




Are we ready for genetic testing for primary open-angle glaucoma?

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Abstract

Following a dramatic reduction in the cost of genotyping technology in recent years, there have been significant advances in the understanding of the genetic basis of glaucoma. Glaucoma patients represent around a quarter of all outpatient activity in the UK hospital eye service and are a huge burden for the National Health Service. A potential benefit of genetic testing is personalised glaucoma management, allowing direction of our limited healthcare resources to the glaucoma patients who most need it. Our review aims to summarise recent discoveries in the field of glaucoma genetics and to discuss their potential clinical utility. While genome-wide association studies have now identified over ten genes associated with primary open-angle glaucoma (POAG), individually, variants in these genes are not predictive of POAG in populations. There are data suggesting some of these POAG variants are associated with conversion from ocular hypertension to POAG and visual field progression among POAG patients. However, these studies have not been replicated yet and such genetic testing is not currently justified in clinical care. In contrast, genetic testing for inherited early-onset disease in relatives of POAG patients with a known genetic mutation is of clear benefit; this can support either regular review to commence early treatment when the disease develops, or discharge from ophthalmology services of relatives who do not carry the mutation. Genetic testing for POAG at a population level is not currently justified.

Introduction

Glaucoma remains the second commonest cause of certifiable visual loss in England and Wales [1]. Given the chronic nature of glaucoma, lifelong follow-up is generally required. Therefore, glaucoma patients form a large proportion of outpatient activity in the UK hospital eye service (an estimated 23% of all follow-up attendances) with over 1 million glaucoma-related visits per year [2, 3]. This represents a huge burden for the National Health Service (NHS) which is likely to grow further given the projected increase in the number of people with glaucoma [4]. Genetic testing offers the promise of personalised glaucoma management and directing limited healthcare resources to the patients that need it most.

There is strong evidence for a genetic contribution to the commonest form of glaucoma, primary open-angle

glaucoma (POAG). One twin study estimated the heritability of POAG to be 13%, though this is a likely underestimate given glaucoma case ascertainment was via prescribing registries and that a considerable proportion of glaucoma in a population is undiagnosed [5]. First-degree relatives of POAG patients were shown to have a 9-fold increased risk of developing glaucoma in their lifetime compared to relatives of controls in the population-based Rotterdam Study [6]. Most convincingly, with the advent of affordable high-throughput DNA genotyping, there have now been multiple genes identified as contributing to susceptibility for POAG [7].

What is the future potential of genetic testing in the management of POAG? For patients already diagnosed with POAG, genetic testing may offer prognostic information, which may guide the intensity of their treatment and follow-up strategy. Genetic testing may also guide which treatments are most suitable for individual patients, predicting the most efficacious treatment and the treatment least likely to induce side effects. Within families with hereditary glaucoma, identifying the genetic cause will allow testing of offspring to determine who requires close monitoring and early treatment. The potential benefits are clear to see, but is our knowledge sufficient or our tools accurate and affordable enough that we are now ready for

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genetic testing in glaucoma management? Our review aims to answer these questions while giving a conceptual overview of POAG genetics and an update on recent advances in the field. The role of genetic testing in congenital glaucomas [8] is established and beyond the scope of this review.

Search strategy

We conducted the following Medline search: 'Genetic Testing' [Mesh] AND 'Glaucoma' [Mesh]. We further considered studies that were referenced in the articles identified in the initial search.

Mendelian versus complex disease

Mendelian disorders are conceptually the simplest demonstration of how genes can be responsible for disease. A single genetic defect alone causes a disease and if this is passed on by parents, their children will potentially inherit the disease. Common forms of inheritance of Mendelian disorders include autosomal dominant, autosomal recessive and X-linked recessive. If the genetic defect responsible for the disease in the family is identified, it is possible to screen offspring to determine their risk of disease and potentially take preventative action. For example, Angelina Jolie famously underwent bilateral mastectomy to prevent breast cancer knowing she had inherited the *BRCA1* gene mutation that had caused breast cancer in her family [9].

A complex disease is generally not caused by a single genetic defect; multiple genetic and/or environmental factors combine to collectively result in disease. Conceptually, it can be considered that each individual risk factor is insufficient to cause disease on its own and that each risk factor may not be present in all cases of disease (Fig. 1a). The fact that the risk factor may not be present in all cases and yet present in some controls makes identifying each individual risk factor challenging in complex disease. Large sample sizes are required to provide adequate power to identify each risk factor. An alternative way of conceptualising complex disease is shown in Fig. 1b–d. It may be that a single genetic factor is sufficient to cause disease, and that another single genetic factor is also sufficient to cause the disease, and these two different 'flavours' of the disease are indistinguishable or have not been separated during analyses. Again, in this situation also, important risk factors may not be present in all cases of the disease, posing a challenge for their identification. Similarly, large sample sizes can help identify each risk factor. Additionally, in this conceptual model, accurate phenotyping and separating

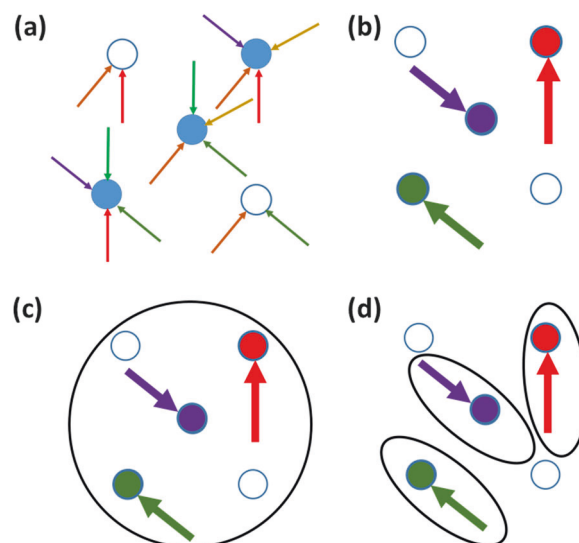


Fig. 1 Conceptual diagrams for complex disease. Each circle represents an individual person; filled in circles are people affected by disease and hollow circles are unaffected controls. **a** and **b–d** are two different concepts for complex disease. First concept: **a** The arrows are risk factors (genetic or environmental); different colours represent different risk factors. It can be seen that none of the risk factors are present in all of the cases, and some of the risk factors that contribute to disease are present in controls. Second concept: **b** In this concept, each individual risk factor is sufficient to cause the disease. The different colours represent different subsets of disease which may or may not be clinically distinguishable. **c** If all cases are examined together, identifying each risk factor can be challenging as they are present only in subset of cases. **d** If the cases are subdivided in a biologically meaningful way, this can increase the power to identify risk factors despite the smaller sample size

cases into biologically meaningful subgroups can help improve power for detection of risk factors.

Genetic mutations versus genetic variants

As stated above, a Mendelian disease is caused by a single genetic alteration which is usually rare and is alone sufficient to cause a gene to malfunction and result in disease. Such genetic alterations are termed 'mutations'. 'Variants', on the other hand, are points in the genome (DNA code) at which we vary from one another. The human genome is >3 billion nucleotides long, but we vary at <1% of these. The commonest form of variation is a nucleotide substitution at a single point in the genome and this is referred to as a single-nucleotide polymorphism (SNP). A genetic variant alone does not cause disease, but may be associated with disease. Possessing a variant may increase or decrease the risk of disease, but alone is insufficient to cause disease and is unlikely to be predictive of who will develop disease (*cf* arrows in Fig. 1a). Complex diseases may have many associated genetic variants. It is the cumulative contribution

of these associations, or potentially interactions between them, that ultimately result in disease (Fig. 1a).

Approaches to discovering genes that contribute to POAG

Identifying a new gene for POAG in a hypothesis-independent manner requires methodology that looks for association across the whole genome. Until recently, it was not feasible to examine all independently inherited SNPs genome-wide. However, this was not necessary if examining genetic factors that segregate with disease in large families with inherited POAG. This approach is called linkage analysis and requires only around 400 markers to cover the whole genome. Linkage studies have identified several genes associated with glaucoma, such as myocilin (*MYOC*) [10], optineurin (*OPTN*) [11] and WD repeat domain 36 (*WDR36*) [12]. Mutations in these genes have been reported to cause autosomal dominant Mendelian POAG in the studied families. Further details on the roles of these genes in POAG have been previously reviewed [7, 13, 14]. While a mutation in one of these genes may completely explain the development of POAG in some families, collectively, mutations in these genes contribute to only around 6% of POAG cases in the general population [15–17]. More recently, family studies have identified TANK binding kinase 1 (*TBK1*) as another cause of Mendelian POAG [18]. Rather than a mutation within the gene, it is duplication of the gene and the resultant increase in function that appears to be causing the glaucomatous process.

The cost of genome-wide genotyping has fallen dramatically in recent years, at a rate much faster than Moore's Law. This has resulted in affordable high-throughput technologies that can measure all common independently inherited genetic variation across the whole genome in individuals. Therefore, it has become possible to investigate genetic associations with POAG, hypothesis independent and genome-wide, without the need for families. Instead, unrelated POAG cases are collected and compared with unrelated controls at several million genetic markers (some directly measured and others imputed based on reference data). This approach is called a genome-wide association study (GWAS). GWAS identifies common variants (with a frequency of over 5% in the general population) associated with disease. Given the number of genetic variants examined, there is a multiple testing statistical issue. For this reason, associations are only considered significant and valid if the *P*-value is very small (a 'hit' is considered to be 'genome-wide significant' if $P < 5 \times 10^{-8}$) and there is evidence for the same association in an independent cohort. The first glaucoma GWAS discovery was the *LOXLI* locus for exfoliation

glaucoma [19]. The first replicated GWAS discovery for POAG was in an Icelandic population which identified a significant locus near *CAVI* and *CAV2* (both of which are expressed in retinal ganglion cells and trabecular meshwork) [20]. Further GWAS of European populations have identified other significant POAG loci in discovery cohorts from the United States [21, 22] (near or at *SIX1/SIX6*, *TXNRD2*, *ATXN2* and *FOXC1*) and Australia [23, 24] (*TMCO1*, *CDKN2B-AS1*, *ABCA1*, *AFAP1* and *GMD5*). A POAG GWAS in people of Chinese descent identified a significant locus in *PMM2* [25]. Despite these identified variants being common, the effect of each one is small, and collectively they explain only a small fraction (<5%) of POAG heritability. It is anticipated many more loci will be identified as the statistical power improves with a larger sample of POAG cases. Following the initial *LOXLI* locus discovery, the combination of cases from a large international consortium has identified further exfoliation glaucoma loci at *CACNA1A* [26], *POMP*, *TMEM136*, *AGPAT1*, *RBMS3* and *SEMA6A* [27]. There has also been some GWAS success for primary angle-closure glaucoma, with eight genetic loci identified to date (near or at *PLEKHA7*, *COL11A1*, *EPDR1*, *PCMTD1-ST18EPDR1*, *CHAT*, *GLIS3*, *FERMT2* and *DPM2-FAM102A*) [28, 29].

There have also been multiple GWAS hits for heritable quantitative traits related to glaucoma (endophenotypes), such as intraocular pressure (IOP), and optic cup-disc ratio (CDR). A large IOP GWAS from the International Glaucoma Genetics Consortium (IGGC) identified *GAS7* as a significant locus for both IOP and POAG [30]. There have been over 40 genetic loci identified for CDR in the largest published GWAS meta-analysis from the IGGC [31]. However, it remains unclear what role these loci have in disease, as the majority do not demonstrate association with POAG when tested in the available cohorts. It is possible these variants are related to developmental processes and associated with optic disc anatomy and not the pathological glaucomatous cupping process. Alternatively, these variants are associated with POAG aetiological processes, but there is insufficient power in the currently available POAG case-control datasets to confirm the associations.

Evidence for clinical utility of genetic testing in POAG

Learning which genes contribute to POAG can inform us about previously unknown biological pathways that are important in disease aetiology and progression. In the longer term, these discoveries can prompt further research into these pathways and potentially lead to new treatments.

In the shorter term, it is possible that genetic markers are of predictive value and can help personalise glaucoma management.

Diagnosis in hereditary POAG

There are situations when genetic testing can be helpful for managing families with inherited POAG. For example, a young member of a family with severe, early onset, autosomal dominant POAG may benefit from knowing their likelihood of developing the disease [32]. If the mutation causing POAG in that family is identified (e.g., by testing for myocilin mutations in affected family members), then the individual concerned can be tested for that mutation. If they do not carry the myocilin mutation, then their risk of developing POAG will be similar to the risk in the general population, and this would allow discharge from routine ophthalmic examinations [32]. Such information may even inform life choices such as occupation, especially if the disease is of early onset. Conversely, if they do carry the mutation, this would warrant regular follow-up for early signs of raised IOP and permit early treatment.

More general screening of relatives for an identified disease-causing mutation is termed cascade genetic testing. There is some evidence that early diagnosis and treatment of myocilin-related POAG following cascade genetic testing may result in a better clinical outcome. In a retrospective study, glaucoma severity parameters were compared between patients who were identified by cascade genetic testing (Genetic cases) and patients who presented through normal clinical pathways and were subsequently found to have a myocilin mutation (Clinical cases) [33]. Clinical cases had significantly higher maximum IOP, larger CDR and worse visual field mean deviation than Genetic cases [33].

It has been suggested there may be benefit in screening patients with advanced POAG for myocilin mutations if they meet certain criteria (young age of onset, high maximum IOP and strong family history) [34]. The prevalence of myocilin mutations in this phenotypically selected group ranged from 16% to 40% depending on the cutoff thresholds. Identification of a myocilin mutation could then prompt cascade genetic testing and early treatment of family members at high risk [34].

Deciding whether to test patients or family members for myocilin mutations may not be straight-forward and genetic counselling should be offered [35]. This may involve referral to a clinical genetics service. Information provided should include details about the condition and its prognosis, its inheritance pattern, and risk to children or other family members. Counselling for at-risk but currently unaffected family members should explore the underlying motivation

for genetic testing, and explain the testing process and potential impact of the test result [35]. Accredited testing for myocilin mutations is currently available to NHS clinicians via the UK Genetic Testing Network [36]. At the time of writing, sequencing the entire myocilin gene to look for any mutation cost £305, whereas testing for one known mutation in a family member cost £180 [36]. There is currently regional variation on whether commissioners will cover the cost of myocilin genetic testing.

Predicting conversion from ocular hypertension (OHT) to POAG

A subset of participants of the Ocular Hypertension Treatment Study (OHTS) were genotyped for variants previously associated with POAG and these variants tested for association with subsequent conversion from OHT to POAG [37]. Among the largest ethnic group in cohort, non-Hispanic Whites, a SNP in *TMCO1* was significantly associated with the development of POAG. *TMCO1* has been strongly associated with IOP [30, 38] and it is assumed that the variant mediates its increased risk of POAG by raised IOP. Remarkably, the association between the *TMCO1* variant and POAG conversion remained highly significant even after adjustment for all parameters in the previously published risk calculator [39], including baseline IOP; the hazard ratio was 1.7 per risk allele (95% confidence interval 1.3–2.3, $P = 0.0004$) [37]. This equates to a 3-fold increased risk of POAG in people with two risk alleles compared to people with no risk alleles, an effect size that is comparable to other established risk factors such as age. It is perhaps surprising that the *TMCO1* effect remains significant even after adjustment for a direct measurement of IOP. This suggests that the *TMCO1* variant provides information regarding the cumulative level of true IOP over and above that provided by a single measurement at baseline. While this is an exciting finding that offers hope for the potential of genetic testing in the management of OHT, replication of this finding in an independent study would provide stronger evidence. It should also be noted that there was no discernible association between the *TMCO1* variant and conversion to POAG in the Black subgroup [37].

Predicting progression of POAG

Examining risk factors for susceptibility to progression of POAG in treated cohorts is challenging, not least because intensity of treatment is difficult to quantify and account for. A study of 469 Singaporean Chinese POAG patients with 5 or more visual fields showed that only one of ten POAG loci tested was associated with visual field progression

(ascertained by pointwise linear regression criteria) [40]. This locus was in the *TGFBR3-CDC7* region and was associated with a 6.7 (95% CI 1.9–23.7, $P = 0.003$) times increased chance of visual field progression. The wide confidence interval suggests uncertainty of this effect estimate and there is a possibility this is a chance finding. Replication in an independent cohort is required before firm conclusions can be made. Unfortunately, data for the *TMCO1* variant that was examined in OHTS were not available for this study.

Predicting response to treatment

There is good evidence that, in general, there may be a genetic basis for effectiveness of treatment in different individuals, as well as for the development of side effects for treatment [41]. However, pharmacogenomic studies for glaucoma treatments have been small and with conflicting results. For example, variants in the prostaglandin *F2 α* gene have been associated with response to prostaglandin analogues in Japanese studies [42, 43] but not in a North American study [44]. A variant in *ADRB2* has been associated with response to timolol drops, but this finding remains unreplicated [45]. Currently, there is no convincing evidence for genetic testing to support the choice of treatment for POAG.

Targeted therapy for Mendelian POAG

Identifying the disease-causing mutation in Mendelian POAG offers the potential for targeted therapy to fix the specific molecular defect caused by the mutation. It has been suggested that myocilin mutations result in misfolded MYOC protein accumulating in trabecular meshwork cells resulting in an adverse effect [46]. Phenylbutyrate, a chemical chaperone that aids proteins folding into their correct conformations, appears to cure myocilin-caused glaucoma in transgenic mice when administered orally or as an eye-drop [46, 47]. While phenylbutyrate has not been tested in humans, this may serve as a proof of concept for targeting treatment to the underlying pathology caused by a specific genetic defect.

More recently, clustered regularly interspaced short palindromic repeats (CRISPR)-mediated genome editing was used to disrupt the mutant myocilin gene in a mouse model, resulting in reduced endoplasmic reticulum stress, lower IOP, and prevention of further glaucomatous damage [48]. Additionally, the investigators demonstrated the potential feasibility of human genome editing in the eye using an ex vivo human organ culture system [48].

Conclusions

The pace of new genetic discoveries for glaucoma has increased significantly in recent years due to the exponential drop in cost of high-throughput genome-wide genotyping platforms. While there is some evidence supporting the clinical utility of this new knowledge, such as *TMCO1* variation being predictive of conversion from OHT to POAG, such studies are small and not replicated to date. Genetic testing for glaucoma is clearly helpful in some specific situations, such as screening of family members in autosomal dominant POAG of early onset. POAG pharmacogenomics is an understudied area that warrants further work in the GWAS-era. However, genetic testing for POAG at a population level is not currently justified. We look forward to further genetic discoveries for glaucoma as statistical power increases, from large cohorts such as the UK Biobank and from global collaborations such as the IGGC. Time will tell if these discoveries will help us manage our patients better, or at least help direct resources to those who need them most.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

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