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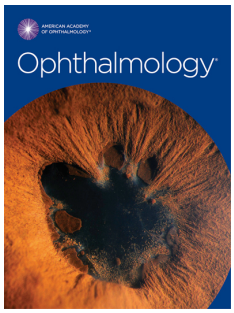

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PII: S0161-6420(21)00189-5

DOI: https://doi.org/10.1016/j.ophtha.2021.03.007

Reference: OPHTHA 11667

To appear in: *Ophthalmology*

Received Date: 8 January 2021

Revised Date: 3 March 2021

Accepted Date: 4 March 2021

Please cite this article as: RRH: Characteristics of Gln368Ter Myocilin variant

Characteristics of Gln368Ter Myocilin variant and influence of polygenic risk on glaucoma penetrance in the UK Biobank

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Meeting presentation: Under consideration for presentation at the ARVO, 2021

Financial support: This work was supported the MEE Institutional Startup Fund (NZ). The funding organization had no role in the design or conduct of this research.

Conflict of Interest: No conflicting relationship exists for any author

Running head: Characteristics of Gln368Ter Myocilin variant

Abbreviations:
POAG – primary open angle glaucoma; DDG – disc defined glaucoma; FP-fundus photo ; IOP-intraocular pressure; GCC – ganglion cell complex ; RNFL – retinal nerve fiber layer ; PRS-polygenic risk score; OCT – optical coherence tomography

Abstract

Objective: *MYOC* (myocilin) mutations account for 3-5% of primary open angle glaucoma (POAG). We aimed to understand the true population-wide penetrance and characteristics of glaucoma among individuals with the most common MYOC variant (p.Gln368Ter) and the impact of a POAG polygenic risk score (PRS) in this population.

Design: Cross-sectional population-based

Methods: Individuals with the p.Gln368Ter variant were identified among 77,959 UK Biobank participants with fundus photographs (FPs). A genome-wide POAG PRS was computed and two masked graders reviewed FPs for disc-defined glaucoma (DDG).

Main Outcome Measures: Penetrance of glaucoma

Results: 200 individuals carried the p.Gln368Ter heterozygous genotype, and 177 had gradable FPs. 132 had no evidence of glaucoma, 45 (25.4%) had probable/definite glaucoma in at least one eye and 19 (10.7%) had bilateral glaucoma. There were no differences in age, race/ethnicity, or gender among groups ($p>0.05$). Of those with DDG, 31% self-reported or had ICD 9/10 code for glaucoma, while 69% were undiagnosed. Subjects with DDG had higher medication-adjusted cornea-corrected intraocular pressure (IOPcc) ($p<0.001$) vs. those without glaucoma. This difference in IOPcc was larger in DDG with prior glaucoma diagnosis vs. those not diagnosed ($p<0.001$). Majority of p.Gln368Ter carriers had IOP in the normal range (<=21 mmHg), though this proportion was lower in those with DDG ($p<0.02$) and those with prior glaucoma diagnosis ($p<0.03$). Prevalence of DDG increased with each decile of the POAG PRS. Subjects with DDG had significantly higher PRS compared to those without glaucoma (0.37 ± 0.97 vs 0.01 ± 0.90, $p=0.03$). Of those with DDG, individuals with prior diagnosis of glaucoma had higher PRS compared to undiagnosed individuals (1.31 ± 0.64 vs 0.00 ± 0.81, $p<0.001$) and had 27.5 times (95%CI 2.5-306.6) adjusted odds of being in the top decile of PRS for POAG.

Conclusion: 1 in 4 individuals with MYOC p.Gln368Ter mutation had evidence of glaucoma, a substantially higher penetrance than previously estimated, with 69% of cases undetected. A large portion of p.Gln368Ter carriers have IOP in the normal range, despite similar age, including those with DDG. PRS increases disease penetrance and severity of disease, supporting the utility of PRS in optimizing risk stratification among MYOC p.Gln368Ter carriers.

Introduction

Glaucoma, a progressive optic neuropathy characterized by retinal ganglion cell degeneration, is a leading cause of blindness worldwide.[1,2] Glaucoma affects 3.54% of the population older than 40 years worldwide, approximately 76 million people, and is projected to increase to 111.8 million by 2040 due to aging of the world's population.[3] Primary open angle glaucoma (POAG) accounts for 75% of glaucoma globally and over 50% of glaucoma-related blindness.[4] Due to relatively slow loss of vision, glaucoma often does not come to clinical attention until significant irreversible vision loss has occurred, with population-based studies suggesting that nearly 50% of glaucoma cases in the US[5,6] and 90% of cases in developing countries[7] are undiagnosed.

Glaucoma is a highly heritable disease, and POAG is one of the most heritable of all complex human diseases.[8] Reconstructed family data have estimated the heritability of glaucoma at 70%.[8] To date, 127 independent common risk variants for POAG have been identified in multi-ethnic populations.[9,10] Of the disease-causing mutations, the *MYOC* (myocilin) p.Gln368Ter variant is the most common rare mutation amongst populations of European ancestry and has been found in 2-7% of patients with clinically diagnosed POAG[11,12] and in 12-20% of patients with increased intraocular pressure (IOP).[13] While the underlying mechanism of this variant remains unclear, it appears that the aggregation of misfolded myocilin proteins leads to trabecular meshwork cell dysfunction and subsequent elevated IOP.[14,15] Though *MYOC* variants have been associated with greater severity of IOP elevation, the p.Gln368Ter variant was recently also associated with normal tension glaucoma (NTG).[16]

MYOC disease-causing alleles are inherited in an autosomal dominant manner, however the reported penetrance is variable and has been noted to be lower in population-based studies compared to family-based studies.[12] While ascertainment bias and aggregation of common environmental risk factors in family-based studies and under sampling in population-based studies likely play a role, accumulation of other common glaucoma-associated risk variants may modify penetrance. For common and complex diseases, such as POAG, a polygenic risk score (PRS) can be calculated using both known (genome-wide significant) common genetic variants and variants of individual small effects, and PRS can be used to identify individuals at high risk of disease.[17] Candidate PRS have previously been calculated for POAG and shown to risk stratify cases, affect age of onset and likelihood of glaucoma progression and modify the penetrance of the MYOC p.Gln368Ter variant.[18]

Recent availability of large-scale genomics data have made it possible to evaluate population-wide effects of the *MYOC* p.Gln368Ter variant. However, prior studies have relied on self-report for identification of cases,[12] which can be inaccurate and does not elucidate clinical disease features. The purpose of our study is to use the available data from the UK Biobank (UKBB), a large population-based study, to understand the true population-wide

mutation penetrance using imaging and IOP data from individuals with *MYOC* p.Gln368Ter variant, to describe the clinical characteristics of glaucoma among these individuals, as well as to understand the impact of underlying polygenic risk in this population.

Methods

Cohort description:

The UKBB is a prospective community-based cohort study of ~500,000 UK residents, aged 40-69 years (http://www.ukbiobank.ac.uk/resources/) who were registered with the National Health Service and includes detailed genotypic and phenotypic information on all participants. Health questionnaires were collected from all participants that included age at recruitment and self-reported race and gender. A subset of ~130,000 people had eye examinations including visual acuity, refraction, keratometry, Goldmann and cornea-corrected IOP (IOPcc, Ocular Response Analyzer; Reichert, Depew, NY). Over 84,000 people underwent retinal imaging with color fundus photographs (FP) and macular optical coherence tomography (OCT) using a Topcon 3D OCT 1000 Mk2 (Topcon, Inc, Japan). 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.

Identification of glaucoma:

Individuals with glaucoma were identified if they self-reported glaucoma on eye problems/disorders (UKBB data field 6148) or noncancer illness (UKBB data field 20002) or had an International Classification of Diseases, Ninth or Tenth Revision (ICD9/10) diagnosis code for POAG, other glaucoma or glaucoma, unspecified (H40.1, H40.8, H40.9). Where available, age at first glaucoma diagnosis was obtained from UKBB data fields 4689 and 20009. Information on treatment with IOP lowering medication and prior laser/surgery was obtained from data fields 20003, 5326 and 5327, respectively. As some participants were already on IOP-lowering medications, and data from pre-treatment was unavailable, we imputed pre-treatment IOP by dividing measured IOP by 0.7, according to the mean IOP reduction achieved by medications.[2]

Two masked graders (NZ and ML) further reviewed color fundus photographs (FPs) from identified p.Gln368Ter carriers for *disc defined glaucoma (DDG)*. Fundus photographs were graded for no glaucoma, probable glaucoma and definite glaucoma and vertical cup-to-disc ratio (vCDR). Probable glaucoma was defined by presence of at least two of the following criteria: vCDR $\geq$0.7 but <0.85, rim width $\leq$0.1 disc diameter, generalized or localized rim thinning, visible RNFL defects or splinter hemorrhage. Definite glaucoma was defined as vCDR $\geq$0.85 or visible RNFL defects corresponding with thinning area of rim or notches.[23] Graders were 80.6%

in agreement, κ=0.66 (p<0.001). The 80 disagreements underwent direct arbitration and were re-graded. **Figure 1** shows an example of an eye with DDG versus one judged to be normal by both graders.

Genotype quality control, p.Gln368Ter carrier identification and polygenic risk score calculation:

The array genotype curation process is described in detail by Bycroft et al.[19] In addition, we applied various quality control steps with PLINK 1.9 on directly genotyped variants of 82,035 UKBB samples with ocular imaging, using best practice approaches refined in GTEx consortium.[20] The pipeline iteratively examines variants (single nucleotide polymorphisms [SNPs] and indels [insertions and deletions]) and sample genotype efficiency, allele frequencies, gender discrepancies and tests of Hardy-Weinberg equilibrium. Additionally, we examined sample duplicates, cryptic relatedness, and contamination. Participants with unresolved differences between genotype-inferred and reported sex were excluded (N=449). Samples with genotyping call rate <97% were removed (229). Additionally, 3,381 individuals with high cryptic relatedness (>0.1875 Pi-hat) and 298 individuals with outlying heterozygosity (4 standard deviations from the mean heterozygosity rate) after accounting for inferred ancestry were also removed. We applied 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 to predict the ancestral background of participants using ancestral labels from the 1000 Genomes Project Phase 3 reference panel. We found good correlation between self-reported and inferred ancestry; for samples with mismatched ancestry, we used the inferred ancestry for quality control (QC) and downstream analyses. Only participants with inferred European ancestry were used in our study, leading to a total of 77,959 participants post QC with both genotype and image data. For variant QC, we removed variants with call rate < 97%, MAF < 0.01 and Hardy-Weinberg equilibrium test p < 1e-5.

Prior studies have demonstrated that *MYOC* c.1102C>T (p.Gln368Ter) can be imputed with high accuracy from genotyping arrays.[12] Similarly, here we identified 200 p.Gln368Ter carriers using the imputation posterior probability for each of the 3 genotypes (GG, AG and AA).

We constructed and tested a POAG PRS for UKBB participants using genome-wide associate study (GWAS) summary statistics from the Caucasian subset of the large cross-ancestry meta-analysis,[9] after exclusion of the UKBB cohort (summary statistics available at https://segrelab.meei.harvard.edu/data/). PRS was computed using *LDpred2* [21] which is implemented in R package *bigsnpr*.[22] The posterior mean effect sizes from GWAS summary statistics were estimated using a point-normal mixture prior for the variant effects and were

adjusted for linkage disequilibrium. The hyperparameters in the model included SNP heritability (h^2) and the fraction of casual variants (p). Hyperparameters to construct the final PRS were chosen based on the best prediction performance measured by the area under the curve (AUC). PRS was calculated using two definitions of glaucoma cases: 1) ICD9/10 diagnosis code (747 cases, 75624 controls, 1,588 missing) and 2) combination of ICD 9/10 diagnosis code and glaucoma self-report as defined above (2001 cases and 75624 controls). Due to better performance of the model using ICD 9/10 diagnosis codes only (supplemental Figure 1), this PRS was used for all further analysis. Calculated PRS were normalized to a mean of zero and standard deviation of 1.

Optical coherence tomography (OCT):

Spectral domain OCT scans of the macula were obtained using Topcon 3D OCT 1000 Mk2 (Topcon, Inc, Japan). Three dimensional macular volume scans were obtained (512 horizontal A-scans/B-scan; 128 B-scans in a 6x6-mm raster pattern).[24] All OCT images were stored in .fda image files without prior analysis of macular thickness. We used the Topcon Advanced Boundary Segmentation (TABS) algorithm to automatically segment all scans, which uses dual-scale gradient information to allow for automated segmentation of the inner and outer retinal boundaries and retinal sublayers.[25] The boundaries segmented include the internal limiting membrane (ILM), nerve fiber layer (NFL), ganglion cell layer (GCL), inner plexiform layer (IPL), inner nuclear layer (INL), external limiting member (ELM), photoreceptor inner segment/outer segment (IS/OS) junction layer, retinal pigment epithelium (RPE), Bruch's membrane (BM) and Chorio-retinal interface (CSI). The software provides an image quality score which was used as quality control measure with scores less than 40 excluded (n=70). Scans with outlying values for the ganglion cell complex (defined as the distance between ILM and GCL) were manually reviewed and excluded if clear segmentation errors were noted (n=2).

The thickness of each retinal sub-layer was determined by calculating the difference between boundaries of interest and averaging this across all scans. For example, NFL thickness was calculated as the difference between ILM and NFL boundary lines. We determined the location of the fovea 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 scan numbers obtained prior to this location were used to calculate average thickness in the superior quadrants while the numbers after were used to calculate inferior quadrant thickness values.

Statistical analyses:

Statistical analyses were performed using STATA software version 15.0 and RStudio v. 4.0.3. Means and standard deviations were calculated for demographic and ocular characteristics and

compared across groups using two tailed Student t-test, and chi-square or Fisher's exact tests for continuous and categorical variables, respectively. Logistic regression models adjusted for age at recruitment and sex were used to estimate odds of falling in the top decile of PRS risk for POAG. Values were considered statistically significant if the P value was less than 0.05.

Results

Among the 73,563 UKBB participants with complete imaging and genotype data, we identified 200 (0.027%) heterozygous carriers of the p.Gln368Ter allele and no homozygous carriers. 177 of the heterozygous carriers had gradable FPs in one or both eyes (160 right eyes and 158 left eyes). Among those with gradable FPs, 132 (74.6%) had no evidence of glaucoma, while 45 (25.4%) had probable or definite glaucoma (DDG) in at least one eye, and 19 (10.7%) had evidence of bilateral disease. Of the 45 individuals with DDG, 14 (31%) self-reported or had an ICD 9/10 code for glaucoma, whereas 31 (69%) were undiagnosed. Conversely, 6 of 132 individuals who were judged to have healthy appearing optic nerves in both eyes self-reported or had ICD 9/10 for glaucoma. Of the 200 individuals with available IOP data, after correction for IOP lowering medications, 27% and 21% had IOP greater than 21 in their right and left eye, respectively, while 25.4% had an average IOP over 21 in both eyes (defined as ocular hypertension, OHTN). There was no difference in age between p.Gln368Ter carriers with and without OHTN (58.0 ± 1.2 vs 57.2 ± 0.6 respectively, p=0.56).

Table 1 outlines the demographic and ocular characteristics among individuals with and without DDG. There were no differences in age at baseline, age at diagnosis, race/ethnicity or gender among individuals with and without DDG (p>0.05 for all). Subjects with DDG had slightly higher myopic refractive error on average compared to those without evidence of glaucoma (-1.3 ± 3.6 D vs 0.1 ± 2.6 D, p=0.005, right eye; -1.7 ± 4.4 D vs 0.1 ± 2.7 D, p=0.001, left eye).

Subjects with DDG also had on average higher IOPcc in both right and left eyes (19.8 ± 6.4 mmHg vs 17.6 ± 4.4 mmHg, p=0.02, right eye; 19.3 ± 5.8 mmHg vs 17.3 ± 4.4 mmHg, p=0.01, left eye). A higher proportion of subjects with DDG (24.4%) were on one or more IOP lowering medications, compared to those without evidence of glaucomatous optic neuropathy (4.6%) on imaging (p<0.001). After correction for use of IOP lowering medication, the difference in IOP between those with and without DDG was larger (22.3 ± 9.8 mmHg vs 18.0 ± 5.6 mmHg, p<0.001, right eye; 21.3 ± 7.7 mmHg vs 17.7 ± 5.3 mmHg, p<0.001, left eye). After adjustment for IOP lowering medications, over half of p.Gln368Ter carriers had IOP in the normal range (<=21 mmHg), though this proportion was significantly lower in those with DDG compared to those without glaucoma (53.3% vs 79.6%, p=0.001, right eye; 64.4% vs 81.8%, p=0.02, left eye). **Figure 2** demonstrates the distribution of medication adjusted IOP in both groups. Individuals

with DDG on average had larger vCDR in both eyes and were more likely to undergo glaucoma surgery or laser based on self-report, though this latter measure was overall rare in this cohort (**Table 1)**.

Individuals with DDG but who were not diagnosed at the time of the study did not differ in age, gender, ethnicity or average refractive error from those with prior glaucoma diagnosis ($p>0.2$ for all) (**Table 2**). Undiagnosed individuals on average had lower IOP both before (18.3 ± 4.2 mmHg vs 23.0 ± 8.9 mmHg, $p=0.02$ right eye; 18.4 ± 5.3 mmHg vs 21.4 ± 6.6 mmHg, $p=0.1$ left eye) and after (18.3 ± 4.2 vs 30.8 ± 12.9, $p<0.001$ right eye, and 18.4 ± 5.3 vs 28.0 ± 8.6, $p=0.001$ left eye) correction for IOP lowering medication, and they were more likely to have IOP in the normal range or less than 21 mmHg (64.5% vs 28.6% $p=0.03$, right eye; 74.2% vs 40.9%, $p=0.04$, left eye). Additionally, individuals with prior diagnosis appeared to have more severe disease compared to those undiagnosed, as evidenced by greater vCDR (0.75 ± 0.15 vs 0.67 ± 0.16, $p=0.1$, right eye; 0.79 ± 0.10 vs 0.67 ± 0.18, $p=0.04$, left eye) (**Table 2)**.

Among p.Gln368Ter carriers, 204 eyes of 124 individuals had OCT scans with an image quality score greater than 40. The average thickness of the ganglion cell complex (GCC) in our cohort was 101.1 ± 8.7 μm and 101.9 ± 9.0 μm in the right and left eye, respectively. Eyes with DDG had on average lower overall GCC thickness (96.5 ± 9.8 μm vs 102.7 ± 8.2 μm, $p<0.001$), inferior GCC thickness (96.0 ± 10.9 μm vs 101.4 ± 10.6 μm, $p=0.01$) and superior GCC thickness (92.1 ± 12.3 μm vs 99.5 ± 13.2 μm, $p=0.005$) compared to eyes judged to be healthy. **Figure 3** shows the GCC thickness distribution amongst eyes with DDG is shifted toward lower values compared to eyes without DDG. Similarly, eyes with DDG displayed lower average RNFL thickness (37.8 ± 5.4 μm vs 40.1 ± 4.9 μm, $p=0.03$) and inferior RNFL thickness (38.0 ± 6.9 μm vs 41.3 ± 6.5 μm, $p=0.01$) compared to eyes without DDG (**Table 3)**. In logistic regression models adjusting for age at recruitment and refractive error, each 10 μm decrease in average GCC thickness predicted 2.1 (95% CI 1.3 to 3.5, $p=0.003$) times higher odds of DDG, and each 10 μm decrease in inferior RNFL thickness similarly predicted a 2.0 (95% CI 1.1 to 3.7, $p=0.03$) times higher odds of DDG.

We computed a POAG PRS for each of the 747 glaucoma cases (defined using ICD 9/10 diagnosis codes) and 75,624 controls with FPs in the UKBB using genome-wide variant associations from the largest to date POAG meta-analysis of European-descendent individuals[9] excluding the UKBB cohort (see Methods). In the full cohort, glaucoma cases had significantly higher PRS (0.51 ± 1.0) compared to controls (0.0 ± 1.0, two-tailed Student's t-test, $p<0.001$).

AUC for glaucoma case detection reached 0.65 for PRS alone and 0.75 with addition of age and sex (supplementary Figure 1).

Among, p.Gln368Ter carriers, individuals with DDG had significantly higher PRS for POAG compared to those without glaucoma (0.37 ± 0.97 vs 0.01 ± 0.90, two-tailed Student's t-test, p=0.03) (**Figure 4)**. When stratified by OHTN, individuals with OHTN and evidence of DDG had slightly higher PRS compared to those without DDG (0.88 ± 1.05 vs 0.52 ± 0.97, p=0.26). Similarly, those with normal IOP who had evidence of DDG had slightly higher PRS compared to those without glaucoma (0.03 ± 0.67 vs -0.14 ± 0.84, p=0.36), though neither reached statistical significance. The prevalence of DDG increased with each decile of PRS (**Figure 5)**. Conversely, individuals with DDG were more likely to have a PRS in the top decile of POAG risk (16.7% vs 8.8% for top decile, p=0.16) (**Figure 6)** and had 2.1 (95% CI 0.7 to 5.7) times higher age and gender-adjusted odds of being in the top decile of PRS for POAG, though this difference did not reach statistical significance.

Of those with DDG, individuals with prior diagnosis of glaucoma had higher PRS compared to undiagnosed individuals (1.31 ± 0.64 vs 0.00 ± 0.81, p<0.001) (**Figure 7)**. Diagnosed individuals were more likely to have a PRS in the top decile of POAG risk (50.0% vs 3.3% for top decile, p=0.001) (**Figure 8)** and had 27.5 (95% CI 2.5 to 306.6) times higher age- and gender-adjusted odds of being in the top decile of PRS for POAG.

In random effects regression models accounting for clustering at the individual level between the right and left eyes, one-point increase in PRS predicted 1.9 μm (95% CI 0.2 to 3.7 μm, p=0.03) decrease in average GCC thickness, 1.0 μm (95% CI 0.1 to 1.9, p=0.047) decrease in average RNFL and 1.4 μm decrease in inferior RNFL thickness (95% CI 0.2 to 2.6, p=0.03). Only the association between PRS and inferior RNFL thickness remained significant after adjustment for age at recruitment and refractive error (p=0.049). The relationship between average GCC and inferior RNFL thickness and PRS was more pronounced among eyes with DDG compared to those without (**Figure 9)**.

Discussion

This is the first large-scale population-based study of imaging and clinical characteristics of glaucoma among individuals with the *MYOC* p.Gln368Ter variant. We demonstrate that nearly 1 in 4 individuals with this mutation has evidence of glaucoma in at least one eye, 70% of whom were likely previously undiagnosed. Our data show that while IOP plays an important role in development and severity of glaucoma in this population, a large portion of patients have IOP in

the normal range, including those with structural signs of glaucomatous optic neuropathy. Importantly, background polygenic risk increases disease penetrance and severity in this population.

Compared to previous estimates in population-based studies, we found a substantially higher penetrance of glaucoma among individuals with *MYOC* p.Gln368Ter, 25.4% in at least one eye and 10.7% with bilateral disease. Using the UKBB and relying on self-report, Han et al,[12] found a penetrance of 7.6% among p.Gln368Ter carriers, while Nag et al[13] found 9.6% POAG penetrance in the Rotterdam study, both substantially lower than our study. Our review of images demonstrated that the majority of glaucoma cases due to *MYOC* p.Gln368Ter in the UKBB were undiagnosed, and conversely, some individuals with healthy optic nerves reported having glaucoma. These data are in line with prior literature that demonstrates over 50% undiagnosed rates of glaucoma in developed countries.[5,6,26,27] In fact, if we rely solely on self-report, the penetrance of glaucoma in our population is only 11.3%, much closer to previously published rates. The Rotterdam study, however, had available visual field and optic disc photos for all participants. The lower penetrance of disease in that population may be partly due to variability in population structure and differences in the p.Gln368Ter minor allele frequency (MAF).[13] Our studies using disease definitions based on image and IOP data provide a more accurate measure of p.Gln368Ter penetrance and show that the penetrance is higher than in prior reports.

It must be noted, however, that even with the higher penetrance of glaucoma among p.Gln368Ter carriers reported here, our estimated penetrance of glaucoma is much lower than that reported in family-based studies. Prior studies have reported glaucoma penetrance ranging from 56-96% in various populations and increasing with age.[11,12,28,29] However, it is likely that these studies over-estimate the true penetrance of glaucoma in this population, as pedigrees are often ascertained from probands with POAG or OHTN, which potentially selects for high penetrance branches. Additionally, aggregation of common environmental and polygenic risk factors among families may lead to inflation of these estimates. Alternatively, population-based studies can underestimate true penetrance due to bias toward recruitment of healthy volunteers. Prior work has shown that the p.Gln368Ter minor allele frequency (MAF) in the entire UKBB dataset is 0.13%, which is similar to the MAF of 0.128% among our UKBB subset with fundus imaging. Additionally, the MAF in the UKBB is similar to reported values from exome sequencing databases in non-Finnish European individuals, suggesting no major ascertainment bias toward healthy individuals in our dataset.[12]

The idea that underlying accumulation of common genetic risk variants can influence risk and penetrance of disease has previously been shown with rare mutations for other diseases, such as

breast and ovarian cancer[30,31] and early-onset myocardial infarction[32] including the p.Gln368Ter variant.[18] We similarly show here that penetrance of glaucoma among p.Gln368Ter carriers varies with underlying polygenic risk and that accumulation of these common genetic variants affect severity of disease and likelihood of diagnosis. We found similar to others that prevalence of DDG increased with each decile of PRS, and individuals with DDG were more likely to be have a PRS in the higher deciles of POAG risk. We further show, for the first time, that higher PRS may increase the likelihood of clinical diagnosis, possibly due to higher disease severity. This is evidenced by higher IOP and greater CDR in those with prior diagnosis. Additionally, PRS was an independent predictor of GCC and RNFL thickness, suggesting more severe disease (thinner GCC and RNFL) in those with higher PRS. Alternatively, it is possible that higher PRS may be linked to stronger family history of glaucoma which may have resulted in earlier and more frequent monitoring of these individuals thus leading to greater likelihood of clinical diagnosis. While, the population-based design of the UKBB precludes this analysis, the link between family history and higher PRS has been previously demonstrated in an Australian population.[18] Further investigation is necessary to understand the link between family history and PRS.

Classically, the p.Gln368Ter variant has been associated with high IOP. The penetrance of OHTN in our study is similar to that reported by Han *et al.*[12] (24.3%) and slightly higher than the 12.5% and 19.4% rates in the TwinsUK and the Rotterdam Study, respectively, likely due to differences in MAF in these populations.[13] IOP clearly plays an important role in development of disease in those with this stop-gained variant and other *MYOC* mutations. Indeed, we found that individuals with DDG had on average higher IOPs than those without structural evidence of disease. Additionally, IOP appears to be an important factor in diagnosis, with individuals with known glaucoma having higher IOPs compared to those with structural disease and no prior diagnosis. Despite this, the majority of individuals with DDG, even those with prior clinical diagnosis, had IOPs in the normal range, even after adjusting for IOP lowering medications. Together, these findings suggest that this *MYOC* mutation may increase susceptibly to optic nerve disease even with IOPs in the normal range. Indeed, prior studies have also found NTG to occur in patients with the p.Gln368Ter variant, albeit at lower rates. These prior studies[16,28] may have been biased towards examination of pedigrees who had already come to clinical attention partly due to high IOP. Our results suggest that NTG may not be as uncommon as previously thought in the p.Gln368Ter carrier population. Importantly, development of glaucoma in both the setting of normal and high IOP, at least in part, appears to be influenced by an individual's underlying polygenic risk. We found a higher PRS among those with evidence of DDG at multiple IOP levels, compared to those without glaucoma, though our sample size is likely too small to reach statistical significance.

Finally, we also found that the inferior RNFL and inferior aspect of the optic nerve is most susceptible to damage in individuals with the MYOC stop mutation with structural evidence of glaucoma. While prior family studies have assessed the clinical features of individuals with p.Gln368Ter related glaucoma,[11,16,33] there is no prior work that has examined glaucoma imaging features in this population. Further investigation may help elucidate clinical phenotypes associated with this mutation.

Our study is subject to a number of limitations. The p.Gln368Ter genotypes are based on imputed and not directly genotyped calls, which may have led to some incorrect identification of carriers. However, prior evidence shows that the p.Gln368Ter variant can be imputed with high accuracy.[34] We identified glaucoma cases using only disc photographs, as functional and other structural data (optic nerve OCT) were unavailable. Though the difference was small, our DDG group had slightly higher myopic refractive error compared to those without glaucoma. It is possible that the myopic appearance of the optic nerve may have led to false positive classifications of DDG. Alternatively, as myopia is a possible risk factor for glaucoma[35] this may simply represent greater underlying disease risk. Our use of two independent masked graders with good agreement mitigated grading problems to some degree. Additionally, as some FPs were ungradable and poor quality, it is possible that we are under- or overestimating the prevalence of disease due to nonrandom missing data. Additionally, we show good association between average GCC thickness and DDG. Approximately 20% of subjects without evidence of DDG had OHTN in our study. The lack of longitudinal data does not allow for determination of exposure time or rate of progression of these eyes to glaucomatous optic neuropathy.

Our study suggests that the *MYOC* p.Gln368Ter variant has a higher penetrance for glaucoma than previously thought among the general European population, and while IOP plays an important role in disease, normal tension glaucoma is not uncommon in this group. Importantly, we demonstrate that background polygenic risk influences disease penetrance and severity in this population. Clinically, our results have important implications by demonstrating that glaucoma occurs across a range of IOPs in this at-risk population and support the utility of PRS in optimizing risk stratification among patients carrying the p.Gln368Ter variant. Clinicians should be aware that polygenic risk scores may increasingly play a role in identification of higher risk individuals, clinical decision-making and guiding earlier treatment.

Figure Legends

Figure 1. Fundus photo example
Figure 2. Distribution of cornea corrected intraocular pressure adjusted for medication use among individuals with and without disc defined glaucoma. Density plots show the distribution of numerical variables. The y-axis is the probability density function for the kernel density estimation and measures the probability per unit on the x-axis.
Figure 3. Ganglion cell complex thickness distribution amongst eyes with and without disc defined glaucoma.
Figure 4. Distribution of polygenic risk score for primary open angle glaucoma among individuals with and without disc defined glaucoma.
Figure 5. Prevalence of disc defined glaucoma by polygenic risk score decile
Figure 6. Percentage of subjects with and without disc defined glaucoma in each decile of polygenic risk score
Figure 7. Primary open angle glaucoma polygenic risk score distribution among individuals with disc defined glaucoma with and without prior diagnosis
Figure 8. Percentage of subjects with disc defined glaucoma with and without prior diagnosis in each decile of primary open angle glaucoma polygenic risk score
Figure 9. Relationship between primary open angle glaucoma polygenic risk score and A) ganglion cell complex thickness and B) inferior retinal nerve fiber layer thickness among eyes with and without disc defined glaucoma. LOWESS (locally weighted scatterplot smoothing) is a non-parametric locally weighted method for scatterplot smoothing which models the interrelationship in data.

Supplemental Figure 1. Area under the receiver operating curve for glaucoma case detection for polygenic risk score (PRS) with and without addition of age and sex. PRS was calculated using two definitions of glaucoma cases: 1) ICD9/10 diagnosis code – red lines and 2) combination of ICD 9/10 diagnosis code and glaucoma self-report - black lines.

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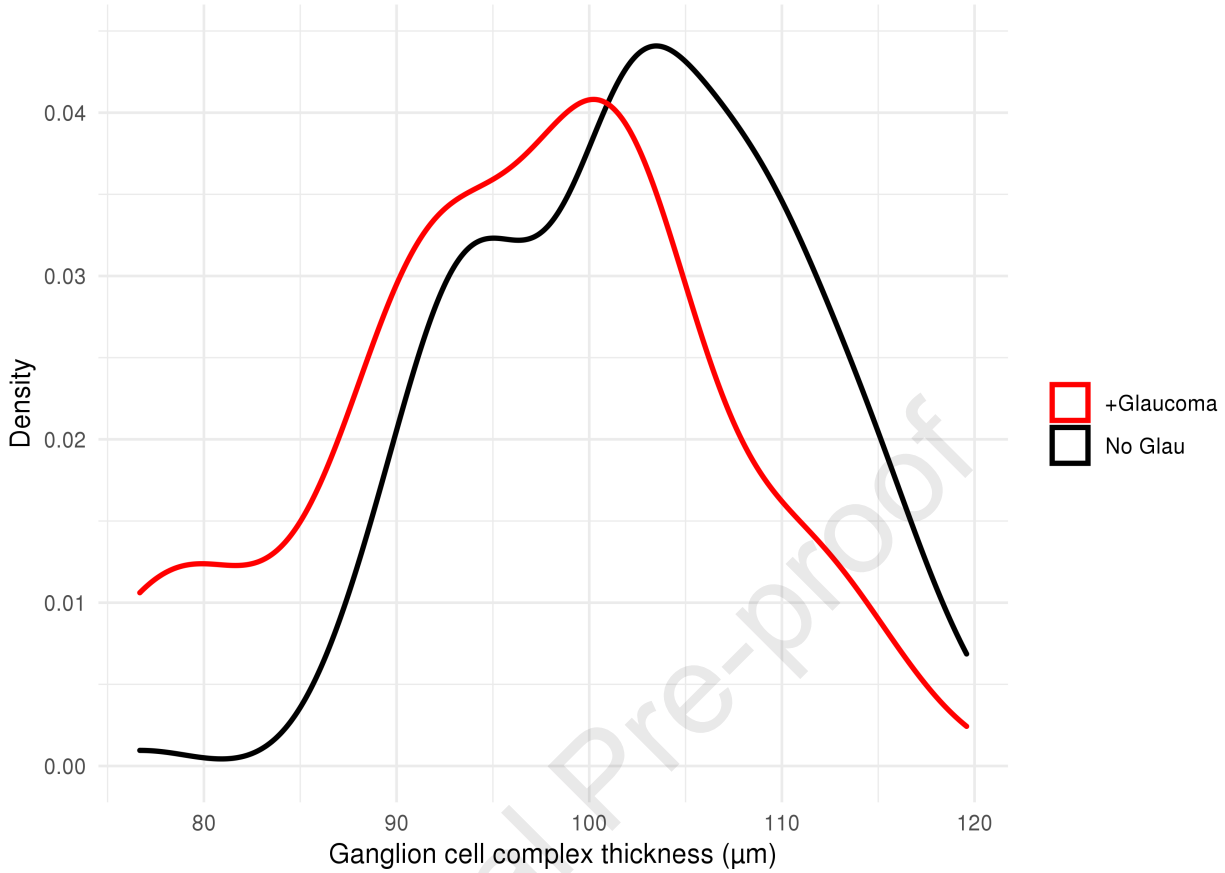

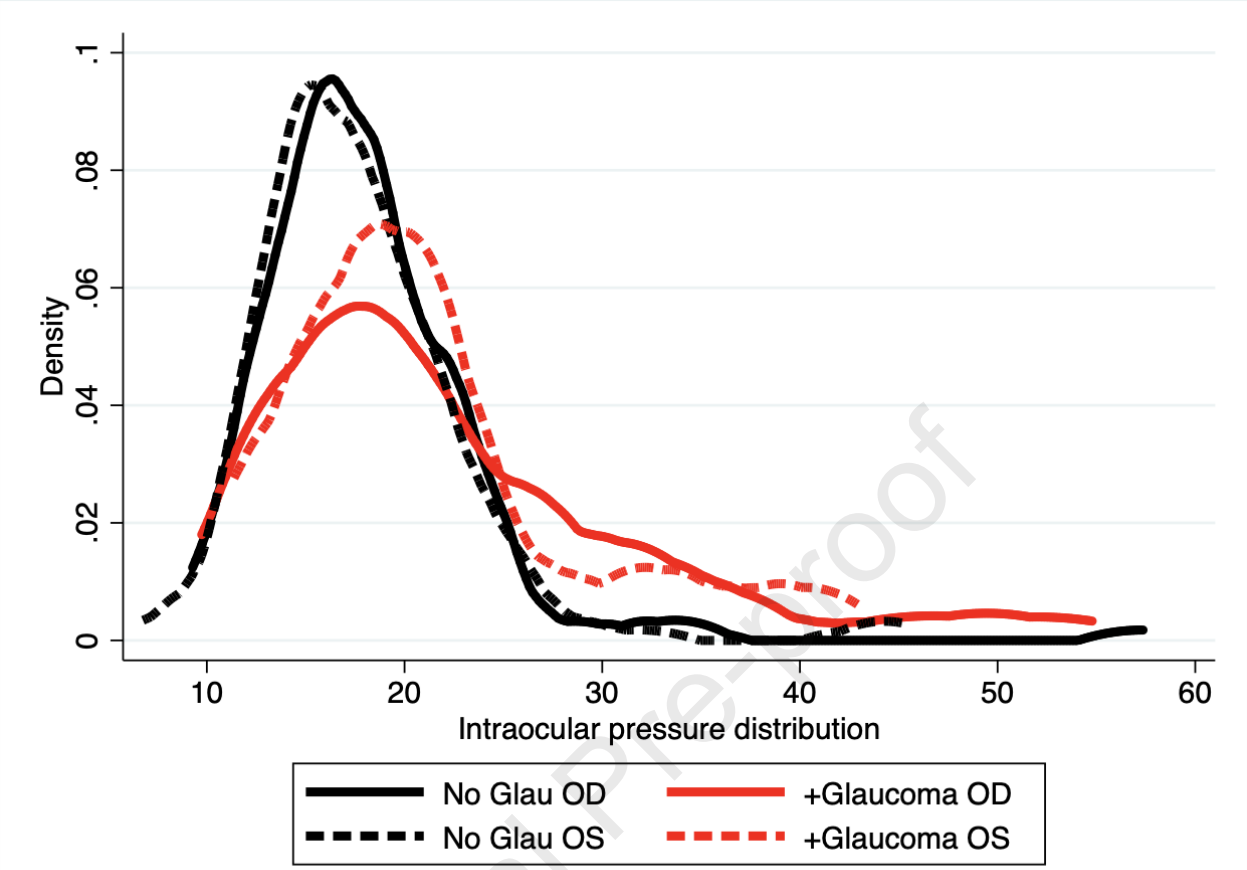

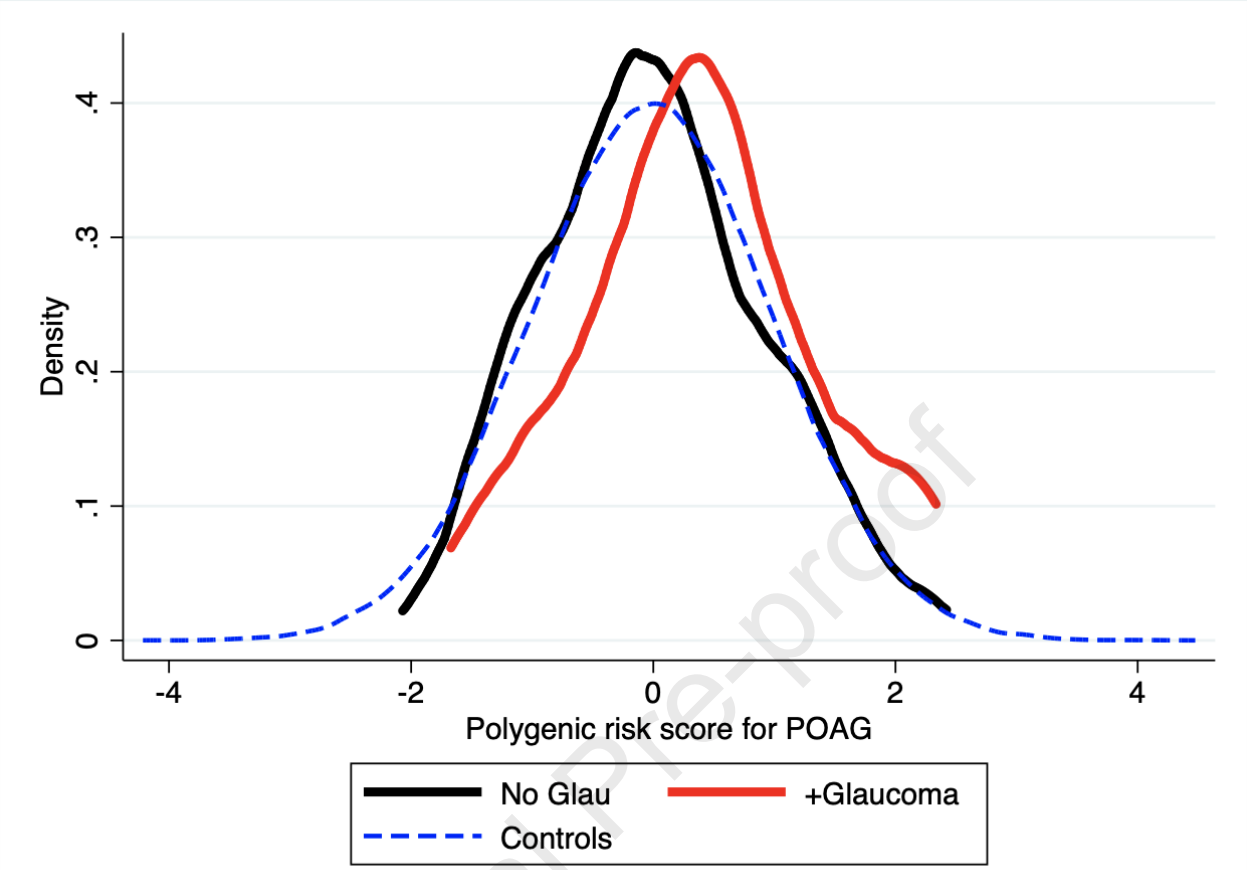

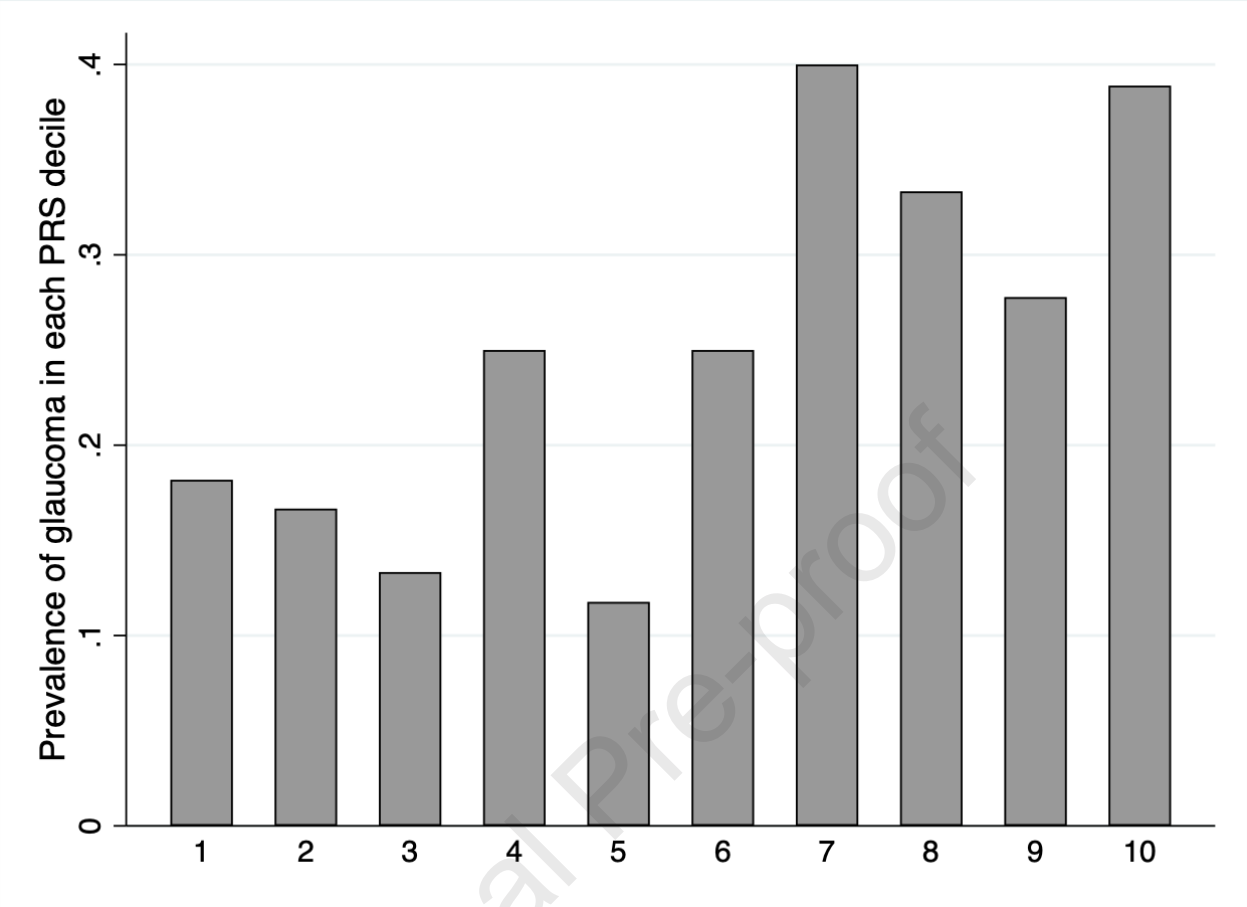

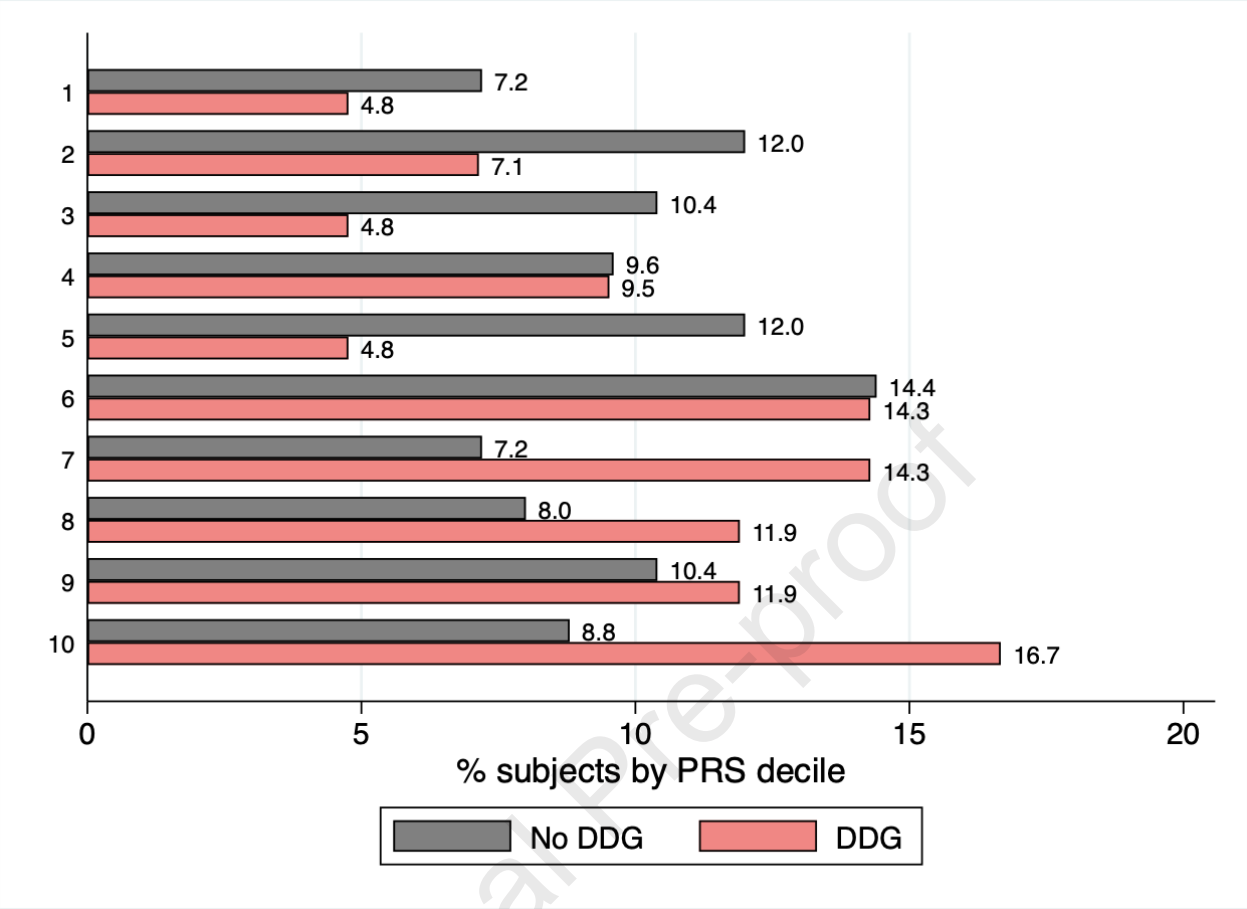

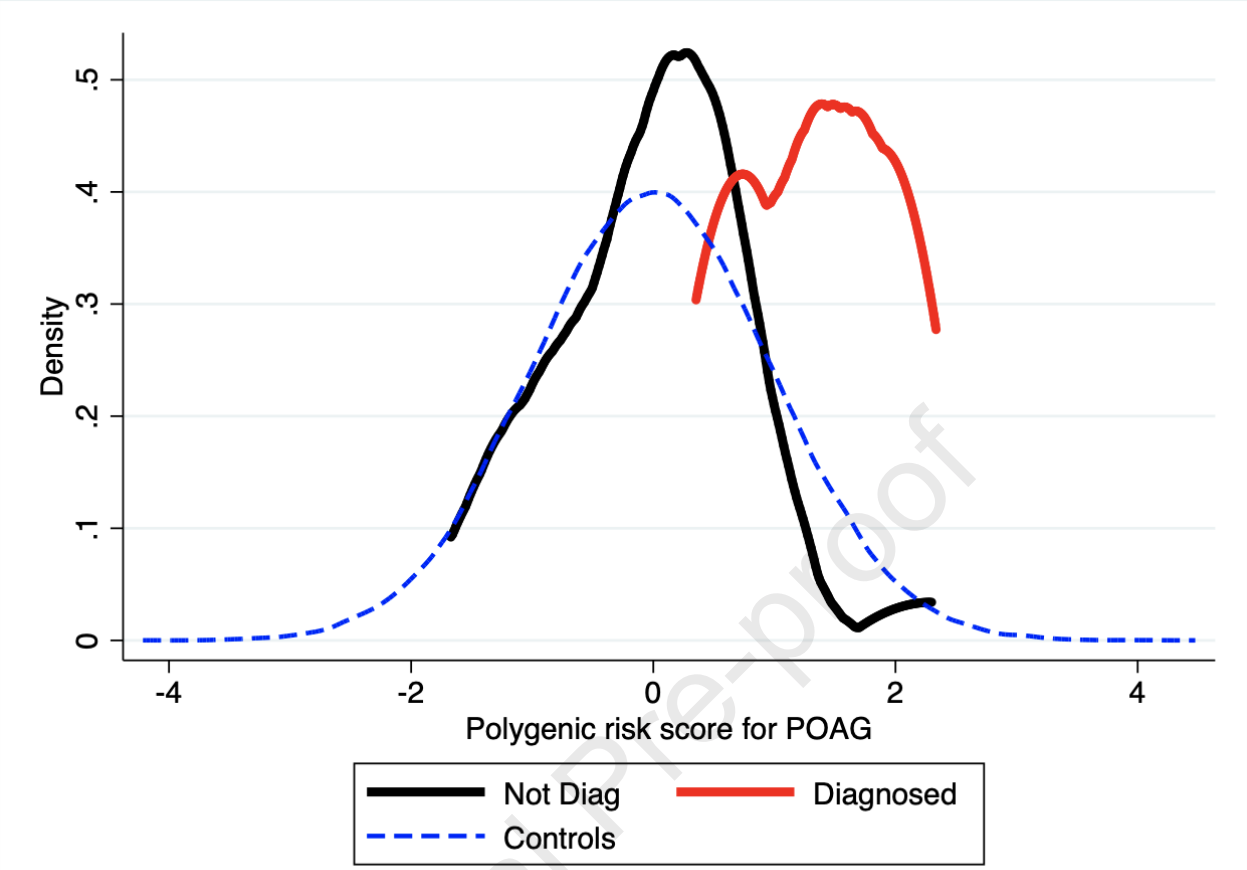

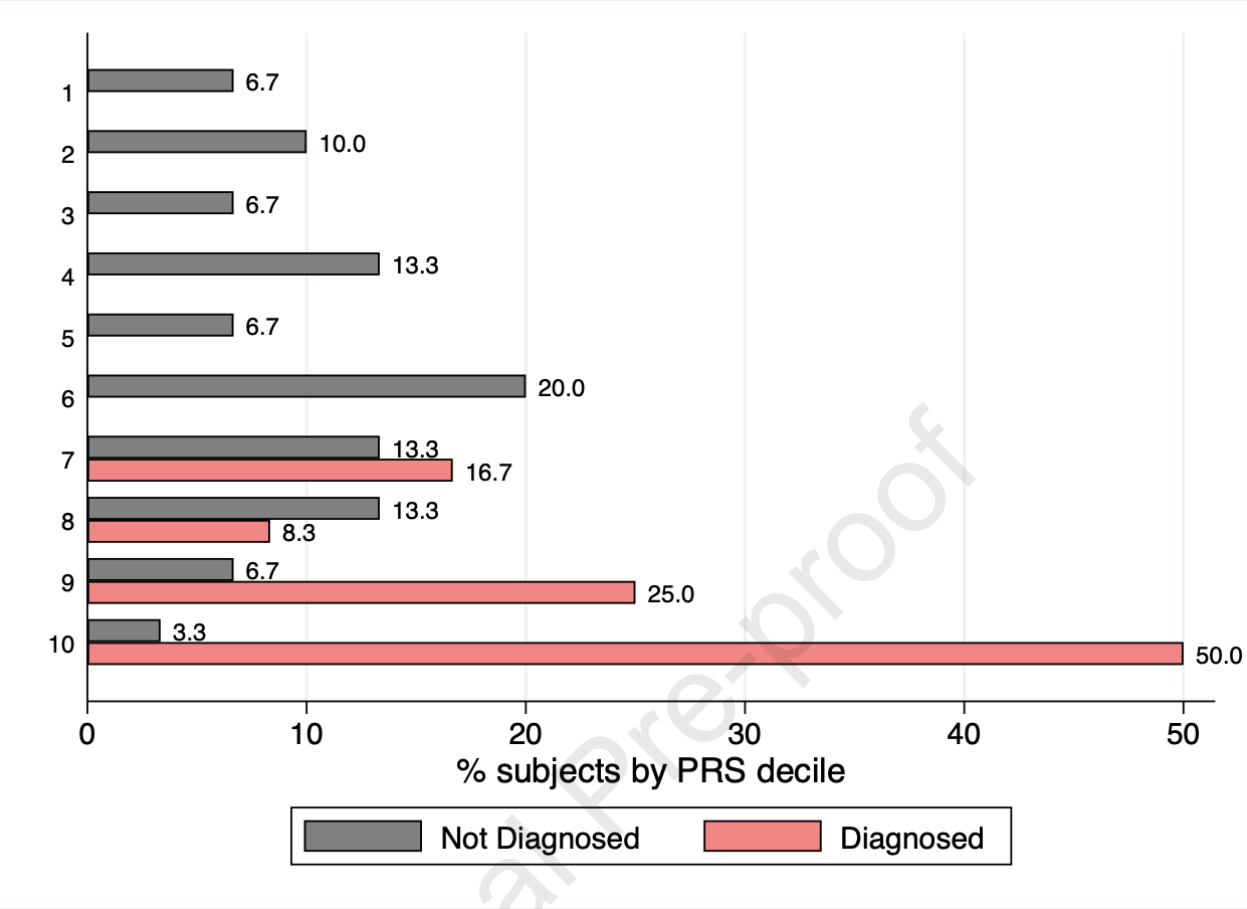

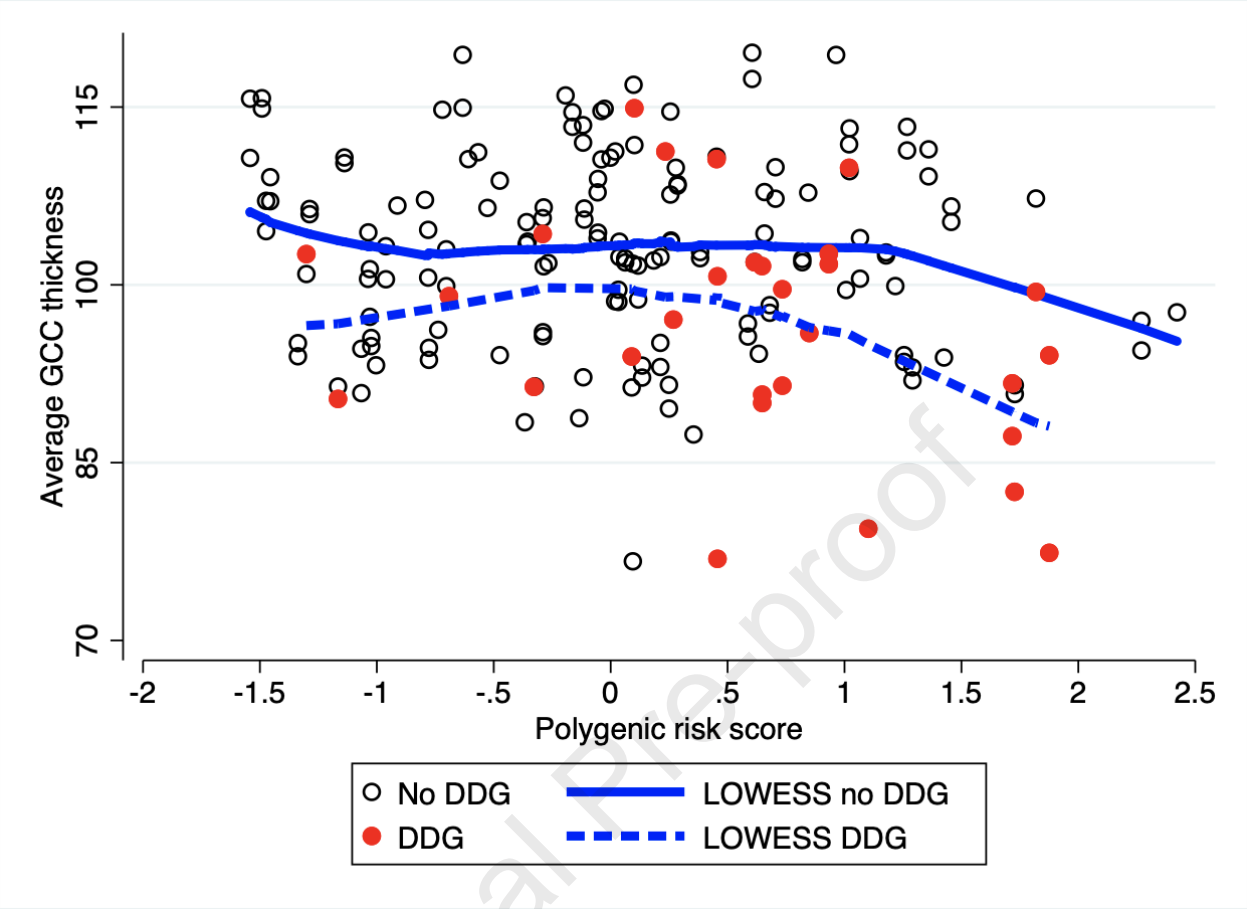

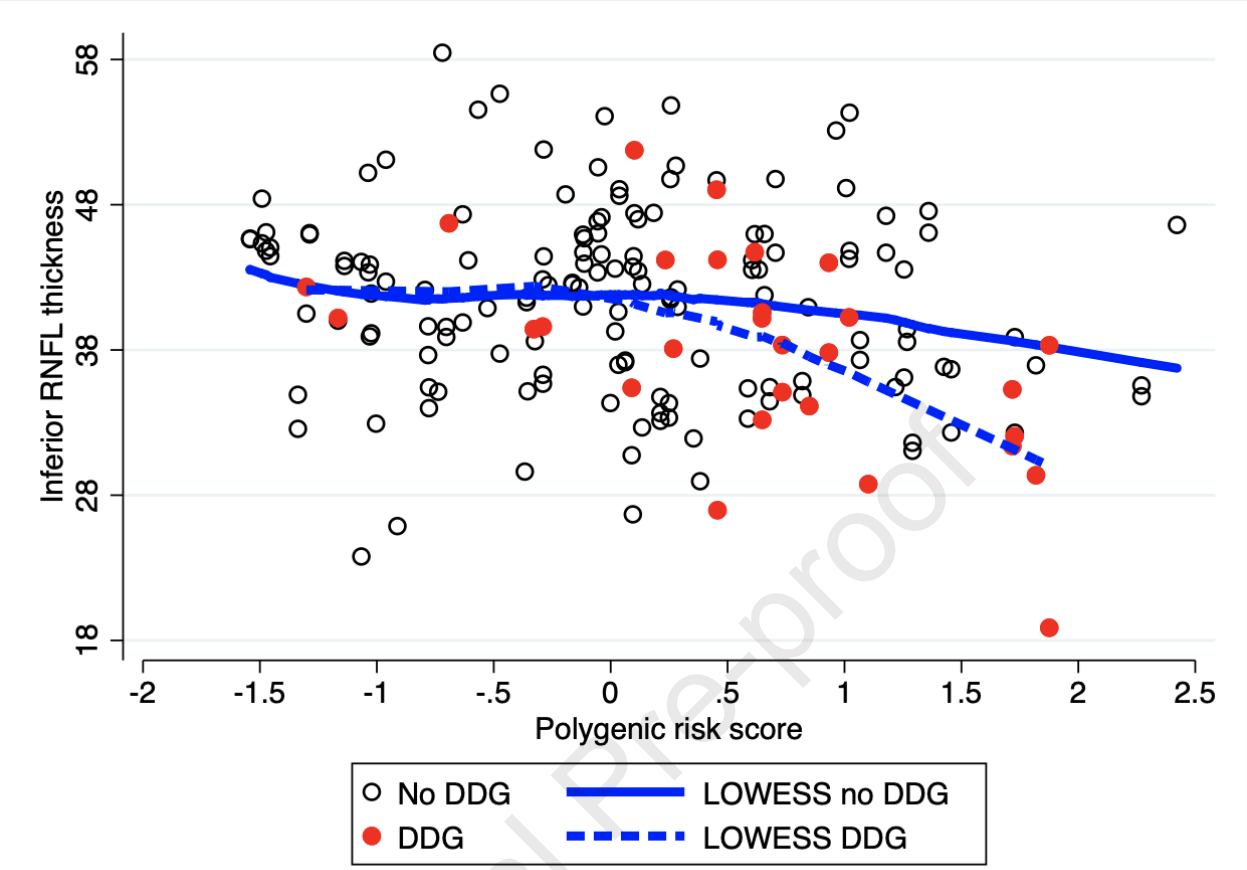

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Precis:

25% of individuals with p.Gln368Ter MYOC mutation have evidence of glaucoma on fundus imaging with 70% of cases undetected. A large portion of p.Gln368Ter carriers have IOP in the normal range. Background polygenic risk increases disease penetrance and severity.