RXRA*; *SLC25A25*; *VAX1*). Rare variants identified by WGS may have much greater positive predictive values in terms of clinical application compared to common SNPs identified by GWAS, as GWAS do not necessarily identify the causal variants.

## **2. Genetics of glaucoma-related traits**

### **A. The endophenotype approach to glaucoma gene discovery**

While case-control GWAS remain the definitive method for identifying genetic variants associated with disease, an alternative approach is to examine a heritable quantitative trait related to the disease. Such traits are termed endophenotypes and examples for glaucoma include IOP and vertical cup-disc-ratio (VCDR). There are several potential advantages of an endophenotype approach. Rather than requiring data from many disease cases, data can be leveraged from healthy population samples as it is the variation of the endophenotype across the whole range of health and disease that drives the association signal. This allows many population cohorts to contribute to analyses, even if the studies have a low prevalence of disease, resulting in large sample sizes and power to detect small associations. Statistical power is also increased by analyzing a continuous outcome trait rather than a binary outcome variable. Additionally, examining individual traits may help better characterize how discovered genetic variants contribute to disease (e.g. IOP-increasing versus IOP-independent mechanisms for glaucoma). However, caution is required in inferring disease-relevance of endophenotype associations and further studies examining association with disease are required.

### **B. Genetic associations with IOP**

IOP is the cardinal modifiable risk factor for POAG(71, 72) and is known to be a heritable trait(73). Understanding what causes variation in level of IOP within the normal range may shed light on the mechanisms

that also contribute to high IOP and, in turn, POAG. Earlier work examining genetic associations with IOP has proven successful at identifying glaucoma risk variants(74, 75). Combining data from 35,296 participants of 18 population studies contributing to the International Glaucoma Genetics Consortium (IGGC) led to the identification of 8 genome-wide significant loci for IOP, the majority of which demonstrated significant association with POAG in independent studies(74). However, these loci explained only a very small proportion of IOP variability. Two recent studies with considerably larger sample sizes have identified over 100 more IOP-loci associated loci, demonstrating the importance of a large sample size in GWAS of complex traits to identify small effect associations(43, 76).

The first of these two studies to report was a multi-ethnic GWAS for IOP in 69,756 individuals of the GERA cohort(43). IOP measurements were taken as part of routine clinical care, and only measurements taken prior to any IOP-lowering treatment were considered. For participants with multiple longitudinal IOP measurements, the median value was considered; this approach was demonstrated to be more effective than just considering IOP measured at one random timepoint(43). The GERA analysis replicated the majority of previously reported IOP-associated loci, demonstrating the validity and utility of using opportunistic IOP measurements in a clinical cohort rather than protocolled measurements in a population-based study. Reporting shortly after GERA was a GWAS of IOP in European participants of the UK Biobank study(76). Results from the UK Biobank GWAS were then meta-analysed with IOP GWAS results from the EPIC-Norfolk Eye Study(77) and the aforementioned IGGC study(75); the combined analysis included 139,555 participants(76). An independent group of investigators also conducted a GWAS for IOP in UK Biobank and found similar results(17).

The GERA analysis identified 47 genome-wide significant loci, 40 of which were novel(75), and the UK Biobank analysis identified 112 genome-wide significant loci, 68 of which were novel(76). The scale of this discovery represents a step-change in our knowledge of IOP genetics. The identified loci explained 17% of the variance of IOP in the EPIC-Norfolk Eye Study(76), which is substantial given the variability of IOP caused by diurnal changes and measurement error alone. There is considerable overlap between the GERA and UK Biobank discovered loci. A meta-analysis of the two studies has not been done to date.

Among the significant results were loci at genes previously associated with POAG, but not previously known to influence IOP (*AFAP1*, *TXNRD2*, *ATXN2*)(43, 76). This strongly suggests that genetic variation at these genes mediate their increased POAG risk via raised IOP, rather than via direct effects on retinal ganglion cells. Also among the significant results were four loci previously reported as conferring susceptibility to primary angle-closure glaucoma (*PLEKHA7*, *HGF*, *FERMT2*, *GLIS3*)(43, 76) suggesting that angle-closure mechanisms may contribute to variation in IOP even within the normal range.

One of the most significant novel findings was a locus at the Diacylglycerol Kinase Gamma (*DGKG*) gene ( $P=8.9 \times 10^{-52}$  in UK Biobank meta-analysis). Diacylglycerol is involved in adenosine receptor signalling, which is known to be involved in IOP regulation and is a reported target for IOP-lowering therapy(78). More generally, *DGKG* is involved in lipid metabolism, adding weight to the growing evidence that lipid metabolism is a key component of IOP regulation(4).



There were multiple IOP-associated loci at genes previously associated with Mendelian childhood glaucoma (*FOXC1*, *PITX2*, *LMX1B*, *LTBP2*)(43, 76). Other IOP-associated loci were at genes involved in ocular development (*SIX3*, *ADAMTS18*, *MEIS1*), eye size (*RSPO1*) and iris architecture (*TRAF3IP1*)(76). Furthermore, genes involved in developmental processes in general were significantly enriched in the UK Biobank results(76). These findings suggest common genetic variation may contribute to developmental or anatomical changes that are insufficient to cause glaucoma in childhood but that may lead to a decompensation of IOP in later adult life and potentially POAG.

### **C. Post-trabecular meshwork IOP regulation**

One of the most striking findings from both IOP GWAS studies was evidence for an important role of vascular endothelial processes in IOP regulation. Genes involved in “vascular endothelial cell morphology” were the most significantly enriched gene-set in GERA(43) and genes involved in “angiogenesis” were the most significantly enriched gene-set in the UK Biobank study(76) (both studies used different approaches for examining enrichment). In contrast to the trabecular meshwork (TM) which is comprised of epithelial cells, Schlemm’s canal and collector channels are composed of endothelial cells which have a similar phenotype to lymphatic vessels(79). The major drivers for the vascular endothelial gene-set enrichment were variants in *ANGPT1*, *ANGPT2* and *VEGFC*. *ANGPT1* and *ANGPT2* are primary *TEK* (Receptor Tyrosine Kinase) ligands. Mutations of *TEK* cause primary congenital glaucoma(80). This suggests that, while rare mutations affecting angiotensin-TEK signalling can cause congenital disease, more common genetic variation with less deleterious functional consequence can cause less severe changes and a decompensation of IOP only manifest in later life. *TEK* receptors are highly expressed in Schlemm’s canal(81), and disruption of angiotensin-TEK signalling in mice causes absent Schlemm’s canal development(82). VEGF-C increases VEGFR-3 tyrosine kinase signalling in lymphatic endothelial cells and anterior chamber delivery of VEGF-C in adult mice induced Schlemm’s canal growth and a sustained reduction in IOP(79). Put together, there is clear emerging evidence that post-TM structures (Schlemm’s canal and collector channels) are critical for IOP regulation, challenging the dogma that POAG is primarily a disease of TM. There also appears to be potential for regulators of lymphangiogenesis as targets for glaucoma therapy.

### **D. Association of IOP loci with POAG**

It is important to determine whether genetic variants associated with higher IOP also confer higher risk for POAG. The GERA investigators tested their IOP-associated loci for association with clinically coded POAG in the same GERA cohort; 89% of variants were directionally consistent with IOP-increasing risk alleles having an odds ratio estimate >1 for POAG(43). In the UK Biobank study(76), association of the IOP loci with POAG was examined in 3,853 cases and 33,480 controls from the independent NEIGHBORHOOD study(13). There was a strikingly linear trend between the effect estimates for IOP from UK Biobank and POAG from NEIGHBORHOOD when plotted(76). Additionally, 48 variants were nominally associated with POAG ( $P < 0.05$ ), of which 14 were significant at a Bonferroni-corrected threshold(76).

### **E. Prediction of POAG using IOP-associated loci**

The UK Biobank investigators also examined whether the IOP loci, together with age, sex and three known POAG-associated polymorphisms showing no evidence of association with IOP (at *MYOC*, *SIX6* and *CDKN2B-AS1*), were predictive of POAG in NEIGHBORHOOD using a regression-based model. The results were particularly striking for high-tension POAG with an area under the receiver operating characteristic curve (AUC) of 76%(76). This suggests that genetic markers, measurable at birth, have a substantial ability to predict later life IOP and risk of POAG, opening up the possibility of targeted population screening to aid earlier POAG detection and prevention of sight loss.

The potential for the UK Biobank IOP loci to aid detection of POAG was also examined in 1,734 cases of advanced POAG and 2,938 controls from the Australian and New Zealand Registry of Advanced Glaucoma (ANZRAG)(17). MacGregor and colleagues derived a polygenic risk score based on their identified IOP loci and the *CDKN2B-AS1* and *SIX6* loci. Participants in the top decile of this score were at 5.6 (95% CI 4.1-7.6) times increased odds of advanced POAG compared to participants in the bottom decile(17).

### **F. Genetic associations with central corneal thickness**

While a thinner central corneal thickness (CCT) has been associated with increased POAG incidence(83), progression(84) and conversion from ocular hypertension(85), it remains uncertain whether the relationship is biologically causal, or whether it is driven by corneal artefact influencing IOP measurement. A landmark GWAS for CCT was reported in 2013 and identified 16 genome-wide significant loci; only one of these loci (at *FNDC3B*) was found to be associated with POAG and in an unexpected direction (the CCT-decreasing allele was protective for POAG)(86). More recently, a larger GWAS of over 25,000 European and Asian participants identified 44 loci associated with CCT at genome-wide significance(87). None of these loci were significantly associated with POAG (comparing 5,008 cases with 35,472 controls) after correction for multiple testing(87). Furthermore, there was no significant correlation between the CCT and POAG effect sizes for the CCT-associated variants ( $r=-0.17$ ,  $P=0.2$ ). This is in contrast to the significant correlation identified between CCT and keratoconus effect sizes ( $r=-0.62$ ,  $P=5.3\times 10^{-5}$ )(87). Another GWAS of CCT and other corneal parameters was recently conducted in an Icelandic population; similarly, no significant CCT-associated loci were associated with glaucoma (either POAG or primary angle-closure glaucoma)(88). Put together, the evidence suggests that CCT is not an endophenotype for POAG and supports the hypothesis that the CCT-glaucoma association observed in studies is due to IOP measurement artefact rather than biological causality.

### **G. Genetic associations with optic disc parameters**

Initial glaucoma-related GWAS suggested that VCDR is a good endophenotype for glaucoma. The *CDKN2B-AS1* locus was first reported in a VCDR GWAS(55) before being identified as POAG-associated subsequently(5). Following this, large VCDR GWAS meta-analyses have identified over 50 associated loci, but only 9 of these were associated with POAG(75). This suggests that some of the genetically-driven variation in population VCDR is reflecting non-glaucomatous processes and may instead reflect baseline anatomy, for

example. Similarly, many genome-wide significant loci have been identified for optic disc rim area and cup area, but the majority of these do not demonstrate significant association with POAG(75). To date, there have been no reported genome-wide significant associations with circumpapillary retinal nerve-fibre layer thickness, though a recent study demonstrated a significant association between a known POAG-risk variant in *SIX6* and retinal nerve fibre layer thickness in a European adult population(89).

### **3. The road to personalized glaucoma management**

#### **A. Risk prediction and screening**

Identifying glaucoma disease-related genes makes it possible to use disease associated or causative genetic variants to assess populations at risk. For glaucoma pre-symptomatic screening is particularly important, as patients are unaware of disease-related visual symptoms until later stages when the optic nerve is severely damaged, and treatment is not optimally effective. Current approaches for population screening involving IOP measurement, and/or optic nerve evaluation, are expensive and may only be effective for targeted screening of high risk groups(90). The discovery of genetic variants related to disease risk allows for development of a gene-based screening approach that could identify patients at increased risk.

Substantial progress has been made toward gene-based screening. For patients with disease onset prior to age 50, disease causing mutations in genes known to cause early-onset forms for glaucoma can be detected by DNA sequencing tests(91, 92). Families and patients found to have a mutation in one of these genes can benefit from informed genetic counseling and treatment and surveillance plans tailored to individual disease risk(93). A limitation is the relatively low diagnostic yield (20%) after testing for the genes currently known to cause early-onset glaucoma(92), suggesting that further work in this area would be fruitful.

Genetic screening to detect patients at high risk for adult-onset (after age 50) POAG also appears promising. Recent GWAS for POAG and related traits have successfully identified over 100 loci associated with disease risk have a receiver operator characteristic of up to 76%, suggesting that genetic risk factors can be an effective tool for discriminating POAG cases from controls(76). More recently genetic risk scores have demonstrated that cases with an excess of risk variants have earlier onset of disease(94) and greater risk of disease development(17) compared to individuals with fewer risk alleles. More comprehensive polygenic risk scores comprised of larger numbers of POAG risk alleles may yield sufficiently accurate tests that screening based on genetics alone may be useful.

#### **B. Gene-based therapies**

Glaucoma gene discovery also makes possible the design of novel therapies that target the actual molecular events responsible for disease and recent advances in methods for gene-replacement and CRISPR/cas gene-editing support the feasibility of gene-based therapies for glaucoma in the relatively near future(95-97). Currently there are no FDA approved gene-based methods for glaucoma, however several strategies have been suggested by recent research.

Genetic defects in *MYOC*, coding for Myocilin are known to cause early-onset open-angle glaucoma inherited as a dominant trait. Disease-causing mutations are known to cause protein misfolding and cell toxicity due to endoplasmic reticulum accumulation(98). Recent studies have shown that removing myocilin by CRISPR/cas gene editing can lower IOP in transgenic mice carrying a deleterious *MYOC* mutation(97). This very promising result suggests that CRISPR/cas could be used to target *MYOC* mutations in humans. Similarly, recent studies identifying *TEK* and *ANGPT1* mutations in humans with early-onset glaucoma and due to abnormal Schlemm's canal development and function suggest that restoring TEK signaling function could be therapeutic(80, 99). As *ANGPT1* has been associated with IOP and POAG in humans(14, 76), therapies targeting TEK signaling could be useful for both early-onset and adult-onset disease. Other early-onset glaucoma genes may also be targets for gene-based therapies including *CYP1B1*, known to cause recessive congenital and juvenile onset glaucoma(100), and *PAX6*, responsible for aniridia(101).

Genetic variants associated with POAG appear to impact a number of different biological processes and pathways and several of these could suggest effective therapeutic approaches. Four POAG and IOP loci include genes known to be involved in lipid metabolism (*ABCA1*, *CAV1*, *DGKG*, *ARHGEF12*)(12, 76). Interestingly recent studies support protective effects of statin therapy on glaucoma(102). Collectively, these results suggest that therapies targeting lipid and cholesterol metabolism could be effective treatment strategies, especially in patients with high tension glaucoma. Another potentially interesting therapeutic target is mitochondrial function. *TXNRD2*, thioredoxin reductase 2, codes for a mitochondrial protein required for reducing oxidative stress and maintaining redox homeostasis. *TXNRD2* genomic variants have been associated with both POAG and IOP(13, 76) and in the UK Biobank IOP GWAS 4 other genomic loci related to mitochondrial function were also associated with IOP (*ME3*, *VPS13C*, *GCAT*, *PTCD2*). These results suggest that mitochondrial function can contribute to IOP variation and that maintaining mitochondrial function could help regulate IOP and reduce POAG risk. Further discovery of POAG associated risk variants will identify additional targets for POAG gene-based therapies with effective and even curative potential.

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## **Conflict of Interest**

The authors declare that they have no competing financial interests.

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**Table 1.** Primary open-angle glaucoma (POAG) loci discovered by GWAS

Population	Case/control (N)		New Loci	Reference
	Discovery	Replication		
Iceland	1,263/34,877	2,175/2,064 (European) 299/1607 (Chinese)	CAV1/CAV2	Thorliefsson <i>et al.</i> Nat. Genet. 2010
Australian (ANZRAG)	615/3,956	892/4,582 (Australian)	CDKN2BAS, TMCO1	Burdon <i>et al.</i> Nat. Genet. 2011
European ancestry (NEIGHBOR)	2,170/2,347	976/1,140 (GLAUGEN)	SIX6, 8q22 (NTG)	Wiggs <i>et al.</i> PLoS Genet. 2012
Japanese	1,394/6,599	1,802/7,212	CDKN2BAS, SIX6	Osman <i>et al.</i> Hum. Mol. Genet. 2012
Australian (ANZRAG)	1,155/1,992	3,548/9,496 (Australian and US European)	ABCA1, AFAP1, GMDS	Gharahkhani <i>et al.</i> Nat. Genet. 2014
Chinese	1,007/1,009	1,899/4,965 (Chinese and Singaporean Chinese)	ABCA1, PMM2	Chen <i>et al.</i> Nat. Genet. 2014
African-American and Hispanic (WHI)	1,720/6,067 (African-American WHI)	489/2,685 (Hispanic WHI)	DNAJC24-ELP4, TRIM9-TMX1, FAM86A-RBFOX1	Hoffmann <i>et al.</i> Hum Mol Genet. 2014
Multiethnic	3,504/9,746	9,173/26,780 (multiethnic)	TGFBR3, FNDC3B	Li <i>et al.</i> Hum. Mol. Genet. 2015
European (Rotterdam)	8,105 (population-based)	7,471 population-based 1,225/4,117	ARHGEF12	Springelkamp <i>et al.</i> Hum. Mol. Genet. 2015
European ancestry (NEIGHBORHOOD)	3,853/33,480	3164/9242 (Australian, European, Singaporean Chinese)	TXNRD2, ATXN2, FOXC1, GAS7	Cooke Bailey <i>et al.</i> Nat. Genet. 2016
Australian (ANZRAG)	3,071/6,750	3,853/33,480 US European ancestry (NEIGHBOR)	MYOF/CYP26A1, LINC02052/CRYGS, LMX1B, LMO7	Gharahkhani <i>et al.</i> Sci Rep. 2018
Japanese	7,378/36,385	1,008/591 (East Asians) 5,008/35,472 (Europeans) 2,341/2,037 (Africans)	FNDC3B, ANKRD55-MAP3K1, LMX1B, LHPP, HMGA2, MEIS2, LOXL1	Shiga <i>et al.</i> Hum Mol Genet. 2018
Multiethnic (GERA and UKB)	4,986/58,426 7,329/169,561	7,329/169,561 (UKB) 4,986/58,426 (GERA)	FMNL2, PDE7B, TMTC2, IKZF2, CADM2, DGKG, ANKH, EXOC2, LMX1B, THSD7A, ANGPT1, CTTNBP2/LSM8, BICC1, ELN, TCF12, PLCE1, LMO4/PKN2-AS1, COL11A1, PNPT1, MEIS1, ACOXL, DGKD, RARB, TSC22D2, LPP, BNIP1, PKHD1, TMEM181, FAM120B, SEMA3C/CACNA2D1, PRKAG2, FBXO32, MADD, NEAT1, LINC00540, VPS13C, CASC20, CHEK2	Choquet <i>et al.</i> Nat Commun. 2018
Multiethnic (UKB and ANZRAG)	11,018/126,068	NA	CADM2, THSD7A, ANGPT1, ANKH, EXOC2, BICC1, CTTNBP2, LOC101929614, LOC105378153, MECOM, CFTR, ETS1, LOC107986141, LOC107986142;	MacGregor <i>et al.</i> Nat Genet. 2018
African (GIGA and BioMe)	1,113/1,826	4,588/4,543	EXOC4	Bonnemaijer <i>et al.</i> Hum Genet. 2018
African (ADAGES)	946 advanced POAG/ 1,709	NA	EN04	Taylor <i>et al.</i> Ophthalmology 2019

Adapted and updated from Wiggs and Pasquale, Hum Mol Genet. 2017. Abbreviations: ANZRAG, Australian and New Zealand Registry of Advanced Glaucoma; NEIGHBOR, National Eye Institute Glaucoma Human Genetics Collaboration; GLAUGEN, Glaucoma Genes and Environment; NTG, normal tension glaucoma; NEIGHBORHOOD, National Eye Institute

Glaucoma Human Genetics Collaboration Heritable Overall Operational Database; WHI, Women's Health Initiative; GERA, Genetic Epidemiology Research in Adult Health and Aging; UKB, UK Biobank; GIGA, Genetics In Glaucoma patients from African descent study; ADAGES, African Descent and Glaucoma Evaluation Study. Note: Loci that are underlined still await validation in external cohorts.