AMERICAN ACADEMY OF OPHTHALMOLOGY®

An Intraocular Pressure Polygenic Risk Score Stratifies Multiple Primary Open-Angle Glaucoma Parameters Including Treatment Intensity

Ayub Qassim, MBBS,[1] Emmanuelle Souzeau, PhD,[1] Owen M. Siggs, MD, DPhil,[1] Mark M. Hassall, MBBS, DPhil,[1] Xikun Han, MSc,[2] Helen L. Griffiths,[3] N. Andrew Frost, FRCOphth, PhD,[4] Neeru A. Vallabh, MBBS,[5] James F. Kirwan, FRCOphth,[6] Geeta Menon, MBBS, FRCOphth,[7] Angela J. Cree, MSc,[3] Anna Galanopoulos, MBBS,[8] Ashish Agar, MBBS, PhD,[9] Paul R. Healey, MMed, PhD,[10] Stuart L. Graham, MBBS, PhD,[11] John Landers, MBBS, PhD,[1] Robert J. Casson, MBBS, DPhil,[12] Puya Gharahkhani, PhD,[2] Colin E. Willoughby, MBChB, MD,[13,14] Alex W. Hewitt, MBBS, PhD,[15] Andrew J. Lotery, MD, FRCOphth,[3,] Stuart MacGregor, PhD,[2,*] Jamie E. Craig, MBBS, DPhil[1,*]*

Purpose: To examine the combined effects of common genetic variants associated with intraocular pressure (IOP) on primary open-angle glaucoma (POAG) phenotype using a polygenic risk score (PRS) stratification.

Design: Cross-sectional study.

Participants: For the primary analysis, we examined the glaucoma phenotype of 2154 POAG patients enrolled in the Australian and New Zealand Registry of Advanced Glaucoma, including patients recruited from the United Kingdom. For replication, we examined an independent cohort of 624 early POAG patients.

Methods: Using IOP genome-wide association study summary statistics, we developed a PRS derived solely from IOP-associated variants and stratified POAG patients into 3 risk tiers. The lowest and highest quintiles of the score were set as the low- and high-risk groups, respectively, and the other quintiles were set as the intermediate risk group.

Main Outcome Measures: Clinical glaucoma phenotype including maximum recorded IOP, age at diagnosis, number of family members affected by glaucoma, cup-to-disc ratio, visual field mean deviation, and treatment intensity.

Results: A dose–response relationship was found between the IOP PRS and the maximum recorded IOP, with the high genetic risk group having a higher maximum IOP by 1.7 mmHg (standard deviation [SD], 0.62 mmHg) than the low genetic risk group ($P = 0.006$). Compared with the low genetic risk group, the high genetic risk group had a younger age of diagnosis by 3.7 years (SD, 1.0 years; $P < 0.001$), more family members affected by 0.46 members (SD, 0.11 members; $P < 0.001$), and higher rates of incisional surgery (odds ratio, 1.5; 95% confidence interval, 1.1–2.0; $P = 0.007$). No statistically significant difference was found in mean deviation. We further replicated the maximum IOP, number of family members affected by glaucoma, and treatment intensity (number of medications) results in the early POAG cohort ($P \leq 0.01$).

Conclusions: The IOP PRS was correlated positively with maximum IOP, disease severity, need for surgery, and number of affected family members. Genes acting via IOP-mediated pathways, when considered in aggregate, have clinically important and reproducible implications for glaucoma patients and their close family members. *Ophthalmology 2020;127:901-907 © 2020 by the American Academy of Ophthalmology*

Supplemental material available at www.aaojournal.org.

See Commentary on page 908.

Glaucoma refers to a group of progressive optic neuropathies with a characteristic pattern of retinal ganglion cell death and visual field loss.[1] Intraocular pressure (IOP) is currently the only proven modifiable risk factor for primary open-angle glaucoma (POAG), in which the iridocorneal angle is open and there is no secondary cause of IOP elevation.[2] Despite this, elevated IOP is not essential for the diagnosis of POAG, nor is it effective for screening for glaucoma.[1,3] The current methods of IOP assessment are limited to the time of measurement and are a poor measure of an individual's IOP profile, maximum, and fluctuations. Additional IOP measurements are more informative for glaucoma management because both diurnal and long-term IOP fluctuations reportedly have been associated with glaucoma progression.[4,5]

https://doi.org/10.1016/j.ophtha.2019.12.025
ISSN 0161-6420/20

Glaucoma is highly heritable, and several genes with a Mendelian pattern of inheritance have been associated with POAG.[6] Monogenic variants causing glaucoma are relatively rare but carry a high risk of the disease developing. Family-based genetic linkage analysis has identified 3 genes associated with Mendelian glaucoma: myocilin (*MYOC*), optineurin, and TANK-binding kinase 1 genes.[7–10] Pathogenic variants in the *MYOC* gene account for 2% to 4% of adult-onset POAG.[10] The most common pathogenic variant in the *MYOC* gene in individuals of European ancestry (p.Gln368Ter) has a minor allele frequency of 0.13% yet carries a significant risk of glaucoma with high IOP in those who carry it (in a population-based setting: odds ratio [OR], 6.76; 95% confidence interval [CI], 4.05–11.29).[11] In family-based studies, the penetrance of p.Gln368Ter to manifest POAG is reported at approximately 80% by the seventh decade of life.[11]

Intraocular pressure in the healthy population is a polygenic trait, with recent large genome-wide association studies (GWASs) discovering more than 100 common loci associated with IOP, accounting for 40% of the heritability.[12–14] Khawaja et al[14] reported that these single nucleotide polymorphisms (SNPs) explained 17% of IOP variance in an independent clinical study and 9% in the UK Biobank Study, which likely reflects the difference in IOP measurement methods. In contrast to the aforementioned monogenic variants, each SNP contributes a very small effect size. For instance, variants in or near the genes *TMC01* and *CAV2*, 2 of the most strongly associated loci with IOP and glaucoma, are present in 10% to 15% of the population, but account for a modest risk of glaucoma individually (OR, 1.1–1.4).[12–14] However, the combined effects of these common SNPs significantly affect the observed clinical phenotype.[12]

To understand the impact of these common variants, we considered the total number of variants an individual is carrying multiplied by their effect size to generate a weighted polygenic risk score (PRS).[15] A genetic risk stratification then was carried out by calculating an aggregate score of all the SNPs an individual has associated with a trait. For instance, a person with most of the discovered IOP variants (a high IOP PRS) was hypothesized to have a higher IOP than someone who has only a few. The PRS model of risk prediction has been used to stratify individualized disease risk in several medical conditions such as coronary artery disease, atrial fibrillation, and breast cancer.[16–18] Recently, a PRS derived from the known IOP variants has been reported to account for a higher risk of glaucoma developing[12]; however, the influence of the IOP PRS on a wider range of glaucoma-related phenotypes has not been described. In this study, we aimed to characterize the clinical features of glaucoma patients with a high burden of IOP-associated variants in a large national Australian glaucoma registry along with ethnically similar cases from the United Kingdom.

Methods

Study Participants

The study adhered to the tenets of the Declaration of Helsinki and followed the National Health and Medical Research Council statement of ethical conduct in research involving humans. Informed consent was obtained from all participants, and the study was approved by the Southern Adelaide Clinical Human Research Ethics Committee.

The study participants were enrolled in the Australian and New Zealand Registry of Advanced Glaucoma (ANZRAG).[19] The study included patients with advanced and nonadvanced glaucoma. Advanced glaucoma was defined by a Humphrey 24-2 visual field mean deviation of less than −15 dB in the worse eye, or loss of at least 2 of the central visual field points on the pattern deviation map.[19] Nonadvanced glaucoma was defined by optic nerve head changes with corresponding visual field defects consistent with glaucoma but not fitting the aforementioned criteria. The study sample included additional ethnically matched patients with advanced glaucoma recruited from the United Kingdom.[20] Only patients of European ancestry with POAG were included to use the currently published IOP SNPs. Patients with variants in the known POAG genes (*MYOC*, optineurin, and TANK-binding kinase 1) were excluded. The highest IOP measurement recorded with Goldmann applanation tonometry by the referring ophthalmologist before treatment of either eye for each participant was recorded. High-tension glaucoma was defined as a maximum recorded IOP of more than 21 mmHg. Other data recorded at the time of recruitment included age at diagnosis, vertical cup-to-disc ratio (VCDR), and glaucoma surgery. Family history was self-reported and recorded for affected relatives up to the fourth degree by the referring clinician. Where applicable, the family tree of affected individuals was recorded and reviewed by the registry staff before recording the number of family members affected by glaucoma in the registry.

An independent cohort of early glaucoma patients enrolled in the Progression Risk of Glaucoma: Relevant SNPs with Significant Association study then were used for replication. Only participants with established perimetric glaucoma, defined by 2 consecutive reliable visual field examinations with Glaucoma hemifield test results outside normal limits, pattern standard deviation of less than 5%, or a cluster of 3 contiguous points depressed less than 5% in the pattern standard deviation map, at least 1 of which was less than 1%, were included. Data recorded included self-reported family history of glaucoma, maximum IOP recorded at any visit, VCDR and visual field at the last visit, number of topical glaucoma medications, and previous selective laser trabeculoplasty. The number of topical medications and selective laser trabeculoplasty status are updated routinely at each visit for this cohort. A small proportion (2.4%) have undergone an incisional surgery for the management of glaucoma, which would alter their medical management significantly. Thus, for the medical treatment analysis, we used the highest number of drops at any 1 appointment for each patient.

Polygenic Risk Score

The IOP-derived PRS comprised 146 statistically independent genome-wide significant SNPs (P value threshold at 5×10^{-8} and linkage disequilibrium [LD] clumping at $r^2 = 0.1$) as reported previously (Table S1, available at www.aaojournal.org).[12] Briefly, SNPs influencing IOP were discovered by a GWAS of cornea-compensated IOP measured by the Ocular Response Analyzer (Reichert, Inc., Depew, NY) in participants of the UK Biobank

Study (n = 103 914).[12,21] This was meta-analyzed with GWAS results from the International Glaucoma Genetics Consortium (n = 29 578) using the inverse variance weighted method (METAL software 2011-03-25 release).[22] A weighted PRS then was derived for each individual in the ANZRAG study cohort using PLINK (version 1.90 beta),[23] taking into account the effect size of each SNP using the UK Biobank Study GWAS summary statistics. None of the study participants in ANZRAG or Progression Risk of Glaucoma: Relevant SNPs with Significant Association were part of the discovery cohort. A percentile score then was derived within the ANZRAG and the Progression Risk of Glaucoma: Relevant SNPs with Significant Association cohorts. We classified patients into 3 risk groups: the top 20% of the genetic risk score were classified as the high-risk group, the middle 60% of the genetic risk score were classified as the intermediate-risk group, and the bottom 20% of the genetic risk score were classified as the low-risk group. Additionally, we calculated the recently published 12-SNP unweighted POAG PRS by Fan et al[24] for our primary cohort for comparison. A detailed comparison between these scores is summarized in Table S2 (available at www.aaojournal.org). Genotyping was carried out in several phases on either Illumina Omni1M, OmniExpress, or HumanCoreExome arrays (Illumina, San Diego, CA) as described previously.[12]

Statistical Analysis

The Shapiro-Wilk test was used to assess for normality. Analysis of variance of continuous variables by PRS groups was carried out using the Kruskal–Wallis test. Count and categorical variables were compared using Pearson's chi-square test. For 2-group comparisons, the Mann–Whitney *U* test was used. Logistic regression models were fitted for binary outcomes, and negative binomial regression was used for count data (number of family members affected). Linear regression using the continuous numerical PRS as the explanatory variable was used to compare the aforementioned 2 scores. All analysis was carried out using *R* software version 3.5.1 (R Foundation for Statistical Computing, Vienna, Austria). The significance level (α) was set at 0.05.

Results

A total of 2154 eligible POAG patients from ANZRAG with mean age at recruitment of 77.4 years (standard deviation [SD], 13.2 years) were included. Most of the study cohort (n = 1664 [77%]) had advanced glaucoma, as defined previously. This included 381 patients recruited from the United Kingdom (n = 290 from Southampton and n = 91 Liverpool) who were ethnically matched to the rest of the cohort. A summary of the glaucoma phenotype across the 3 genetic risk groups is summarized in Table 1.

The high IOP genetic risk group showed a significantly higher maximum IOP by 1.3 mmHg (95% CI, 0.32–2.7 mmHg; $P = 5.5\times10^{-3}$) compared with the intermediate and low genetic risk groups. The maximum IOP was not statistically significantly different in the intermediate group relative to the low-risk group (mean difference, 0.54 mmHg; 95% CI, −1.5 to 0.47 mmHg; $P = 0.08$). Similarly, the high genetic risk group was more likely to demonstrate high-tension glaucoma, defined by a maximum IOP of more than 21 mmHg (OR, 1.9; 95% CI, 1.3–2.8; $P = 7.9\times10^{-4}$ relative to the low-risk group). Further analysis by decile groups of the IOP PRS showed a continuous variant dose–response relationship between higher IOP PRS and maximum IOP, signifying the cumulative effects of the common IOP variants (Fig 1A).

The mean age at glaucoma diagnosis was significantly different across the genetic risk groups ($P = 1.3 \times 10^{-4}$). The high genetic risk group was diagnosed with glaucoma on average 2.2 years (SD, 0.80 years) earlier than the intermediate group ($P = 5.5\times10^{-3}$) and 3.7 years (SD, 1.0 years) earlier than the low genetic risk group ($P = 2.4\times10^{-4}$). The high-risk group was more likely to have family members affected by glaucoma relative to the low-risk group (OR, 1.6; 95% CI, 1.2–2.1; $P = 1.1\times10^{-3}$). The number of self-reported family members affected by glaucoma also was higher in the high IOP PRS group compared with the intermediate group (mean, 0.29 family member; SD, 0.1 family member; $P = 5.2\times10^{-3}$) and low-risk group (mean, 0.46 family member; SD, 0.11 family member; $P = 1.8\times10^{-4}$). Furthermore, a linear relationship was found between the IOP PRS and the number of family members affected by glaucoma, which highlights the importance of these variants and their impact on the development of glaucoma (Fig 1B).

Table 1. Summary of the Glaucoma Phenotype across Genetic Risk Groups in the Australian and New Zealand Registry of Advanced Glaucoma

	Low	Intermediate	High	*P* Value
No.	410	1313	431	—
Male gender, no. (%)	192 (46.8)	622 (47.4)	185 (43.0)	0.286
HTG, no. (%)	251 (74.3)	867 (79.1)	298 (84.7)	**0.003**
Age at diagnosis (yrs), mean (SD)	62.90 (15.32)	61.39 (14.16)	59.16 (13.80)	**<0.001**
No. of family members affected, mean (SD)	0.99 (1.56)	1.16 (1.53)	1.45 (1.89)	**0.001**
Maximum recorded IOP (mmHg), mean (SD)	25.54 (9.17)	26.08 (8.63)	27.25 (9.14)	**0.005**
VCDR, mean (SD)	0.87 (0.11)	0.86 (0.12)	0.87 (0.13)	0.32
Visual field MD (dB), mean (SD)	−16.87 (9.04)	−16.12 (9.15)	−16.91 (9.25)	0.179
Incisional surgery rate, no. (%)*	151 (39.8)	507 (43.7)	186 (49.5)	**0.026**
Bilateral incisional surgery, no. (%)*	65 (17.2)	259 (22.3)	100 (26.6)	**0.007**

HTG = high-tension glaucoma; IOP = intraocular pressure; MD = mean deviation; SD = standard deviation; VCDR = vertical cup-to-disc ratio.
The low-risk group represents the first quintile of the IOP genetic risk score, and the high-risk is the highest quintile. Number of family members is self-reported up to the fourth degree. Vertical cup-to-disc ratio, MD, and treatment values were recorded at the time of referral. *P* values represent the statistical significance of the analysis of variance (difference between any 2 groups) for continuous variables (Kruskal–Wallis test) or chi-square test for categorical variables. High-tension glaucoma is defined by a maximum IOP of more than 21 mmHg.
Boldface indicates $P < 0.05$.
*Most incisional surgeries in this dataset were trabeculectomies. Other surgeries include tube shunt surgery, deep sclerectomy, and 2 cases of Xen Gel implants.

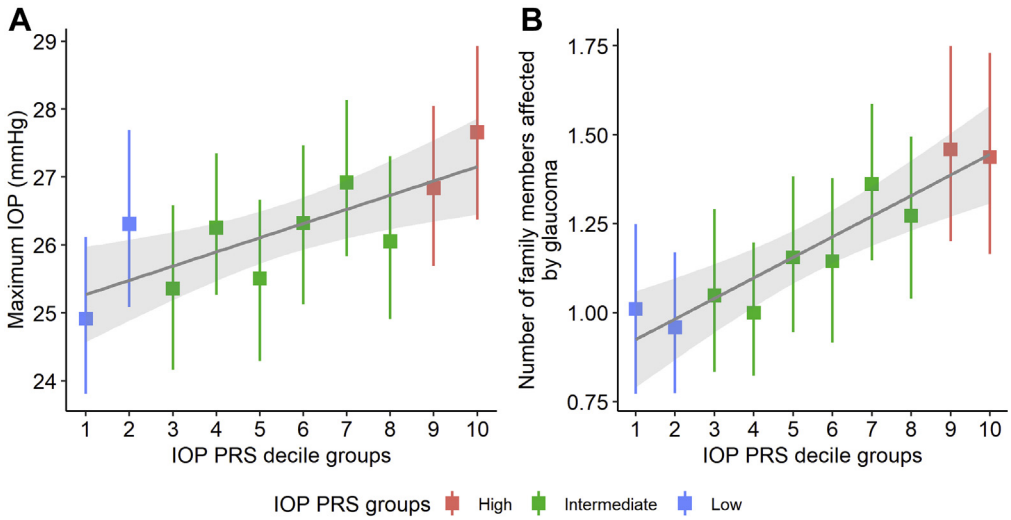

Figure 1. Graphs showing a continuous variant dose–response relationship between intraocular pressure (IOP) polygenic risk score (PRS) and (**A**) the maximum recorded IOP in the Australian and New Zealand Registry of Advanced Glaucoma cohort ($P = 1.9\times10^{-3}$ for linear model trend) and (**B**) the mean number of family members affected by glaucoma ($P = 1.3\times10^{-5}$ for negative binomial generalized linear model trend). The squares represent the mean values for each PRS decile group, and the error bars represent the 95% confidence interval of the mean. The grey line is the line of best fit with the 95% confidence interval lightly shaded around the line.

No significant difference was found between the Humphrey visual field mean deviation between the IOP PRS groups ($P = 0.18$). However, the high genetic risk group was more likely to require incisional surgery for the management of glaucoma relative to the intermediate- and low-risk groups (OR, 1.3 [95% CI, 1.0–1.6; $P = 0.049$] and OR, 1.5 [95% CI, 1.1–2.0; $P = 7.9\times10^{-3}$], respectively). Further, the high IOP PRS group was more likely to require bilateral incisional surgery than the intermediate- and low-risk groups (OR, 1.4; 95% CI, 1.0–1.8; $P = 0.02$).

For replication, we stratified an independent cohort of early perimetric POAG patients (n = 624), with an average age of 69.5 years (SD, 10 years), into 3 risk groups based on the same absolute numerical IOP PRS cutoff used previously. A similar association of increasing maximum IOP, number of family members affected, and treatment intensity was found (Table 2; Fig 2). The high-risk group had more than twice as many family members affected as the low-risk group and was more likely to require more intensive medical therapy to control the disease ($P \leq 0.01$). No significant association was found between the PRS and the length of follow-up ($P = 0.65$).

A recently reported PRS associated with POAG in European white populations was associated with a younger age at glaucoma diagnosis.[24] For comparison, we calculated this PRS in our primary cohort (ANZRAG, n = 2154; Table S2). The IOP PRS presented in this study was associated more strongly with the age at glaucoma diagnosis ($P = 2.0\times10^{-5}$) than the 12-SNP PRS reported by Fan et al[24] ($P = 2.6\times10^{-4}$) and explained a greater variance of this outcome (R^2 of linear regression, 0.89% vs. 0.65%, respectively; Table S3, available at www.aaojournal.org). The 12-SNP PRS was not associated with the maximum IOP recorded ($P = 0.45$) and explained less variance in the need for incisional surgery outcome compared with the IOP PRS (R^2 of linear regression, 0.53% vs. 0.79%, respectively; Table S3). Because of the inclusion of 2 VCDR-associated POAG risk variants near CDKN2B-AS1 and SIX6, the 12-SNP PRS was associated with a higher VCDR but not the IOP PRS (Table S3).

Table 2. Summary of the Glaucoma Phenotype across Genetic Risk Groups in the Replication Cohort (Progression Risk of Glaucoma: Relevant SNPs with Significant Association)

	Low	Intermediate	High	P Value
No.	144	378	102	—
Male gender, no. (%)	68 (47.2)	159 (42.1)	37 (36.3)	0.228
No. of family members affected, mean (SD)	0.60 (1.21)	0.88 (1.19)	1.27 (1.81)	**0.001**
Maximum recorded IOP (mmHg), mean (SD)	19.31 (5.36)	21.08 (5.80)	21.03 (5.20)	**<0.001**
VCDR, mean (SD)	0.73 (0.10)	0.74 (0.10)	0.72 (0.10)	0.119
Visual field MD (dB), mean (SD)	−3.17 (2.94)	−3.35 (3.10)	−2.79 (2.73)	0.442
No. of glaucoma drops or SLT (%)*	1.06 (1.18)	1.36 (1.35)	1.35 (1.26)	**0.033**
Required 2 or more medications or SLT, no. (%)*	33 (22.9)	133 (35.2)	39 (38.2)	**0.013**

IOP = intraocular pressure; MD = mean deviation; SD = standard deviation; SLT = selective laser trabeculoplasty; VCDR = vertical cup-to-disc ratio. Risk group stratification is based on IOP genetic risk score cutoffs as calculated and used in the primary cohort (Australian and New Zealand Registry of Advanced Glaucoma). Vertical cup-to-disc ratio, MD, and treatment intensity are from the last clinic visit recorded in the study database. *P* values represent the statistical significance of the analysis of variance for continuous variables (Kruskal–Wallis test) or chi-square test for categorical variables.
Boldface indicates $P < 0.05$.
*Selective laser trabeculoplasty was counted as equivalent to 1 glaucoma medication.

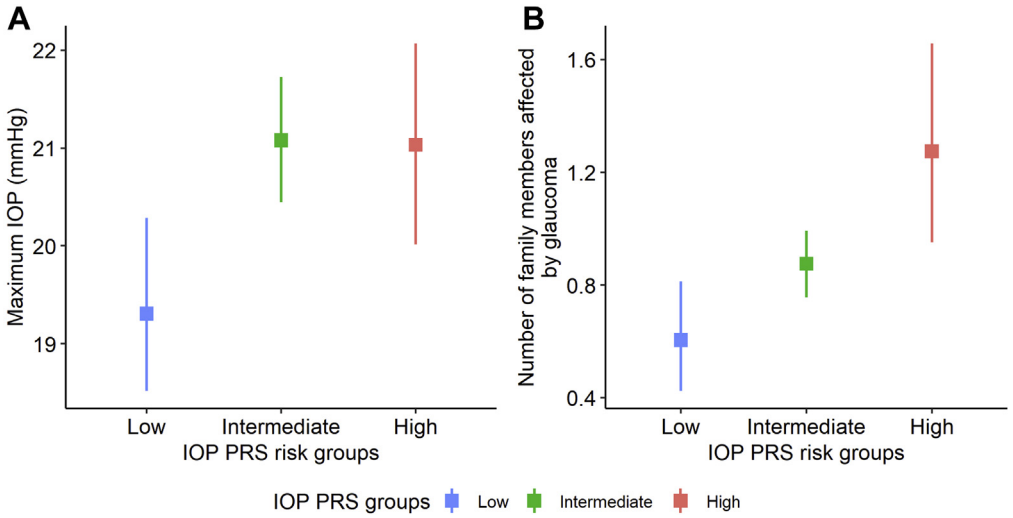

Figure 2. Graphs showing the replication of the (**A**) maximum intraocular pressure (IOP) recorded ($P = 5.0\times10^{-4}$ for 1-way analysis of variance) and (**B**) the number of family members affected by glaucoma ($P = 1.0\times10^{-3}$ for 1-way analysis of variance) in an independent cohort of early primary open-angle glaucoma patients (n = 624). The squares represent the mean values for each polygenic risk score (PRS) group, and the error bars represent the 95% confidence interval of the mean.

Discussion

Common genetic variants associated with both glaucoma and IOP have been identified via GWASs. Genetic risk score stratification can be used to estimate the combined effect size of these variants on the patient. In this study, glaucoma patients in the high IOP genetic risk group showed a higher maximum (pretreatment) IOP and younger age at glaucoma diagnosis and were more likely to require incisional surgery to control the disease than those in the intermediate or low IOP genetic risk groups. We further replicated these results in an independent cohort of early glaucoma patients and observed a similar association with the higher genetic risk group requiring more intensive medical therapy for glaucoma management.

Interestingly, despite the clinically modest difference in the maximum IOP between the high and low IOP genetic risk groups (between 1 and 2 mmHg in 2 independent cohorts), we observed a stronger relationship in treatment intensity. In the ANZRAG cohort, the incisional surgery rate was 50% in the high genetic risk group compared with 38% in the low-risk group. Similarly, in the early glaucoma cohort, 38% of the high genetic risk group required 2 or more medications or selective laser trabeculoplasty for glaucoma management compared with 23% in the low genetic risk group. Thus, IOP genetic risk variants and stratification may offer further insight into an individual's chronic exposure to higher IOP than sporadic clinic measurements. Furthermore, these risk variants confer increased risk of POAG developing in carriers[12]; thus, patients with higher PRSs had significantly more family members affected by glaucoma.

TMC01 was one of the earliest reported genes to be associated with POAG in common variant studies and remains one of the most strongly associated variants with IOP and POAG.[12,14,25] A variant in the *TMC01* gene reportedly is associated with conversion from ocular hypertension to glaucoma in non-Hispanic white persons.[26] In another study, individuals homozygous for a variant near *TMC01* were reported to have a younger age at POAG onset.[27] However, the clinical usefulness of genetic risk scores is expanding because of the accelerated discovery of disease-associated loci as larger genome-wide association studies are conducted. Although early studies on the use of genetic risk scores for POAG are limited,[28,29] MacGregor et al[12] recently reported an IOP-based genetic risk score accounting for a significant risk of glaucoma developing (OR, 5.6 in the highest decile of the score relative to the lowest). Fan et al[24] reported a PRS inclusive of 12 SNPs associated with POAG to be associated with a younger age at glaucoma diagnosis. This PRS was inclusive of 2 variants near CDKN2B-AS1 and SIX6 associated with POAG and VCDR, but not IOP,[7,24,25] which, in addition to the low number of variants used in the score, may account for why this PRS was not associated with the maximum IOP phenotype in our study cohort. This supports the fact that inclusion of additional low-impact variants leads to better PRS models for complex traits.[30] Further research is needed on a more comprehensive PRS inclusive of variants associated with POAG and its endophenotypes.

Conversely, the effects of Mendelian variants on glaucoma phenotype have been well described. Pathogenic variants in the *MYOC* gene are associated most commonly with high IOP and more advanced disease.[31] In contrast, duplications and triplications involving TANK-binding kinase 1 and missense variants in optineurin cause familial normal-tension glaucoma and typically are not found in high-tension glaucoma.[7–9] Although these genes are important in familial glaucoma and are highly predictive of disease risk, they are a relatively rare cause of POAG in the general population. Thus, genetic risk stratification

using common variants of IOP is more widely applicable to most POAG patients. Our results show that the cumulative effect of IOP-associated genetic variants may predict an individual's lifetime IOP exposure and support the usefulness of genetic risk scores in POAG monitoring. Further, PRS risk stratification can be carried out before the clinical presentation of the disease, and therefore may be useful for identifying high-risk individuals for screening.

This study has several strengths. We used the large UK Biobank cohort to derive a genetic risk score of corneal-compensated IOP. Using this score inclusive of variants at a strict genome-wide threshold, we characterized the clinical glaucoma phenotype that is attributable to the genetic biomarkers of IOP and its associated pathways. Inclusion of additional POAG risk and other endophenotype variants may yield a better glaucoma risk profiling. Our study cohort also was independent, allowing validation of the discovered variants. We further replicated our findings in another independent POAG cohort with mild glaucoma, allowing further generalizability across the glaucoma severity spectrum. Our study also has some limitations. Interclinician variability in the rate of incisional surgeries may exist, because this was not carried out per protocol. A mixed-effects model with the referring clinician as a random-effect intercept yielded similar results in the estimated effect size of the IOP PRS on incisional surgery risk. Patient-reported number of family members affected has not been validated in a glaucoma setting and may lack sensitivity and specificity. Although our replication of this finding in an independent sample suggests plausible correlation, the effect size may be underestimate or overestimated because of recall and survival biases and community underdiagnosis of glaucoma. Genetic risk scores are limited by the genetic pool of the discovery cohort. Our results are limited to the ethnicities of the European ancestry individuals of the UK Biobank Study, which matched our prediction target cohort. Validation is needed in other ethnicities. We have used only SNPs that reached genome-wide significance in the GWAS to calculate the PRS. Although the inclusion of additional SNPs would include further low-impact susceptibility SNPs, it also would introduce further noise to the PRS and may not improve risk stratification.[32]

In conclusion, our IOP PRS correlated with the maximum recorded IOP and glaucoma severity of POAG patients in a national glaucoma registry. Our results support the clinical usefulness of PRS in POAG risk stratification.

References

1. Casson RJ, Chidlow G, Wood JP, et al. Definition of glaucoma: clinical and experimental concepts. *Clin Exp Ophthalmol*. 2012;40:341–349.
2. Conlon R, Saheb H, Ahmed IIK. Glaucoma treatment trends: a review. *Can J Ophthalmol*. 2017;52:114–124.
3. Tielsch JM, Katz J, Singh K, et al. A population-based evaluation of glaucoma screening: the Baltimore Eye Survey. *Am J Epidemiol*. 1991;134:1102–1110.
4. Caprioli J, Coleman AL. Intraocular pressure fluctuation a risk factor for visual field progression at low intraocular pressures in the advanced glaucoma intervention study. *Ophthalmology*. 2008;115:1123–1129.e3.
5. Asrani S, Zeimer R, Wilensky J, et al. Large diurnal fluctuations in intraocular pressure are an independent risk factor in patients with glaucoma. *J Glaucoma*. 2000;9:134–142.
6. Wang K, Gaitsch H, Poon H, et al. Classification of common human diseases derived from shared genetic and environmental determinants. *Nat Genet*. 2017;49:1319–1325.
7. Wiggs JL, Pasquale LR. Genetics of glaucoma. *Hum Mol Genet*. 2017;26:R21–R27.
8. Aung T, Rezaie T, Okada K, et al. Clinical features and course of patients with glaucoma with the E50K mutation in the optineurin gene. *Invest Ophthalmol Vis Sci*. 2005;46:2816–2822.
9. Awadalla MS, Fingert JH, Roos BE, et al. Copy number variations of TBK1 in Australian patients with primary open-angle glaucoma. *Am J Ophthalmol*. 2015;159:124–130.e1.
10. Fingert JH, Héon E, Liebmann JM, et al. Analysis of myocilin mutations in 1703 glaucoma patients from five different populations. *Hum Mol Genet*. 1999;8:899–905.
11. Han X, Souzeau E, Ong J-S, et al. Myocilin gene Gln368Ter variant penetrance and association with glaucoma in population-based and registry-based studies. *JAMA Ophthalmol*. 2019;137(1):28–35.
12. MacGregor S, Ong J-S, An J, et al. Genome-wide association study of intraocular pressure uncovers new pathways to glaucoma. *Nat Genet*. 2018;50:1067–1071.
13. Gao XR, Huang H, Nannini DR, et al. Genome-wide association analyses identify new loci influencing intraocular pressure. *Hum Mol Genet*. 2018;27:2205–2213.
14. Khawaja AP, Cooke JB, Wareham NJ, et al. Genome-wide analyses identify 68 new loci associated with intraocular pressure and improve risk prediction for primary open-angle glaucoma. *Nat Genet*. 2018;50:778–782.
15. Torkamani A, Wineinger NE, Topol EJ. The personal and clinical utility of polygenic risk scores. *Nat Rev Genet*. 2018;19(9):581–590.
16. Mavaddat N, Michailidou K, Dennis J, et al. Polygenic risk scores for prediction of breast cancer and breast cancer subtypes. *Am J Hum Genet*. 2019;104:21–34.
17. Inouye M, Abraham G, Nelson CP, et al. Genomic risk prediction of coronary artery disease in 480,000 adults. *J Am Coll Cardiol*. 2018;72:1883–1893.
18. Khera AV, Chaffin M, Aragam KG, et al. Genome-wide polygenic scores for common diseases identify individuals with risk equivalent to monogenic mutations. *Nat Genet*. 2018;50:1219–1224.
19. Souzeau E, Goldberg I, Healey PR, et al. Australian and New Zealand Registry of Advanced Glaucoma: methodology and recruitment. *Clin Exp Ophthalmol*. 2012;40:569–575.
20. Ennis S, Gibson J, Griffiths H, et al. Prevalence of myocilin gene mutations in a novel UK cohort of POAG patients. *Eye*. 2010;24:328–333.
21. Sudlow C, Gallacher J, Allen N, et al. UK Biobank: an open access resource for identifying the causes of a wide range of complex diseases of middle and old age. *PLOS Med*. 2015;12:e1001779.
22. Loh P-R, Tucker G, Bulik-Sullivan BK, et al. Efficient Bayesian mixed-model analysis increases association power in large cohorts. *Nat Genet*. 2015;47:284–290.
23. Purcell S, Neale B, Todd-Brown K, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet*. 2007;81:559–575.

24. Fan BJ, Bailey JC, Igo RP, et al. Association of a primary open-angle glaucoma genetic risk score with earlier age at diagnosis. *JAMA Ophthalmol*. 2019;137:1190−1194.
25. Burdon KP, Macgregor S, Hewitt AW, et al. Genome-wide association study identifies susceptibility loci for open angle glaucoma at TMC01 and CDKN2B-AS1. *Nat Genet*. 2011;43:574−578.
26. Scheetz TE, Faga B, Ortega L, et al. Glaucoma risk alleles in the Ocular Hypertension Treatment Study. *Ophthalmology*. 2016;123:2527−2536.
27. Sharma S, Burdon KP, Chidlow G, et al. Association of genetic variants in the TMC01 gene with clinical parameters related to glaucoma and characterization of the protein in the eye. *Invest Ophthalmol Vis Sci*. 2012;53:4917−4925.
28. Mabuchi F, Mabuchi N, Sakurada Y, et al. Additive effects of genetic variants associated with intraocular pressure in primary open-angle glaucoma. *PLoS One*. 2017;12(8): e0183709. https://doi.org/10.1371/journal.pone.0183709. eCollection 2017.
29. Tham Y-C, Liao J, Vithana EN, et al. Aggregate effects of intraocular pressure and cup-to-disc ratio genetic variants on glaucoma in a multiethnic Asian population. *Ophthalmology*. 2015;122:1149−1157.
30. Boyle EA, Li YI, Pritchard JK. An expanded view of complex traits: from polygenic to omnigenic. *Cell*. 2017;169: 1177−1186.
31. Souzeau E, Tram KH, Witney M, et al. Myocilin predictive genetic testing for primary open-angle glaucoma leads to early identification of at-risk individuals. *Ophthalmology*. 2017;124: 303−309.
32. Chatterjee N, Shi J, García-Closas M. Developing and evaluating polygenic risk prediction models for stratified disease prevention. *Nat Rev Genet*. 2016;17:392−406.

Footnotes and Financial Disclosures

Originally received: October 23, 2019.
Final revision: December 19, 2019.
Accepted: December 20, 2019.
Available online: January 6, 2020. Manuscript no. D-19-00560.

[1] Department of Ophthalmology, Flinders University, Flinders Medical Centre, Bedford Park, Australia.

[2] QIMR Berghofer Medical Research Institute, Brisbane, Australia.

[3] Clinical and Experimental Sciences, Faculty of Medicine, University of Southampton, Southampton, United Kingdom.

[4] Department of Ophthalmology, Torbay Hospital, Torquay, Devon, United Kingdom.

[5] Department of Eye and Vision Science, Institute of Ageing and Chronic Disease, University of Liverpool, Liverpool, United Kingdom.

[6] Department of Ophthalmology, Portsmouth Hospitals, Portsmouth, United Kingdom.

[7] Department of Ophthalmology, Frimley Park Hospital NHS Foundation Trust, Frimley, United Kingdom.

[8] South Australian Institute of Ophthalmology, Royal Adelaide Hospital, Adelaide, Australia.

[9] Department of Ophthalmology, Prince of Wales Hospital, Randwick, Australia.

[10] Centre for Vision Research, Westmead Institute for Medical Research, University of Sydney, Sydney, Australia.

[11] Faculty of Medicine and Health Sciences, Macquarie University, Sydney, Australia.

[12] South Australian Institute of Ophthalmology, University of Adelaide, Adelaide, Australia.

[13] Biomedical Sciences Research Institute, Ulster University, Coleraine, United Kingdom.

[14] Royal Victoria Hospital, Belfast Health and Social Care Trust, Belfast, United Kingdom.

[15] Menzies Institute for Medical Research, University of Tasmania, Hobart, Australia.

*These authors contributed equally as senior authors.

Financial Disclosure(s):

The author(s) have no proprietary or commercial interest in any materials discussed in this article.

This work was conducted using the UK Biobank Resource (application no., 25331) and publicly available data from the International Glaucoma Genetics Consortium.

Supported by the National Health and Medical Research Council of Australia (grant nos.: 1107098, 1116360, 1116495, and 1023911; fellowships to S.M., J.E.C., and A.W.H.); the Ophthalmic Research Institute of Australia; the BrightFocus Foundation; the International Glaucoma Association; the UK and Eire Glaucoma Society; and the Mason Medical Research Foundation. The funding organizations had no role in the design or conduct of this research.

HUMAN SUBJECTS: Human subjects were included in this study. The human ethics committees at the Southern Adelaide Clinical Human Research Ethics Committee approved the study. All research adhered to the tenets of the Declaration of Helsinki. All participants provided informed consent.

No animal subjects were included in this study.

Author Contributions:

Conception and design: Qassim, Souzeau, Siggs, Hassall, Hewitt, Craig

Analysis and interpretation: Qassim, Souzeau, Siggs, Hassall, Han, Craig

Data collection: Qassim, Souzeau, Siggs, Han, Griffiths, Frost, Vallabh, Kirwan, Menon, Cree, Galanopoulos, Agar, Healey, Graham, Landers, Casson, Gharahkhani, Willoughby, Hewitt, Lotery, MacGregor, Craig

Obtained funding: N/A

Overall responsibility: Qassim, Souzeau, Siggs, Hassall, Willoughby, Hewitt, Lotery, MacGregor, Craig

Abbreviations and Acronyms:
ANZRAG = Australian and New Zealand Registry of Advanced Glaucoma; **CI** = confidence interval; **GWAS** = genome-wide association study; **IOP** = intraocular pressure; **OR** = odds ratio; **POAG** = primary open-angle glaucoma; **PRS** = polygenic risk score; ***MYOC*** = myocilin; **SD** = standard deviation; **SNP** = single nucleotide polymorphism; **VCDR** = vertical cup-to-disc ratio.

Correspondence:
Ayub Qassim, MBBS, Department of Ophthalmology, Flinders University, Level 2 Car Park Tenancies, 1 Flinders Drive, Bedford Park, SA, 5042, Australia. E-mail: ayub.qassim@flinders.edu.au.