# Journal Pre-proof

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

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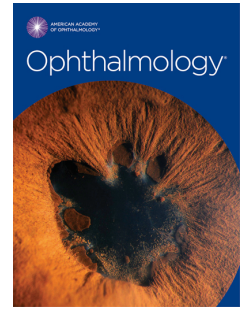
Please cite this article as: RRH: Characteristics of Gln368Ter Myocilin variant

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1   **Characteristics of Gln368Ter Myocilin variant and influence of polygenic risk on glaucoma**
2   **penetrance in the UK Biobank**
3

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20   Meeting presentation: Under consideration for presentation at the ARVO, 2021
21

24

25   Conflict of Interest: No conflicting relationship exists for any author
26

27   Running head: Characteristics of Gln368Ter Myocilin variant
28

29   Abbreviations:
30   POAG – primary open angle glaucoma; DDG – disc defined glaucoma; FP-fundus photo ; IOP-
31   intraocular pressure; GCC – ganglion cell complex ; RNFL – retinal nerve fiber layer ; PRS-
32   polygenic risk score; OCT – optical coherence tomography
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34
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36
37

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

112   mutation penetrance using imaging and IOP data from individuals with *MYOC* p.Gln368Ter

113   variant, to describe the clinical characteristics of glaucoma among these individuals, as well as to

114   understand the impact of underlying polygenic risk in this population.

115

116   **Methods**

117   *Cohort description:*

118   The UKBB is a prospective community-based cohort study of ~500,000 UK residents, aged 40-

119   69 years (http://www.ukbiobank.ac.uk/resources/) who were registered with the National Health

120   Service and includes detailed genotypic and phenotypic information on all participants. Health

121   questionnaires were collected from all participants that included age at recruitment and self-

122   reported race and gender. A subset of ~130,000 people had eye examinations including visual

123   acuity, refraction, keratometry, Goldmann and cornea-corrected IOP (IOPcc, Ocular Response

124   Analyzer; Reichert, Depew, NY). Over 84,000 people underwent retinal imaging with color

125   fundus photographs (FP) and macular optical coherence tomography (OCT) using a Topcon 3D

126   OCT 1000 Mk2 (Topcon, Inc, Japan). The National Research Ethics Service Committee

127   NorthWest–Haydock approved the study, and it was conducted in accordance with the

128   Declaration of Helsinki. All participants provided written informed consent.

129

130   *Identification of glaucoma:*

131   Individuals with glaucoma were identified if they self-reported glaucoma on eye

132   problems/disorders (UKBB data field 6148) or noncancer illness (UKBB data field 20002) or

133   had an International Classification of Diseases, Ninth or Tenth Revision (ICD9/10) diagnosis

134   code for POAG, other glaucoma or glaucoma, unspecified (H40.1, H40.8, H40.9). Where

135   available, age at first glaucoma diagnosis was obtained from UKBB data fields 4689 and 20009.

136   Information on treatment with IOP lowering medication and prior laser/surgery was obtained

137   from data fields 20003, 5326 and 5327, respectively. As some participants were already on IOP-

138   lowering medications, and data from pre-treatment was unavailable, we imputed pre-treatment

139   IOP by dividing measured IOP by 0.7, according to the mean IOP reduction achieved by

140   medications.[2]

141

142   Two masked graders (NZ and ML) further reviewed color fundus photographs (FPs) from

143   identified p.Gln368Ter carriers for *disc defined glaucoma (DDG)*. Fundus photographs were

144   graded for no glaucoma, probable glaucoma and definite glaucoma and vertical cup-to-disc ratio

145   (vCDR). Probable glaucoma was defined by presence of at least two of the following criteria:

146   vCDR ≥0.7 but <0.85, rim width ≤0.1 disc diameter, generalized or localized rim thinning,

147   visible RNFL defects or splinter hemorrhage. Definite glaucoma was defined as vCDR ≥0.85 or

148   visible RNFL defects corresponding with thinning area of rim or notches.[23] Graders were 80.6%

4

149   in agreement, κ=0.66 (p<0.001). The 80 disagreements underwent direct arbitration and were re-

150   graded. **Figure 1** shows an example of an eye with DDG versus one judged to be normal by both

151   graders.

152

153   *Genotype quality control, p.Gln368Ter carrier identification and polygenic risk score*

154   *calculation:*

155   The array genotype curation process is described in detail by Bycroft et al.[19] In addition, we

156   applied various quality control steps with PLINK 1.9 on directly genotyped variants of 82,035

157   UKBB samples with ocular imaging, using best practice approaches refined in GTEx

158   consortium.[20] The pipeline iteratively examines variants (single nucleotide polymorphisms

159   [SNPs] and indels [insertions and deletions]) and sample genotype efficiency, allele frequencies,

160   gender discrepancies and tests of Hardy-Weinberg equilibrium. Additionally, we examined

161   sample duplicates, cryptic relatedness, and contamination. Participants with unresolved

162   differences between genotype-inferred and reported sex were excluded (N=449). Samples with

163   genotyping call rate <97% were removed (229). Additionally, 3,381 individuals with high

164   cryptic relatedness (>0.1875 Pi-hat) and 298 individuals with outlying heterozygosity (4 standard

165   deviations from the mean heterozygosity rate) after accounting for inferred ancestry were also

166   removed. We applied Principal Component Analysis (PCA) to linkage disequilibrium (LD)-

167   pruned (r^2<0.1 in 200kb windows) genetic markers with minor allele frequency (MAF)>1% and

168   the k-nearest neighbors algorithm to predict the ancestral background of participants using

169   ancestral labels from the 1000 Genomes Project Phase 3 reference panel. We found good

170   correlation between self-reported and inferred ancestry; for samples with mismatched ancestry,

171   we used the inferred ancestry for quality control (QC) and downstream analyses. Only

172   participants with inferred European ancestry were used in our study, leading to a total of 77,959

173   participants post QC with both genotype and image data. For variant QC, we removed variants

174   with call rate < 97%, MAF < 0.01 and Hardy-Weinberg equilibrium test p < 1e-5.

175

176   Prior studies have demonstrated that *MYOC* c.1102C>T (p.Gln368Ter) can be imputed with high

177   accuracy from genotyping arrays.[12] Similarly, here we identified 200 p.Gln368Ter carriers using

178   the imputation posterior probability for each of the 3 genotypes (GG, AG and AA).

179

180   We constructed and tested a POAG PRS for UKBB participants using genome-wide associate

181   study (GWAS) summary statistics from the Caucasian subset of the large cross-ancestry meta-

182   analysis,[9] after exclusion of the UKBB cohort (summary statistics available at

183   https://segrelab.meei.harvard.edu/data/). PRS was computed using *LDpred2* [21] which is

184   implemented in R package *bigsnpr*.[22] The posterior mean effect sizes from GWAS summary

185   statistics were estimated using a point-normal mixture prior for the variant effects and were

186   adjusted for linkage disequilibrium. The hyperparameters in the model included SNP heritability

187   (h^2) and the fraction of casual variants (p). Hyperparameters to construct the final PRS were

188   chosen based on the best prediction performance measured by the area under the curve (AUC).

189   PRS was calculated using two definitions of glaucoma cases: 1) ICD9/10 diagnosis code (747

190   cases, 75624 controls, 1,588 missing) and 2) combination of ICD 9/10 diagnosis code and

191   glaucoma self-report as defined above (2001 cases and 75624 controls). Due to better

192   performance of the model using ICD 9/10 diagnosis codes only (supplemental Figure 1), this

193   PRS was used for all further analysis. Calculated PRS were normalized to a mean of zero and

194   standard deviation of 1.

195

196   *Optical coherence tomography (OCT):*

197   Spectral domain OCT scans of the macula were obtained using Topcon 3D OCT 1000 Mk2

198   (Topcon, Inc, Japan). Three dimensional macular volume scans were obtained (512 horizontal A-

199   scans/B-scan; 128 B-scans in a 6x6-mm raster pattern).[24] All OCT images were stored in .fda

200   image files without prior analysis of macular thickness. We used the Topcon Advanced

201   Boundary Segmentation (TABS) algorithm to automatically segment all scans, which uses dual-

202   scale gradient information to allow for automated segmentation of the inner and outer retinal

203   boundaries and retinal sublayers.[25] The boundaries segmented include the internal limiting

204   membrane (ILM), nerve fiber layer (NFL), ganglion cell layer (GCL), inner plexiform layer

205   (IPL), inner nuclear layer (INL), external limiting member (ELM), photoreceptor inner

206   segment/outer segment (IS/OS) junction layer, retinal pigment epithelium (RPE), Bruch's

207   membrane (BM) and Chorio-retinal interface (CSI). The software provides an image quality

208   score which was used as quality control measure with scores less than 40 excluded (n=70). Scans

209   with outlying values for the ganglion cell complex (defined as the distance between ILM and

210   GCL) were manually reviewed and excluded if clear segmentation errors were noted (n=2).

211

212   The thickness of each retinal sub-layer was determined by calculating the difference between

213   boundaries of interest and averaging this across all scans. For example, NFL thickness was

214   calculated as the difference between ILM and NFL boundary lines. We determined the location

215   of the fovea by calculating the minimum thickness of the 3 inner-most segments across all B

216   scans and identifying the location where this thickness value approached zero. All B scan

217   numbers obtained prior to this location were used to calculate average thickness in the superior

218   quadrants while the numbers after were used to calculate inferior quadrant thickness values.

219

220   *Statistical analyses:*

221   Statistical analyses were performed using STATA software version 15.0 and RStudio v. 4.0.3.

222   Means and standard deviations were calculated for demographic and ocular characteristics and

223 compared across groups using two tailed Student t-test, and chi-square or Fisher's exact tests for
224 continuous and categorical variables, respectively. Logistic regression models adjusted for age at
225 recruitment and sex were used to estimate odds of falling in the top decile of PRS risk for
226 POAG. Values were considered statistically significant if the P value was less than 0.05.
227
228 **Results**
229 Among the 73,563 UKBB participants with complete imaging and genotype data, we identified
230 200 (0.027%) heterozygous carriers of the p.Gln368Ter allele and no homozygous carriers. 177
231 of the heterozygous carriers had gradable FPs in one or both eyes (160 right eyes and 158 left
232 eyes). Among those with gradable FPs, 132 (74.6%) had no evidence of glaucoma, while 45
233 (25.4%) had probable or definite glaucoma (DDG) in at least one eye, and 19 (10.7%) had
234 evidence of bilateral disease. Of the 45 individuals with DDG, 14 (31%) self-reported or had an
235 ICD 9/10 code for glaucoma, whereas 31 (69%) were undiagnosed. Conversely, 6 of 132
236 individuals who were judged to have healthy appearing optic nerves in both eyes self-reported or
237 had ICD 9/10 for glaucoma. Of the 200 individuals with available IOP data, after correction for
238 IOP lowering medications, 27% and 21% had IOP greater than 21 in their right and left eye,
239 respectively, while 25.4% had an average IOP over 21 in both eyes (defined as ocular
240 hypertension, OHTN). There was no difference in age between p.Gln368Ter carriers with and
241 without OHTN (58.0 ± 1.2 vs 57.2 ± 0.6 respectively, p=0.56).
242
243 **Table 1** outlines the demographic and ocular characteristics among individuals with and without
244 DDG. There were no differences in age at baseline, age at diagnosis, race/ethnicity or gender
245 among individuals with and without DDG (p>0.05 for all). Subjects with DDG had slightly
246 higher myopic refractive error on average compared to those without evidence of glaucoma (-1.3
247 ± 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).
248
249 Subjects with DDG also had on average higher IOPcc in both right and left eyes (19.8 ± 6.4
250 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,
251 left eye). A higher proportion of subjects with DDG (24.4%) were on one or more IOP lowering
252 medications, compared to those without evidence of glaucomatous optic neuropathy (4.6%) on
253 imaging (p<0.001). After correction for use of IOP lowering medication, the difference in IOP
254 between those with and without DDG was larger (22.3 ± 9.8 mmHg vs 18.0 ± 5.6 mmHg,
255 p<0.001, right eye; 21.3 ± 7.7 mmHg vs 17.7 ± 5.3 mmHg, p<0.001, left eye). After adjustment
256 for IOP lowering medications, over half of p.Gln368Ter carriers had IOP in the normal range
257 (<=21 mmHg), though this proportion was significantly lower in those with DDG compared to
258 those without glaucoma (53.3% vs 79.6%, p=0.001, right eye; 64.4% vs 81.8%, p=0.02, left eye).
259 **Figure 2** demonstrates the distribution of medication adjusted IOP in both groups. Individuals

260 with DDG on average had larger vCDR in both eyes and were more likely to undergo glaucoma

261 surgery or laser based on self-report, though this latter measure was overall rare in this cohort

262 (**Table 1**).

263

264 Individuals with DDG but who were not diagnosed at the time of the study did not differ in age,

265 gender, ethnicity or average refractive error from those with prior glaucoma diagnosis (p>0.2 for

266 all) (**Table 2**). Undiagnosed individuals on average had lower IOP both before (18.3 ± 4.2

267 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

268 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,

269 p=0.001 left eye) correction for IOP lowering medication, and they were more likely to have IOP

270 in the normal range or less than 21 mmHg (64.5% vs 28.6% p=0.03, right eye; 74.2% vs 40.9%,

271 p=0.04, left eye). Additionally, individuals with prior diagnosis appeared to have more severe

272 disease compared to those undiagnosed, as evidenced by greater vCDR (0.75 ± 0.15 vs 0.67 ±

273 0.16, p=0.1, right eye; 0.79 ± 0.10 vs 0.67 ± 0.18, p=0.04, left eye) (**Table 2**).

274

275 Among p.Gln368Ter carriers, 204 eyes of 124 individuals had OCT scans with an image quality

276 score greater than 40. The average thickness of the ganglion cell complex (GCC) in our cohort

277 was 101.1 ± 8.7 μm and 101.9 ± 9.0 μm in the right and left eye, respectively. Eyes with DDG

278 had on average lower overall GCC thickness (96.5 ± 9.8 μm vs 102.7 ± 8.2 μm, p<0.001),

279 inferior GCC thickness (96.0 ± 10.9 μm vs 101.4 ± 10.6 μm, p=0.01) and superior GCC

280 thickness (92.1 ± 12.3 μm vs 99.5 ± 13.2 μm, p=0.005) compared to eyes judged to be healthy.

281 **Figure 3** shows the GCC thickness distribution amongst eyes with DDG is shifted toward lower

282 values compared to eyes without DDG. Similarly, eyes with DDG displayed lower average

283 RNFL thickness (37.8± 5.4 μm vs 40.1± 4.9 μm, p=0.03) and inferior RNFL thickness (38.0 ±

284 6.9 μm vs 41.3 ± 6.5 μm, p=0.01) compared to eyes without DDG (**Table 3**). In logistic

285 regression models adjusting for age at recruitment and refractive error, each 10 μm decrease in

286 average GCC thickness predicted 2.1 (95% CI 1.3 to 3.5, p=0.003) times higher odds of DDG,

287 and each 10 μm decrease in inferior RNFL thickness similarly predicted a 2.0 (95% CI 1.1 to 3.7,

288 p=0.03) times higher odds of DDG.

289

290 We computed a POAG PRS for each of the 747 glaucoma cases (defined using ICD 9/10

291 diagnosis codes) and 75,624 controls with FPs in the UKBB using genome-wide variant

292 associations from the largest to date POAG meta-analysis of European-descendent individuals[9]

293 excluding the UKBB cohort (see Methods). In the full cohort, glaucoma cases had significantly

294 higher PRS (0.51 ± 1.0) compared to controls (0.0 ± 1.0, two-tailed Student's t-test, p<0.001).

295 AUC for glaucoma case detection reached 0.65 for PRS alone and 0.75 with addition of age and

296 sex (supplementary Figure 1).

297

298 Among, p.Gln368Ter carriers, individuals with DDG had significantly higher PRS for POAG

299 compared to those without glaucoma (0.37 ± 0.97 vs 0.01 ± 0.90, two-tailed Student's t-test,

300 p=0.03) (**Figure 4**). When stratified by OHTN, individuals with OHTN and evidence of DDG

301 had slightly higher PRS compared to those without DDG (0.88 ± 1.05 vs 0.52 ± 0.97, p=0.26).

302 Similarly, those with normal IOP who had evidence of DDG had slightly higher PRS compared

303 to those without glaucoma (0.03 ± 0.67 vs -0.14 ± 0.84, p=0.36), though neither reached

304 statistical significance. The prevalence of DDG increased with each decile of PRS (**Figure 5**).

305 Conversely, individuals with DDG were more likely to have a PRS in the top decile of POAG

306 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

307 higher age and gender-adjusted odds of being in the top decile of PRS for POAG, though this

308 difference did not reach statistical significance.

309

310 Of those with DDG, individuals with prior diagnosis of glaucoma had higher PRS compared to

311 undiagnosed individuals (1.31 ± 0.64 vs 0.00 ± 0.81, p<0.001) (**Figure 7**). Diagnosed individuals

312 were more likely to have a PRS in the top decile of POAG risk (50.0% vs 3.3% for top decile,

313 p=0.001) (**Figure 8**) and had 27.5 (95% CI 2.5 to 306.6) times higher age- and gender-adjusted

314 odds of being in the top decile of PRS for POAG.

315

316 In random effects regression models accounting for clustering at the individual level between the

317 right and left eyes, one-point increase in PRS predicted 1.9 μm (95% CI 0.2 to 3.7 μm, p=0.03)

318 decrease in average GCC thickness, 1.0 μm (95% CI 0.1 to 1.9, p=0.047) decrease in average

319 RNFL and 1.4 μm decrease in inferior RNFL thickness (95% CI 0.2 to 2.6, p=0.03). Only the

320 association between PRS and inferior RNFL thickness remained significant after adjustment for

321 age at recruitment and refractive error (p=0.049). The relationship between average GCC and

322 inferior RNFL thickness and PRS was more pronounced among eyes with DDG compared to

323 those without (**Figure 9**).

324

325 **Discussion**

326

327 This is the first large-scale population-based study of imaging and clinical characteristics of

328 glaucoma among individuals with the *MYOC* p.Gln368Ter variant. We demonstrate that nearly 1

329 in 4 individuals with this mutation has evidence of glaucoma in at least one eye, 70% of whom

330 were likely previously undiagnosed. Our data show that while IOP plays an important role in

331 development and severity of glaucoma in this population, a large portion of patients have IOP in

9

332 the normal range, including those with structural signs of glaucomatous optic neuropathy.

333 Importantly, background polygenic risk increases disease penetrance and severity in this

334 population.

335

336 Compared to previous estimates in population-based studies, we found a substantially higher

337 penetrance of glaucoma among individuals with *MYOC* p.Gln368Ter, 25.4% in at least one eye

338 and 10.7% with bilateral disease. Using the UKBB and relying on self-report, Han et al,[12] found

339 a penetrance of 7.6% among p.Gln368Ter carriers, while Nag et al[13] found 9.6% POAG

340 penetrance in the Rotterdam study, both substantially lower than our study. Our review of images

341 demonstrated that the majority of glaucoma cases due to *MYOC* p.Gln368Ter in the UKBB were

342 undiagnosed, and conversely, some individuals with healthy optic nerves reported having

343 glaucoma. These data are in line with prior literature that demonstrates over 50% undiagnosed

344 rates of glaucoma in developed countries.[5,6,26,27] In fact, if we rely solely on self-report, the

345 penetrance of glaucoma in our population is only 11.3%, much closer to previously published

346 rates. The Rotterdam study, however, had available visual field and optic disc photos for all

347 participants. The lower penetrance of disease in that population may be partly due to variability

348 in population structure and differences in the p.Gln368Ter minor allele frequency (MAF).[13] Our

349 studies using disease definitions based on image and IOP data provide a more accurate measure

350 of p.Gln368Ter penetrance and show that the penetrance is higher than in prior reports.

351

352 It must be noted, however, that even with the higher penetrance of glaucoma among

353 p.Gln368Ter carriers reported here, our estimated penetrance of glaucoma is much lower than

354 that reported in family-based studies. Prior studies have reported glaucoma penetrance ranging

355 from 56-96% in various populations and increasing with age.[11,12,28,29] However, it is likely that

356 these studies over-estimate the true penetrance of glaucoma in this population, as pedigrees are

357 often ascertained from probands with POAG or OHTN, which potentially selects for high

358 penetrance branches. Additionally, aggregation of common environmental and polygenic risk

359 factors among families may lead to inflation of these estimates. Alternatively, population-based

360 studies can underestimate true penetrance due to bias toward recruitment of healthy volunteers.

361 Prior work has shown that the p.Gln368Ter minor allele frequency (MAF) in the entire UKBB

362 dataset is 0.13%, which is similar to the MAF of 0.128% among our UKBB subset with fundus

363 imaging. Additionally, the MAF in the UKBB is similar to reported values from exome

364 sequencing databases in non-Finnish European individuals, suggesting no major ascertainment

365 bias toward healthy individuals in our dataset.[12]

366

367 The idea that underlying accumulation of common genetic risk variants can influence risk and

368 penetrance of disease has previously been shown with rare mutations for other diseases, such as

369   breast and ovarian cancer[30,31] and early-onset myocardial infarction[32] including the p.Gln368Ter

370   variant.[18] We similarly show here that penetrance of glaucoma among p.Gln368Ter carriers

371   varies with underlying polygenic risk and that accumulation of these common genetic variants

372   affect severity of disease and likelihood of diagnosis. We found similar to others that prevalence

373   of DDG increased with each decile of PRS, and individuals with DDG were more likely to be

374   have a PRS in the higher deciles of POAG risk. We further show, for the first time, that higher

375   PRS may increase the likelihood of clinical diagnosis, possibly due to higher disease severity.

376   This is evidenced by higher IOP and greater CDR in those with prior diagnosis. Additionally,

377   PRS was an independent predictor of GCC and RNFL thickness, suggesting more severe disease

378   (thinner GCC and RNFL) in those with higher PRS. Alternatively, it is possible that higher PRS

379   may be linked to stronger family history of glaucoma which may have resulted in earlier and

380   more frequent monitoring of these individuals thus leading to greater likelihood of clinical

381   diagnosis. While, the population-based design of the UKBB precludes this analysis, the link

382   between family history and higher PRS has been previously demonstrated in an Australian

383   population.[18] Further investigation is necessary to understand the link between family history and

384   PRS.

385

386   Classically, the p.Gln368Ter variant has been associated with high IOP. The penetrance of

387   OHTN in our study is similar to that reported by Han *et al.*[12] (24.3%) and slightly higher than the

388   12.5% and 19.4% rates in the TwinsUK and the Rotterdam Study, respectively, likely due to

389   differences in MAF in these populations.[13] IOP clearly plays an important role in development of

390   disease in those with this stop-gained variant and other *MYOC* mutations. Indeed, we found that

391   individuals with DDG had on average higher IOPs than those without structural evidence of

392   disease. Additionally, IOP appears to be an important factor in diagnosis, with individuals with

393   known glaucoma having higher IOPs compared to those with structural disease and no prior

394   diagnosis. Despite this, the majority of individuals with DDG, even those with prior clinical

395   diagnosis, had IOPs in the normal range, even after adjusting for IOP lowering medications.

396   Together, these findings suggest that this *MYOC* mutation may increase susceptibly to optic

397   nerve disease even with IOPs in the normal range. Indeed, prior studies have also found NTG to

398   occur in patients with the p.Gln368Ter variant, albeit at lower rates. These prior studies[16,28] may

399   have been biased towards examination of pedigrees who had already come to clinical attention

400   partly due to high IOP. Our results suggest that NTG may not be as uncommon as previously

401   thought in the p.Gln368Ter carrier population. Importantly, development of glaucoma in both the

402   setting of normal and high IOP, at least in part, appears to be influenced by an individual's

403   underlying polygenic risk. We found a higher PRS among those with evidence of DDG at

404   multiple IOP levels, compared to those without glaucoma, though our sample size is likely too

405   small to reach statistical significance.

406

407 Finally, we also found that the inferior RNFL and inferior aspect of the optic nerve is most

408 susceptible to damage in individuals with the MYOC stop mutation with structural evidence of

409 glaucoma. While prior family studies have assessed the clinical features of individuals with

410 p.Gln368Ter related glaucoma,[11,16,33] there is no prior work that has examined glaucoma imaging

411 features in this population. Further investigation may help elucidate clinical phenotypes

412 associated with this mutation.

413

414 Our study is subject to a number of limitations. The p.Gln368Ter genotypes are based on

415 imputed and not directly genotyped calls, which may have led to some incorrect identification of

416 carriers. However, prior evidence shows that the p.Gln368Ter variant can be imputed with high

417 accuracy.[34] We identified glaucoma cases using only disc photographs, as functional and other

418 structural data (optic nerve OCT) were unavailable. Though the difference was small, our DDG

419 group had slightly higher myopic refractive error compared to those without glaucoma. It is

420 possible that the myopic appearance of the optic nerve may have led to false positive

421 classifications of DDG. Alternatively, as myopia is a possible risk factor for glaucoma[35] this may

422 simply represent greater underlying disease risk. Our use of two independent masked graders

423 with good agreement mitigated grading problems to some degree. Additionally, as some FPs

424 were ungradable and poor quality, it is possible that we are under- or overestimating the

425 prevalence of disease due to nonrandom missing data. Additionally, we show good association

426 between average GCC thickness and DDG. Approximately 20% of subjects without evidence of

427 DDG had OHTN in our study. The lack of longitudinal data does not allow for determination of

428 exposure time or rate of progression of these eyes to glaucomatous optic neuropathy.

429

430 Our study suggests that the *MYOC* p.Gln368Ter variant has a higher penetrance for glaucoma

431 than previously thought among the general European population, and while IOP plays an

432 important role in disease, normal tension glaucoma is not uncommon in this group. Importantly,

433 we demonstrate that background polygenic risk influences disease penetrance and severity in this

434 population. Clinically, our results have important implications by demonstrating that glaucoma

435 occurs across a range of IOPs in this at-risk population and support the utility of PRS in

436 optimizing risk stratification among patients carrying the p.Gln368Ter variant. Clinicians should

437 be aware that polygenic risk scores may increasingly play a role in identification of higher risk

438 individuals, clinical decision-making and guiding earlier treatment.

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443 **Figure Legends**

444 **Figure 1.** Fundus photo example
445 **Figure 2.** Distribution of cornea corrected intraocular pressure adjusted for medication use
446 among individuals with and without disc defined glaucoma. Density plots show the distribution
447 of numerical variables. The y-axis is the probability density function for the kernel density
448 estimation and measures the probability per unit on the x-axis.
449 **Figure 3.** Ganglion cell complex thickness distribution amongst eyes with and without disc
450 defined glaucoma.
451 **Figure 4.** Distribution of polygenic risk score for primary open angle glaucoma among
452 individuals with and without disc defined glaucoma.
453 **Figure 5.** Prevalence of disc defined glaucoma by polygenic risk score decile
454 **Figure 6.** Percentage of subjects with and without disc defined glaucoma in each decile of
455 polygenic risk score
456 **Figure 7.** Primary open angle glaucoma polygenic risk score distribution among individuals with
457 disc defined glaucoma with and without prior diagnosis
458 **Figure 8.** Percentage of subjects with disc defined glaucoma with and without prior diagnosis in
459 each decile of primary open angle glaucoma polygenic risk score
460 **Figure 9.** Relationship between primary open angle glaucoma polygenic risk score and A)
461 ganglion cell complex thickness and B) inferior retinal nerve fiber layer thickness among eyes
462 with and without disc defined glaucoma. LOWESS (locally weighted scatterplot smoothing) is a
463 non-parametric locally weighted method for scatterplot smoothing which models the
464 interrelationship in data.
465
466 **Supplemental Figure 1**. Area under the receiver operating curve for glaucoma case detection for
467 polygenic risk score (PRS) with and without addition of age and sex. PRS was calculated using
468 two definitions of glaucoma cases: 1) ICD9/10 diagnosis code – red lines and 2) combination of
469 ICD 9/10 diagnosis code and glaucoma self-report - black lines.
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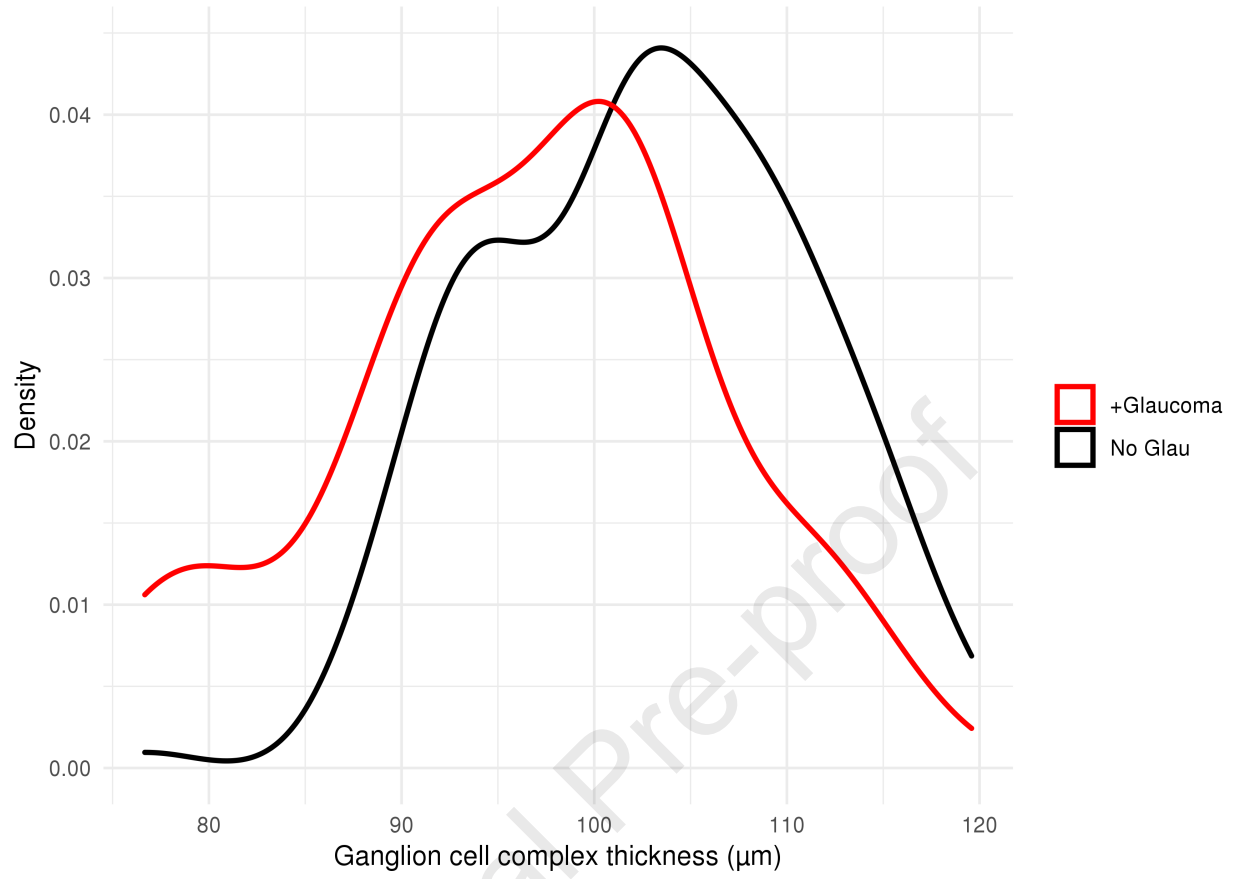481
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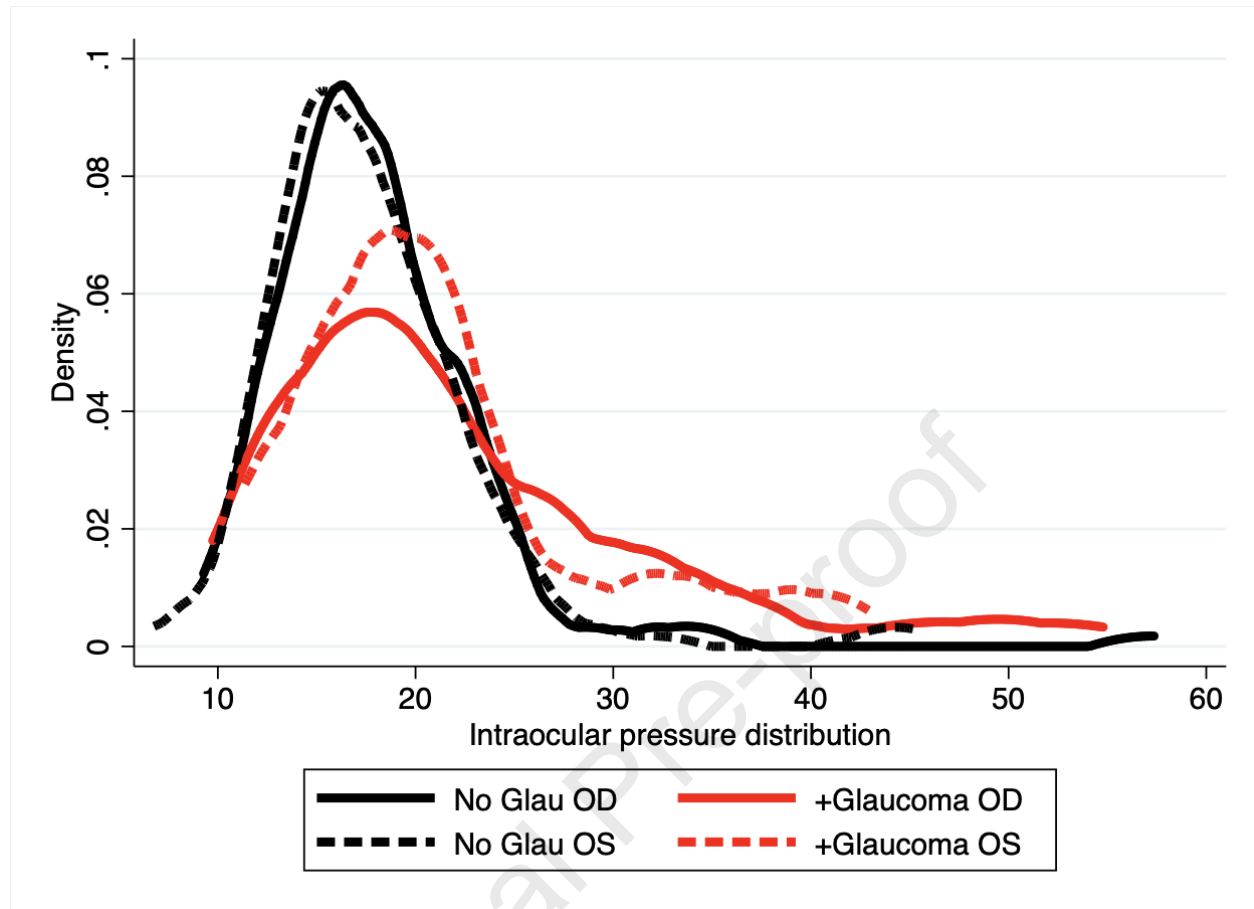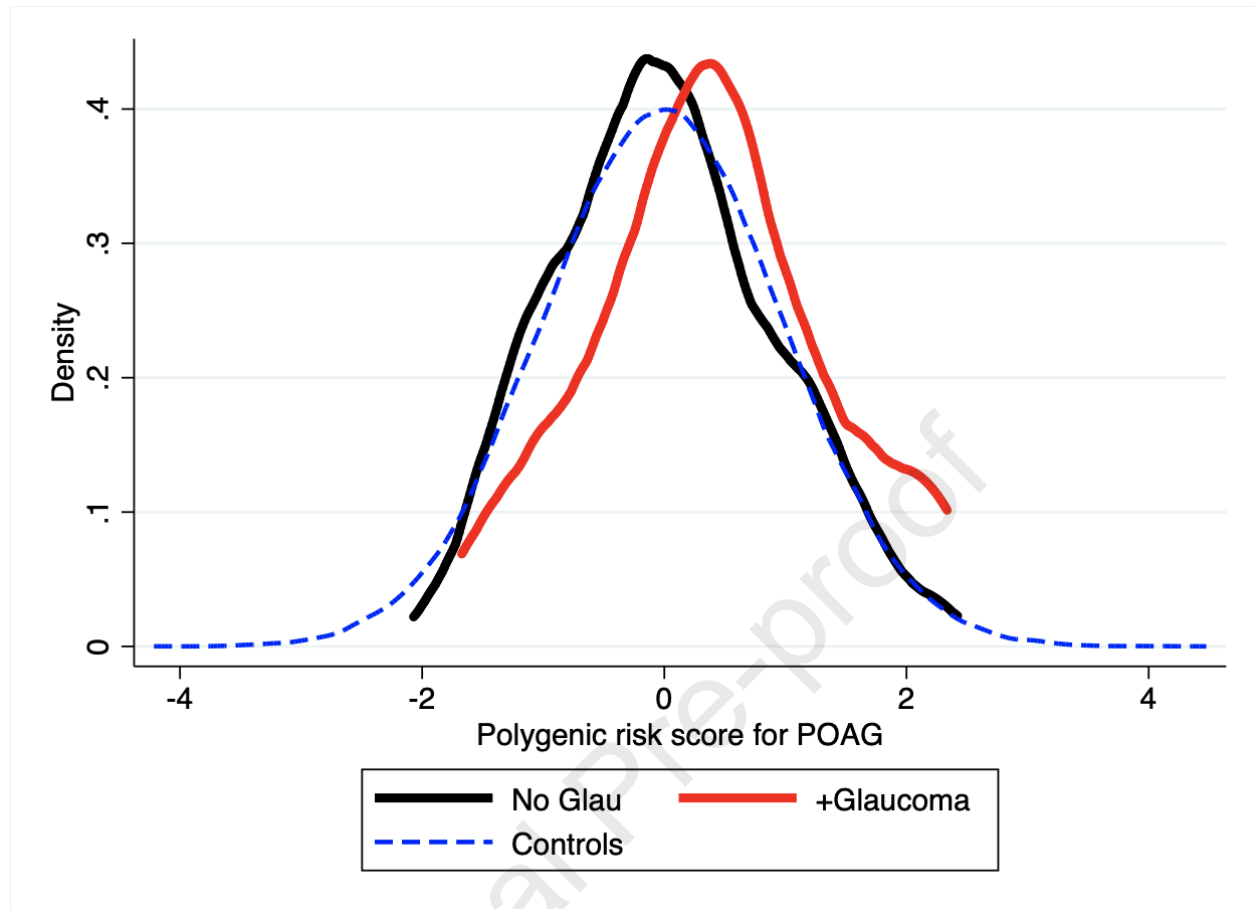
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Disc defined glaucoma
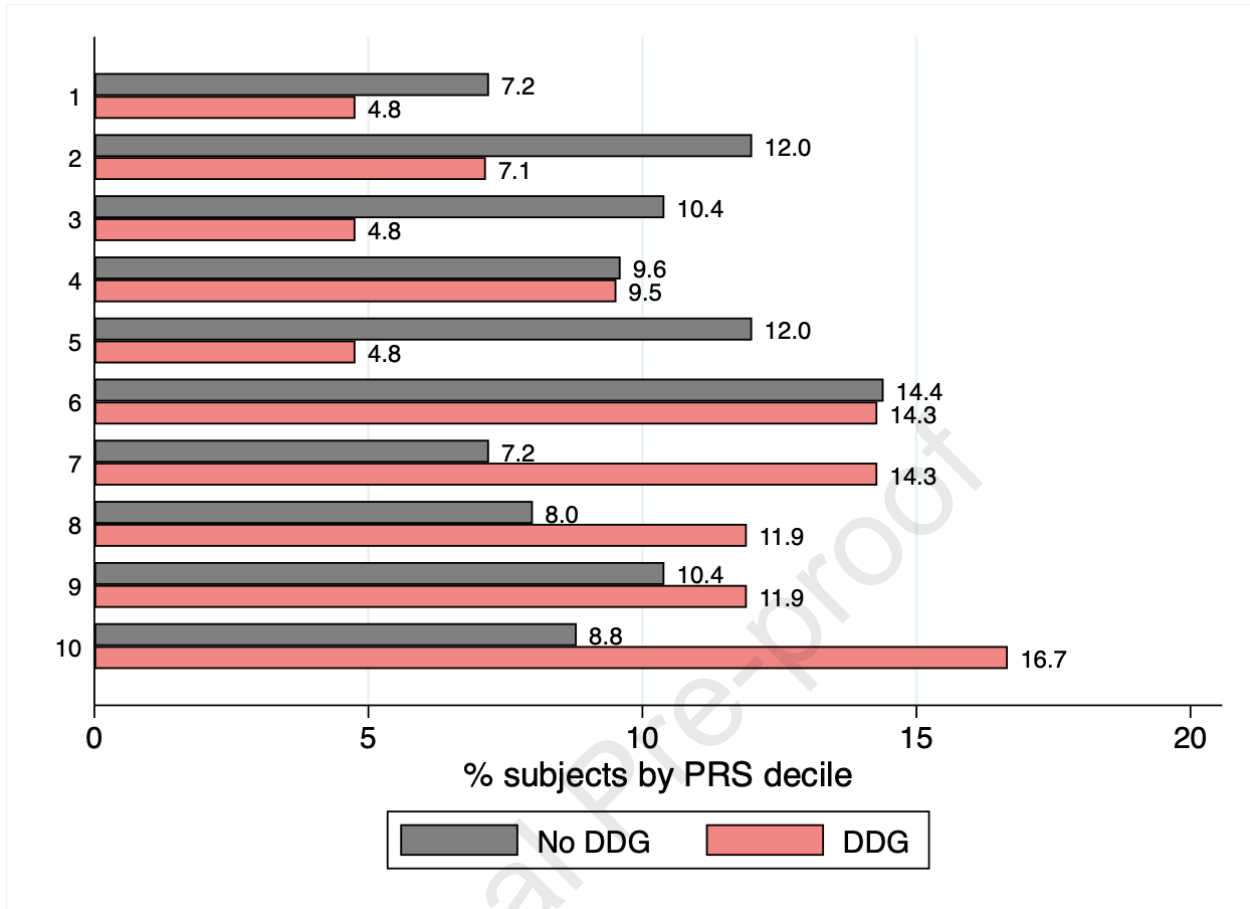
No glaucoma

% subjects by PRS decile

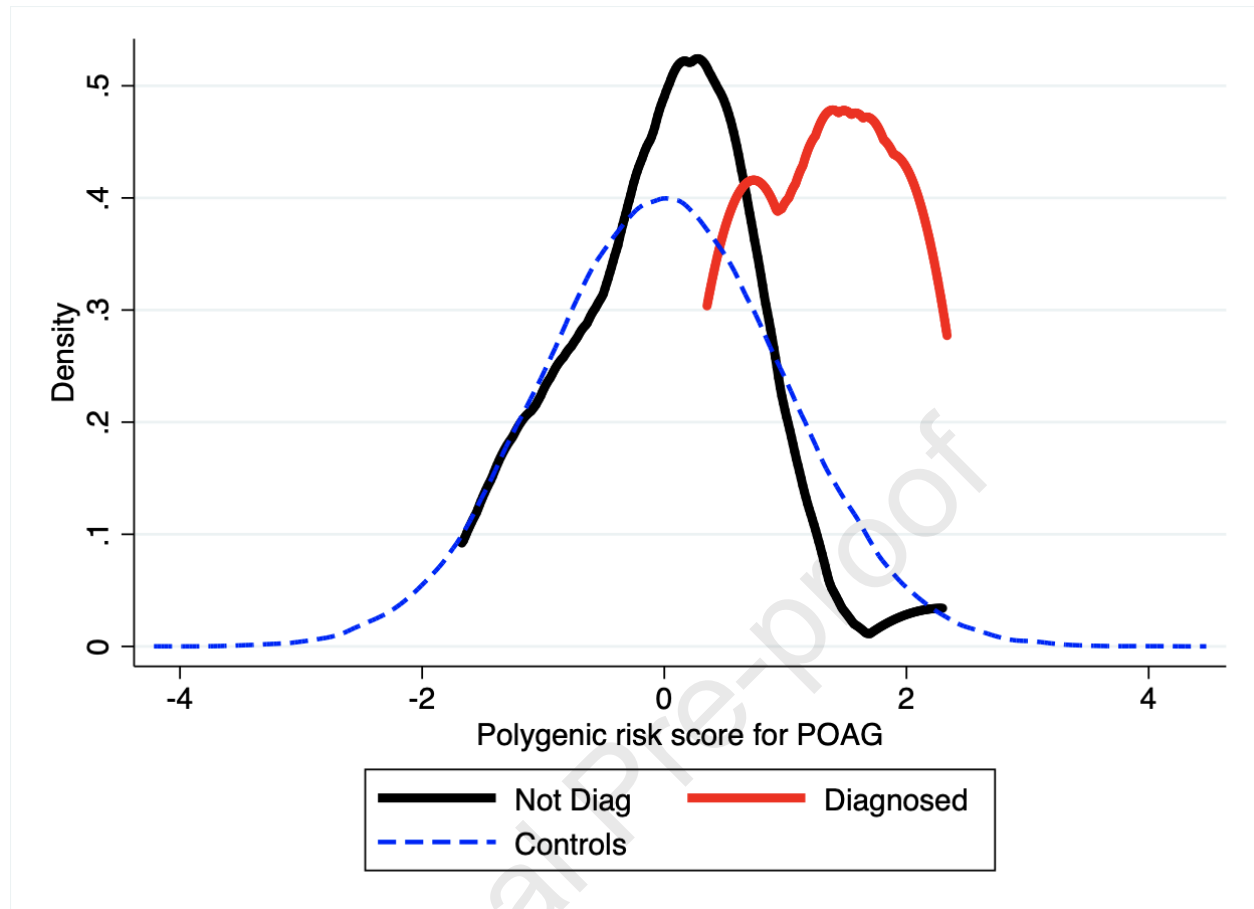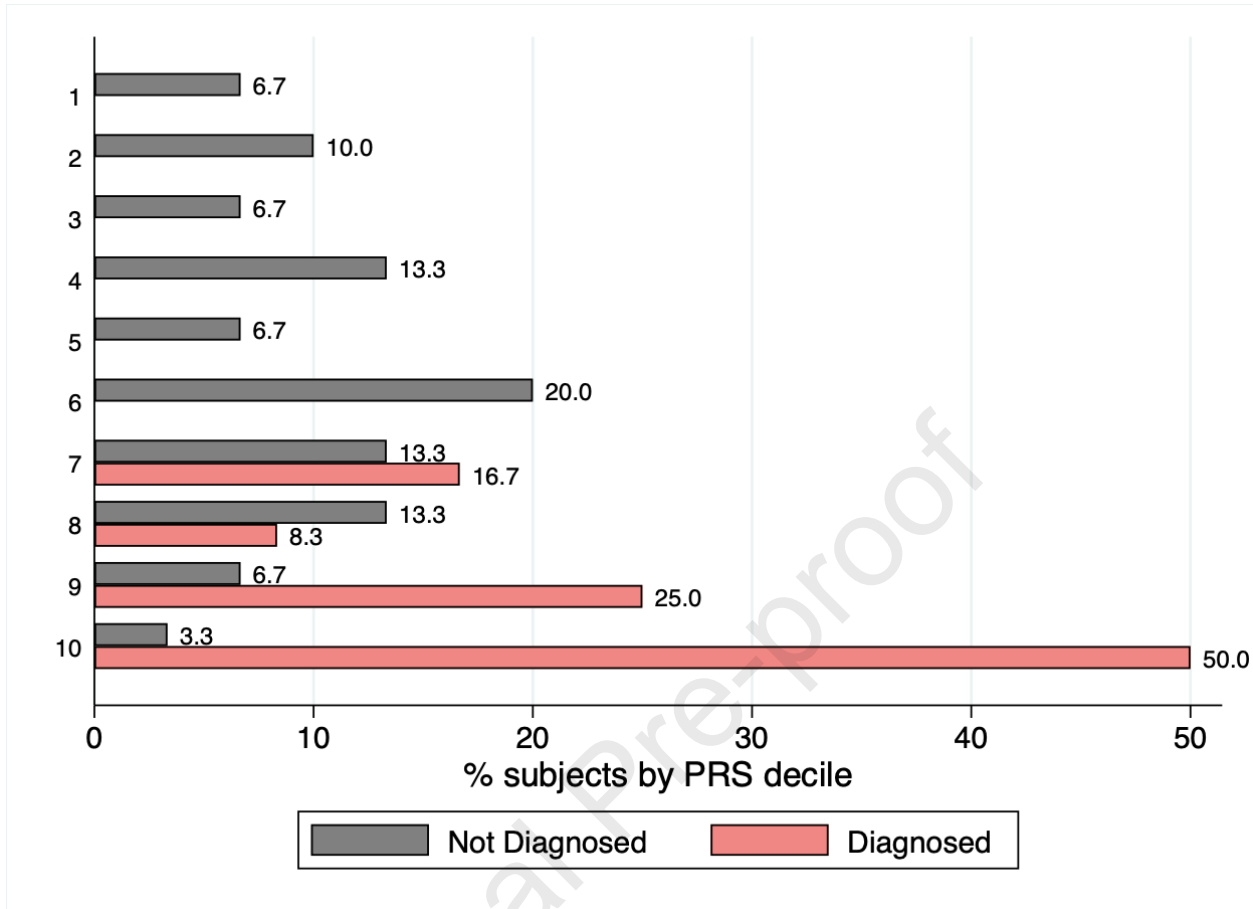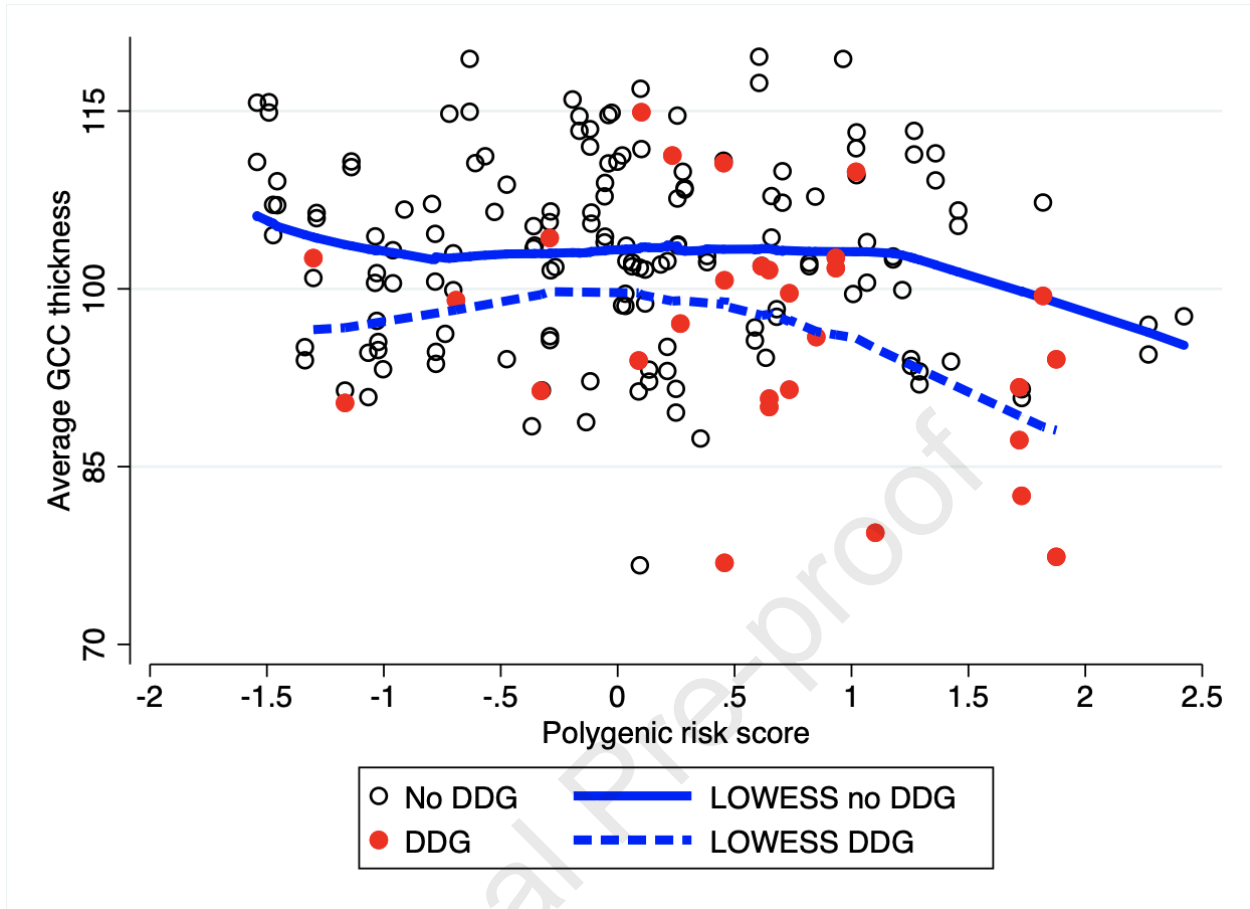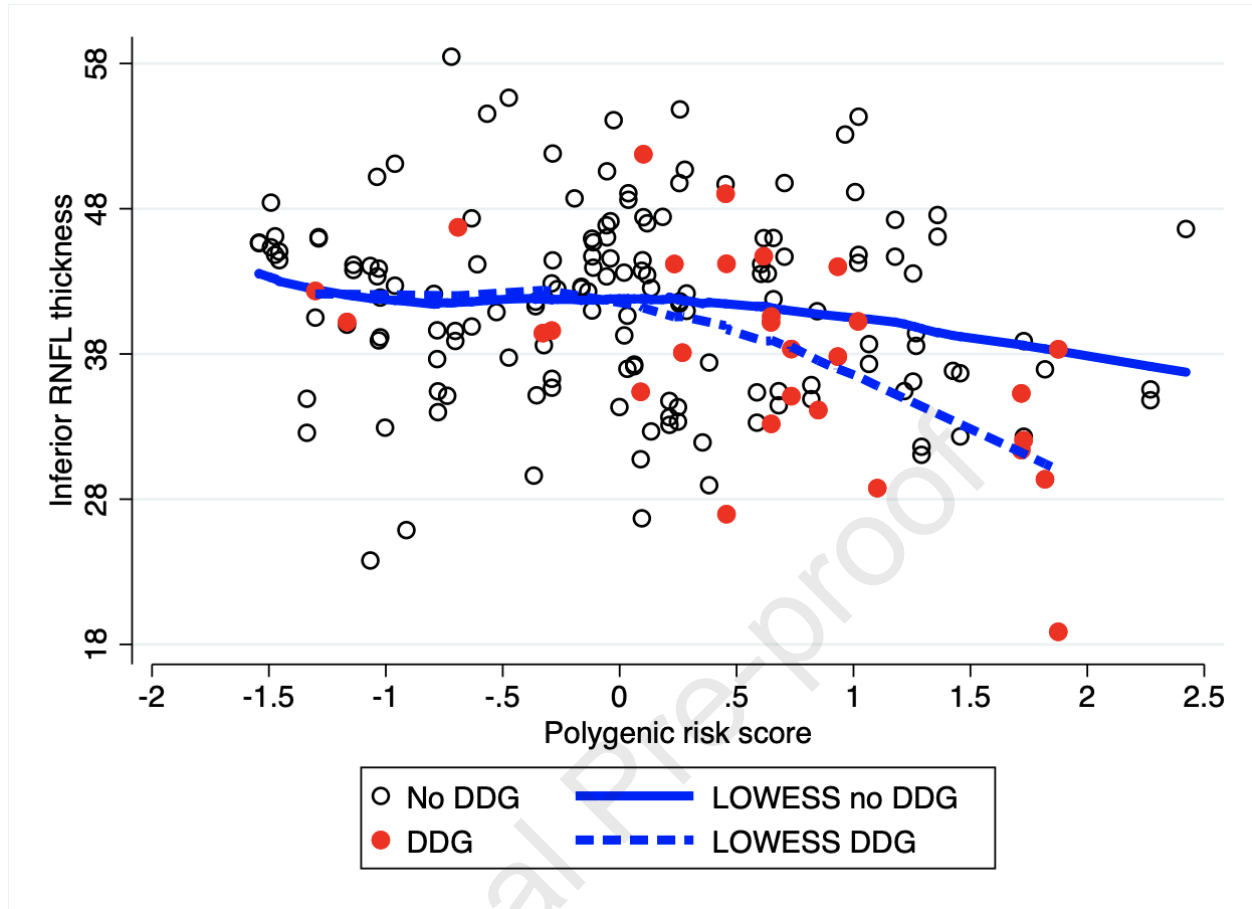| PRS decile	Not Diagnosed	Diagnosed
1	6.7	
2	10.0	
3	6.7	
4	13.3	
5	6.7	
6	20.0	
7	13.3	16.7
8	13.3	8.3
9	6.7	25.0
10	3.3	50.0

% subjects by PRS decile
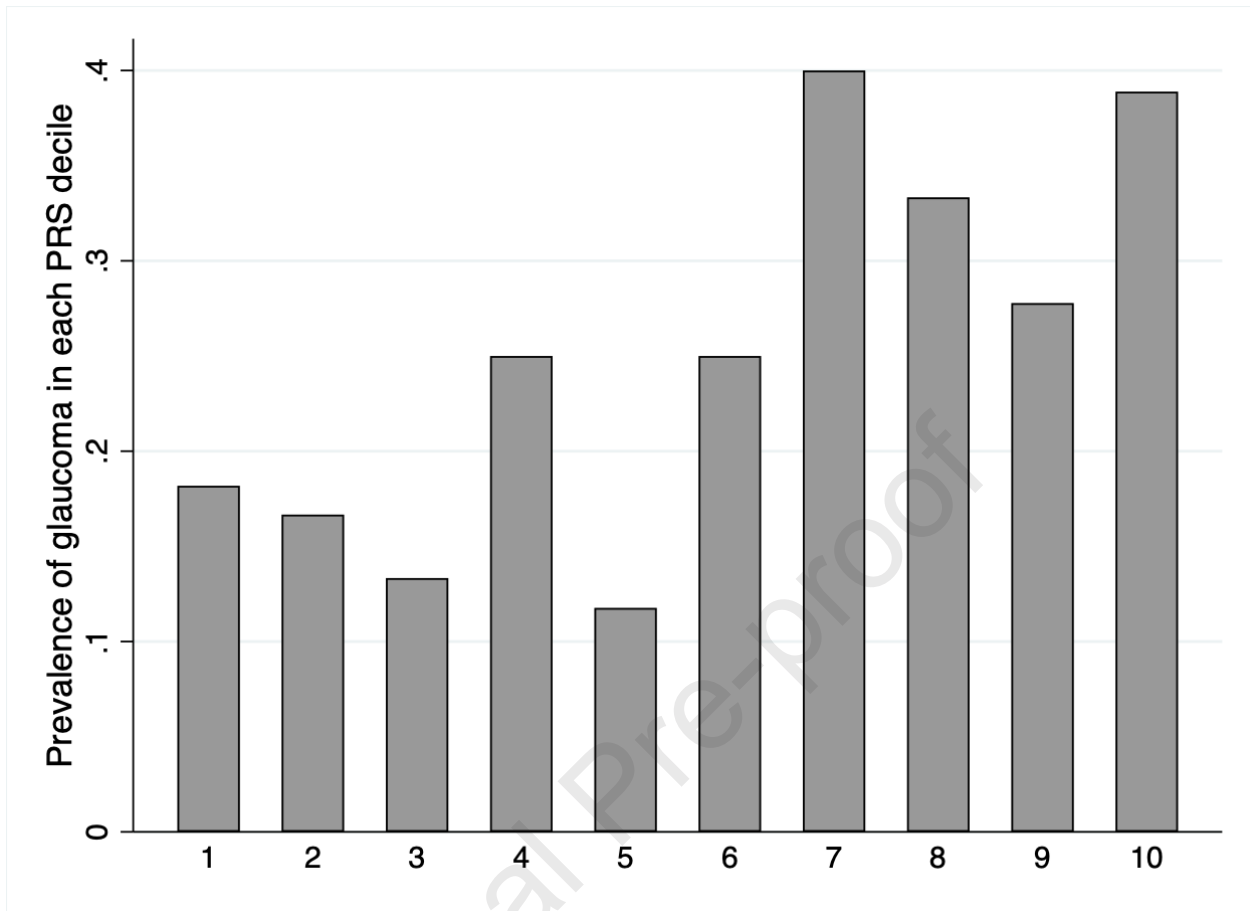
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Precis:

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.