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Genome-wide meta-analysis identifies 22 loci for normal tension glaucoma with significant overlap with high tension glaucoma

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Primary open-angle glaucoma typically presents as two subtypes. This study aimed to elucidate the shared and distinct genetic architectures of normal-tension (NTG) and high-tension glaucoma (HTG), motivated by the need to develop intraocular pressure (IOP)-independent drug targets for the disease. We conducted a comprehensive multi-ethnic meta-analysis, prioritized variants based on functional annotation, and explored drug-gene interactions. We further assessed the genetic overlap between NTG and HTG using pairwise GWAS analysis. We identified 22 risk loci associated with NTG, 17 of which have not previously been reported for NTG. Two loci, *BMP4* and *TBKBP1*, have not previously been associated with glaucoma at the genome-wide significance level. Our results indicate that while there is a significant overlap in risk loci between tension subtypes, the magnitude of the effect tends to be lower in NTG compared to HTG, particularly for IOP-related loci. Additionally, we identified a potential role for biologic immunomodulatory treatments as neuroprotective agents.

Primary open-angle glaucoma (POAG) is a chronic and progressive optic neuropathy that is characterized by damage to the optic nerve and loss of vision[1]. It is a leading cause of irreversible blindness, and its worldwide prevalence was estimated to be around 2.4% in 2021[2]. POAG is one of the most heritable diseases[3] and typically presents as two subtypes: high-tension glaucoma (HTG) and normal-tension glaucoma (NTG).

HTG is defined as POAG in patients with intraocular pressure (IOP) ≥ 21 mmHg, and it is usually associated with the ocular hypertension process, in which high IOP is the main risk factor in the disease progression[4]. NTG is defined as POAG in patients with IOP consistently measured <21 mmHg, and is believed to be more related to specific processes such as vascular dysregulation and ischemia[5,6]. However, the stratification of POAG by IOP is controversial whereby different tension subtypes may be part of the same pathogenic process[7] versus the claim that primary neurodegeneration features are more prominently in NTG[8].

The current treatments for NTG/HTG aim to lower IOP, which can slow disease progression[9,10]. Yet, over 50% of POAG cases are not diagnosed until there is irreversible optic nerve damage[11]. This highlights the importance of developing more accurate screening methods to identify individuals at high risk for NTG earlier in the disease process. In addition, a deeper understanding of the genetics of the neurodegenerative process of glaucoma could help develop

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neuroprotective treatments, which are currently unavailable[12], by inhibiting the pathogenic cascade that leads to nerve damage.

In this work, we aimed to untangle the shared and specific genetic architecture between the glaucoma tension subtypes and identify specific loci for NTG, which could lead to a more accurate risk assessment and screening prioritization. We also used our gene-discovery approach to highlight possible neuro-protective drug targets for POAG.

Results

Multitrait meta-analysis

Throughout our study, we used a two-stage meta-analysis approach. The first stage included European data from the IGGC, UK Biobank, CLSA, and VCDR. Upon validating the initial results on Asian participants from IGGC and Europeans using Finngen data, the second stage incorporated these datasets along with the results from the first stage.

This study identified 22 independent loci associated with NTG by meta-analyzing multiple ethnicities and traits (Fig. 1). The first stage MTAG analysis found 11 genome-wide significant risk loci, nine of which replicated after Bonferroni correction for multiple testing ($P < 0.05/11$) and the remaining two at nominal significance ($P < 0.05$) in Finngen; see Supplementary Data 1. In the IGGC Asian meta-analysis, three were replicated after Bonferroni correction and five at nominal significance (Supplementary Data 1). Three loci did not replicate in IGGC Asians (rs6054248, rs57831033, and rs6539772), and two, rs66998222 and rs2971831, did not replicate after correction for multiple testing in FinnGen, however, all of them maintained the same direction of effect. The resulting meta-analysis demonstrated low genetic inflation (λGC = 1.08; see Q–Q plot in Supplementary Fig. 1), an LDSC intercept of 1.02 (SE = 0.007), and an array heritability estimate of 0.22 (SE = 0.05) for NTG in Europeans. We further validated the consistency of the results between stage one and stage two of the meta-analysis using an IVW approach ($r = 0.99$, $P = 1.7e\text{-}11$); as per Supplementary Fig. 2. Examination of all the loci across the genome using the GWAS pairwise method (GWAS-PW) indicated that the risk loci are shared across NTG and HTG (PPA > 0.8), consistent with the high genetic correlation (rg) between HTG and NTG (rg = 0.84, se = 0.07 $P = 5.1e{-}33$).

For the genome-wide significant NTG loci from our stage-one model, we found a high correlation ($r = 0.89$, $P = 0.003$) between the effect size on NTG for the European discovery sample datasets and the effect sizes estimated in the IGGC Asian NTG datasets (Fig. 2A). The correlation was even higher ($r = 0.99$, $P = 1.5e{-}8$) when comparing the European discovery sample NTG datasets and NTG in FinnGen (Fig. 2B). We further tested the correlation of effect sizes between the MTAG-independent NTG genome-wide significant loci and the published IGGC POAG GWAS, which revealed a lower correlation than the results obtained from NTG in Finngen (Fig. 2C). Similarly, the correlation between the effect sizes of the risk loci associated with POAG[13] and NTG in FinnGen was smaller ($r = 0.53$, $P < 0.001$); as shown in Supplementary Fig. 3.

The second stage of the meta-analysis across ethnicities (i.e., joint analysis of the discovery and validated datasets from stage 1) found 22 independent genome-wide significant loci associated with NTG (Supplementary Data 2), 17 have not been identified for NTG and two loci (*MIR5580/BMP4* and *KPNB1/TBKBP1*) have not been associated at the genome-wide significant level with any glaucoma subtype. However, some of these loci have been previously associated at a genome-wide significant level with primary open-angle glaucoma (16/22), high-tension glaucoma (7/22), and some glaucoma endophenotypes such as IOP (9/22) and VCDR (21/22); VCDR has been previously associated with *MIR5580* and *KPNB1*.

The effect size of the genome-wide significant loci tends to be lower in NTG when compared with HTG (Fig. 3); on average, the HTG effects on the log(OR) scale were 1.6 times higher (SE = 0.13) based on

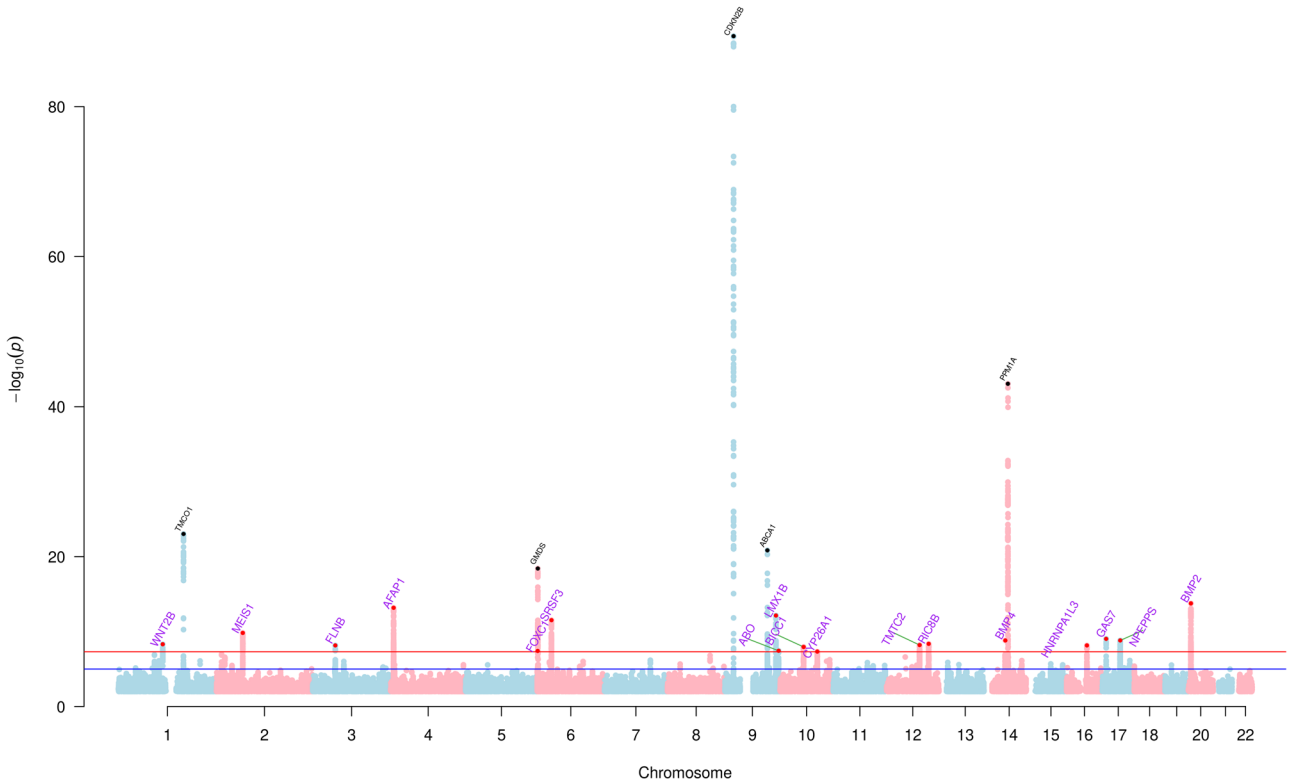

Fig. 1 | Manhattan plot based on the meta-analysis of normal tension glaucoma (NTG). Each dot ($N = 22$) represents a single nucleotide polymorphism (SNP), and the red line represents the threshold for multiple testing correction ($p < 5 \times 10^{-8}$) and blue line $p < 5 \times 10^{-6}$; p-values derived from logistic regression models are two-sided. Previously unidentified loci are highlighted in purple and represented in the plot as red dots, while known loci are in black.

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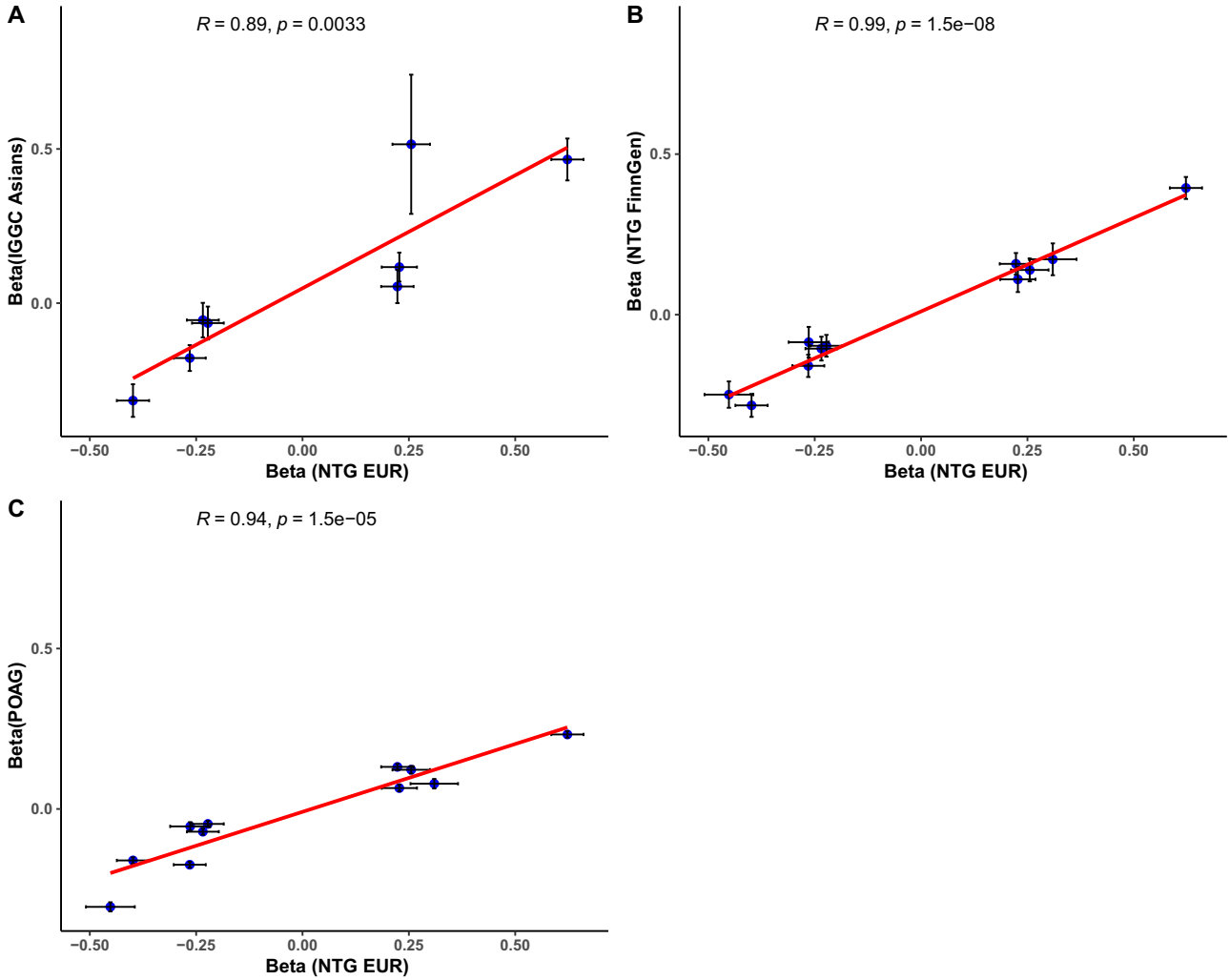

Fig. 2 | Correlation of the allele effect estimates using a two-sided linear model between multitrait analysis of European ancestry (EUR) and two independent cohorts, FinnGen and the Asians in the International Genetics of Glaucoma Consortium (IGGC). Effects estimate (Beta) are presented as center points and black crosses represent 95% confidence intervals. **A** Correlation of the effect estimates between the NTG of EUR and IGGC Asians ($N = 8$), **B** NTG of EUR and FinnGen ($N = 11$) and **C** NTG of EUR and POAG ($N = 11$).

an inverse variance weighting regression of the effect sizes. This difference was larger for IOP loci ($P < 0.05$ for IOP), with the HTG effects being 1.9 times higher (SE = 0.2) than NTG compared with non-IOP loci ($P > 0.05$ for IOP) where the HTG effects were only 1.2 times higher (SE = 0.09). Seven loci showed statistically significantly different magnitudes of effect between NTG and HTG after multiple testing corrections ($P < 0.002$; 0.05/22 loci), as shown in Supplementary Data 2. Four loci (rs2472493, nearest gene is *ABCA1*, rs12602519, nearest gene is *GAS7*, rs4657477, nearest gene is *TMCO1*, and rs2745572, nearest gene is *FOXC1*) were consistently using SNP effect sizes from clinically diagnosed NTG in FinnGen and IGGC.

Functional annotation and drug-gene interaction

Gene-based analysis in mBAT-combo on 18,766 genes identified 25 genes associated with NTG ($P < 0.05/18{,}766$) (Supplementary Data 3), and gene-set enrichment analysis in MAGMA identified a biological process "go_response_to_laminar_fluid_shear_stress" that is likely associated with the etiology of NTG ($P < 2.7e{-}6$). Although gene-based analysis did not identify additional risk loci over and above the SNP-based analysis, it provided gene-based support for the association of genes within the two loci (*MIR5580/BMP4* and *KPNB1/TBKBP1/EFCAB13)*, which were not previously associated at the genome-wide significant level with any glaucoma phenotype. The results of the conditional analysis in GCTA-COJO for the top SNP of *KPNB1* (rs7220935) and *EFCAB13* (rs9894179) suggest that this could be part of the same signal at the *TBKBP1* locus, as the signal for *TBKBP1* did not remain in the conditional analysis. Similarly, the signal for *MIR5580* did not remain after the analysis was conditioned for *BMP4*, indicating that it is likely the same locus, as shown in Supplementary Figs. 12–15. *KPNB1* has been previously associated with snoring[14], hemoglobin levels[15], and Body Mass Index (BMI)[16].

Single-cell expression results showed a statistically significant association between the expression of *BMP4* and NTG in serotonin transporter neurons that were exposed to rotenone-induced oxidative stress. Expression of two other genes, *ALDH9A*1 and *MGST3*, were also associated with NTG in neuron or neuron-like cells; however, these associations were nominally significant or had a HEIDI $P < 0.05$, likely depicting heterogeneity in the association; as per Supplementary Data 5. Results for *BMP4* were also consistent with multi-omics analysis where *MIR5580/BMP4* (rs12893484) was associated with methylation at a DNA promoter region (cg16720578), with a posterior probability of association greater than 0.9 for a combined effect of changes in gene expression and methylation of *BMP4* in development of NTG. Results from eQTL on peripheral blood using the SMR framework also found

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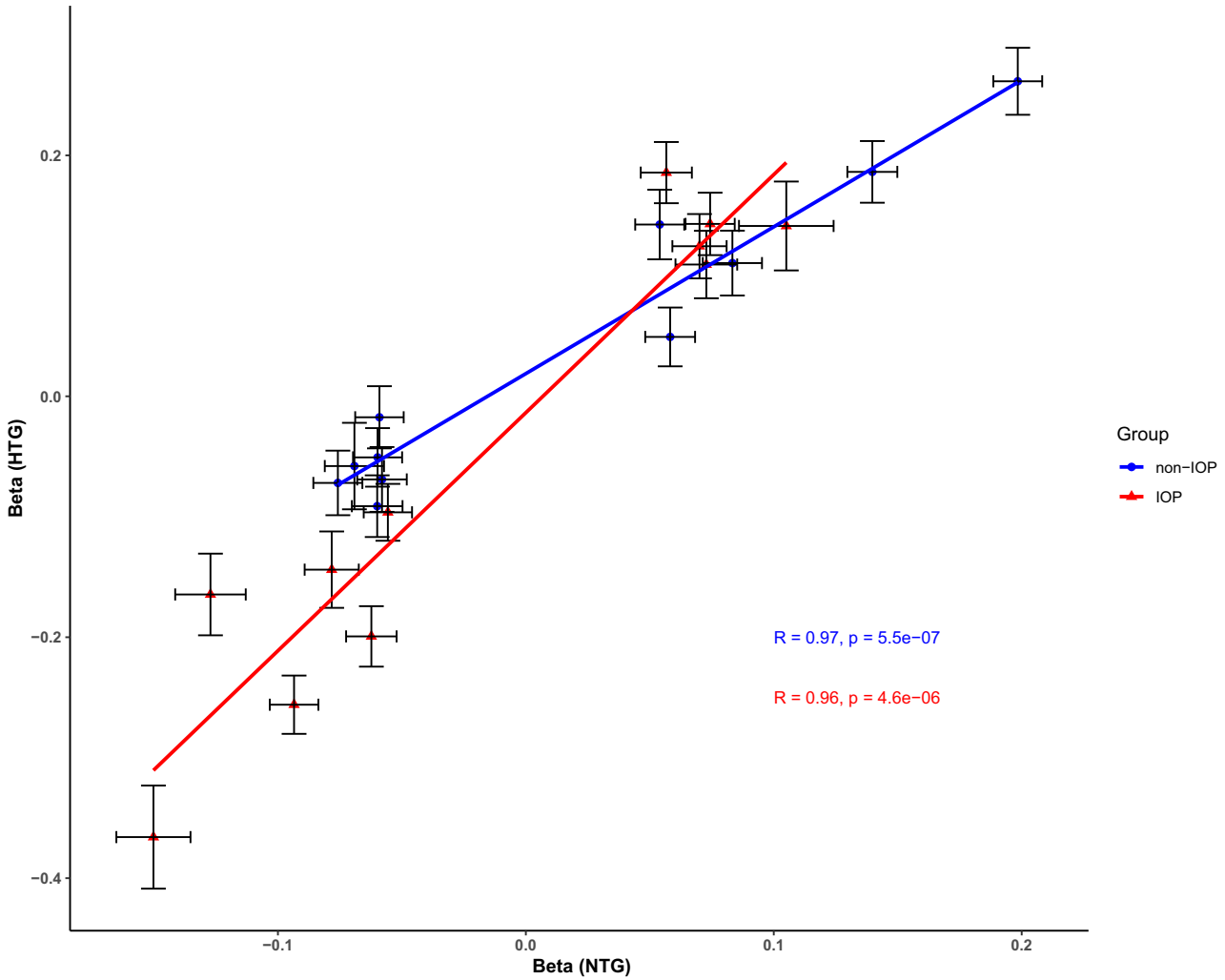

Fig. 3 | Inverse weighted variance (IVW) correlation between the effect sizes (*N* = 22), estimated in the log(OR), of normal tension glaucoma (NTG) and high tension glaucoma (HTG). The slope for IOP loci ($P < 0.05$ for IOP; red line) shows that the effect sizes on average are 1.9 times higher in HTG compared to NTG whereas the slope for non-IOP loci ($P > 0.05$ for IOP; blue line), shows that the effect sizes on average are more similar (only 1.2 times higher in HTG); *P*-values derived from IVW are two-sided. Effects estimate (Beta) are presented as center points and black crosses represent 95% confidence intervals.

an association with NTG and 6 genes, as per the supplementary Data 6, including *TBKBP1*, however, only *ITGB3* association remained significant after assessing for heterogeneity ($P > 0.05$ [HEIDI]). We further assessed the expression profile using retinal eQTL data, but the results were inconclusive, likely due to a lower sample size of the retinal eQTL data.

Results from DBIbd found interactions between four genes (i.e., *ABCA1, CDKN2A, CDKN2B*, and *ITGB3*) and 42 drugs, as shown in Supplementary Data 4. Of interest, *CDKN2A, CDKN2B*, and *ITGB3*, are genes that have been previously associated with the neurodegeneration of the optic nerve and had the majority of the drug-gene interactions (36/42). Highlighting a potential role for biologic immunomodulatory treatments as neuroprotective agents; as per Supplementary Data 4.

Discussion

We conducted a GWAS and identified 22 independent loci associated with NTG, thereby expanding our understanding of the genetic architecture underlying the condition. Gene enrichment analysis and SNP-based tests identified two loci, *BMP4* and *TBKBP1*, which had not previously been associated at a genome-wide significant level with any glaucoma subtype.

TBKBP1 is an adaptor protein that binds to TANK-binding kinase 1 (*TBK1*), a gene known to be involved in various cellular processes, including inflammation, autophagy, and the innate immune response[17]. Previous studies have shown an association between *TBK1* mutations and NTG, where gene duplications and triplications have been associated with early-onset familial NTG[1,18,19]. Consistent with these studies, our results highlight an association between *TBKBP1* in peripheral blood and NTG. These findings support the potential involvement of the adaptor protein *TBKBP1* in the development of NTG.

Through this study, we also found an association between *BMP4* and NTG, an association that was confirmed through multi-omics analysis. Bone Morphogenetic Protein 4 (*BMP4*) has been associated with retinal ganglion cell integrity in mouse models[20]. There is a well-known interaction between *BMP4* and *TGF-β* that could play a significant role in NTG pathogenesis through effects on the trabecular meshwork and optic nerve head[21,22]. *TGF-β2* is significantly upregulated in patients with glaucoma[21], and increased concentrations of *TGF-β* have been observed in the aqueous humor and reactive optic nerve astrocytes of individuals diagnosed with POAG[23]. It has also been suggested that *BMP4* acts as an endogenous inhibitor of *TGF-β2* within the human trabecular meshwork and aids in controlling the increased

accumulation of extracellular matrix protein deposits[24], thereby maintaining IOP within normal limits.

Our European meta-analysis revealed a consistent loci effect when compared to two independent cohorts, Finngen and the Asian meta-analysis of IGGC. Although our first stage meta-analysis used proxy phenotype information for NTG (based on IOP < 21 mmHg, as well as VCDR), the results replicated well in IGGC and Finngen with clinically diagnosed NTG. Notably, three loci were not present in the Asian population, one of which corresponded to the *TMCO1* region where the key SNP (rs4657477) is monomorphic in Asians. Given the higher prevalence of NTG in Asian populations[25], the absence of a *TMCO1*-related variant could be expected as this gene is likely associated with higher IOP[26].

Genes such as *FOXC1* are likely to have a primary effect on IOP but also a secondary effect on VCDR, as a result of the pressure effect. In our study, we adjust for IOP to focus our efforts on identifying loci that act on VCDR in a way that is relatively unaffected by IOP. In the second stage of the meta-analysis, we sought to leverage the effect of known variants in genes such as *CDKN2BAS* and *SIX1*, which affect VCDR with little or no impact on IOP, across the genome. These results still show a high genetic correlation between HTG and NTG, with shared risk loci across the genome. However, further analysis revealed that NTG loci could be divided into two groups, 'neurodegenerative-driven' (VCDR-related) and 'IOP-driven'. Genes in the IOP-driven group (e.g., *TMCO1, GAS7, ABCA1*) had a much larger effect on HTG than on NTG, while genes in the neurodegenerative-driven group (e.g., *SIX6/BICC1, CDKN2BAS, MTMC2, CASC20*) had a similar effect on both HTG and NTG risk. This suggests that the observed 1.6-fold increased effect on HTG compared to NTG is a result of a combination of two types of genetic variants, where the neurodegenerative-driven loci have a similar effect size on both HTG and NTG, while the IOP-driven loci have a much larger effect size on HTG than on NTG. Future studies of NTG may be able to leverage this further to estimate more accurate effect sizes, which could benefit some types of analysis, such as polygenic risk scores.

The genetic analysis of drug-gene interactions revealed a significant interaction between four genes and several drugs. Specifically, *ABCA1* had an interaction with some cholesterol-lowering medications such as Probucol, Simvastatin, Pravastatin, and Atorvastatin. These drugs have been studied for their potential neuroprotective effects in animal models of glaucoma, and some have been found to lower IOP and improve retinal ganglion cell survival[27,28]. However, no drug-gene interactions associated with the neurodegeneration of the optic nerve (*CDKN2A, CDKN2B* and *ITGB3*) have been specifically proposed or studied as treatments for glaucoma and further research is necessary to evidence if these could be potential targets for neuroprotective treatments.

It is important to highlight that our results were limited by statistical power to identify loci with a small effect, warranting larger studies to identify such loci. We have shown that the VCDR-driven loci are shared across NTG and HTG, with similar effect sizes. For the IOP-driven loci, the largest ones affect NTG, although the effect size on NTG for such loci is much smaller than it is on HTG. Additionally, while our results were replicated in Asians, where NTG is more prevalent, further research is needed to validate our proposed explanations in other ethnic groups.

A limitation of our study is the reliance on IOP measurements, which included participants who had tonometry up to five years before or after the diagnosis of glaucoma. This introduces a risk of misclassification, particularly given that glaucoma diagnoses were self-reported in CLSA. While our two-stage approach to analysis mitigates some of these concerns by reducing the likelihood of false positives, it may increase the heterogeneity of the sample and the potential for false negatives. Consequently, while the loci identified in this study are likely robust, additional loci associated with NTG may remain undetected.

To conclude, our study identified 22 independent loci associated with NTG and revealed a high genetic correlation between NTG and HTG. We also found an association between two genes, NTG, *BMP4*, and *TBKBP1*, that have not been previously associated at a genome-wide level with any type of glaucoma. This highlights an important consistent association of the TANK-binding kinase 1 (*TBK1*) in the development of NTG and a role of *BMP4* in NTG etiology, as underlined by our multi-omic analysis.

Despite the high genetic overlap, we have shown that IOP-related loci tend to have smaller effect sizes for NTG compared to HTG while, loci that are independent of IOP have similar effect sizes for NTG and HTG. This suggests that, even though shared mechanisms may lead to the development of major POAG subtypes, certain genetic profiles may be more sensitive to the ocular hypertension process. However, the presence of specific loci in NTG that are not associated with HTG at a genome-wide significant level might imply the existence of distinct mechanisms that primarily contribute to neurodegeneration, leading to ocular neuropathy and that are exacerbated by a comorbid increase in ocular pressure.

Methods

Study design and participants

To identify risk loci specific to NTG, we conducted a large multi-trait meta-analysis of genome-wide association studies (GWAS) across individuals of European and Asian descent. The European ancestry NTG data included the International Genetics of Glaucoma Consortium (IGGC; 3247 cases and 47,997 controls), UK Biobank (UKB; 2184 cases and 7000 controls), Canadian Longitudinal Study on Aging (CLSA; 755 cases and 3000 controls), FinnGen (Release 8, 1756 cases and 326,434 controls), and a structural measurement of the integrity of the optic nerve, vertical cup-to-disk ratio (VCDR, $N = 97{,}939$ participants). VCDR estimates were obtained from UKB and CLSA participants of European ancestry[29]. The Asian NTG data included a GWAS meta-analysis of 4418 cases and 34,303 controls from Hong Kong, Singapore, and Japan.

Glaucoma was self-reported in CLSA following diagnosis by a clinician as per the report in the item 'ICQ_GLAUC_COM' (Has a doctor ever told you that you have glaucoma?)[30,31]. UK Biobank was restricted to glaucoma diagnosed under the International Classification of Diseases (ICD) 10 criteria, without specific classification for HTG or NTG. Thus, participants with a diagnosis of glaucoma and IOP under 21 mmHg were considered NTG cases in UKB and CLSA, provided that the tonometry to measure IOP was performed within 5 years of the glaucoma diagnosis. However, to ensure consistency in results when IOP measurements are taken closer to the time of glaucoma diagnosis, we re-ran the analysis to include participants who had tonometry within one year of their diagnosis. The results were consistent, but the power decreased due to a reduction in the number of 'probable NTG' participants by approximately 4.5-fold, as shown in Supplementary Fig. 16. Maximum IOP was used when multiple measurements were present. Patients receiving glaucoma medication or those who had undergone surgery to decrease the IOP at the time of the tonometry were excluded from the analysis in UKB and CLSA. Controls were randomly selected from UKB and CLSA datasets, maintaining a ratio of 1:3, from a pool of individuals who had no reported ocular conditions. Given that UKB and CLSA cases were not clinically diagnosed with specificity for tension subtypes, we considered them as "probable NTG cases" and used them as a separate phenotype in the multi-trait meta-analysis. NTG was clinically diagnosed (in person or confirmed with medical records) in IGGC and FinnGen. Detailed information regarding recruitment, genotyping, and quality control are described within their respective publications[32,33].

Genome-wide association studies (GWAS)

GWAS analysis was performed using UKB and CLSA participants of European ancestry determined through principal component analysis. We used Regenie v 2.3.4, a machine-learning method[34], to run the GWAS. We included sex, age, and 10 principal components as covariates in the model. We meta-analyzed the UKB and CLSA results using METAL, a software to meta-analyze genome-wide association scans[35]. For IGGC NTG and VCDR data, we used the previously published GWAS summary statistics[13,29]. The VCDR GWAS was adjusted for IOP using the mtCOJO, a method to adjust estimates for the effect of another trait using GWAS summary statistics. For FinnGen, we used the release 8 normotensive glaucoma GWAS (https://r8.finngen.fi/pheno/H7_GLAUCOMA_NTG).

Multitrait meta-analysis

We used multi-trait analysis of GWAS (MTAG), version 2020080, a method for the joint analysis of summary statistics from genome-wide association studies (GWAS) of correlated traits[36], and an inverse variance weighting meta-analysis approach through METAL, version 20211102, to enhance the power for discovering NTG risk loci. MTAG leverages the genetic correlation between correlated traits to boost the statistical power for the GWAS of each input trait. We conducted a two-stage multi-trait meta-analysis.

The first stage entailed a meta-analysis using METAL, incorporating NTG probable cases from the UKB and CLSA. Subsequently, the results of the UKB and CLSA meta-analysis, vertical cup-disc ratio (VCDR) adjusted for intraocular pressure (IOP), and clinically diagnosed NTG cases of European ancestry from the International Glaucoma Genetics Consortium (IGGC) were integrated into a multi-trait meta-analysis using MTAG. This first stage aimed to enhance the statistical power of IGGC NTG cases of European ancestry.

Loci identified as independent and genome-wide significant ($P < 5e{-}8$) in the first-stage analysis were validated using an inverse variance weighting approach in independent datasets (i.e., FinnGen and Asian IGGC). Upon validation of the first-stage results, we jointly analyzed phenotypes that were clinically diagnosed (i.e., IGGC in Europeans and Asians, FinnGen) using METAL and then used MTAG to incorporate the probable NTG cases of UKB and CLSA, and IOP-adjusted VCDR. The second stage resulted in an overall sample size of 7942 cases and 384,431 controls, with VCDR values available for 97,939 participants. VCDR data from UKB and CLSA were used for their high correlation with the clinically diagnosed phenotype, aiming to boost the statistical power of the associations.

To compare HTG and NTG, we used the HTG GWAS published by Gharahkhani et al. [13] based on 5144 HTG cases and 76,997 controls. We further compared the magnitude of the effect of the risk loci between NTG and IOP[26], vertical cup-to-disk ratio[29] and POAG[13]. The difference in magnitude of effect between the MTAG NTG results and previously published results for HTG and between FinnGen NTG and HTG was estimated using the equation below. Given the strong correlation between the two phenotypes, we decided to include the method described in Randall et al. [37], which accounted for differences between NTG and HTG effect estimates β_{htg}, β_{ntg} and corresponding standard errors SE_{htg}, SE_{ntg} using t statistics and adjusting for HTG and NTG correlation, where r was computed as the genetic correlation coefficient across all the loci; effects were estimated on the log(OR) scale:

$$t = \frac{\beta_{htg} - \beta_{ntg}}{\sqrt{SE_{htg}^2 + SE_{ntg}^2 - 2r * SE_{htg} * SE_{ntg}}} \quad (1)$$

The genetic correlation between HTG and NTG was evaluated using linkage disequilibrium score regression (LDSC). LDSC is a technique that estimates the genetic correlation between phenotypes by analyzing GWAS summary statistics, while also taking into consideration factors such as overlapping samples and polygenicity[38]. We used the 1000 Human Genome Project reference panel for LDSC estimations. Additionally, LDSC was utilized to estimate the LD-Score intercept and heritability of NTG in individuals with European ancestry.

We then compared differences in the genetic architecture of NTG and HTG using the GWAS pairwise method (GWAS-PW)[39]. GWAS-PW is a method that evaluates the genetic overlap over specific genomic regions by splitting the genome into 1703 segments and estimating the posterior probability of four different models: (1) the region is unique to NTG, (2) unique to HTG, (3) shared with both with a common causal variant and (4) shared with both without a common causal variant.

Functional annotation and drug–gene interaction

To improve our power to detect genes associated with NTG, we employed a Gene-based association test, mBAT-combo v 1.94.1, a method that is considered robust to detect SNPs with masking effects[40]. We corrected for multiple testing using a Bonferroni approach given by the number of genes tested in the analysis ($\alpha = 0.05/18{,}766$ [genes], $P < 2.7e{-}06$). We further performed conditional analysis using GCTA-COJO v 1.91.7 to identify independent genetic signals in low linkage disequilibrium[41]. To find biological pathways associated with NTG, we used gene-set tests in the program Meta-Analysis Gene-set Mining of GWAS (MAGMA) v 1.08. We applied Bonferroni correction for multiple testing, accounting for the total number of gene-sets tested ($\alpha = 0.05/15{,}483$ [gene-sets], $P < 3.3e{-}06$).

We then leveraged omics data to explore the functional relevance of the genes identified in the mBAT-combo gene-based analysis. First, we used summary-data-based Mendelian randomization (SMR) (Zhu et al.[42]), v 1.3.1, to identify putative causal associations between gene expression and NTG based on eQTL data from peripheral blood of 2765 individuals from the Consortium for the Architecture of Gene Expression (CAGE)[43] and retinal eQTL data from 453 individuals[44]. We then conducted an in-depth analysis of cell-type specific transcriptomic profiles in retinal ganglion cells, the most relevant cells implicated in the development of NTG. This analysis employed single-cell RNA-sequencing data derived from 23 distinct sub-populations of retinal ganglion cells, which collectively constituted a sample comprising 247,520 cells[45] and over one million neuron-like cells, including cells that had been exposed to rotenone to induced oxidative stress[46]. These neurons encompassed a diverse range, including dopaminergic neurons, serotonin transporters, astrocyte-like cells, ependymal cells, and cell clusters undergoing neuronal differentiation. For the assessment of gene expression across multiple cell lines in relation to NTG, we employed the SMR method. Additionally, to account for multiple testing, we applied the Bonferroni correction technique, considering the effective number of independent genes being analyzed ($\alpha = 0.05/25$ [genes], $p < 0.002$).

We further assessed the multi-omic profile of genes that were consistent between the gene-based and sc-RNA Seq-based approach through the Omics Pleiotropic Association (OPERA), v 1.0.0. OPERA is a Bayesian method that integrates the SMR and HEIDI approach for multi-omics analysis, aiming to provide further interpretation of the biological mechanisms underlying GWAS signals and to prioritize molecular phenotypes[47]. This analysis incorporates the single-cell RNA-sequencing data, described in the previous paragraph, methylation profile based on mQTL of peripheral blood samples from 1980 participants[48] and eQTL data from the peripheral blood of 2765 individuals from the Consortium for the Architecture of Gene Expression (CAGE)[43].

Genes that reached genome-wide significance in the mBAT-combo gene-based tests were included for drug–gene interaction using the Drug–Gene Interaction Database (DBIbd) v 4.0[49] to identify genes that could be prioritized for targeted therapy or drug

development. DBIbd is a scientifically curated database that provides information on known and predicted interactions between drugs and genes. It integrates data from multiple sources to provide a comprehensive overview of drug–gene interactions and is commonly used as a resource for evaluating the potential impact of genetic variation on drug response.

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

The GWAS summary data for the meta-analysis generated in this study is available on Zenodo [https://doi.org/10.5281/zenodo.14010557]. Individual-level data from the UK Biobank were accessed under application number 25331, and CLSA data under application number 190225. Researchers may obtain access to these datasets by applying directly to each cohort: UK Biobank (https://www.ukbiobank.ac.uk/) and CLSA. GWAS data from the IGGC in European and Asian populations used in this study are available upon request to the corresponding authors of Gharahkhani et al. [13]; the POAG GWAS data used in this study can be accessed via the GWAS Catalog (GCST90011766). FinnGen normotensive glaucoma GWAS data used in this study is accessible at https://r8.finngen.fi/pheno/H7_GLAUCOMA_NTG. The GWAS summary statistics for vertical cup-disc ratio (VCDR) GWAS used in this study are available at https://xikunhan.github.io/site/publication/. Bulk and single-cell eQTL data used in this study, including peripheral blood and retinal datasets, are accessible at https://yanglab.westlake.edu.cn/software/smr/#DataResource, with additional retinal single-cell RNA-seq data accessible upon request to the corresponding authors of Daniszewski et al. [45]. Neuronal single-cell RNA-seq data from Jerber et al. [46] can be found on Zenodo (https://zenodo.org/record/3625024). Methylation profile datasets used in this study are accessible at https://yanglab.westlake.edu.cn/software/smr/#DataResource, and drug target data were obtained from DGIdb (https://www.dgidb.org/). Supplementary data for Figs. 1–3 are included in Supplementary Data 7–9.

Code availability

The code used in this study is available upon request to the corresponding authors. Scripts will be provided to the requester within one to five business days.

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Author contributions

P.G. and S.M. conceived the study and designed the analyses. S.D.-T. carried out the analyses with support from W.H. and wrote the manuscript with input and support from R.Y., A.P.K., C.J.H., P.G.H., L.R.P., Y.W., M.K., M.A., T.A., C.-Y.C., C.C.K., P.K., J.H.K., A.W.H., D.A.M., J.E.C., J.L.W., and J.-S.O.

Competing interests

A.P.K. has acted as a paid consultant or lecturer to Abbvie, Aerie, Allergan, Google Health, Heidelberg Engineering, Novartis, Reichert, Santen and Thea. J.H.K. has received research funding from Pfizer, Inc. S.M., J.E.C. and A.W.H. are co-founders of and hold stock in Seonix Pty Ltd. The remaining authors report no other competing interests.

Additional information

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