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Genome-wide analysis of multi-ancestry cohorts identifies new loci influencing intraocular pressure and susceptibility to glaucoma

Elevated intraocular pressure (IOP) is an important risk factor in developing glaucoma, and variability in IOP might herald glaucomatous development or progression. We report the results of a genome-wide association study meta-analysis of 18 population cohorts from the International Glaucoma Genetics Consortium (IGGC), comprising 35,296 multi-ancestry participants for IOP. We confirm genetic association of known loci for IOP and primary open-angle glaucoma (POAG) and identify four new IOP-associated loci located on chromosome 3q25.31 within the *FNDC3B* gene ($P = 4.19 \times 10^{-8}$ for rs6445055), two on chromosome 9 ($P = 2.80 \times 10^{-11}$ for rs2472493 near *ABCA1* and $P = 6.39 \times 10^{-11}$ for rs8176693 within *ABO*) and one on chromosome 11p11.2 (best $P = 1.04 \times 10^{-11}$ for rs747782). Separate meta-analyses of 4 independent POAG cohorts, totaling 4,284 cases and 95,560 controls, showed that 3 of these loci for IOP were also associated with POAG.

POAG is the leading cause of irreversible blindness in the world[1]. The only modifiable risk factor for the development and progression of glaucoma is high IOP[2], and lowering IOP is currently the only therapy that can reduce glaucomatous progression, even in forms of glaucoma that have IOP close to the statistical norm for the population (normal-tension glaucoma, or NTG)[3,4]. POAG and IOP are highly heritable: the lifetime risk of developing POAG is 22% among first-degree relatives of cases[5], which is approximately ten times higher than the risk for the rest of the population[1]. Heritability for IOP is estimated to be approximately 55% (ref. 6). Genetic studies have shown that the genetic risks for POAG and IOP are partly shared; polymorphisms within the *TMCO1* gene are associated with both POAG risk[7] and IOP[8]. Studying genetic determinants of IOP is therefore likely to provide critical insights into the genetic architecture of POAG and open new avenues for therapeutic intervention.

In this study, we present the results from a meta-analysis of genome-wide association studies (GWAS) of IOP from 18 studies participating in IGGC and an assessment of the importance of the genetic findings for susceptibility to POAG (**Fig. 1**). The IOP meta-analysis included 35,296 subjects (7,738 Asians and 27,558 individuals of European descent) drawn from the general populations of 7 countries. The demographic characteristics of these population-based cohorts are given in **Supplementary Table 1**. Genotyping assays and imputation to HapMap 2 haplotypes were performed at individual sites. Association analyses were performed using an additive model with IOP as the outcome and the number of alleles at each polymorphic site as the predictor, adjusting for age and sex. IOP levels for participants who were receiving IOP-lowering therapy at the time of the study and for whom data on baseline, pretreatment levels were not available were imputed as previously described[8]. Subjects who had undergone surgery or had other eye diseases that could affect IOP were excluded (**Supplementary Note**). Secondary analyses were carried out adjusting for central corneal thickness (CCT), which is known to influence IOP measurements[9].

After applying conventional quality control filters, we performed a fixed-effects meta-analysis of the 22 autosomes across the cohorts with approximately 2.5 million markers. Within-study genomic inflation factors[10] ranged between 0.992 and 1.043 (**Supplementary Fig. 1** and **Supplementary Table 2**), indicating a lack of major population stratification bias within each study. SNPs available in fewer than 16 cohorts or showing large effect heterogeneity (defined as $I^2 > 75\%$)[11] were removed. We found 145 SNPs (**Supplementary Table 3**) whose associations crossed the conventional genome-wide significance threshold for association ($P < 5 \times 10^{-8}$)[12]. All of these SNPs clustered around seven separate regions of the genome (**Fig. 2** and **Supplementary Figs. 2** and **3**). Two of the regions associated with IOP in our meta-analysis had previously been implicated in IOP variability: the regions near

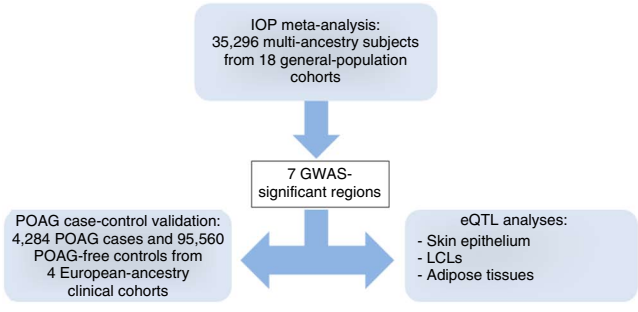

Figure 1 Flow chart of the analyses. Associated SNPs in a meta-analysis of IOP in participants from 18 general-population cohorts were validated in 4 clinical case-control cohorts and examined for transcription regulation activity in 3 tissues from 856 white British subjects.

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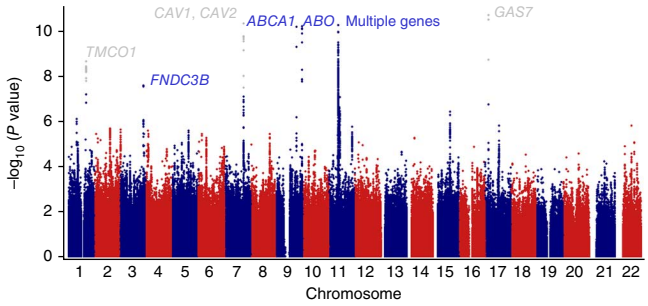

Figure 2 Manhattan plot of the results from the meta-analysis of data from 18 multi-ancestry cohorts from IGGC. The 22 autosomes are plotted along the *x* axis, and the values on the *y* axis denote the $-\log_{10}$-transformed *P* values from the meta-analysis of association with IOP observed for each of the SNPs. Loci previously associated with IOP or glaucoma are shown in gray.

the *TMCO1* locus[7,8] ($P = 2.19 \times 10^{-9}$ for rs7555523) and near the *GAS7* gene[8] ($P = 1.03 \times 10^{-11}$ for rs9913911). A third associated locus, new for IOP, was near the *CAV1* and *CAV2* genes ($P = 1.87 \times 10^{-11}$ for rs10258482), which had previously been associated with POAG[13].

New associations were identified within a large linkage disequilibrium (LD) block on chromosome 11 encompassing, among other genes, *AGBL2*, *SPI1* and *PTPRJ* (best $P = 1.04 \times 10^{-11}$ for rs747782) (**Supplementary Fig. 1**). Two additional associated loci were mapped on chromosome 9: one at 9q31.1 upstream of *ABCA1* ($P = 2.80 \times 10^{-11}$ for rs2472493) and the other at 9q34.2 within the *ABO* blood group gene ($P = 3.08 \times 10^{-11}$ for rs8176743). A fourth associated region was detected at 3q25.31, within *FNDC3B* ($P = 4.19 \times 10^{-8}$ for rs6445055).

Interestingly, of all the loci previously associated with glaucoma or related quantitative traits[14], *CDKN2B-AS1* and *SIX1/SIX6* were not associated with IOP in the meta-analysis. It is possible that these two loci exert their influence on POAG through mechanisms unrelated to IOP.

Genome-wide significant SNPs from the IOP meta-analysis were then investigated for their effect on the clinical outcome of POAG in 4 independent cohorts representing a combined 4,284 POAG cases (normal-tension and high-tension glaucoma) and 95,560 controls (details about these cohorts are provided in the **Supplementary Note**). Associations with POAG were found for the newly discovered regions near *ABCA1* ($P = 4.15 \times 10^{-9}$ for rs2472493), near *FNDC3B* ($P = 0.03$ for rs6445055) and at the chromosome 11 cluster ($P = 0.008$ for rs12419342). We did not find significant statistical evidence of association of POAG with the *ABO* locus. The case-control analyses reinforced association evidence at the previously identified loci at *TMCO1* ($P = 1.34 \times 10^{-16}$ for rs7555523), *CAV1-CAV2* ($P = 6.27 \times 10^{-9}$ for rs10258482) and *GAS7* ($P = 5.22 \times 10^{-13}$ for rs12150284). All alleles associated with higher IOP levels also increased glaucoma risk (**Table 1**).

We then examined whether the effect sizes of SNPs on IOP levels (β_{IOP}) were linearly related to their effect sizes on POAG (β_{POAG}) using a causal inference framework as previously described[15]. In a linear regression analysis, we observed a significant association between β_{IOP} and β_{POAG} ($P = 0.03$; **Supplementary Table 4**), suggesting that the strength of a SNP's effect on IOP levels is correlated with its effect on risk for POAG.

We subsequently investigated the relationship between variants within the seven regions associated with IOP and *cis* regulation of mRNA expression in 3 tissues (adipose, lymphoblastoid cell lines (LCLs) and skin) from a sample of 856 UK subjects[16]. The most significant expression quantitative trait locus (eQTL) associations were generally observed in LCLs for most loci, except for *CAV1*, where effects were strongest in adipose and skin tissues (**Table 2**). Significant eQTL association was observed for rs4656461 and rs7555523 ($P = 0.003$ and 0.0001 with *TMCO1* and *ALDH9A1* transcript levels in skin and LCLs, respectively), rs2024211 ($P = 5.43 \times 10^{-16}$ and 3.84×10^{-13} with *CAV1* transcript levels in adipose and skin tissues, respectively), rs2472493 ($P = 3.67 \times 10^{-5}$ with *ABCA1* transcript levels in LCLs) and rs1681630 on chromosome 11 ($P = 2.72 \times 10^{-10}$ with *SPI1* transcript levels in LCLs), among others (**Table 2** and **Supplementary Table 5a**). These SNPs also had the strongest eQTL effects for their respective transcripts (**Supplementary Table 5b**).

We measured the mRNA expression levels of the identified genes in adult ocular tissues using RT-PCR. We found that most of the identified genes, including *TMCO1*, *FDNC3B*, *CAV1-CAV2*, *ABCA1* and *GAS7*, were expressed in most ocular tissues (**Supplementary Table 6**). The genes within the chromosome 11 locus showed varied expression levels across ocular tissues. Gene-based tests or enrichment for Gene Ontology terms did not identify any new genes or pathways after correction for multiple testing (**Supplementary Tables 7** and **8**).

Table 1 Results for association with IOP from the general-population cohorts for SNPs significant at a multiple-testing correction level ($P < 5 \times 10^{-8}$) and their association with POAG in case-control validation meta-analyses

SNP					Association with IOP in the discovery cohort					Association in POAG case-control cohorts	
Chr.	Position (bp)	rsID	A1/A2	Nearest gene	β	SE	*P*	Heterogeneity *P*	I^2	OR (95% CI)	*P*
1	165,687,205	rs4656461	G/A	*TMCO1*	0.228	0.039	6.51×10^{-9}	0.46	0.00	1.38 (1.28–1.50)	2.55×10^{-15}
1	165,718,979	rs7555523	C/A	*TMCO1*	0.235	0.039	2.19×10^{-9}	0.55	0.00	1.40 (1.30–1.52)	1.34×10^{-16}
3	171,992,387	rs6445055	A/G	*FNDC3B*	−0.177	0.030	4.19×10^{-8}	0.17	0.24	0.92 (0.85–0.99)	0.03
7	116,150,095	rs10258482	A/C	*CAV1*	0.196	0.029	1.87×10^{-11}	0.81	0.00	1.20 (1.13–1.28)	6.27×10^{-9}
7	116,150,952	rs10262524	A/C	*CAV1*	0.186	0.029	9.69×10^{-11}	0.67	0.00	1.20 (1.13–1.28)	1.39×10^{-8}
9	107,695,848	rs2472493	G/A	*ABCA1*	0.159	0.024	2.80×10^{-11}	4×10^{-5}	0.66	1.24 (1.16–1.34)	4.15×10^{-9}
9	136,131,415	rs8176743	T/C	*ABO*	0.261	0.039	3.08×10^{-11}	0.53	0.00	1.07 (0.96–1.19)	0.2
11	47,468,545	rs12419342	C/T	*RAPSN*	0.153	0.026	4.77×10^{-9}	0.75	0.00	1.09 (1.02–1.16)	0.008
11	47,940,925	rs747782	C/T	*NUP160*, *PTPRJ*	0.203	0.030	1.04×10^{-11}	0.95	0.00	1.03 (0.96–1.11)	0.36
11	47,969,152	rs1681630	T/C	*PTPRJ*	0.144	0.026	1.69×10^{-8}	0.60	0.00	1.06 (0.99–1.12)	0.08
11	48,004,369	rs7946766	T/C	*PTPRJ*	0.230	0.035	2.71×10^{-11}	0.35	0.09	1.03 (0.95–1.12)	0.43
17	10,031,183	rs9913911	G/A	*GAS7*	−0.179	0.026	1.03×10^{-11}	4×10^{-4}	0.61	0.80 (0.75–0.85)	2.98×10^{-13}

Chr., chromosome; A1/A2, reference/alternative alleles; β, linear regression coefficient (mm Hg); SE, standard error of the regression coefficient; OR, odds ratio; 95% CI, 95% confidence interval for OR.

Table 2 Summary of eQTL effects observed in 3 different tissues extracted from 849 individuals for SNPs associated with IOP

SNPs					eQTL effect *P* values				
Chr.	Position (bp)	rsID	A1/A2	Nearest gene	Adipose	LCLs	Skin	Probe ID	Gene
1	165,687,205	rs4656461	G/A	*TMCO1*	0.004	0.12	0.003	ILMN_1793829	*TMCO1*
1	165,718,979	rs7555523	C/A	*TMCO1*	0.39	0.0001	0.05	ILMN_1761804	*ALDH9A1*
3	171,992,387	rs6445055	A/G	*FNDC3B*	NS	NS	NS	–	–
7	116,150,095	rs10258482	A/C	*CAV1*	NS	NS	NS	–	–
7	116,150,952	rs10262524	A/C	*CAV1*	5.79×10^{-16}	8.54×10^{-5}	3.91×10^{-13}	ILMN_1687583	*CAV1*
9	107,695,848	rs2472493	G/A	*ABCA1*	0.19	3.67×10^{-5}	0.36	ILMN_1766054	*ABCA1*
9	136,131,415	rs8176743	T/C	*ABO*	NS	NS	NS	–	–
11	47,468,545	rs12419342	C/T	*RAPSN*	0.002	4.32×10^{-8}	0.0003	ILMN_1696463	*SPI1*
11	47,940,925	rs747782	C/T	*NUP160, PTPRJ*	NS	NS	NS	–	–
11	47,969,152	rs1681630	T/C	*PTPRJ*	0.006	2.72×10^{-10}	0.002	ILMN_1696463	*SPI1*
11	48,004,369	rs7946766	T/C	*PTPRJ*	0.66	2.02×10^{-5}	0.0066	ILMN_1688627	*AGBL2*
17	10,031,183	rs9913911	G/A	*GAS7*	NS	NS	NS	–	–

The SNPs listed are the same as those in **Table 1**. LCLs, lymphoblastoid cell lines; NS, no significant association detected.

Altogether, these SNPs explained approximately 1.2% of the heritability for IOP in the TwinsUK cohort[17], 1.5% of the phenotypic variability in IOP in the Rotterdam Study[18] and between 0.6 and 1.2% of the phenotypic variability in IOP in Asians. *FNDC3B* has been associated with CCT[19], and, as CCT has a significant effect on IOP measurements[20], we performed an additional meta-analysis of IOP adjusted for age, sex and CCT in a smaller subsample that had CCT measures (19,563 subjects from 13 population cohorts). The association for rs6445055 remained nominally significant although it was weaker ($P = 9.87 \times 10^{-4}$, $\beta = -0.121$ in comparison to −0.177 before adjustment for CCT). This finding suggests that this locus has at least some CCT-independent effect over IOP levels. The association evidence remained consistent, although slightly weaker, for the other loci (**Supplementary Table 9**).

We report association of variants within the *ABCA1* gene with IOP and POAG. A strong eQTL effect was observed in LCLs ($P = 3.67 \times 10^{-5}$) for the most highly associated SNP (rs2472493) in our analyses. *ABCA1* is expressed in many tissues[21], and its expression in leukocytes is significantly upregulated in individuals with glaucoma[22].

Associations for a number of SNPs within the *ABO* blood group gene and IOP, although statistically significant and homogeneous across the participating cohorts, were not observed in the glaucoma case-control meta-analysis. This might be owing to type I error in the initial meta-analysis or insufficient power to detect a primarily IOP-led effect in cases that included individuals with NTG, resulting in a type II error in the latter analysis. Four of the nine GWAS polymorphisms associated at genome-wide significance in the *ABO* locus were nonsynonymous variants, determining the B blood group[23]. This finding might be relevant, given previous observations that the B blood group is epidemiologically associated with glaucoma, including POAG[24], although the mechanisms remain unclear.

Association was found between IOP and variants lying over a large region on chromosome 11. Of the many genes in that region, eQTL analyses singled out *SPI1* and *AGBL2* as possible candidates for prioritization in future studies. eQTL analyses also raised the possibility of *ALDH9A1* as a candidate for IOP regulation, given its strong expression in the ciliary body [25] and location just downstream of the *TMCO1*-associated variant. The eQTL results also suggest that *CAV1* is a stronger candidate than *CAV2*, although transcription regulation might not be the only mechanism influencing IOP at this locus.

Although IOP and POAG are strongly genetically correlated[26], we further explored their shared genetic backgrounds. Using independent SNPs (not in LD) with association $P < 1 \times 10^{-6}$ in the IOP GWAS meta-analysis as described elsewhere[27], we found a statistically significant polygenic overlap between IOP and POAG in the ANZRAG cohort of advanced blinding glaucoma cases and controls (**Supplementary Note**) ($P = 4.33 \times 10^{-5}$). The variance explained in POAG was 0.7%, which changed little if less significant SNPs were progressively included in the model (**Supplementary Table 10**).

There are potential limitations to this study. First, there was variability across the studies in terms of IOP measurement methods, although the differences are likely to be small[28]. In addition, we maximized power to discover genetic variants of small effect size by including multi-ancestry cohorts, at the risk of introducing heterogeneity into the study. Heterogeneity was, however, generally low (**Table 1**) for most of the loci reported, so we consider our results to be conservative. Second, assessment of clinical importance using panels of POAG cases is not equivalent to a formal replication. Even in this case, we expect our results to be overly conservative at the price of reduced sensitivity, which could be a possible reason for non-validation of our associations with IOP in the *ABO* blood group locus. Finally, we based our eQTL analysis on sample tissue availability rather than analyzing the ideal ocular tissue types. Tissues such as trabecular meshwork would have been preferable, but they are impractical to obtain because they are generally less accessible. We tried to circumvent this limitation by studying three different tissues, but caution is required when interpreting eQTL results.

Despite these considerations, our report of seven loci associated with IOP and glaucoma, of which four are newly discovered, is a key step toward better understanding the mechanisms of IOP regulation, currently the only modifiable risk factor for POAG.

METHODS

Methods and any associated references are available in the online version of the paper.

Note: Any Supplementary Information and Source Data files are available in the online version of the paper.

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AUTHOR CONTRIBUTIONS

P.G.H., C.-Y.C., H.S., S.M., J.N.C.B. and R.W. performed analyses and drafted the manuscript. S.M., A.J.L., J.E.B.-W., V.V., L.R.P., N.P., C.D., A.V., D.A.M., J.E.C., J.L.W., C.M.v.D., C.J.H. and T.A. jointly conceived the project and supervised the work. P.G.H., H.S., R.W., A.N., A.W.H., A.M., C.V., R.H., G.T., B.A.O., S.-M.S.,

W.D.R., E.V., C.-C.K., D.D.G.D., J.L., J.L.H., Y.X.W., F.R., P.S.W., H.G.L., A.B.O., J.Z.L., B.W.F., R.C.W.W., T.Z., S.E.S., Y.-Y.T., G.C.-P., X.L., R.R.A., J.E.R., A.S., Y.Z., C.B., A.I.I., L.X., J.F.W., J.H.K., L.C.K., K.S., V.J., A.G.U., N.M.J., U.T., J.R.V., N.A., S.E., S.E.M., N.G.M., S.Y., E.-S.T., E.M.v.L., P.A., J.K., M.A.H., F.J., P.L., A.H., S.J.L., R.F., L.K., P.T.V.M.d.J., K.J.L., C.L.S., C.E.P., L.M.E.v.K., C.M., C.C.W.K., K.P.B., T.D.S., T.-Y.W., D.A.M., J.E.C. and A.B.S. were responsible for cohort-specific activities, data generation and analyses. T.L.Y. and K.S.S. were responsible for expression and eQTL work. P.G.H., C.-Y.C., H.S., S.M., T.-Y.W., D.A.M., J.L.W., C.M.v.D. and C.J.H. critically reviewed the manuscript.

COMPETING FINANCIAL INTERESTS

The authors declare competing financial interests: details are available in the online version of the paper.

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ONLINE METHODS

IGGC participants. All studies participating in this meta-analysis are part of the International Glaucoma Genetics Consortium. The discovery cohorts included 27,558 individuals of European ancestry from 14 studies (ALIENOR, BATS, BMES[29,30], ERF[31,32], Framingham Family Study[33], GHS1, GHS2, ORCADES[34], RAINE[35–37], RS-I, RS-II, RS-III (ref. 38), TEST[39] and TwinsUK[40]). In addition, 7,738 individuals of Asian ancestry from 4 cohorts (BES[41], SCES[42], SiMES[43] and SINDI[42]) were included. In addition, four case-control population panels were used, all of European ancestry: ANZRAG[7], MEEI, NEIGHBOR and deCODE. General methods, demographics and phenotyping of the study cohorts have previously been described extensively, and details are provided in the **Supplementary Note** and **Supplementary Table 1**. All studies were performed with the approval of their local medical ethics committees, and written informed consent was obtained from all participants in accordance with the Declaration of Helsinki.

Phenotype measurements. Eligible participants underwent an ophthalmologic examination including measurements of IOP and, for most but not all studies, measurements of CCT. Each participating cohort was phenotyped separately, and the IOP measurement methods used by each are described in **Supplementary Table 1**.

Genotyping and imputation. Study samples were genotyped on either Illumina or Affymetrix platforms. Each study performed SNP imputation using the genotype data, together with HapMap Phase 2 ancestry-matched reference panels (CEU (Utah of residents of Northern and Western European ancestry), JPT + CHB (Japanese in Tokyo and Han Chinese in Beijing) or the four HapMap populations) on the basis of the Build 36 databases (release 22 or 24). Markov Chain Haplotyping software, IMPUTE[44,45] or MACH[46], were adopted for imputation. A detailed description regarding genotyping platforms and imputation procedures for each study is provided in **Supplementary Table 1**.

Stringent quality control of genotype data was applied in each cohort. Samples with low call rates (<95%) or with sex discrepancies were excluded. Cryptically related samples and outliers in population structure from principal-component analyses were also excluded. SNPs flagged with missingness of >5%, gross departure from Hardy-Weinberg equilibrium ($P < 1 \times 10^{-6}$) or minor allele frequency (MAF) of <1% were removed from further analyses.

Statistical analysis. For each study, an allele dosage regression model at each directly genotyped or imputed SNP was conducted to determine its association with IOP. Data for eyes with previous glaucoma surgery or laser treatment were excluded. For subjects receiving IOP-lowering medication, we added 25% to the measured IOP levels to estimate pretreatment IOP, on the basis of a reported average of a 17–33% reduction in IOP caused by IOP-lowering medication in a meta-analysis of clinical trails[47]. The mean of the right and left IOP measurements was used. When data from only one eye were available, the IOP measurement from the available eye was used.

For the analyses, we assumed an additive genetic model where the dosage of each SNP was a continuous variable ranging from 0 to 2 for the minor alleles carried. Primary analysis for IOP was adjusted for age and sex. Additional adjustment for principal components was carried out by a few participating cohorts to correct for subtle population substructure.

Per-SNP meta-analyses were performed using GWAMA software with the weighted inverse variance approach, assuming fixed effects, as for initial discovery purposes the fixed-effects model was preferred for increased statistical power[48]. A Cochran's Q test and I^2 values were used to assess heterogeneity across studies[49]. For each participating cohort, only SNPs with sufficient imputation quality scores (proper-info of IMPUTE or R^2 of MACH > 0.3) were included in the meta-analysis.

Gene-based testing was conducted using VEGAS software[50] on the European-ancestry and Asian-ancestry meta-analysis results separately. VEGAS incorporates information from the full set of markers in a gene and accounts for LD between markers using simulations from the multivariate normal distribution. For samples of European descent, we used the HapMap 2 CEU population as the reference to estimate patterns of LD. For Asian-ancestry groups, we used the combined HapMap 2 JPT and CHB populations as the reference population to approximate LD patterns. To include gene-regulatory regions, SNPs were included if they fell within 50 kb of a gene. We performed meta-analysis on the two sets of gene-based P values using Fisher's method.

VEGAS-Pathway analysis[19,50] was carried out using prespecified pathways from Gene Ontology. Pathways with 10 to 1,000 components were selected, yielding 4,628 pathways. Pathway analysis was based on combining gene-based test results from VEGAS. Pathway P values were computed by summing χ^2 test statistics derived from VEGAS P values. Empirical VEGAS-Pathway P values for each pathway were computed by comparing the summed χ^2 test statistics from real data with those generated in 500,000 simulations where the relevant number (according to the size of the pathway) of randomly drawn χ^2 test statistics was summed. To ensure that clusters of genes did not adversely affect the results, within each pathway, gene sets were pruned such that each gene was >500 kb away from all other genes in the pathway. Where required, all but one of the clustered genes was dropped at random when genes were clustered. We performed meta-analysis on the two sets of pathway P values using Fisher's method.

To investigate shared genetic background using a large number of autosomal SNPs, we performed a systematic evaluation of the overlap between IOP and POAG on the basis of profile scores, following previously described approaches[27]. We estimated the relative risk for each SNP of interest on the basis of a discovery set (IOP), with a profile score computed for every individual in a target set of interest (POAG). For each target set individual, the profile score was computed as the number of risk alleles weighted by the effect size estimated in the discovery set. The discovery set comprised the European ancestry–derived samples from our meta-analysis, and the target set was a set of 590 glaucoma cases and 3,956 controls, as previously described[7]. To ensure that there was not a high degree of dependence between the SNPs included in the profile score, we filtered the set of SNPs used in the profile score so that only a set of 149,571 SNPs in LD ($r^2 < 0.5$) was used. We constructed models progressively including more SNPs by lowering the threshold of inclusion (i.e., $P < 0.000001$, $P < 0.00001$, $P < 0.0001$, $P < 0.001$, $P < 0.01$, $P < 0.1$, $P < 0.5$). Profiles derived from IOP SNP effects were tested for association with the phenotype (here, POAG) using logistic regression. Variance explained was assessed using Nagelkerke's pseudoR^2 measure[51].

To assess whether and to what degree IOP levels confer POAG risk, we performed a causal inference analysis using an instrumental variable framework as previously described[15]. In brief, we obtained estimates of effect size (β_{IOP}) for the association of a given SNP with IOP from the meta-analysis of the 18 discovery cohorts. For the association of a given SNP with POAG, we obtained estimates of the effect size (β_{POAG}) from the four case-control panels as described above. We selected the SNP with the strongest association from each of the loci with genome-wide significant association with IOP that we identified. To assess whether the strength of a SNP's association with IOP predicted risk of POAG, we conducted linear regression analysis using the effect size of each SNP for IOP (β_{IOP}) as an independent variable and the effect size for POAG (β_{POAG}) as a dependent outcome variable. A total of seven independent IOP-associated SNPs were used for this analysis, including rs7555523 (*TMCO1*), rs6445055 (*FNDC3B*), rs10258482 (*CAV1*), rs2472493 (*ABCA1*), rs8176743 (*ABO*), rs747782 (*NUP160-PTPRJ*) and rs9913911 (*GAS7*).

Gene expression in human tissues. Adult ocular samples were obtained from the normal eyes of an 82-year-old European-ancestry female from the North Carolina Eye Bank. All adult ocular samples were stored in RNAlater (Qiagen) within 6.5 h of collection and shipped on dry ice overnight to the laboratory. Isolated tissues were snap frozen and stored at −80 °C until RNA extraction. RNA was extracted from each tissue sample independently using the Ambion mirVana total RNA extraction kit. Tissue samples were homogenized in Ambion lysis buffer using an Omni Bead Ruptor Tissue Homogenizer according to the provided protocol. Reverse-transcription reactions were performed with the Invitrogen SuperScript III First-Strand Synthesis kit. Expression of the identified genes was assessed by running 10-µl reactions with Qiagen PCR products consisting of 1.26 µl of water, 1.0 µl of 10× buffer, 1.0 µl of dNTPs, 0.3 µl of $MgCl_2$, 2.0 µl of Q-Solution, 0.06 µl of Taq polymerase, 1.0 µl of forward primer, 1.0 µl of reverse primer and 1.5 µl of cDNA. Reactions were run on an Eppendorf MasterCycler Pro S thermocycler with touchdown PCR decreasing the annealing temperature by 1 °C per cycle from 72 °C to

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55 °C followed by 50 cycles of 94 °C for 30 s, 55 °C for 30 s and 72 °C for 30 s with a final elongation of 7 min at 72 °C. All primer sets were designed using Primer3 (ref. 52). Products were run on a 2% agarose gel at 70 V for 35 min. Primer sets were run on a custom tissue panel including Human MTC Panel I and Fetal MTC Panel I (Clontech) and an ocular tissue panel.

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