Genome-wide analyses identify 68 new loci associated with intraocular pressure and improve risk prediction for primary open-angle glaucoma

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Glaucoma is the leading cause of irreversible blindness globally. Despite its gravity, the disease is frequently undiagnosed in the community. Raised intraocular pressure (IOP) is the most important risk factor for primary open-angle glaucoma (POAG). Here we present a meta-analysis of 139,555 European participants, which identified 112 genomic loci associated with IOP, 68 of which are novel. These loci suggest a strong role for angiopoietin-receptor tyrosine kinase signaling, lipid metabolism, mitochondrial function and developmental processes underlying risk for elevated IOP. In addition, 48 of these loci were nominally associated with glaucoma in an independent cohort, 14 of which were significant at a Bonferroni-corrected threshold. Regression-based glaucoma-prediction models had an area under the receiver operating characteristic curve (AUROC) of 0.76 in US NEIGHBORHOOD study participants and 0.74 in independent glaucoma cases from the UK Biobank. Genetic-prediction models for POAG offer an opportunity to target screening and timely therapy to individuals most at risk.

IOP is strongly associated with POAG, and population-based studies have suggested a 16% increase in risk for every mm Hg increase in IOP. Lowering of IOP remains the only proven therapy to slow the progression of vision loss in POAG. IOP heritability is estimated at 55% (ref. 1), and, to date, genome-wide association study (GWAS) meta-analyses have identified several loci associated with IOP1–5 and POAG6–12, which explain a minor proportion of disease heritability and have provided only limited insight into the underlying biological mechanisms. This relative lack of knowledge is partially due to the insufficient statistical power of previous association studies.

Here we present the largest GWAS of IOP to date, in 139,555 participants from three cohorts: UK Biobank13, EPIC-Norfolk14 and the previously reported combined results from 14 European studies in the International Glaucoma Genetics Consortium (IGGC)15. Additionally, we examined associations of 120 significant IOP loci with glaucoma among independent UK Biobank participants (not included in the IOP discovery GWAS) and with clinically diagnosed POAG among participants in a large multicenter case–control study (NEIGHBORHOOD)16.

First, a linear-mixed-model GWAS for IOP was carried out in UK Biobank participants (n = 103,382). The results were replicated in, then meta-analyzed with, the results from EPIC–Norfolk (n = 6,595) and the IGGC meta-analysis17 (n = 29,578). Cohort summary details are presented in Supplementary Table 1. All participants were of European descent (Supplementary Fig. 1 and 2). The meta-analysis results had a genomic inflation factor of 1.28 (Supplementary Fig. 3) but a linkage disequilibrium (LD)-score regression intercept18 of 1.06 (s.e.m. = 0.011) along with (intercept−1)/(mean(χ²)−1) = 0.12, which was consistent with IOP polygenicity rather than population structure.

The UK Biobank analysis alone identified 74 unique autosomal genomic regions meeting genome-wide significance (P < 5 × 10⁻⁸), of which 45 were novel (not previously associated with IOP, glaucoma or related endophenotypes). The results across the three studies were directionally consistent (Supplementary Table 2): 49 loci were replicated in IGGC with a false discovery rate < 0.05, and 27 loci were replicated in either of the replication cohorts (IGGC or EPIC–Norfolk) at a Bonferroni-corrected threshold (P < 6.8 × 10⁻⁸).

Combining the three separate study results into a meta-analysis of 139,555 participants revealed genome-wide-significant associations...
### Table 1 | List of novel SNPs most significantly associated with IOP or POAG in our study

| SNP ID      | Chromosome | Position | Nearest gene | Effect allele | Effect-allele frequency | IOP GWAS meta-analysis | NEIGHBORHOOD POAG association |
|-------------|------------|----------|--------------|---------------|-------------------------|------------------------|--------------------------------|
| rs4074961   | 1          | 38092723 | RSP01        | C             | 0.56                    | -0.09 (-0.11, -0.06)   | 4.4 × 10⁻¹²              |
| rs6781336   | 3          | 66858050 | KBTBD8, LRG1 | A             | 0.70                    | 0.12 (0.09, 0.15)      | 2.7 × 10⁻¹⁸              |
| rs9853115   | 5          | 186131600| DKGK         | T             | 0.50                    | 0.20 (0.17, 0.22)      | 8.9 × 10⁻¹⁵              |
| rs368503    | 6          | 14820471 | ANKH         | C             | 0.72                    | 0.11 (0.08, 0.14)      | 5.1 × 10⁻¹⁰              |
| rs113985657 | 8          | 597203   | EKOC2        | A             | 0.85                    | -0.15 (-0.18, -0.11)   | 1.2 × 10⁻¹⁵              |
| rs17752199  | 9          | 51406848 | PKHD1        | A             | 0.90                    | 0.16 (0.12, 0.20)      | 2.2 × 10⁻¹⁵              |
| rs9494457   | 10         | 13644794 | PEDEB        | A             | 0.62                    | -0.12 (-0.14, -0.09)   | 3.7 × 10⁻⁷               |
| rs10230941  | 11         | 11763611 | CTTNBP2      | C             | 0.64                    | -0.09 (-0.11, -0.06)   | 4.6 × 10⁻¹⁵              |
| rs62520913  | 14         | 12461432 | FBXO32       | T             | 0.93                    | 0.22 (0.17, 0.27)      | 3.6 × 10⁻⁷               |
| rs12377624  | 16         | 12937310 | LMX1B        | G             | 0.63                    | 0.15 (0.13, 0.18)      | 1.3 × 10⁻¹⁵              |
| rs2433414   | 16         | 86410241 | ME3          | T             | 0.80                    | 0.13 (0.10, 0.16)      | 6.9 × 10⁻¹⁶              |
| rs7924522   | 17         | 12830074 | ET5I         | A             | 0.34                    | 0.11 (0.08, 0.14)      | 3.1 × 10⁻¹⁶              |
| rs4775427   | 18         | 69591235 | VPS13C       | T             | 0.43                    | -0.11 (-0.09, 0.04)    | 4.1 × 10⁻¹⁶              |
| rs1874458   | 20         | 65080730 | CDH11        | A             | 0.64                    | -0.10 (-0.13, -0.08)   | 2.9 × 10⁻⁹               |
| rs3743860   | 22         | 89818491 | FANCA        | T             | 0.58                    | 0.10 (0.08, 0.013)     | 4.2 × 10⁻¹⁰              |

Results are presented for the IOP GWAS meta-analysis (UK Biobank, IGGC and EPIC-Norfolk; n = 139,555) and for the association with POAG in the NEIGHBORHOOD study (3,853 cases and 33,480 controls). All IOP-association P values are genome-wide significant (P < 5 × 10⁻⁸) and are in bold if not previously reported to be associated with IOP. POAG-association P values are in bold if novel and significant. A Bonferroni-corrected threshold of P < 4.2 × 10⁻⁴. A full list of all genome-wide-significant loci from the IOP GWAS is given in Supplementary Table 2 (including 68 novel loci), and their associations with POAG in NEIGHBORHOOD are shown in full in Supplementary Table 9. CI, confidence interval; OR, odds ratio.

POAG vs. IOP genetic risk

Fig. 1 | Scatter plot demonstrating the correlation of effect estimates for SNP associations with IOP in our GWAS meta-analysis with effect estimates for SNP associations with POAG in the NEIGHBORHOOD study. Each point represents one SNP from the 120 independent IOP-associated SNPs (derived from the conditional analysis of our IOP GWAS meta-analysis; 13 of 133 SNPs were not available in NEIGHBORHOOD). The color of each point represents the statistical significance of the SNP association with IOP (indicated in the key). The P values were calculated from linear models as described in Supplementary Table 2. Effect estimates are per risk allele.

I–hypersensitivity cluster region 51 kb upstream from the DKGK (diacylglycerol kinase gamma) gene. Diacylglycerol is involved in for 112 unique autosomal genomic regions (Supplementary Fig. 4 and Supplementary Table 2), of which 68 were novel (Table 1). A conditional analysis traced the origin of association signals to 133 SNP loci; when included together in a linear-regression model, these SNPs collectively explained 17% of the IOP variance in the EPIC-Norfolk cohort and 9% of the IOP variance in UK Biobank. The difference in variance explained between the studies may be partly due to less measurement error in EPIC-Norfolk, in which three measurements were taken per eye, as compared with just one measurement per eye in UK Biobank. Among the significant regions were previously reported IOP-associated loci, including TMCO1 (rs10918274, P = 2.4 × 10⁻⁶), GAS7 (rs9913911, P = 4.0 × 10⁻⁴), ABCA1 (rs2472493, P = 6.2 × 10⁻⁵) and CAV1/CAV2 (P = 2.5 × 10⁻³⁶ for rs10281637). Additionally, four of the ten previously reported POAG-associated loci not known to also be associated with IOP were among the significant regions: AFAP1 (rs28649910, P = 8.9 × 10⁻⁴), FOXC1 (rs2745572, P = 1.8 × 10⁻¹⁸), TXNRD2/GNB1L (rs17534001, P = 5.2 × 10⁻¹²) and ATXN2/SH3B3 (rs10774624, P = 3.4 × 10⁻¹⁰⁻¹²). These results strongly suggest that these genes mediate POAG risk via raised IOP.

Interestingly, four loci previously associated with primary angle-closure glaucoma, a form of glaucoma distinct from POAG, were also among the significant regions for IOP, namely HGF (rs3277716, P = 6.1 × 10⁻¹³), PLEKHA7 (rs4141194, P = 7.2 × 10⁻¹²), FERM2 (rs8009633, P = 7.1 × 10⁻¹¹) and GLIS3 (rs6476827, P = 1.2 × 10⁻¹⁰⁻¹¹), thus suggesting that mechanisms underlying angle closure may also contribute to variation in IOP within the normal range. Three IOP-significant loci were in genes previously associated with the optic disc cup area (a structural quantitative trait associated with glaucoma) but not with IOP or POAG, namely BCA3 (rs3785855, P = 4.0 × 10⁻¹⁴), EFEMP1 (rs4672075, P = 1.9 × 10⁻¹¹) and RARB (rs1286771, P = 4.7 × 10⁻¹¹); these results suggest that a proportion of optic disc structural variability in a population may be IOP mediated.

Among the significant IOP loci, a strong association was observed for rs9853115 (P = 8.9 × 10⁻¹²), a SNP located in an ENCODE DNase

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stimulates VEGFR-3 tyrosine kinase signaling in lymphatic endothelial cell subsets versus controls (n = 1,298) and NTG (right; n = 2,606) from a subset of the NEIGHBORHOOD study with individual-level genotype data available. AUC, area under the curve.

Adenosine-receptor signaling, which is important in IOP regulation and a potential target for IOP-lowering therapy\(^\text{16}\). More broadly, DGKG is involved in lipid metabolism, a function shared with other IOP-influencing genes\(^\text{28}\). Very recently, DGKG has also been associated with IOP in a multiancestral study of individuals residing in the United States\(^\text{28}\).

In addition, two loci containing angiopoietin genes (ANGPT1, \(P = 2.7 \times 10^{-10}\) for rs4496939 and ANGPT2, \(P = 1.7 \times 10^{-11}\) for rs76020419) were significantly associated; both encode primary TEK receptor tyrosine kinase ligands whose alteration causes primary congenital glaucoma\(^\text{22}\). In addition, significant association was also found for LRIG1 (rs6781336, \(P = 2.7 \times 10^{-10}\)), an endogenous feedback regulator of receptor tyrosine kinases, and FER (tyrosine kinase (rs73220177, \(P = 1.6 \times 10^{-11}\))). These results suggest a critical role of angiopoietin-receptor tyrosine kinase (ANG-TEK) signaling in IOP regulation. ANG-TEK signaling is an established key mediator of blood and lymphatic vessel development\(^\text{21}\), and gene-set enrichment analysis of our meta-analysis results suggested a strong role of developmental genes whose rare mutations cause congenital or childhood glaucoma\(^\text{25}\).

Two of the IOP-associated SNPs were missense coding (rs2433414, \(P = 6.9 \times 10^{-16}\)), which has previously been implicated in POAG through a mitochondrial gene-set analysis\(^\text{25}\); VPS13C (rs4775427, \(P = 4.1 \times 10^{-14}\)), which is necessary for mitochondrial transmembrane potential; GCAI (rs6000889, \(P = 2.2 \times 10^{-12}\)), which regulates mitochondrial glycine production; and PTCD2 (rs10036789, \(P = 7.7 \times 10^{-10}\)), which is involved in maturation of mitochondrial RNA.

Many of the IOP-associated SNPs reported here were previously associated with other ocular and systemic phenotypes (Supplementary Table 4). A subsequent systematic comparison of all significantly associated SNPs from the current IOP meta-analysis with all the previously published and currently public-domain GWAS data\(^\text{1}\) showed that IOP significantly shares genetic risk factors with other traits; the most significant correlations were with traits previously epidemiologically linked to IOP or glaucoma, such as heart rate\(^\text{3}\), sleep duration\(^\text{3}\) and cholesterol level\(^\text{3}\) (Supplementary Table 5).

Two of the IOP-associated SNPs were missense coding (rs12923138, ELMO3 and rs61755579, SOS2); the rest were outside gene coding regions. Querying of the effects of expression quantitative loci on the GTEx database confirmed that many of these SNPs alter the efficiency of the transcription of genes in their immediate vicinity (Supplementary Table 6). Genes in the vicinity of the IOP-associated SNPs were highly expressed in human trabecular meshwork and ciliary body (Supplementary Table 7), tissues important in IOP homeostasis\(^\text{1}\). Furthermore, S-PrediXcan analyses supported a role of the IOP-associated SNPs in regulation of gene expression, especially for GAS7 (\(P = 1.7 \times 10^{-39}\) and AFAP1 (\(P = 6.1 \times 10^{-20}\)) (Supplementary Table 8).

To evaluate the disease relevance of the IOP-significant SNPs, we tested for association with clinically diagnosed POAG in participants in the NEIGHBORHOOD study\(^\text{1}\) (3,853 cases and 33,480 controls). In total, 48 SNPs were nominally associated with POAG (\(P < 0.05\)), 14 of which were significant at a Bonferroni-corrected threshold of \(P < 4.2 \times 10^{-4}\). For all SNPs, we observed a remarkable correlation between the effect sizes for IOP and POAG (Fig. 1). Analysis of the high-tension glaucoma (HTG) and normal-tension glaucoma (NTG) subgroups suggested that although the association was stronger in HTG, it was still evident in NTG even though the IOP was within normal limits (Supplementary Table 9). Additionally, we identified similar associations between the IOP-significant SNPs and glaucoma (whose status was ascertained by self-reporting and hospital episode statistics data) among UK Biobank participants for whom IOP data was not available and who...
therefore were not part of the IOP GWAS (1,500 cases and 331,078 controls; Supplementary Table 10). There was no evidence of association between IOP-significant SNPs and age at glaucoma diagnosis in either cohort (Supplementary Tables 11 and 12).

Using 120 significant variants from the conditional analysis (Supplementary Table 2) for which genotypes were available in NEIGHBORHOOD participants, and three known POAG-associated polymorphisms showing no evidence of association with IOP in our meta-analysis (rs74315329 within MYOC, rs2157719 near SIX6 and rs8015152 within CDKN2B–AS1), we built and evaluated the performance of a regression-based POAG-prediction model. This model, in addition to including the associated alleles’ predisposing or protective effects on glaucoma, also included age and sex. Despite being limited to a smaller number of significant SNPs, the prediction model performed well in a subset of the NEIGHBORHOOD study with individual-level genotype data available, in particular for HTG (AUROC=0.76) (Fig. 2). This model also performed well in predicting glaucoma in UK Biobank participants not previously included in the IOP GWAS, with an AUROC=0.74 (Supplementary Fig. 5).

In summary, our analysis identified 112 loci, including 68 novel loci, associated with IOP and the development of POAG. Several loci support an important role of ANG-TEK signaling in IOP regulation, and ANG-TEK may thus be a therapeutic target. Together with other genetic factors previously known to affect POAG risk, the loci explained and predicted a substantial portion of POAG with other genetic factors previously known to affect POAG risk, and half of all community glaucoma cases are undiagnosed, genetic prediction models offer an opportunity for improved case detection, earlier treatment and prevention of morbidity from the leading cause of irreversible blindness. The genetic loci identified in this study not only increase understanding of the pathways involved in IOP and glaucoma but also open the possibility of using genetic markers to improve disease screening or even prediction of the natural history of disease in people at risk of glaucoma.

URLs

UK Biobank protocols, http://www.ukbiobank.ac.uk/resources/ and http://biobank.ctsu.ox.ac.uk/crystal/docs.cgi; BOLT-LMM, http://data.broadinstitute.org/alkesgroup/BOLT-LMM/downloads/; LD Score, https://github.com/bulik/lscat; R programming language and software environment for statistical computing, https://cran.r-project.org/; GTEx Portal, https://www.gtexportal.org/home/; EPIC-Norfolk, http://www.epic-norfolk.org.uk/; UK Biobank Access Management System, http://www.ukbiobank.ac.uk/register-ap/. Methods

Methods, including statements of data availability and any associated accession codes and references, are available at https://doi.org/10.1038/s41588-018-0126-8.

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Author contributions
A.P.K., J.N.C.B., M.S., R.P.I., Y.E.S. and P.G.H. conducted the analyses. A.P.K., J.N.C.B., P.J.F., J.L.W., C.J.H. and P.G.H. jointly wrote the manuscript. P.T.K. and P.J.F. designed the ophthalmic component of the UK Biobank study. N.J.W. and R.A.S. led genotyping of the EPIC-Norfolk study. R.W., C.-Y.C., L.R.P. and J.L.H. critically appraised the analyses and critically reviewed the manuscript. The UK Eyes and Vision Consortium critically appraised the analyses. The NEIGHBORHOOD Consortium carried out or critically appraised the genome-wide analyses in the NEIGHBORHOOD study.

Competing interests
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Additional information
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Methods
Study methods. UK Biobank. The UK Biobank is a very large multisite cohort study established by the Medical Research Council, Department of Health, Wellcome Trust medical charity, Scottish Government and Northwest Regional Development Agency. Detailed study protocols are available online (see URLs). A baseline questionnaire was administered, and measurements and biological samples were collected in 22 assessment centers across the UK between 2006 and 2010. All UK residents 40 to 69 years of age who were registered with the National Health Service (NHS) and living within 25 miles from a study center were invited to participate. The study was conducted with the approval of the North-West Research Ethics Committee (ref 06/MRE08/65), in accordance with the principles of the Declaration of Helsinki, and all participants gave written informed consent.

Ophthalmic assessment was not part of the original baseline assessment and was introduced as an enhancement in 2009 for six assessment centers spread across the UK (Liverpool, two sites in Birmingham in the West Midlands, Swanses in Wales, and Croydon and Hounslow in Greater London). Participants completed a touch-screen self-administered questionnaire. The response options for ancestry included White (English/Irish or other White background), Asian or British Asian (Indian/Pakistani/Bangladesh or other Asian background), Black or Black British (Caribbean, African or other Black background), Chinese, mixed (White and Black Caribbean or African, White and Asian, or other mixed background) or other ancestral group (not defined). Self-reported glaucoma status was ascertained by participant selection of ‘glaucoma’ from a list of eye disorders in response to the question “Has a doctor told you that you have any of the following problems with your eyes?”

Participant IOP was measured once for each eye with an Ocular Response Analyzer (ORA; Reichert). Participants reporting eye surgery within the previous 4 weeks or participants reporting an eye infection were precluded from having IOP measured. The ORA is a noncontact tonometer that measures the force required to flatten the cornea with a jet of air. Unlike conventional noncontact tonometry, the ORA measures two pressures: first, when the cornea flattens on inward motion and second when the cornea flattens on outward motion. The average of these two pressures has been calibrated to derive a Goldmann-correlated IOP, and the difference between these two pressures has been shown to be related to the biomechanical properties of the cornea\(^\text{37}\). A linear combination of these two pressures has been developed to derive a corneal-compensated IOP (IOPcc)\(^\text{38}\). We used IOPccs in analyses, because it is thought to provide the most accurate assessment of true IOP and to be least affected by corneal properties\(^\text{41}\).

We excluded participants with a history of laser or surgery for glaucoma, eye injury, corneal graft surgery or refractive laser surgery, because those participants were likely to have IOP altered from physiological levels as a result of nongenetic causes. To handle extreme values of IOP, we excluded IOP measurements in the top and bottom 0.5 percentiles.

A substantial proportion of participants with the highest IOPs in the cohort were diagnosed and treated with IOP-lowering medication in the community before entering the current study. Data for pretreatment IOP were not available, and excluding these participants would have truncated the study IOP distribution, thereby reducing statistical power for detecting associations with IOP. We therefore imputed pretreatment IOP: in study participants reporting current IOP-lowering medication (n = 1,151), the measured IOP was divided by 0.7, according to the mean IOP reduction achieved by medication\(^\text{39}\). This method has been used in previous studies published for IOP\(^\text{40}\). Pretreatment IOP was calculated as the mean of the right- and left-eye values for each participant with data available for both eyes. If data were available for only one eye, we considered that value to be the participant’s IOP. Figures presenting the cleaning and derivation flow for IOP and glaucoma status are shown in the Supplementary Note.

Details for DNA extraction and genotyping of UK Biobank participants are given in the Supplementary Note. The basic model tested was the average of IOP measured in the left and right eye as an outcome of a regression model whose predictor is the allele dosage at a given polymorphic locus, adjusted for age, sex and the first five principal components (further details in the Supplementary Note). Because there was, at the time of writing, evidence of cryptic population relatedness among the UK Biobank participants, a linear mixed model controlling for population structure was used\(^\text{41}\), as implemented in the program BOLT-LMM (see URLs).

International Glaucoma Genetics Consortium. The IGGC study was a meta-analysis of 37,930 participants from 19 studies of European (14 studies) and Asian (5 studies) descent\(^\text{42}\). In that study, similarly to our study, the mean IOP of the right and left eyes was considered, and pretreatment IOP was imputed for participants using IOP-lowering medication. A variety of genotyping arrays were used across the different studies, and genotypes were imputed with 1,000 Genomes Phase 1 reference samples. SNPs with MAF <0.01 and imputation quality scores <0.3 were excluded. Linear-regression analyses were adjusted for age, sex and the first five principal components for population-based studies, or family structure for family-based studies. For the purposes of the current study, we used publicly available summary results for the European subset of the IGGC study (n = 29,578).

EPIC-Norfolk. The European Prospective Investigation into Cancer (EPIC) study is a pan-European prospective cohort study designed to investigate the etiology of major chronic diseases\(^\text{43}\). EPIC-Norfolk, one of the UK arms of EPIC, recruited a total of 1,151,933 individuals between 1993 and 1997, during a single in-person examination\(^\text{44}\). Recruitment was via general practices in the city of Norwich and the surrounding small towns and rural areas, and methods have been described in detail previously\(^\text{45}\). Because virtually all residents in the UK are registered with a general practitioner through the National Health Service, general practice lists served as population registers. Ophthalmic assessment formed part of the third health examination, and this has been termed the EPIC-Norfolk Eye Study\(^\text{46}\).

In total, 8,623 participants were seen for the Eye Study between 2004 and 2011, and IOP was measured with an ORA. Three measurements were taken per eye, and the best signal value was used. The mean IOP of the right and left eyes was calculated and used in analyses. 97.9% of EPIC-Norfolk participants are of European descent, and we excluded non-White participants. The EPIC-Norfolk Eye Study was carried out in accordance with the principles of the Declaration of Helsinki and the Research Governance Framework for Health and Social Care. The study was approved by the Norfolk Local Research Ethics Committee (05/Q0101/191) and East Norfolk & Waveney NHS Research Governance Committee (2005E0701). All participants gave written, informed consent.

Details for genotyping and imputation of EPIC-Norfolk participants are given in the Supplementary Note.

Similarly to the UK Biobank GWAS, we examined the relationship between allele dosage and mean IOPcc by using linear regression adjusted for age, sex and the first five principal components. Analyses were carried out in SNPTEST version 2.5.1.

NEIGHBORHOOD Study. All cases and controls met the clinical criteria previously used by the NEIGHBOR and GLAUGEN studies previously described\(^\text{47,48}\). The study subjects were enrolled under a protocol approved by the Massachusetts Eye and Ear Infirmary institutional review board, and all local institutional review board present approved by the local institutional review board before enrolling in the study.

Briefly, POAG cases were defined as individuals for whom reliable visual field (VF) tests showed characteristic VF defects consistent with glaucomatous optic neuropathy. Individuals were classified as affected if the VF defects were reproduced in a subsequent test or if a single qualifying VF was accompanied by a cup-disc ratio (CDR) of 0.7 or more in at least one eye. Most cases (>90%) met this definition, including 96% of the NEIGHBOR cases\(^\text{49}\) and all of the Massachusetts Eye and Ear Infirmary (MEEI), Nurses’ Health Study, Health Professionals Follow-up Study (HPFS) and Women’s Genomes Health Study (WGHS) cases. A small percentage (less than 10%) of the NEIGHBOR, Mayo, Marshfield and Iowa cases were defined by CDR only because VF data were not available, in some cases because of advanced disease (poor visual acuity) or other medical conditions. The CDR definition was >0.7 in both eyes or a CDR asymmetry between the two eyes of 0.2 (Supplementary Table 2). In the OHTS study, an alternative case definition based on progression rates of central retinal nerve fiber layer thickness and mean deviation (MD) (described below). Patients with signs of secondary causes for elevated IOP, such as exfoliation syndrome or pigment dispersion syndrome or critically narrow filtration structures, were excluded. Elevation of IOP was not a criterion for inclusion of cases or controls; however, 1,868 cases did have a history of elevated IOP (>22 mm Hg) measured in a clinical setting (typically between the hours of 8:00 AM and 10:00 AM) and were classified as having IOP and were classified as NTG. For 1,260, cases, peak IOP data were not available. The controls were selected to be representative of the age range and sex of the cases. Although the average age of cases and controls was not statistically different for any dataset included in the NEIGHBORHOOD, some datasets included cases and controls younger than age 55, thus potentially decreasing the power of the study. Controls had IOP <21 mm Hg, as measured in a clinical setting, and CDR <0.6, and did not have a family history of glaucoma.

NEIGHBORHOOD used different genotyping chips and imputation methods, as specified elsewhere\(^\text{50}\). The quality controlled genotypes (1,000 Genomes panel, March 2012, INFO score >0.9) for 3,853 cases and 33,480 controls from eight independent datasets were used as the discovery cohort for the NEIGHBORHOOD genome-wide association study (GWAS)\(^\text{51}\). Quality control was performed for each dataset, as described in Bailey et al.\(^\text{52}\). Overall sample and genotype call rates were ≥95% for each site. Samples with log R ratio (LRR) and B allele frequency (BAF) values suggestive of copy number variants were removed before analysis. Principal components (eigenvectors) were computed for all participants in EIGENSTRAT\(^\text{14}\). For each dataset, logistic regression was performed in ProbABEL\(^\text{55}\) for all analyses (POAG overall, HTG and NTG), controlling for age, sex and study-specific covariates including study-specific eigenvectors. Each analysis was evaluated separately for overall genotyping inflation (implementing the package GenABEL (G value ≤1.05 for each dataset)). Results were meta-analyzed in METAL\(^\text{56}\) through implementing the inverse-variance-weighted method and applying genomic control correction.

For the prediction models and assessment of their performance, a balanced dataset of the cases and controls (n\(_{\text{cases}} = n_{\text{controls}} = 2,068\)) were used from only two subcohorts: NEIGHBOR and MEEI. The choice of the two largest subcohorts
within NEIGHBORHOOD ensured that the prediction dataset was fully balanced and minimized the risk of stratification among the samples, because the genotyping and imputation pipelines followed were largely compatible.

**Statistical analyses.** Details of our statistical analyses are below and in the accompanying Reporting Summary.

**Meta-analysis.** Summary statistics from each stratum (UK Biobank, the International Glaucoma Genetics Consortium meta-analysis53 and from the participants in the EPIC study who were not included in the IGGC meta-analysis) were combined through fixed-effects inverse-variance-weighted meta-analysis in METAL54. Random-effects meta-analyses results were also obtained with GWAMA55, but the results did not differ significantly from those from the fixed-effect model; hence, the results shown are from only the latter. No genomic control adjustment was applied during the meta-analysis.

**Conditional and explained heritability analyses.** The program GCTA52 was used for the conditional analyses76 to identify independent effects within associated loci and to calculate the phenotypic variance explained77 by all polymorphisms, genotyped or imputed, associated with the trait after the conditional analyses. The threshold of significance was set at $5 \times 10^{-8}$, and the collinearity threshold was set at $r^2 = 0.9$.

The LD estimates were derived from the UKBB cohort.

**Calculation of genomic inflation factor.** To assess the potential inflation of association probabilities, genomic inflation factors78 were calculated, and Q–Q plots were drawn in the package ‘gap’ in R (see URLs).

**Multiple testing correction.** Two methods of correcting for multiple testing were used. The first was a classic Bonferroni correction, in which the threshold of significance ($0.05$) was divided by the number of experiments ($n$): $n_e = 0.05 / n$

Given the large number of loci for which replication was needed, we additionally calculated the false discovery rates, using the Benjamini–Hochberg method81.

**LD Score analyses.** For intertrait genetic correlation, bivariate genetic correlations between IOP and other complex traits whose summary statistics are publicly available were assessed according to previously described methods82, in the program LD Score (see URLs).

For calculating the LD-score regression intercepts, to distinguish between the effect of polygenicity and those arising from sample stratification or uncontrolled population admixture, we followed previously suggested approaches83, using the program LD Score (see URLs).

**Prediction analyses.** To assess the potential value of the loci associated with IOP to predict POAG, regression-based models were deliberately trained and tested separately in two different groups. The first was the set of UK Biobank participants for whom IOP measurements were not available (thus making them ineligible for participation in the meta-analysis of the IOP regression analysis; Supplementary Note). Because this information was questionnaire derived, for those patients it was impossible to stratify the diagnosis of glaucoma into NTG or HTG subgroups. This dataset was not balanced, because it included 1,500 cases of glaucoma and 331,078 individuals with no self-reported diagnosis of glaucoma. The second group comprised the clinical cases and controls from two of the NEIGHBORHOOD subcohorts (NEIGHBOR and MEEI). Patients and controls in this group were clinically characterized. They were a mixture of NTG and HTG cases ($n = 561$ and $n = 1,298$ respectively), a further 747 subjects of uncertain POAG and 2,606 controls.

We built the same model in all cases, which included age, sex and the major genetic variants associated with IOP after the conditional analysis. We additionally included three known POAG-associated polymorphisms showing strong evidence of association with IOP in our meta-analysis ($rs74315329$ within MYOC, $rs12577719$ near SIX6 and $rs8015152$ within CDKN2B-AS1). To minimize bias, we did not use the effect sizes observed for IOP to weigh the effects in other cohorts. Instead, in each group separately, logistic regressions were trained with a random subset of 80% of cases and controls. The ability of these trained models to correctly predict the presence of POAG (whether self-reported or doctor diagnosed, depending on the group) was assessed in the remaining 20% of the subjects. A receiver operating characteristic curve was drawn for each case, and an area under the curve was calculated. R programming language and software environment for statistical computing (see URLs) was used for the logistic regression models (glm) and to evaluate the performance of the model (ROC).

**SNP and gene annotations.** Polymorphisms associated at a GWAS level ($P < 5 \times 10^{-8}$) were clustered within an ‘associated genomic region’ defined as a contiguous genomic region where GWAS-significant markers were within 1 million bp from each other. Significant polymorphisms were annotated with the gene within whose transcript-encoding region they were located, or alternatively, for polymorphisms located between two genes, with the nearest gene. The associated genomic regions were collectively annotated with the gene overlapping or nearest to the most significantly associated variant within that region.

In addition, the polymorphic sites were functionally annotated in SNPnexus84.

GTEx. Because of unavailability of tissues extracted from human eyes, the influence of our significant SNPs on transcription of adjacent genes was assessed in all other tissues available to the GTEx Project85 and was queried in the GTEx Portal (see URLs).

**Ocular gene expression.** Gene expression in the human trabecular meshwork and ciliary body tissue of genes at loci significant in the IOP GWAS was examined by using results from a published RNA-sequencing study86. The expression of each gene (adjusted for gene length and the number of sequencing reads in a given sample) is presented in fragments per kilobase of transcript per million mapped reads (FPKM). On the basis of the overall gene expression distribution, genes with an FPKM $\geq 1$, an FPKM $\geq 4.7$ (thirty-third percentile) and an FPKM $\geq 15.9$ (sixty-seventh percentile) were classified as weakly, moderately or highly expressed, respectively.

*S-PrediXcan*. We used S-PrediXcan87 to estimate genetically regulated gene expression by using whole-genome tissue-dependent prediction models trained with GTEx reference transcriptome data, and then to correlate the results with IOP to identify gene sets involved in IOP regulation. S-PrediXcan is related to PrediXcan87 but GWAS summary statistics at input. Through the GTEx analysis described above, we examined correlations by using the following reference tissues: whole blood, adipose-omentum, brain-cortex, artery-aorta and artery-coronary. Results are presented in Supplementary Table 8 for all genes significant after Bonferroni correction for all genes tested in all tissues.

**Gene-set enrichment.** To identify pathways or other gene sets that were over- or underrepresented among our results, we used a gene-set enrichment analysis, as implemented in Meta-Analysis Gene Set Enrichment of Variant (MAGENTA) software88. This program assigns scores to each gene on the basis of the strength of association with IOR adjusting for potential confounders such as gene length and linkage disequilibrium. Enrichment for any gene set was assessed within genes above the cutoff of the highest seventy-fifth centile of significant gene scores. For the current study, the most recent versions of the Gene Ontology (GO), Panther, KEGG, Biocarta and MSigDB databases were used. A permuted procedure and false discovery rates were used to calculate the significance of enrichment and to control for multiple testing.

**Reporting Summary.** Further information on experimental design is available in the Nature Research Reporting Summary linked to this article.

**Data availability.** UK Biobank data are available through the UK Biobank Access Management System (see URLs). The data sharing and preservation strategy in EPIC-Norfolk and full details about the study, including contact information, are reviewed and approved; there is no serious risk to the viability of continuing the cohort study, for example, through offense to the participants from use of the data supplied; the science of the proposal has been satisfactorily peer reviewed; and the proposal does not duplicate work already being done.

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### Experimental design

1. Sample size
   
   Describe how sample size was determined.

   The UK Biobank is a very large multisite cohort study of around 500,000 participants. The design of the study aimed to be large enough to detect associations of small effect, even in sub-group analyses. The very large sample size was not calculated for one particular trait. Intraocular pressure (IOP), our primary trait of interest, was available for around 103,000 participants which makes this study the largest IOP analysis to date.

2. Data exclusions
   
   Describe any data exclusions.

   Other than data cleaning for IOP, exclusion of non-European descent participants and genotyping quality control, we did not exclude participants from our analysis. The IOP cleaning process undertaken is presented in Supplementary Figure A so that this process may be repeated by future investigators. Of 502,631 UK Biobank participants, 128,964 participants had an IOP measurement in at least one eye. The top and bottom 0.5% of left and right eye measurements were excluded to remove values that were likely artifact, leaving 128,723 participants with at least one IOP measurement. There were 124,463 participants remaining after exclusion for (in order): eye injury (n = 905), corneal graft (n = 174), glaucoma laser (n = 357), glaucoma surgery (n = 131), corneal refractive surgery (n = 2,693). Primary analysis was carried out using 103,382 participants remaining following exclusion of: non-European descent by using principal component plots, related individuals, UK Biobank recommended exclusions.

3. Replication
   
   Describe the measures taken to verify the reproducibility of the experimental findings.

   UK Biobank IOP significant results were tested in two independent studies: EPIC-Norfolk (n=6,595) and previously reported combined results from 14 European studies in the International Glaucoma Genetics Consortium (IGGC, n=29,578). The discovery analysis in UK Biobank identified 74 regions meeting genome-wide significance. Results across the three studies were directionally consistent (Supplementary Table 1); 49 loci replicated in IGGC with a false discovery rate (FDR) of <0.05, and 27 loci replicated in either of the replication cohorts (IGGC or EPIC-Norfolk) at a Bonferroni-corrected threshold (P<6.8E-4).

4. Randomization
   
   Describe how samples/organisms/participants were allocated into experimental groups.

   Our study was observational and participants were not allocated into experimental groups.

5. Blinding
   
   Describe whether the investigators were blinded to group allocation during data collection and/or analysis.

   Our study participants were not allocated into different groups and therefore blinding was not required.

Note: all in vivo studies must report how sample size was determined and whether blinding and randomization were used.
6. Statistical parameters

For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

| n/a | Confirmed |
|-----|-----------|
| ☐   | ☒         |
| ☐   | ☒         |
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| ☐   | ☒         |

- The exact sample size ($n$) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)
- A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
- A statement indicating how many times each experiment was replicated
- The statistical test(s) used and whether they are one- or two-sided
- Only common tests should be described solely by name; describe more complex techniques in the Methods section.
- A description of any assumptions or corrections, such as an adjustment for multiple comparisons
- Test values indicating whether an effect is present
- Provide confidence intervals or give results of significance tests (e.g. $P$ values) as exact values whenever appropriate and with effect sizes noted.
- A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)
- Clearly defined error bars in all relevant figure captions (with explicit mention of central tendency and variation)

See the web collection on statistics for biologists for further resources and guidance.

7. Software

Policy information about availability of computer code

Describe the software used to analyze the data in this study.

The UK Biobank team performed imputation from Haplotype Reference Consortium reference panel; phasing was performed using SHAPEIT3 and imputation was carried out via the IMPUTE3 program. Since there was, at the time of writing, evidence of cryptic relatedness among the UK Biobank participants, a linear mixed model that controls for population structure was used and implemented using the program BOLT-LMM (http://data.broadinstitute.org/alkesgroup/BOLT-LMM/downloads/). In EPIC-Norfolk, Data were pre-phased using SHAPEIT version 2 and imputed to the Phase 3 build of the 1000 Genomes project (October 2014) using IMPUTE version 2.3.2; linear regression analysis was carried out using SNPTST version 2.5.1. Results from UK Biobank, EPIC-Norfolk and IGGC were combined using fixed-effects inverse variance weighted meta-analysis, using METAL.

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). Nature Methods guidance for providing algorithms and software for publication provides further information on this topic.

8. Materials availability

Policy information about availability of materials

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a third party.

No unique materials were used in our study.

No antibodies were used in our study.

No eukaryotic cell lines were used.

No eukaryotic cell lines were used.

No eukaryotic cell lines were used.

No commonly misidentified cell lines were used.
Animals and human research participants

Policy information about studies involving animals; when reporting animal research, follow the ARRIVE guidelines

11. Description of research animals
   Provide all relevant details on animals and/or animal-derived materials used in the study.
   No animals were used in our study.

Policy information about studies involving human research participants

12. Description of human research participants
   Describe the covariate-relevant population characteristics of the human research participants.
   The 103,382 participants in the UK Biobank IOP analysis had a mean age of 57.4 years (SD 7.8) and 53.1% were women. The 6,595 participants of EPIC-Norfolk had a mean age of 68.8 years (SD 8.0) and 54.9% were women.