

## Deep ocular phenotyping across primary open-angle glaucoma genetic burden

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### **Key Points**

**Question:** How do phenotypic features of patients vary across genetic burden for primary open-angle glaucoma (POAG)?

**Findings:** In a population-based cross-sectional study, individuals at higher risk of glaucoma were identified using a genome-wide polygenic risk score. Higher polygenic risk was associated with more advanced disease (higher cup-to-disc ratio, intraocular pressure, thinner retinal nerve fiber layers/ganglion cell complex layers, or greater medication requirements, laser or surgery treatment).

**Meaning:** Polygenic risk for POAG identified individuals at higher risk for POAG, supporting PRS risk stratification to identify individuals at higher risk of severe disease, potentially informing healthcare resource allocation and clinical decisions.

**Abstract:**

**Importance:** Better understanding of primary open angle glaucoma (POAG) genetics could enable timely screening and promote individualized disease risk prognostication.

**Objective:** Evaluate phenotypic features across genetic burden for primary open-angle glaucoma (POAG).

**Design:** Cross-sectional study

**Setting:** Population-based

**Participants:** 407,667 individuals from the UK Biobank aged 40-69.

**Main Outcomes and Measures:** POAG prevalence based on structural coding, self-reports and glaucoma-related traits.

**Results:** Among 407,667 participants were 14,171 POAG cases. Area under receiver operator curve for POAG detection was 0.748 in a model including PRS, age, sex, and ancestry. POAG prevalence in the highest decile of PRS was 7.39% vs. 1.33% in lowest decile ( $p < .001$ ). A one-SD increase in PRS was associated with 1.42 times higher odds of POAG (95% CI: 1.69, 1.75), a 0.61mmHg increase in corneal-compensated intraocular pressure (IOPcc) (95% CI: 0.59, 0.64), a -0.09mmHg decrease in corneal hysteresis (CH) (95% CI: -0.10, -0.08), a 0.08mmHg increase in corneal resistance factor (95% CI 0.06, 0.09), and a -0.08D decrease in spherical equivalent (95% CI: -0.11, -0.07) ( $p < .001$  for all). A one-SD increase in PRS was associated with a thinning of the macula-region retinal nerve fiber layer (mRNFL) of 0.14 $\mu$ m and macular ganglion cell complex (GCC) of 0.26  $\mu$ m ( $p < .001$  for both). In the subset of individuals with FPs, a one-SD increase in PRS was associated with 1.42 times higher odds of suspicious optic disc features (SOD) (95% CI: 1.18, 1.69) and a 0.013 increase in cup-to-disc ratio (CDR) (95% CI: 0.012, 0.014,  $p < .001$  for both). 0.39% of FPs in decile 10 had disc hemorrhages and 0.58% had SOD, compared to 0.18% and 0.18% in decile 1 ( $p < .001$  for both). CDR in decile 10 was 0.46, compared to 0.41 in decile 1 ( $p < .001$ ).

**Conclusion:** This PRS identified a group of individuals at substantially higher risk for POAG. Higher genetic risk was associated with more advanced disease, namely higher CDR and IOPcc, thinner mRNFL and thinner GCC. Associations with POAG PRS and corneal hysteresis and greater prevalence of disc hemorrhages were identified. These results suggest genetic risk is an increasingly important parameter for risk stratification to consider in clinical practice.

## **Introduction**

Primary open-angle glaucoma (POAG), the most common form of glaucoma, is a highly heritable complex disease.<sup>1,2</sup> POAG heritability is estimated to be approximately 70%<sup>3</sup> and a population-based study demonstrated that first-degree relatives of POAG patients had a 9-fold increased risk of developing glaucoma.<sup>4,5</sup> While genome wide association studies (GWAS) have identified at least 127 disease risk loci to date, POAG genetic architecture remains incompletely explained and individual POAG genetic risk variants have relatively small effects and poor predictive value.<sup>6</sup>

For complex diseases, polygenic risk scores (PRS) can be utilized to measure the cumulative risk from contributions of many disease-associated DNA variants reflecting aggregate genetic risk. Accurate, generalizable PRS can potentially inform clinical practice and influence disease-screening recommendations, as previously demonstrated in other common complex disease processes such as coronary heart disease, prostate cancer, and breast cancer.<sup>7-10</sup> Prior POAG genetic risk scores and MTAG (multi-trait analysis of GWAS)-derived PRS for POAG have been generated, demonstrating that higher POAG genetic risk is associated with a higher risk of advanced glaucoma, higher intraocular pressure (IOP), earlier age of diagnosis, increased probability of disease progression in early-stage disease, and modulate the effect of myocilin mutations.<sup>11-13</sup> Prior glaucoma-related PRS used in many of these studies have either been derived primarily from variants associated with glaucoma-related traits or a small number of disease-associated genetic variants. A genome-wide PRS for glaucoma that can be utilized to better understand the cumulative genetic burden for POAG as well as ocular features that may be associated with higher genetic risk for POAG could be employed to help guide glaucoma management decisions.

The purpose of our study was to use available data in the UK Biobank (UKBB) to understand the impact of background polygenetic risk for POAG on disease diagnosis as well as ocular and image-based features within a large population. Our results will contribute to a better understanding of how a POAG PRS may affect POAG disease features and ultimately be incorporated into individualized disease risk prognostication.

## **Materials and methods**

### *The UK Biobank*

We used the UKBB dataset, a prospective cohort study of 502,506 UK residents aged 40-69 (application number: 50211). The dataset includes detailed genotypic and phenotypic information on all participants. Over 130,000 participants underwent eye examinations, including cornea-corrected intraocular pressure

(IOPcc; Ocular Response Analyzer, Reichert, Depew, NY), autorefractometry (RC-5000, Tomey, USA), corneal hysteresis (CH; Ocular Response Analyzer, Reichert, Depew, NY), and corneal resistance factor (CRF; Ocular Response Analyzer, Reichert, Depew, NY). The National Research Ethics Service Committee NorthWest–Haydock approved the study, and it was conducted in accordance with the Declaration of Helsinki. All participants provided written informed consent. Participants did not receive financial incentive to participate in this study.

### *Assessment of POAG*

Individuals with POAG were identified by the International Classification of Diseases, Ninth or Tenth Revision (ICD9/10) diagnosis code for POAG (ICD9: 365.2; ICD10: H40.1, H40.8, H40.9) from UKBB data field 41271/41270 or if they self-reported glaucoma on the eye problems/disorders (UK Biobank data field 6148) or noncancer illness fields (UK Biobank data field 20002), henceforth represented as ICD/SR-POAG. Individuals without glaucoma were identified if they had no ICD 9/10 diagnosis for POAG, no self-reported glaucoma, no glaucomatous features on fundus photographs (cup-to-disc ratio  $< 0.7$ , no hemorrhage or suspicious optic disc features), medication-adjusted IOPcc  $< 21$ mmHg, and no history of glaucoma treatment (e.g., glaucoma medications, glaucoma surgery or laser). Individuals with non-POAG forms of glaucoma (e.g., PACG, secondary forms of glaucoma) were excluded from analysis. The ICD/SR-POAG case vs. control definition was used for the receiver under the operating curve analysis; the remainder of the analysis included the entire cohort.

### *Fundus photographs (FP)*

Fundus photographs were obtained for a subset of participants ( $n=52,672$ ) using Topcon 3D OCT 1000 Mk2 (Topcon, Inc, Japan, stored in a .png image file). These images were evaluated by trained and certified ophthalmic image graders of NetwORC UK for a measurement of cup/disc ratio and the presence of disc hemorrhage or other suspicious optic disc features (e.g., notch, inferior rim thinning). FP assessments were made masked to POAG PRS. FPs assessed to be ungradable were excluded ( $n=8,222$ ). Per Warwick et al., evidence of POAG on FP, henceforth represented as FP-POAG, was present if the vertical cup-to-disc ratio (vCDR) was greater than 0.7 or if there was evidence of hemorrhage or suspicious optic disc features.<sup>14</sup> Similarly, control individuals were identified if they had no ICD 9/10 diagnosis for POAG, no self-reported glaucoma, no glaucomatous features on fundus photographs (vCDR  $< 0.7$ , no hemorrhage or suspicious optic disc features), medication-adjusted IOPcc  $< 21$ mmHg, and no history of glaucoma treatment (e.g., glaucoma medications, glaucoma surgery or laser).

### *Assessment of ocular factors*

Corneal-compensated intraocular pressure (IOPcc), corneal hysteresis (CH), and corneal resistance factor (CRF) for the right and left eye were obtained from UKBB data fields 5254, 5262, 5256, 5264, 5257, and 5265 respectively. Information on IOPcc-lowering medication use was obtained from UKBB data field 20003; pre-treatment IOPcc was imputed by dividing IOPcc by 0.7 for those on any IOP-lowering medication.<sup>15</sup> IOPcc less than 5mmHg or greater than 60mmHg were excluded from analysis. Spherical power and cylindrical power for the right and left eye were obtained from UKBB data fields 5084, 5085, 5087, and 5086 respectively. Spherical equivalent (SE) was calculated by adding half the cylindrical power to the spherical power. CRF, CH, and SE greater than 3 standard deviations away from mean were excluded from analysis.

#### *Assessment of glaucoma medications and glaucoma surgery*

Individuals using glaucoma medications were identified if they reported glaucoma medication use (UKBB data field 20003, see **eTable 1** for included glaucoma medications). Individuals who had previously undergone surgery or laser treatment for glaucoma were identified if they reported previous surgery or laser treatment for glaucoma (UKBB data field 5326/5327).

#### *Optical coherence tomography (OCT)*

Spectral-domain OCT scans of the macula were obtained on a subset of participants (n=37,851) using Topcon 3D OCT 1000 Mk2 (Topcon, Inc, Japan). Three-dimensional macular volume scans were obtained (512 horizontal A-scans/B-scans; 128 B-scans in a 6x6-mm raster pattern). All OCT images were stored in .fda image files without prior analysis of macular thickness. The Topcon Advanced Boundary Segmentation (TABS) algorithm was used to automatically segment all scans, using dual-scale gradient information to allow for automated segmentation of the inner and outer retinal boundaries and retinal sublayers.<sup>16,17</sup> Segmented boundaries include the internal limiting membrane, nerve fiber layer, ganglion cell layer, inner plexiform layer, inner nuclear layer, external limiting membrane, photoreceptor inner segment/outer segment, retinal pigment epithelium, Bruch's membrane, and the chorioid-scleral interface. The thickness of each sublayer was calculated as the difference between boundaries of interest and averaging across all scans. The location of the fovea was determined by calculating the minimum thickness of the 3 inner-most segments across all B scans and identifying the location where this thickness value approached zero. All B scans obtained before this location were used to calculate average thickness in the superior quadrants while the numbers after were used to calculate inferior quadrant thickness values. Sublayers include the retinal nerve fiber layer (RNFL), ganglion cell layer (GCL), inner plexiform layer (IPL), ganglion cell complex (GCC, defined as the total thickness of RNFL, GCL, and IPL), inner nuclear layer (INL), outer plexiform layer (OPL), photoreceptor segment (PS), retinal pigment

epithelium (RPE), and choroid scleral interface (CSI) (see **eTable 2** for more each layer's boundaries of interest).

The software provides an image quality score and segmentation indicators which were used for quality control. Segmentation indicators included the Inner Limiting Membrane (ILM) Indicator, a measure of the minimum localized edge strength around the ILM boundary across the scan, which can be used to identify blinks, scans that contain regions of signal fading, and segmentation errors. We excluded all images with image quality less than 40 and images representing the poorest 10% using the ILM indicator.<sup>18</sup> To exclude outliers, we also excluded any image with a layer thickness greater than 2.5 standard deviations away from the mean.

#### *POAG polygenic risk score calculation*

The polygenic risk score (PRS) for POAG was computed using GWAS summary statistics from the largest cross-ancestry meta-analysis<sup>6</sup>, after exclusion of the UK Biobank cohort (summary statistics available at <https://segrelab.meei.harvard.edu/data/>). Participants' imputed genetic data were utilized as previously described.<sup>19</sup> To predict the ancestral background of participants using ancestral labels from the 1000 Genomes Project Phase 3 reference panel, Principal Component Analysis (PCA) to linkage disequilibrium (LD)-pruned ( $r^2 < 0.1$  in 200kb windows) genetic markers with minor allele frequency (MAF)  $> 1\%$  and the k-nearest neighbors algorithm were used.<sup>13</sup> The Lassosum method, a regression-based model that shrinks the variants via variable selection and retains the best set of variants by adjusting the tuning parameters, was used to compute the PRS using 9,705,359 imputed variants from 408,463 participants (14,171 ICD/SR-POAG patients and 394,292 controls).<sup>20</sup> Parameter settings included a sample of 5000 and cluster of cl. Calculated PRSs were normalized to a mean of 0 and standard deviation of 1 within each ancestry group.

#### *Statistical analysis*

For both cases and controls, participant-level IOPcc, CH, CRF, SE, FP features, and OCT values were calculated for the more severely affected eye. We defined the worse eye as the eye with the larger cup/disc ratio if FP was available, thinner GCC if FP was not available and higher IOPcc if neither FP nor GCC were available. If data were available for only one eye, data for that eye were used. As visual field data was not available, higher vCDR, IOPcc, thinner mRNFL and GCC, and greater requirements for medication, laser and/or surgery to treat glaucoma was used as a proxy for more advanced disease.



Statistical analyses were performed using R version 4.0.4 and RStudio v1.4.1106. Means and standard deviations were calculated for demographic and ocular characteristics. Means/frequencies were compared across groups using two-tailed Student's t-tests and Chi-square tests/exact Fisher tests for continuous and categorical variables, respectively. We used logistic regression models adjusted for age, age<sup>2</sup>, sex, and ancestry to evaluate associations between PRS and POAG diagnosis, as well as PRS and glaucoma features on FP. Linear regression models adjusted for age, age<sup>2</sup>, sex, and ancestry were used to estimate associations between POAG PRS and ocular factors (IOPcc, CH, CRF, SE), POAG PRS and retinal thicknesses, and POAG PRS and cup-to-disc ratios. P values were two-sided. For retinal thickness analyses with 9 non-overlapping retinal layers, the threshold for significance was defined using a Bonferroni adjustment ( $p < .05/9 = 0.0055$ ). STROBE reporting guidelines were utilized for this paper.

## **Results**

### *Study population*

Among the 407,667 UKBB participants included in this analysis, 14,171 (3.48%) were identified as ICD/SR-POAG cases. 87,812 participants had ocular data, including IOPcc, CH, CRF, and SE; 44,450 participants had FPs; and 37,851 participants had OCTs available for analysis. Of the 44,450 individuals with gradable FPs, 710 (1.60%) were identified as FP-POAG cases. Additionally, of the 44,411 individuals with gradable FPs, 1,559 (3.51%) were ICD/SR-POAG cases and 188 were identified as both FP-POAG and ICD/SR-POAG cases. Further study population characteristics can be found in **eTable 3**.

### *POAG PRS performance*

A POAG PRS was computed for the 14,171 ICD/SR-POAG cases and 393,496 controls. Individuals with ICD/SR-POAG had higher mean PRS for POAG compared to those without ICD/SR-POAG ( $0.50 \pm 1.02$  SD vs.  $-0.02 \pm 0.99$  SD,  $p < .001$ ). AUC receiver operative curve for ICD/SR-POAG case detection was 0.646 for PRS alone, and 0.748 with the addition of age, sex, and inferred ancestry (**Figure 1**). The prevalence of ICD/SR-POAG in the entire cohort was 3.48%; this prevalence increased progressively with each ICD/SR-POAG PRS decile (**Figure 2**). The prevalence of ICD/SR-POAG in decile 10 (those at highest genetic risk) was more than five times the prevalence of ICD/SR-POAG in decile 1 (those at lowest genetic risk) (1.33% vs. 7.39%). ICD/SR-POAG prevalence was higher with increased genetic risk at all ages; this effect was most pronounced in older individuals (**Figure 3**). In a logistic regression model adjusting for age, age<sup>2</sup>, and sex, a one-SD increase in PRS was associated with 1.74 times higher odds of ICD/SR-POAG (adjusted OR (aOR) = 1.74, 95% CI: 1.71, 1.77,  $p < .001$ ).

Similarly, in the subset of 44,411 individuals with available FPs, individuals at higher POAG genetic risk were more likely to have FP-POAG. AUC receiver operative curve for FP-POAG case detection was 0.614 for PRS alone, and 0.683 with the addition of age, sex, and inferred ancestry (**Figure 1**). The prevalence of FP-POAG amongst individuals with available FPs was 1.60%. Though there was some variability, there was a progressive increase of FP-POAG prevalence from decile 1 to decile 10 (**Figure 2**) and FP-POAG prevalence increased with genetic risk at all ages (**Figure 3**). In an adjusted logistic regression, a one-SD increase in PRS was associated with 1.46 times higher odds of FP-POAG (aOR = 1.46, 95% CI: 1.36, 1.58,  $p < .001$ ).

#### *Use of glaucoma medications or prior glaucoma surgery*

Amongst the 407,667 participants included in this analysis, 4,299 (1.05%) reported glaucoma medication use. A subset ( $n=5617$ ) had available data on previous glaucoma surgery or laser use; of this subset, 148 (2.63%) reported previous glaucoma surgery or laser use. Glaucoma medication use and previous glaucoma surgery or laser use increased with PRS decile (**eFigure 1**). In adjusted logistic regression, a one- ICD/SD increase in PRS was associated with 1.95 times higher odds of glaucoma medication use (aOR = 1.95, 95% CI 1.89, 2.01,  $p < .001$ ) and 1.67 times higher odds of previous glaucoma surgery or laser (aOR = 1.67, 95% CI: 1.42, 1.97,  $p < .001$ ).

#### *Ocular factors*

87,512 individuals in this analysis had complete ocular data. Mean medication-adjusted IOPcc was  $16.60 \pm 4.17$  mmHg, mean CH was  $10.40 \pm 1.91$  mmHg, mean CRF was  $10.55 \pm 1.98$  mmHg, and mean SE was  $-0.21 \pm 2.34$  D. Higher POAG PRS decile was associated with higher medication adjusted IOPcc and CRF and lower SE and CH (**eFigure 2, eTable 4**). In adjusted models, a one-SD increase in PRS was associated with 0.61 mmHg higher IOPcc (95% CI: 0.59, 0.64,  $p < .001$ ), -0.09 mmHg lower CH (95% CI: -0.10, -0.08,  $p < .001$ ), 0.08 mmHg higher CRF (95% CI: 0.06, 0.09,  $p < .001$ ), and 0.08 D more myopic SE (95% CI: -0.11, -0.07,  $p < .001$ ) (**Table 1**).

Additionally, 4.0% of individuals had IOPcc greater than 24mmHg and 0.9% of individuals had IOPcc greater than 30mmHg. The prevalence of eyes with high IOPcc greater than 24mmHg and 30mmHg increased with PRS decile. 2.1% of individuals in decile 1 had IOPcc greater than 24mmHg, compared to 7.7% in decile 10 ( $p < .001$ ) and 0.5% of individuals in decile 1 had IOPcc greater than 30mmHg, compared to 1.9% in decile 10 ( $p < .001$ ).

#### *Imaging features*

Of the 44,411 FPs available for analysis, 0.25% had a hemorrhage on the disc, 0.28% had glaucomatous optic disc features and 0.71% had a vCDR greater than 0.7. Mean vCDR was  $0.43 \pm 0.10$ . Prevalence of optic disc hemorrhage and glaucomatous optic disc features were highest in POAG PRS decile 10. 0.42% of FPs in decile 10 had a hemorrhage on the disc, compared to 0.17% in decile 1 ( $p=.07$ ). Similarly, 0.51% of FPs in decile 10 had a suspicious optic disc feature, compared to 0.19% in decile 1 ( $p=.03$ ). vCDR increased progressively with POAG PRS decile (0.47 in decile 10 vs 0.41 in decile 1,  $p<.001$ ) (**eFigure 3**). In an adjusted logistic regression, a one-SD increase in PRS was associated with 1.42 times higher odds of glaucomatous optic disc features (aOR = 1.42, 95% CI: 1.19, 1.69,  $p<.001$ ). The association between PRS and disc hemorrhage did not reach significance (aOR = 1.19, 95% CI: 0.99, 1.43,  $p=.07$ ). In an adjusted linear regression, a one-SD increase in PRS was associated with a 0.013 increase in vCDR (adjusted beta (aB) = 0.013, 95% CI: 0.012, 0.014,  $p<.001$ ).

**eTable 5** summarizes the mean thicknesses for each retina layer from 37,818 available OCTs. A one-SD increase in POAG PRS was associated with a 0.14 $\mu$ m thinner RNFL (95% CI -0.19, -0.1), a 0.05 $\mu$ m thinner GCL (95% CI -0.08, -0.02), a 0.06 $\mu$ m thinner IPL (95% CI -0.09, -0.04), and a 0.26 $\mu$ m thinner GCC (95% CI -0.34, -0.17) ( $p<.001$  for all) (**eTable 6**). Similarly, decreases in INL (aB = -0.03, 95% CI -0.05, -0.01,  $p=.003$ ) were observed per one-SD increase in POAG PRS (**eTable 6**). While most layers had consistent changes in superior and inferior layer thickness per one-SD increase in POAG PRS, the inferior RNFL had an adjusted beta value that was more than double that of the superior RNFL (-0.2 $\mu$ m vs. -0.1 $\mu$ m,  $p<.001$  for both, **eTable 6**). Amongst individuals with ICD/SR-POAG, those in the decile 10 of POAG PRS had thinner inferior RNFL compared to those in decile 1 of POAG PRS (35.9 $\mu$ m vs. 39.2 $\mu$ m,  $p<.001$ ),

The association between PRS and RNFL and GCC thickness (**Figure 4**) appeared to be largely driven by individuals with POAG. When our cohort was stratified by ICD/SR-POAG vs. controls, individuals with ICD/SR-POAG had a relationship between PRS and thinner RNFL (aB = -0.88 $\mu$ m, 95% CI: -1.21, -0.55,  $p<.001$ ) while controls had no relationship ( $p=.13$ ). Similarly, individuals with ICD/SR-POAG had a relationship between PRS and thinner GCC (aB = -1.4 $\mu$ m, 95% CI: -1.98, -0.23,  $p<.001$ ), while controls had a diminished relationship (aB = -0.14 $\mu$ m, 95% CI: -0.23, -0.05,  $p=.002$ ) (**Figure 5**). These findings are replicated when stratified by FP-POAG case vs. control.

## **Discussion**

We were able to identify individuals at substantially higher risk of glaucoma using a genome-wide PRS. The risk increased across deciles and in all age groups, with the effect most pronounced in older

individuals. The PRS was associated with having more advanced disease, specifically higher vCDR, IOPcc, thinner mRNFL, and GCC, and greater requirements medication, laser and/or surgery to treat glaucoma. We also identified novel associations with background genetic risk and corneal hysteresis, greater prevalence of disc hemorrhages, and preponderance for decreased inferior RNFL thickness.

The prevalence of POAG in the highest PRS decile was more than five times the prevalence of POAG in the lowest decile indicating that those with high PRS are truly at risk of developing glaucoma. While there is limited work on this topic, a prior study using MTAG-derived PRS constructed based on glaucoma disease status, vCDR, and IOP found that individuals in the top PRS decile had 14.9 times higher risk of POAG compared to those in the lowest decile. Overall, the AUC using the PRS was somewhat useful, but not likely high enough for population-based screening. Adding age, sex, and inferred ancestry increased the AUC and resulted in similar findings to those reported previously with traditionally-used risk factors (age, sex, and self-reported family history).<sup>11</sup> Differences in performance of our PRS and prior literature is likely due to differing methods of PRS calculation used in each study as well as the definition of glaucoma. Prior MTAG-derived PRS have been tested using datasets with clinically confirmed glaucoma cases,<sup>11</sup> while here we applied a different PRS derived using Lassosum penalized regression in a population where there was not systematic confirmation of case status, and our PRS still performed well.

We show here that the AUC receiver operator curve for POAG case detection was similar for individuals with available fundus photographs and without fundus photographs. The prevalence of POAG in both individuals with fundus photographs and without fundus photographs progressively increased with each POAG PRS decile. This suggests that the usage of ICD diagnosis codes and self-reported glaucoma is a valid way to identify individuals with glaucoma when assessing the utility of PRS in large population-based or registry studies. Indeed, prior studies have found high accuracy of ICD codes for the diagnosis of glaucoma.<sup>21–23</sup>

Those with higher PRS scores likely had more advanced disease, specifically they had higher vCDR, IOPcc, thinner mRNFL, and GCC, and higher glaucoma medication use and were more likely to have prior glaucoma surgery and/or laser procedures. A previous study assessing an IOP-based PRS constructed from SNPs demonstrated similar results in a sample of White European study participants from the UK Biobank cohort, with higher PRS associated with higher likelihood of increased IOP.<sup>24</sup> A prior PRS stratification that assessed IOP using a registry of patients in Australia and New Zealand also demonstrated a significant relationship between high genetic risk groups having a higher maximum IOP

as compared to lower genetic risk groups and demonstrated that treatment intensity (including the number of medications used and number of glaucoma operations) increased with higher PRS.<sup>25</sup> Even with elevated treatment intensity, patients with higher PRS may have worse visual outcomes. In a longitudinal cohort study of individuals with early or suspected glaucoma, Siggs et al. found that people in the top 5% of their MTAG-derived PRS had a greater likelihood of visual field progression despite receiving significantly more drops and laser trabeculoplasty procedures.<sup>26</sup> Due to higher medication, surgery, and laser use, the true strength of association between PRS and glaucoma severity may be underestimated.

Thinning of the RNFL has been shown in the literature to be associated with progressive functional loss.<sup>27,28</sup> Our results showed that higher PRS was associated with thinner macular RNFL, particularly in the inferior sector. Amongst individuals with glaucoma, those with higher polygenic risk for POAG had thinner inferior RNFL. It is possible that individuals without inferior thinning have non-genetic or not yet identified genetic causes of glaucoma. This association may also be a result of more frequent inferior RNFL thinning in early glaucoma. Prior studies have found that the AUC tends to be greater for the inferior area compared to other quadrants, suggesting that the inferior area of the optic nerve is most affected in glaucoma.<sup>29,30</sup> Although the inferior RNFL seems to undergo the most thinning in glaucoma, this region may also have a greater capacity for thinning before visual field loss, making it an optimal parameter for detecting early glaucoma. A previous retrospective cross-sectional study of 108 glaucoma study participants found that in the inferior quadrant, a greater percentage of RNFL thinning is required to detect functional loss of vision compared to the superior quadrant.<sup>31</sup> Further work is required to understand imaging phenotypes associated with POAG genetic risk and how these may be combined to improve risk stratification.

We demonstrate a novel association between higher PRS and prevalence of disc hemorrhages on FP. It is possible our result did not reach statistical significance to the rarity of this event and the possibility that eyes with disc hemorrhage secondary to causes other than glaucoma may have been included. While the exact mechanism underlying disc hemorrhages remains unclear, multiple studies have demonstrated strong association with disc hemorrhage and glaucoma progression.<sup>32</sup> This suggests that accumulated genetic risk burden may predispose individuals to glaucoma visual field progression. While it is not possible to assess progression rates in a population-based study, prior work has demonstrated an association between PRS and visual field progression in glaucoma patients.<sup>26</sup> The association between higher PRS and prevalence of disc hemorrhages may point to alternate ischemic or vascular etiology in high PRS glaucoma compared to glaucoma associated with low PRS. POAG is a complex disease with

both genetic and environmental factors, so disc hemorrhages may represent specific biological pathways that may help us better elucidate the mechanism of disease.

Higher PRS was also associated with lower CH in our study. While central corneal thickness (CCT) has been utilized classically to assess glaucoma risk, the association with higher PRS and CH here demonstrates that increased clinical attention should also be given to measuring CH.<sup>33</sup> In separate unpublished analyses, our groups found CCT did not correlate with POAG genetic risk in study participants from the Ocular Hypertension Treatment Study (OHTS), suggesting that CH may be a better marker of POAG risk (*unpublished data*). While the association between CH and glaucoma has been less thoroughly examined in previous literature, multiple studies have demonstrated that CH is strongly associated with glaucoma presence, risk of progression, and effectiveness of certain treatments.<sup>34–40</sup> Even in glaucoma patients with well-controlled intraocular pressure, lower CH was associated with a higher risk of global visual field progression.<sup>41</sup> Low CH has also been found to be a risk factor for central visual field progression which is a major concern for vision-related quality of life.<sup>42,43</sup> It has been proposed that low CH may be associated with glaucoma progression because CH measurements may indirectly provide information about the characteristics of posterior ocular tissue extracellular matrix that make an eye more susceptible to glaucomatous damage.<sup>41</sup> Our findings thus reinforce the clinical significance of CH in the diagnosis and management of glaucoma, especially in patients with higher PRS.

This study has several strengths, including its use of genetically-inferred ancestry, large sample size, and exploration of viable glaucoma endophenotypes using IOP and OCT-derived retinal layer thicknesses. We also used not only diagnosis and self-report-based definitions of glaucoma, but also explored fundus photogram-based definitions of glaucoma. We were also able to demonstrate that individuals with the highest POAG PRS also had the lowest CH and highest myopia, the latter factors increasing propensity for developing severe disease.

However, this study is subject to several limitations that should be considered. First, 95.7% of the UKBB participants that met the inclusion criteria for our study are of European ancestry. In addition, though we used cross-ancestry summary statistics to construct our PRS, these weights are derived from prior GWAS with mostly European participants. Although the prior GWAS found that the majority of POAG loci had generally consistent effects across different ancestries, this highlights an issue of equity in representation in data.<sup>6</sup> Further investigation is required to improve the generalizability of our PRS. Secondly, UKBB participants are aged 40-69. The prevalence of POAG increases with age, and people over the age of 80 are at highest risk of having POAG.<sup>44</sup> Despite the younger population and likely lower prevalence of

POAG in the UKBB cohort, we observed a large effect size. Third, our dataset is subject to influence from possible inaccuracies in medication self-reporting and medical documentation. These inaccuracies likely explain the limited overlap between study participants with ICD/SR-POAG and FP-POAG. However, the size of the dataset likely diminishes this effect. Fourth, this study utilizes macular RNFL thicknesses, though in clinical practice, peripapillary RNFL are more often utilized. Fifth, we used a definition of POAG with lower specificity than other population-based studies; despite this limitation, our PRS performed well. Our definition of ICD/SR-POAG inferred that all self-reported cases of glaucoma had POAG which may not be true, but we compensated by using alternative definitions and objective endophenotypes. Sixth, we used a vCDR cutoff of 0.7 to categorize FP-POAG. This may have resulted in false categorization of some subjects with large discs as having FP-POAG. Conversely, it is possible that this cutoff may have missed some true POAG cases. Finally, this study included individuals with ICD codes for POAG; diagnosis codes of secondary causes of glaucoma including exfoliation syndrome glaucoma and pigmentary glaucoma were excluded. Similarly, this dataset and its conclusions may not apply to a population with normal tension glaucoma.

This investigation identified individuals at higher risk of POAG, and found that higher PRS was associated with markers for more severe disease. We also identified associations of POAG PRS with disc hemorrhages and corneal hysteresis. This study supports the increasing clinical importance of PRS risk stratification to identify individuals at higher risk of severe disease to help inform healthcare resource allocation and clinical decision making. Continuing to investigate the genetic markers contributing to our PRS may further our understanding of glaucoma pathology and reveal biomarkers useful for treatment development and disease monitoring.

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**Table 1. Ocular Factors Logistic Regression per 1 point increase in POAG PRS.**

	Beta (95% CI)	P-value	Adjusted Beta (95% CI)	P-value
<b>Intraocular pressure (mmHg)</b>	0.61 (0.59, 0.64)	<.001	0.62 (0.59, 0.64)	<.001
<b>Corneal hysteresis (mmHg)</b>	-0.09 (-0.10, -0.08)	<.001	-0.09 (-0.10, -0.08)	<.001
<b>Corneal resistance factor (mmHg)</b>	0.08 (0.06, 0.09)	<.001	0.08 (0.06, 0.09)	<.001
<b>Spherical equivalent (D)</b>	-0.09 (-0.11, -0.07)	<.001	-0.08 (-0.10, -0.07)	<.001

Note: adjusted model includes age, age<sup>2</sup>, sex, and ancestry as covariates.

**Figure 1. ROC curve for ICD / Self-Report POAG**

Note: ROC = receiver under operator curve.

**Figure 2. POAG prevalence per POAG PRS decile.**

Note: POAG = primary open angle glaucoma, PRS = polygenic risk score.

**Figure 3. POAG prevalence by POAG PRS and age.**

Note: POAG = primary open angle glaucoma, PRS = polygenic risk score.

**Figure 4. RNFL and GCC layer thickness amongst individuals with POAG, decile 1 vs. decile 10 of POAG PRS.**

Note: RNFL = retinal nerve fiber layer, GCC = ganglion cell complex, POAG = primary open angle glaucoma, PRS = polygenic risk score.

**Figure 5. Relationship between RNFL and GCC thickness with POAG PRS for POAG cases vs. controls**

Note: RNFL = retinal nerve fiber layer, GCC = ganglion cell complex, POAG = primary open angle glaucoma, PRS = polygenic risk score.