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REVIEW

Genes, pathways, and animal models in primary open-angle glaucoma

AI Iglesias[1,2], H Springelkamp[1,3], WD Ramdas[3], CCW Klaver[1,3], R Willemsen[2] and CM van Duijn[1]

Abstract

Glaucoma is an optic neuropathy characterized by loss of retinal ganglion cells (RGCs) and consequently visual field loss. It is a complex and heterogeneous disease in which both environmental and genetic factors play a role. With the advent of genome-wide association studies (GWASs), the number of loci associated with primary open-angle glaucoma (POAG) have increased greatly. There has also been major progress in understanding the genes determining the vertical cup–disc ratio (VCDR), disc area (DA), cup area (CA), intraocular pressure (IOP), and central corneal thickness (CCT). In this review, we will update and summarize the genetic loci associated so far with POAG, VCDR, DA, CA, IOP, and CCT. We will describe the pathways revealed and supported by genetic association studies, integrating current knowledge from human and experimental data. Finally, we will discuss approaches for functional genomics and clinical translation.

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Introduction

Glaucoma is a complex, heterogeneous disease and the leading cause of irreversible blindness worldwide. In 2013, ~64.3 million people were affected by glaucoma, and it is expected that 76 million will be affected in 2020.[1] Primary open-angle glaucoma (POAG), the most common form of glaucoma in all populations, is characterized by visual field loss because of progressive death of retinal ganglion cells (RGCs). The known risk factors for POAG include a higher age, African ancestry, high intraocular pressure (IOP), decreased central corneal thickness (CCT), high myopia, and a positive family history for glaucoma.[2–4] There is growing evidence that diabetes mellitus and hypertension, both components of the metabolic syndrome, may be potential risk factors.[2]

The genetic contribution to POAG has long been recognized. First-degree relatives of glaucoma patients are estimated to have a 10-fold increased risk of POAG compared with the general population.[5] Various disease-causing mutations in *optineurin* (*OPTN*), *myocilin* (*MYOC*), and *WD-repeat domain 36* (*WDR36*) genes have been long identified as the cause of familial forms of POAG.[6] In the past decade, genome-wide association studies (GWASs, see Figure 1 for details) have successfully identified over 70 single-nucleotide polymorphism (SNPs) associated with POAG and related quantitative traits or endophenotypes. The description of the candidate genes tagged by these SNPs is shown in Supplementary Table 1.

Endophenotypes are measurable traits with a strong genetic component that are related to a disease, POAG in this case (see Figure 2). Complex diseases, such as POAG, are generally linked to various (in)dependent endophenotypes and vice versa; one endophenotype can be related to multiple other diseases apart from POAG. Endophenotypes for POAG include IOP, CCT, cup area (CA), vertical cup–disc ratio (VCDR), and disc area (DA). The genetic component of endophenotypes is usually quantified by the heritability, a proportion that ranges from 0 (no genetic effect) to 1 (a phenotype that is completely determined by genes). The heritability of POAG endophenotypes is very high: 0.55 for IOP, 0.85 for CCT, 0.72 for DA, 0.75 for CA, and between 0.48 and 0.66 for VCDR.[7]

Here we review the genes that GWASs have brought to surface in the context of the question to what extent GWASs have improved the understanding of the specific pathways and biological processes implicated in the pathophysiology of POAG. This review aims to integrate current knowledge from human and experimental data in POAG. First, we will provide an update of the loci associated with POAG and its endophenotypes and then we will

[1]Department of Epidemiology, Erasmus University Medical Center, Rotterdam, The Netherlands

[2]Department of Clinical Genetics, Erasmus University Medical Center, Rotterdam, The Netherlands

[3]Department of Ophthalmology, Erasmus University Medical Center, Rotterdam, The Netherlands

Correspondence:
CM van Duijn, Department of Epidemiology, Erasmus Medical Center, PO Box 2040, 3000 CA Rotterdam, The Netherlands
Tel: +31 10 7043 394;
Fax: +31 10 7044 657.
E-mail: c.vanduijn@erasmusmc.nl

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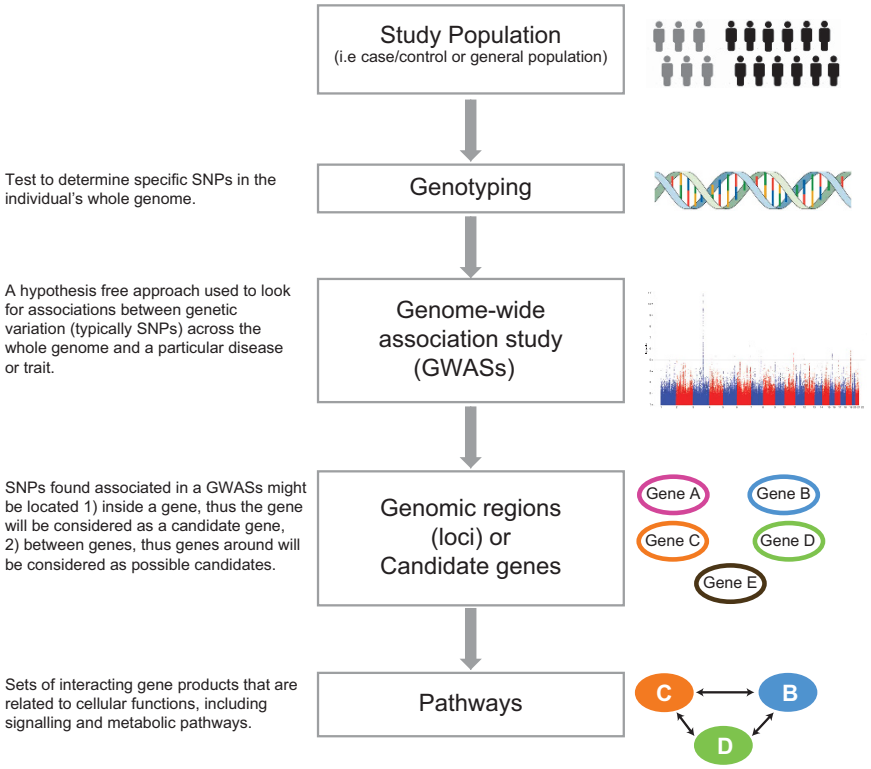

Figure 1 Schematic representation of the stages involved in genome-wide association studies (GWASs). GWASs are based on either a case/control or a population-based design in which single-nucleotide polymorphisms (SNPs) across the whole genome are genotyped. Outcome of GWASs are associated SNPs that are tagging candidate genes or genomic regions (loci). Identified genes may lead to identification of pathways involved in the disease.

describe the molecular pathways and biological functions pointed by genetic association studies. Furthermore, we will evaluate the role of animal models for this disease in the era of functional genomics. Finally, we will discuss the potential uses of GWASs findings in the clinical practice.

Search strategy

We searched the MEDLINE/PubMed database for articles from December 2009 to March 2015. Search terms covered the keywords: 'glaucoma', 'genetic association studies', 'single nucleotide polymorphism', 'animal models', and 'pathway'. A manual search was also based on references from retrieved articles.

Rare and common variants associated with POAG

Linkage studies have identified at least 20 genomic regions linked to POAG. In three of these genomic regions, the causal genes have been identified: *MYOC*,[8] *OPTN*,[9] and *WDR36*.[10] In addition, copy number variations in *TBK1* gene were linked to normal-tension glaucoma (NTG) in an African-American pedigree[11] that was confirmed in Caucasians and Asians populations.[12–14] Mutations in *MYOC*, *OPTN*, and *WDR36* account for <10% of POAG cases,[15] and copy number variations in *TBK1* explained 0.4 to 1.3% of NTG cases.[12] POAG is a complex disease in which multiple genetic variants with small effects and environmental factors may play a role. GWASs is a powerful approach to identify common SNPs or genetic variants with modest effects, and have consistently identified 13 genetic regions as glaucoma susceptibility loci: *ATOH7*, *CAV1/CAV2*, *CDKN2B/CDKN2B-AS1*, *GAS7*, *SIX6*, *TMCO1*, a regulatory region at 8q22, and recently *ABCA1*, *AFAP1*, *ARHGEF12*, *CDC7/TGFβR3*, *GMDS*, and *PMM2* were added to the list.[16–25] In addition to SNPs, copy number variants (ie, submicroscopic chromosomal deletions and duplications) have been shown to play a role in POAG. Besides copy number variations in *TBK1*, genomic duplications (in *CDKN2B-AS1*, near *GAS7* and *TMCO1)* and deletions (in *SIX6* and *ATOH7*) have been reported in POAG cases. In Figure 3 the genes associated with POAG in general, NTG, and high-tension glaucoma (HTG) are shown.

Common variants associated with POAG endophenotypes

Research of endophenotypes has been a successful strategy to elucidate the genetics of POAG.

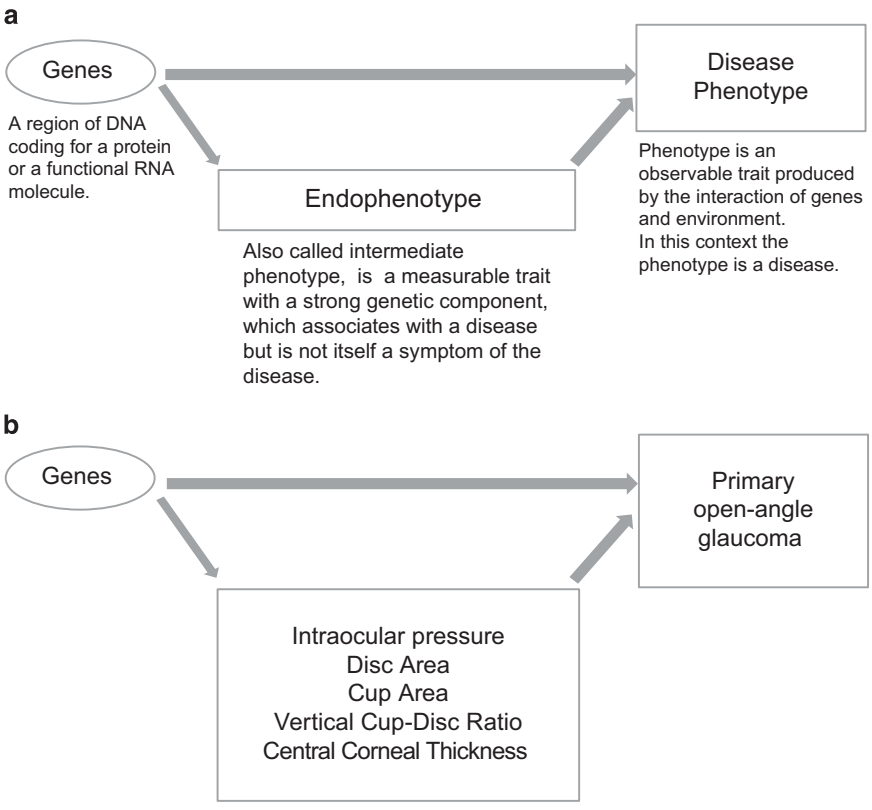

Figure 2 Endophenotype. (a) Definition and relation of endophenotype, phenotype, and gene. (b) Use of endophenotype in the context of primary open-angle glaucoma.

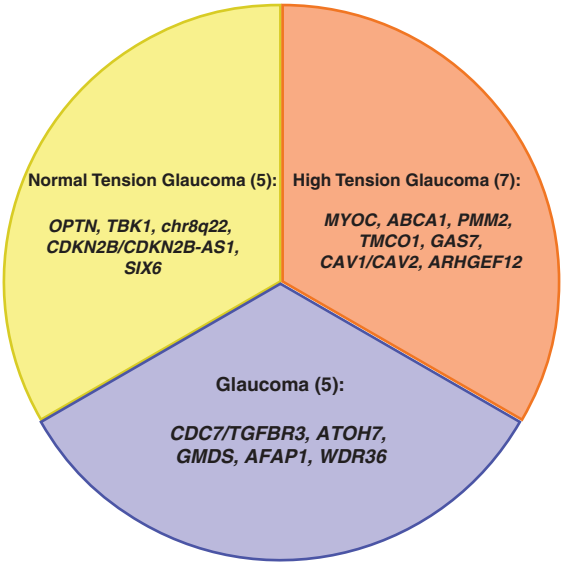

Figure 3 Genes implicated in POAG. The pie diagram illustrates the number of loci that show association with POAG including the familial forms. Three big groups are defined based on whether loci show association with normal-tension glaucoma, high-tension glaucoma, or POAG in general.

The endophenotype strategy allows individuals to be ranked along the continuum of risk as opposed to ranking individuals as patients and controls. Selection of controls in a late-onset disease like POAG can be difficult because of the fact that any control may later become a case. Figure 4 shows the variants identified so far for optic disc parameters (VCDR, CA, and DA), IOP, and CCT in GWASs.

Optic nerve parameters

A total of 38 loci have been found associated with optic disc parameters. Of these, 18 loci have been found for VCDR: *ADAMTS8, ATOH7, BMP2, CDC7/TGFβR3, CARD10, CDKN2B/CDKN2B-AS1, CHEK2, COL8A1, DUSP1, EXOC2, HSF2, PLCE1, RPAP3, SSSCA1, SIX1/6, SALL1, TMTC2,* and *RERE*.[26–28] For DA, 14 loci have been identified, including 5 loci also associated with VCDR (*ATOH7, CARD10, CDC7/TGFβR3, SALL1,* and *TMTC2*) and 9 exclusively DA loci: *ABI3BP, CDC42BPA, DCAF4L2, F5, DIRC3, ELP4, HORMAD2, NR2F2,* and *RARB*.[26,29,30] Finally, for CA, 22 loci have been found, including 11 loci overlapping with VCDR. VCDR and CA are two highly correlated endophenotypes ($r = 0.78$),[30] and thus the large overlap between genetic variants is

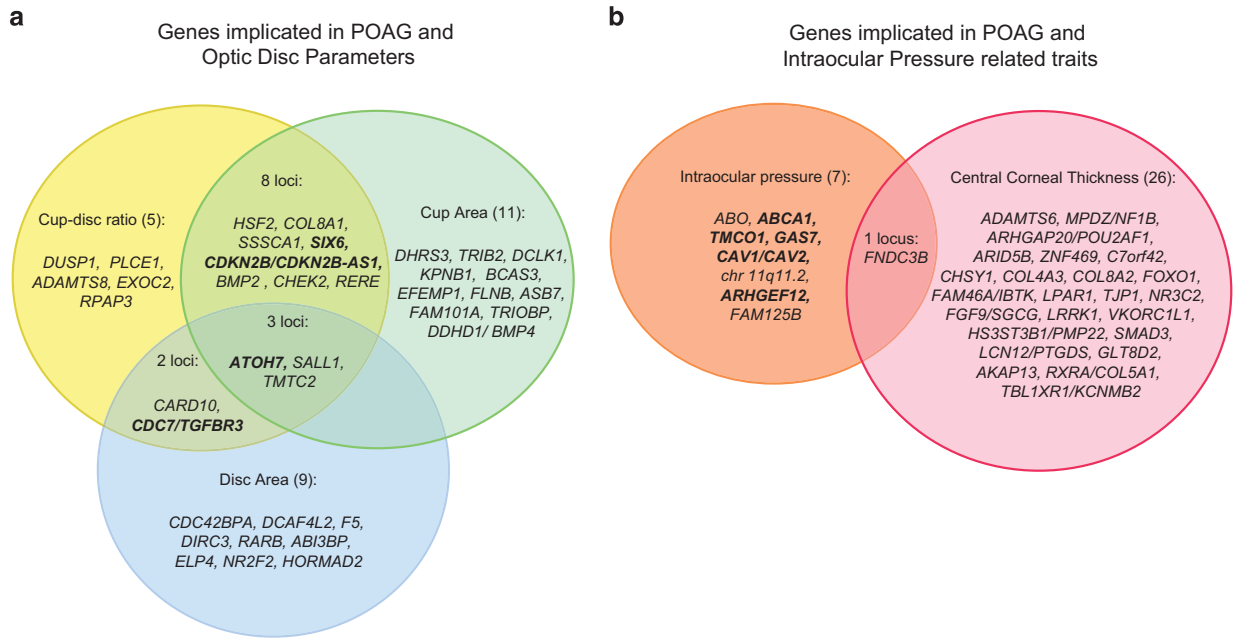

Figure 4 Genes implicated in glaucoma and endophenotypes. The Venn diagram illustrates the number of loci that show association with (a) optic disc parameters and (b) intraocular pressure and central corneal thickness. The number of loci associated with only one trait is mentioned in parentheses after the trait name, and genes names are listed below. Loci that show association with two or more traits are shown in the appropriate segment. Genes in bold have also been associated with POAG.

expected (see Figure 4a). In the case of VCDR, there are 5 loci associated with CA that have not reached yet genome-wide significance. For CA there are 11 loci not shared with VCDR: *ASB7*, *BCAS3*, *DCLK1*, *DDHD1/BMP4*, *DHRS3*, *EFEMP1*, *FAM101*, *FLNB*, *KPNB1*, *TRIB2*, and *TRIOBP*[30] (see Figure 4a). These CA-associated loci are not genome-wide significant yet with VCDR that may be attributable to a lack of power. Only larger study sizes can overcome this.

Among the optic nerve parameters (VCDR, CA, and DA) there are three overlapping loci, shown in Figure 4a. These loci include *ATOH7*, *SALL1*, and *TMTC2*. The first one is a key player in the differentiation of RGCs, and long known for its role in the determination of the disc size rather than the cup size or VCDR.[29,31] *SALL1* is involved in development[32] and *TMTC2* is implicated in calcium homeostasis in the endoplasmic reticulum.[33]

IOP and CCT

So far, nine loci have been found associated with IOP: *TMCO1*, *FNDC3B*, *CAV1/CAV2*, *ABCA1*, *ABO*, *ARHGEF12*, *FAM125B*, *GAS7*, and a region of ~3 Mb at 11p11.2 containing multiple genes[21,34–36] (Figure 4b). Variants in *TMCO1*, *CAV1/CAV2*, and *ABCA1* were found first in POAG studies and subsequently associated with IOP.[16,17,19,20] CCT is a risk factor for POAG in individuals with high IOP, and over 26 loci have been identified.[37] So far, the genetic overlap in loci involved in IOP and CCT is limited. Only one gene is overlapping with IOP (ie, *FNDC3B*) that regulates cell motility and appears to activate the TGF-β pathway in cancer.[38]

From endophenotypes to glaucoma

Analyses of quantitative traits in glaucoma have proven to be fruitful: seven loci found in association studies of endophenotypes showed a significant genome-wide association with POAG. Of these, four were found in association studies of optic disc parameters *ATOH7*, *CDC7/TGFβR3*, *CDKN2B/CDKN2B-AS1*, and *SIX6*,[26,28–30] representing 10.5% of the loci identified for optic disc parameters by GWASs. Although the overall number of genetic variants involved in IOP is much lower ($n=9$), 3 out of the 9 (33.3%) are also involved in POAG: *ABCA1*, *ARHGEF12*, and *GAS7*.[21,34,35] There is no evidence to date that GWASs has reached its limits. Expanding the sample size virtually always leads to identification of new variants. Furthermore, improvements in sequencing technologies and the emerging of expanded reference panels as 1000 genomes is expected to increase the number of variants in the coming years.

Pathways and biological mechanisms revealed by GWASs

Genetic variants identified through GWASs opened new perspectives in the understanding of known potential

pathways involved in the pathogenesis of POAG. In a recent review, four pathways were described on the basis of genes discovered up to 2013:[39] (1) extracellular matrix metabolism (ECM), (2) transforming growth factor-β (TGF-β) signalling, (3) tumour necrosis factor α (TNF-α) signalling, and (4) oestrogen metabolism (here integrated with 'vascular tone pathway'). In the next section, we will discuss how the new discovered genes contribute to these and other pathways.

Extracellular matrix

The ECM, a key component of multicellular organisms, is mainly composed of proteoglycans and fibrous proteins such as collagens, elastins, and fibronectins. It provides structural support for cells and participates in the regulation of many cellular functions.[40] The ECM is a highly dynamic structure that undergoes controlled remodelling in response to environmental stimuli or physiological challenges, such as elevated IOP.[40] Changes in the ECM composition and turnover are speculated to play an important role in the outflow resistance in the trabecular meshwork (TM) that leads to high IOP and to the fibrotic phenotype observed at the optic nerve head, particularly in the lamina cribrosa region, of glaucomatous eyes.[40,41]

There are no genetic variants genome-wide significantly associated with POAG linked directly to the ECM. However, various genetic variants in genes encoding collagens, key players in the ECM metabolism, are genome-wide significantly associated with the endophenotypes; *COL4A3*, *COL5A1*, and *COL8A2* have been found associated with CCT[37] and variants in *COL8A1* with CA and VCDR.[28,30] Mutations in different members of the ADAMTS proteins, involved in ECM remodelling, have been found in human and animal studies. Mutations in *ADAMTS10* were found in a dog model of POAG, whereas mutations in *ADAMTS10* and *ADAMTS17* cause glaucoma, myopia, and ectopic lentis in humans.[42] Genetic variants in *ADAMTS8* have been associated with VCDR, whereas variants in *ADAMTS6* have been associated with CCT,[37] supporting a role of ECM in both IOP regulation and optic nerve degeneration.

Transforming growth factor-β

The TGF-β and its signalling effectors have been previously implicated in glaucoma.[43,44] Of the three TGF-β isoforms (TGF-β1, TGF-β2, and TGF-β3), TGF-β2 is the predominant form in the eye.[45,46] Analyses of the aqueous humour (AH) of glaucoma patients have consistently found increased levels of TGF-β2 in both AH and TM,[47] leading to an increase in ECM protein deposition in the TM and a rise in IOP.[48] Furthermore, elevation of TGF-β2 has also been found in the optic nerve head of glaucoma patients[49] that may induce ECM changes in the lamina cribrosa that cause deprivation of the neurotrophic factors required for the maintenance of ganglion cells.[50] Bone morphogenetic proteins (BMPs) represent a subfamily of the TGF-β superfamily. It has been shown that increased levels of TGF-β2 inhibits BMP4 and/or BMP7, counterbalancing the effect of TGF-β2.[51]

Genetic variants in TGF-β have not been associated with POAG yet. Instead, variants near to *TGF-βR3* have been found associated with VCDR, DA, and POAG in GWASs. *TGF-βR3* is a coreceptor for the TGF-β superfamily; it binds to all three TGF-β isoforms and is considered necessary for TGF-β2 signal transduction.[43,52] Furthermore, two members of the BMP subfamily have been implicated into POAG endophenotypes: *BMP2* (VCDR and CA) and *BMP4* (CA). The *BMP4* gene plays a role in optic cup morphogenesis, and is essential for lens formation. In humans, deletions of chromosome 14q including *BMP4* have been identified in patients with anophthalmia. Furthermore, *CDKN2B*, one of the first genes implicated in POAG and VCDR/CA by GWASs, is induced by TGF-β.[53]

TNF-α and ubiquitination

The TNF-α signalling pathway has been implicated as mediator of neurodegeneration in POAG. Increased levels of TNF-α have been found in the retina and optic nerve head of glaucomatous eyes.[54] Two genes involved in POAG, *OPTN* and *TBK1*, are activated in response to TNF-α. Up until now, no other genetic variants implicated in TNF-α signalling have been associated with POAG. Of interest, these two genes play a key role in amyotrophic lateral sclerosis (ALS), in which the role of *OPTN* was long recognized but for which rare mutations in *TBK1* were recently identified.[55] *TBK1* is involved in neuroinflammation and interacts with *OPTN* that is involved in autophagy. *OPTN* and *TBK1* seem to be critical components of the pathway required for removal of pathological ribonucleoprotein inclusions.[55] Dysfunction of these genes may lead to protein aggregates that is one of the main characteristic of neurodegenerative disorders including ALS, Alzheimer's, Parkinson's, and Huntington's diseases. In addition *LRRK1*, a gene implicated in CCT, is involved in Parkinson's disease.

Degradation of misfolded and aggregated proteins is important in cellular homeostasis, not only as protein quality control but also for the regulation of protein levels. This links POAG to the main pathway involved in protein degradation: the ubiquitin–proteasome system (UPS). The UPS is responsible for the elimination of

misfolded or damaged proteins, and is implicated in important cellular processes including cell cycle, differentiation, proliferation, development, and synaptic plasticity.[56] In the eye, ubiquitin is present in RGCs and the retinal pigmented epithelium.[56] Post-translational processing of two known glaucoma genes, *OPTN* and *MYOC*, involves UPS.[57–59]

The most common mutation in *OPTN* that causes glaucoma (E50K) leads to malfunction of the UPS,[57] and subsequently to apoptosis in cultured cells.[58] In a study using induced pluripotent stem cells (iPSCs) from NTG patients with the E50K mutation, it was demonstrated that the E50K mutant OPTN is insoluble, generating abnormal protein deposits in the retina. Insolubility of the E50K mutant protein was attributed to a strong interaction between TBK1 and mutant OPTN that in wild-type conditions only occurs under infection–response processes.[60] Interestingly, copy number variants in *TBK1* have also been associated with NTG.[11–14] The mechanism of the OPTN–TBK1 cross-talk and its role in neurodegeneration as observed in POAG and ALS needs to be elucidated. Alterations of the UPS have also been observed when *MYOC* is overexpressed or mutated.[59] Of note is that mutations in *OPTN* are associated with NTG whereas mutations in *MYOC* are suspected to alter AH outflow,[59] suggesting that the UPS system is involved in both NTG and HTG.

To date, 101 disease-causing mutations have been documented in the *MYOC* gene (see www.myocilin.com).[61] The protein encoded by *MYOC* is a secreted glycoprotein highly express in the TM. The proposed pathogenic mechanism involves aggregation of misfolded mutant *MYOC* in the TM, resulting in decreased AH outflow and early-onset high IOP.[61] In cell culture studies it has been observed that cells with mutations in *MYOC* showed protein aggregation in the endoplasmic reticulum, leading to stress and death of the TM cells. It has been proposed that the reduced ability of the UPS to eliminate misfolded mutant MYOC may result in endoplasmic reticulum stress with pathological consequences.[62]

Six of the genetic variants associated with optic nerve degeneration (*ASB7*, *GMDS*, *HSF2*, *OPTN*, *RPAP3*, and *TBK1*), three to IOP (*MYOC*, *PMM2*, *TMCO1*) and one to CCT (*TBL1XR1*) are involved in UPS, raising the question of whether UPS failure is a key step in the (early) pathogenesis of POAG, either as an independent pathway or interacting with neuroinflammation. This implies that abnormalities of the UPS may be related not only with familial but also with general POAG. UBC seems to be a hub in pathway analysis including candidate genes identified in association studies,[63] although no genetic variants in UBC have been associated yet with POAG and its phenotypes.

Vascular tone

Oestrogen metabolism has been proposed as possible mechanism involved in POAG, mainly because of the effect of the 17β-estradiol hormone in the regulation of *CAV1* and *NOS3*.[39] However, the consistent association of *CAV1/CAV2* region also points to vascular tone. *CAV1* and *CAV2* encode caveolin-1 and caveolin-2, respectively. This region has been genome-wide significantly associated with POAG and IOP, and has been implicated in dysregulation of the vascular tone particularly through their interaction with endothelial nitric oxide synthase (eNOS)[35] and production of nitric oxide (NO) in the vascular endothelia. NO regulates the vascular tone in ocular arteries[64] and has also been implicated in AH outflow regulation;[65] that is, high levels of NO in a mouse model lead to increased AH outflow and thus decreased IOP.[66] This finding is consistent with data showing that patients with POAG have less NO production in the TM and Schlemm's canal compared with age-matched controls.[65] Variants in the *NOS3* gene that codes for eNOS have been found associated with HTG in women,[67] POAG patients with migraines,[68] and familial POAG.[69] This has led to the hypothesis that it is an oestrogen-related pathway. However, these associations have not been found yet in the large meta-analyses. *Cav-1* knockout mice show several abnormalities, including reduced retinal function due to changes in retinal microenvironment[70] and possible impairment in sensing mechanical forces in the retinal veins.[71] Furthermore, mice show insulin resistance, alteration in lipid metabolism, and pulmonary hypertension,[72] also supporting the role of caveolins in atherosclerosis and metabolic syndrome. Up until now, there are no reports about the IOP levels in these mice.

Recent studies have shown that CAV1 binds directly to ABCA1, possibly regulating the ABCA1-mediated cholesterol efflux.[73] Genetic variants in *ABCA1* have been found associated with POAG and IOP. However, positive regulation by CAV1 on ABCA1 seems to be cell type specific. Further in-depth examination of the interaction of ABCA1 with CAV1 in eye is needed to elucidate its possible role in IOP and POAG. In particular, as the ABCA1 protein may interact with ARHGEF12 that is involved in the RhoA/ROCK pathway that is a current target for drug development.

RhoA/Rho-associated kinase pathway

The Rho family includes three members of small G-proteins (RhoA, RhoB, and RhoC). G-proteins are inactive when bound to guanosine diphosphate (GDP) and active when bound to guanosine triphosphate (GTP). Rho-associated kinases or ROCKs are serine/threonine

kinases activated by Rho GTPases. The RhoA/ROCK pathway is known to regulate cell contractility via actin–myosin interactions,[74] and its role in IOP regulation was first demonstrated in rabbit eyes exposed to a specific ROCK inhibitor.[75] Inhibition of ROCK caused a significant reduction in IOP and an increase in AH outflow.[75] In the past years, selective ROCK inhibitors have been proposed as a new category of IOP-lowering medication.[76,77]

ROCKs can be activated not only by Rho G-proteins, but also in response to lipids such as arachindonic acid.[78] This may be the link between statin use and reduced glaucoma risk.[77] However, the exact mechanism of how statins reduce the risk of glaucoma is not clear. Recently, an intronic variant in *ARHGEF12* (Rho guanine nucleotide exchange factor (GEF) 12) was found to be associated with IOP and glaucoma, particularly in HTG.[21] ARHGEF12 activates RhoA.[79,80] Therefore, a possible mechanism might be that activation of RhoA via ARHGEF12 leads to ROCK activation, causing a decrease in AH outflow and IOP elevation (see Figure 5). This provides a link between common IOP variants and known IOP regulation mechanisms. Furthermore, it has been shown that ARHGEF12 can extend the half-life of the ABCA1 protein,[81] known to be essential in the synthesis of HDL cholesterol. When ARHGEF12 binds to the C terminus of ABCA1 it activates RhoA that in turn prevents ABCA1 degradation.[81] Moreover, analysis of proteins binding to APP, the first gene identified in Alzheimer's disease, demonstrated that both RhoA and GAS7 are part of the APP interactome.[82] In addition, it has been shown that TGF-β has an effect on *GAS7* expression.[83] As is the case for the *CAV1/CAV2*, *ABCA1* and *ARGHEF12*, variants in *GAS7*, have been found associated with both IOP and POAG, asking for further research evaluating the interaction between these proteins in the eye to elucidate the underlying mechanisms.

Eye development

There is strong evidence for the role of eye development genes in POAG, this is remarkable for a late-onset disorder. Eye development is a multistep process, starting with the formation of the optic vesicle that ultimately gives rise to the fully mature retina. Among essential transcription factors required for eye formation, *PAX6*, *ATOH7*, and *SIX6* have been associated with optic disc parameters,[26,28,30,84] of which *ATOH7* and *SIX6* have also been associated with POAG.

Variants in *ELP4* close to *PAX6* were associated with DA in the Rotterdam Study.[84] *PAX6*, a transcription factor considered as a master regulator gene in eye development, is required for lens differentiation and controls the expression of key regulatory transcription factors, such as *ATOH7* and *SIX6*.[85] In turn, *ATOH7* is essential in the differentiation of the RGCs, the firstborn neurons in the retina. Studies have shown that *ATOH7* plays a more important role in the determination of the DA than in the loss of RGC,[29,31] the main characteristic of POAG. The relation between optic disc area and POAG is controversial. However, it has been suggested that individuals with larger optic discs may suffer more from IOP-related stress.[86]

SIX6 has been associated with VCDR and NTG.[17,87] In zebrafish, knockdown of both zebrafish orthologs (*six6a* and *six6b*) lead to small eye phenotype[88,89] and possible abnormal differentiation of retinal progenitor cells. Moreover, it is known that *SIX6* acts as transcriptional repressor of CDK inhibitors. Knockdown of *six6b* increases the expression of *cdkn2b in vivo*. This not only provides a link between both loci but also between the eye development and TGF-β pathway.[88] In addition, variants in the antisense of *SIX6* (*SIX6-AS*) have been associated with myopia,[90] being together with *BMP2*, one of the two overlapping loci between myopia and glaucoma. Myopia is a known risk factor for POAG, and further studies evaluating the genetic overlap between both conditions will extend the understanding of the genetic mechanism that determines the relation between myopia and glaucoma.

Genes associated with the endophenotypes and their role in the reviewed pathways

Table 1 shows the role of the loci associated with POAG endophenotypes in the context of the six biological processes discussed in this review (see pathways section). Of note is that genes may be attributed to multiple pathways, for example, *ABCA1*, *CAV1/CAV2* (ECM and vascular tone), *CDKN2B-AS1*, and *BMP4* (TGF-β signalling and eye development). Genes associated with CCT seem to play an important role in ECM metabolism, whereas genes associated with optic disc parameters play a key role in eye development pathways. Genes involved in IOP are the major players in RhoA/ROCK signalling and vascular tone. It is noteworthy that many of the genes associated with POAG endophenotypes have been implicated in different type of cancers, in particular in gliomas. The relation between cancer and glaucoma seems stronger with genes associated with optic disc parameters, which are indicators of optic nerve degeneration; this is in line with emerging theories supporting the relation between neurodegeneration and cancer.[91] Although neurodegeneration is caused by death of neurons and cancer cells are characterized by an 'immortal' phenotype, both are age-related conditions, and it has been observed that the same molecules play a role in processes such as cell proliferation, differentiation,

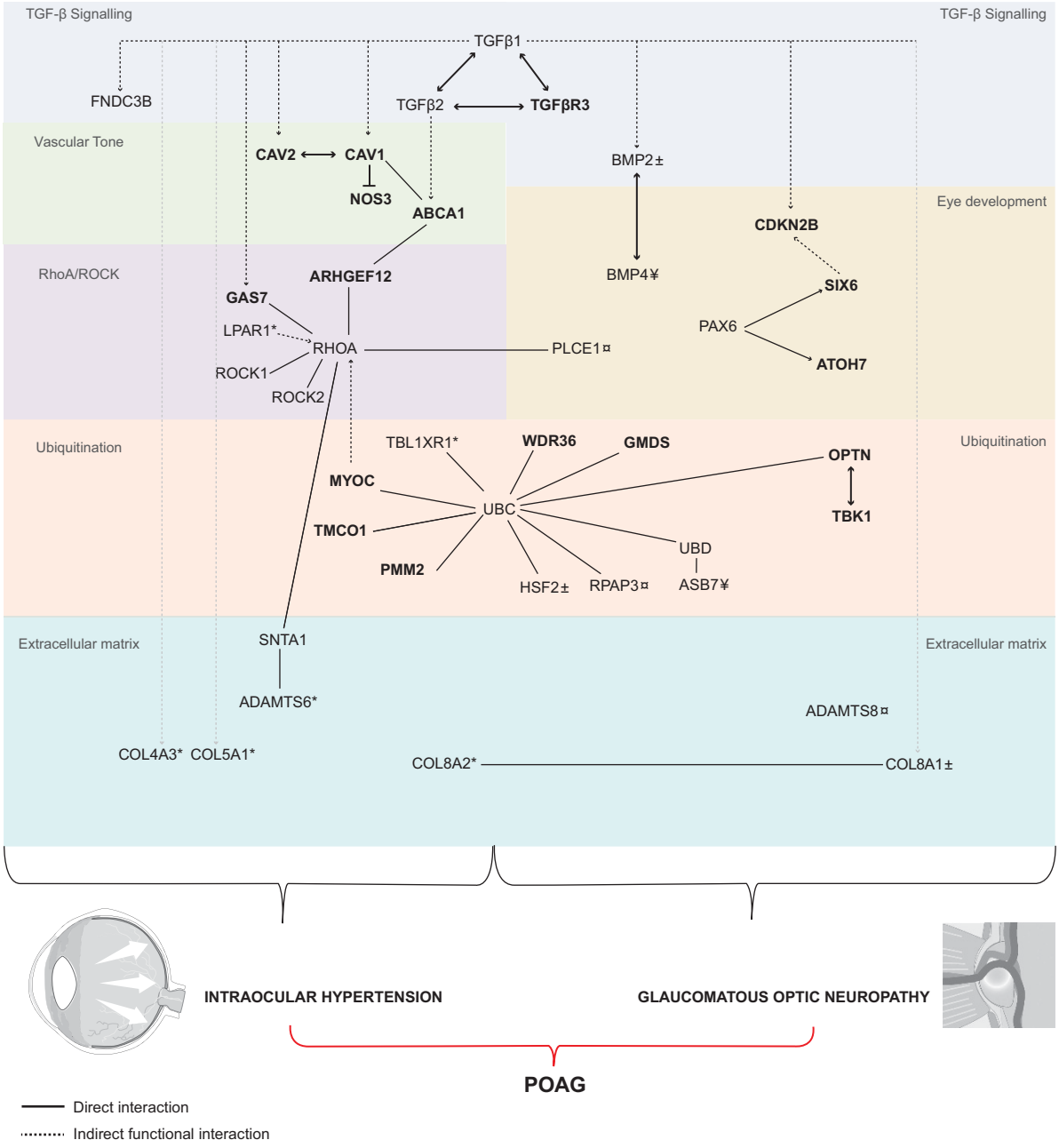

Figure 5 Pathways possibly implicated in POAG revealed by linkage and association studies. The six different biological processes possibly implicated in POAG reviewed are shown in the figure. Map was built using Ingenuity Pathway Analysis (IPA). Solid lines imply direct relationships between proteins (eg, physical protein–protein interaction or enzyme–substrate); dotted lines imply indirect functional relationships, such as coexpression, phosphorylation/dephosphorylation, activation/deactivation, transcription, or inhibition. Proteins in bold correspond to known glaucoma genes. Genes genome-wide associated with CCT (*), VCDR and CA (±), CA only (¥), and VCDR only (¤).

and apoptosis. It has been proposed that protein alterations that predispose cells to undergo apoptosis might lead to a decreased risk of cancer and increased risk of neurodegeneration, whereas protein modifications that facilitate cell growth might be protective for neurodegeneration but a risk factor for cancer.[91]

So far, at least six of the genes associated with VCDR, CA, or DA have been implicated in gliomas. These include the chromosome 9p21.3 locus in which *CDKN2A*, *CDKN2B*, and *CDKN2B-AS1* are located. This locus has also been associated with coronary artery disease, type 2 diabetes mellitus, and lymphoblastic leukaemia.[92]

Table 1 Role of the genes associated with POAG endophenotypes in the context of the six biological processes implicated in POAG

Pathway /endophenotype	*RhoA/ROCK*	*Vascular tone*	*TGF-β signalling*	*Ubiquitination*[a]	*Development*[b]	*Extracellular matrix*	*Cancer*	*Other*
VCDR/CA	—	—	*BMP2, CDKN2B/ CDKN2B-AS1, BMP4*	*HSF2, RPAP3, ASB7*	*SIX6, RERE, BMP4, CDKN2B/ CDKN2B-AS1, HSF2, FLNB1, FAM101,*	*COL8A1, ADAMTS8*	*COL8A1, CHEK2*[c]*, DUSP1*[c]*, ADAMTS8*[c]*, BCAS3*[c]*, EFEMP1*[c]*, EXOC2, TRIB2, DCLK1, KPNB1, PLCE1*	*SSSCA1, DHRS3, DDHD1, TRIOBP*
VCDR/DA	—	—	*TGF-βR3*	—	—	—	*CARD10*[c]*, CDC7, ABI3BP*	
VCDR/CA/DA	—	—	—	—	*ATOH7, SALL1*	—		*TMTC2*
DA only	—	—	—	—	*ABI3BP, RARB, NR2F2*	—	*DIRC3, NR2F2, HORMAD2*	*CDC42BPA, DCAF4L2, F5, ELP4*
IOP only	*ARHGEF12, ABCA1, GAS7*	*ABCA1, CAV1/CAV2*	—	*TMCO1*	—	*CAV1/ CAV2*	*ABO,CAV1/CAV2*	*FAM125B*
CCT only	*LPAR1, AKAP13*	—	*SMAD3, FOXO1*	—	*FGF9*	*ADAMTS6, COL4A3, COL8A2, COL5A1, ZNF469, CHSY1, SGCG, HS3ST3B1*	*NF1B, ARHGAP20, ARID5B, FOXO1*[c]*, FGF9*[c]*, TBL1XR1*	*MPDZ, POU2AF1, TMEM248, FAM46A, IBTK, TJP1, NR3C2, LRRK1, VKORC1L1, PMP22, LCN12, PTGDS, GLT8D2, RXRA, KCNMB2*
IOP/CCT	—	—	*FNDC3B*	—	—	—	*FNDC3B*	—

[a] Either play a central role in ubiquitination (RPAP3, ASB7) or are processed by the ubiquitin–proteasome system (HSF2, TMCO1). [b] Genes listed here are involved in development in general, not only eye development. [c] Genes also involved in glioma.

Some variants are associated with various diseases beyond POAG. Variants in *ABCA1, CAV1*, and *CAV2* have been associated with IOP and POAG; these genes are well known for their association with HDL cholesterol, atherogenenesis, and pulmonary hypertension, whereas variants in *ABO* have been associated with IOP and also with venous thromboembolism, atherosclerosis, stroke, myocardial infraction, and diabetes. Furthermore, *PAX6*, known for its role in eye development, is also a key player in the development of the endocrine pancreas. *Pax6* knockout mutants showed not only anophtalmia but also decreased production of insulin.[93]

Building bridges between the various pathways identified in genetics research

The six pathways discussed above may be linked to each other in several ways. Three out of the six pathways (TGF-β, RhoA/ROCK, and the Caveolins-1 and -2) have emerged as potential mechanism leading to the ECM changes observed in POAG or its early stages (endophenotypes). The TGF-β signalling alters ECM production and turnover in both the TM and lamina cribrosa. Studies have demonstrated that high levels of TGF-β2 promote a fibrotic phenotype in the TM.[44,48] Interestingly, TGFβR3 (associated with VCDR, DA, and POAG) is considered necessary for TGF-β2 signal transduction. In addition, the RhoA/ROCK signalling has been also identified as significant player in the fibrogenic activity induced by TGF-β2.[43,44,48] However, not only increased production of ECM proteins may lead to decreased outflow of AH, but also alterations in their recycling and degradation may have this effect. CAV1 and CAV2 have been implicated in the endocytosis and degradation of ECM fibronectin, a glycoprotein of the ECM.[40,41] Thus, dysregulation of CAV function could also contribute to the changes observed in the ECM in glaucoma. Furthermore, *CDKN2B*, one of the first genes implicated in POAG by GWAS and involved in cell cycle and growth regulation, is induced by TGF-β.[53] Upregulation of *CDKN2B* leads to a cell cycle arrest and has been found overexpressed in a rat model of glaucoma[17] and in a *six6b* knockdown zebrafish model.[88] TGF-β has also been implicated in the activation of RhoA, as part of the non-smad signalling pathway.[94] Both experimental and genetic data point to the TGF-β signalling pathway as an important contributor to the complex pathogenesis of glaucoma. Figure 5 shows the different connections between the pathways emerging from combining the genetics and experimental findings.

Interestingly, two of the genes associated with POAG, *GMDS* (*GDP-mannose 4,6-dehydratase*) and *PMM2* (*phosphomannomutase 2*), that are processed by the

ubiquitin–proteasome system are also involved in the fructose and mannose metabolism. Mutations in *PMM2* lead to congenital disorders involving a variety of clinical features related to the role of N-glycoproteins during embryonic development. These include defects in the nervous system development, cerebellar hypoplasia, peripheral neuropathy, as well as abnormal eye movement and retinitis pigmentosa. In POAG, the fructose and mannose pathways have also been found associated with disease status in a case/control study.[95] A combination of genetics and metabolomics studies will help to clarify the role of carbohydrate metabolism in the pathophysiology of POAG.

There will be an important role of system biology and functional genomics research to disentangle the pathways. Systems biology aims to combine genomic, transcriptomic, proteomic, and metabolomic approaches along with computational models, whereas functional genomics aims to integrate multiple lines of evidence, for example, genetic data from human studies, gene expression, and animal models to identify, characterize, and prioritize candidate genes implicated in complex diseases. With the advent of GWASs, the amount of loci associated with glaucoma and its endophenotypes have increased significantly. So far, at least 70 loci have been associated with optic disc parameters, IOP, CCT, and POAG. Although these loci explain a small part of the variance in POAG endophenotypes, these loci have revealed and supported the role of several possible pathways implicated in the pathogenesis of POAG (discussed in this review). However, there is a lack of functional validation and detailed analysis of the *in vivo* function of these genes in the anterior chamber. More experimental data based on the animal models that are already available and new models are necessary.

There is a wide variety of animal models of different species in POAG, including monkeys, dogs, rabbits, rats, mice, chickens, and zebrafish.[96] Different approaches have been used to study the possible molecular events leading to POAG. The first animal models in glaucoma focussed on non-human primates in which high IOP was induced, posteriorly high IOP was induced in other species, such as rabbits and mice. In addition, two other broad approaches have been used: (1) natural-occurring glaucoma models, or animal models with an induced glaucoma-like phenotype generated by the use of radiation or mutagens, in which subsequently the gene causing the phenotype is identified and (2) animal models in which a known candidate gene is mutated to evaluate the causal role of the gene or a specific mutation.

Among examples of the first category are: (1) an inherited form of POAG in dogs that led to the identification of mutations in *ADAMTS10*, which also belongs to the ADAMTS family, involved in ECM remodelling[40] and (2) analysis of the bugeye zebrafish led to the identification of a nonsense mutation in *lrp2* as cause of a glaucoma-like phenotype, including severe myopia, high IOP, and progressive RGC death. Examples of the second category are: genetically modified animal models in which *OPTN*, *MYOC*, and *WDR36* were either knocked-out or mutated.[97] These studies have helped in the understanding of the molecular events implicated in the development of glaucoma. Although the numbers of loci and candidate genes have increased dramatically in the past years, functional validation of the loci found associated with glaucoma needs to be performed to elucidate their role in the pathophysiology of the disease.

Zebrafish, for example, provides an excellent tool for high-throughput functional validation of GWASs loci. Recently, it has been demonstrated that substantial knockdown of *six6a* and *six6b* during early embryonic development causes a small eye phenotype.[88,89] Analysis of the effect of *six6* depletion at later age as well will probably give more insight into the role of *SIX6* in POAG and myopia.

Despite the fact that there are available mouse models for POAG genes such as *cav1*, *abca1*, and *atoh7*, and CCT genes such as *col5a1* and *col8a2*, its role in the development of the disease has not been studied in depth yet. Although these have been developed for other diseases, they offer an opportunity to understand the pathogenesis of POAG-related phenotypes. For example, no studies focussed on IOP assessment in *cav1* or *abca1* mutants.

Discussion

In this paper we integrate findings from different organisms and different levels from molecular to (patho) physiological. We show that the genes involved in POAG and its endophenotypes can be linked to six pathways. These pathways can be studied jointly in cellular and animal models. There are still major gaps in our knowledge: many genes cannot be linked to the pathways that we proposed, and experimental studies will play a key role to link these genetic variants to the pathways described here or others. Whereas experimental studies show a key role of TGF-β2, the genetic studies point to *TGFβR3*. The genetic studies show evidence for a partly genetic overlapping aetiology with myopia (related to the eye development pathway), ALS, and Parkinson's and Alzheimer' disease (through ubiquitination pathway). This warrants off-target effect of therapeutic research addressing these disorders. Many genes that have been implicated in the ECM are not yet linked to POAG: more genetic studies are needed to do so. An important question is whether larger and more extensive studies will bring into view new pathways or whether these will fill in

the missing genes in the pathways described here. We have not reached the state that we can answer this question. Without doubt, finding new variants will improve the translation of the genetic findings into clinical prediction and diagnosis.

Although to date the translation of genetic findings to clinical practice is limited, genetic findings will be of clinical value in the near future, particularly in the context of precision medicine, an emerging approach for prevention and disease treatment that takes into account individual variability in genes, environment, and lifestyle.[98] In the case of POAG, strategies in precision medicine will aim to target, for instance, the specific pathways involved in the pathogenesis of POAG in each patient individually. A more in-depth knowledge of the pathways disturbed in glaucoma will help elucidating whether, for example, HTG patients have one particularly altered pathway, such as the RhoA/ROCK pathway, compared with NTG patients. This may change the treatment selection for these patients, who probably will benefit more from a selective ROCK inhibitor. Genetic profiling will be a time- and cost-effective approach to elucidate the underlying pathology. Further applications include the assessment of genetic variants related to drug response. Translation of these findings to clinical practice requires not only functional validation but also assessment of the clinical utility, that is, the use of test results (genetic test result) to inform clinical decision making. Knowledge integration across a wide range of biomedical fields is needed to translate genomic findings into clinical practice.

Conflict of interest

The authors declare no conflict of interest.

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