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New insights into the genetics of primary open-angle glaucoma based on meta-analyses of intraocular pressure and optic disc characteristics.

Authors

Henriët Springelkamp(1, 2)*, Adriana I. Iglesias(1-3)*, Aniket Mishra(4, 5)*, René Höhn(6, 7)*, Robert Wojciechowski(8-10)*, Anthony P. Khawaja(11), Abhishek Nag(12), Ya Xing Wang(13, 14), Jie Jin Wang(15), Gabriel Cuellar-Partida(4), Jane Gibson(16), Jessica N. Cooke Bailey(17), Eranga N. Vithana (18-20), Puya Gharahkhani(4), Thibaud Boutin(21), Wishal D. Ramdas(2), Tanja Zeller(22), Robert N. Luben(23), Ekaterina Yonova-Doing(12), Ananth C. Viswanathan(11), Seyhan Yazar(24), Angela J. Cree(25), Jonathan L. Haines(17), Jia Yu Koh(18), Emmanuelle Souzeau(26), James F. Wilson(21, 27), Najaf Amin(1), Christian Müller(22), Cristina Venturini(12), Lisa S. Kearns(28), Jae Hee Kang(29), NEIGHBORHOOD Consortium**, Yih Chung Tham(18, 20), Tiger Zhou(26), Elisabeth M. van Leeuwen(1), Stefan Nickels(7), Paul Sanfilippo(24, 28), Jiemin Liao(18, 20), Herma van der Linde(3), Wanting Zhao(18), Leonieke M.E. van Koolwijk(1), Li Zheng(18, 30), Fernando Rivadeneira(1, 31, 32), Mani Baskaran(18-20), Sven J. van der Lee(1), Shamira Perera(18, 19), Paulus T.V.M. de Jong(33-35), Ben A. Oostra(3), André G. Uitterlinden(1, 31, 32), Qiao Fan(18), Albert Hofman(1, 32), E-Shyong Tai(19, 36, 37), Johannes R. Vingerling(2), Xueling Sim(37), Roger C.W. Wolfs(2), Yik Ying Teo(37, 38), Hans G. Lemij(39), Chiea Chuen Khor(18, 30), Rob Willemsen(3), Karl J Lackner(6), Tin Aung(18, 20), Nomdo M. Jansonius(40), Grant Montgomery(41), Philipp S Wild(42-44), Terri L. Young(45), Kathryn P. Burdon(46), Pirro G. Hysi(12), Louis R. Pasquale(29, 47), Tien Yin Wong(18-20), Caroline C.W. Klaver(1, 2), Alex W. Hewitt(28, 46), Jost B. Jonas(48), Paul Mitchell(15), Andrew J Lotery(25), Paul J. Foster(11), Veronique Vitart(21), Norbert Pfeiffer(7), Jamie E. Craig(26), David A. Mackey(24, 46) ±, Christopher J. Hammond(12) ±, Janey L. Wiggs(47) ±, Ching-Yu Cheng(18-20) ±, Cornelia M. van Duijn(1) ±, Stuart MacGregor(4) ±.

1. Department of Epidemiology, Erasmus Medical Center, Rotterdam, the Netherlands.
2. Department of Ophthalmology, Erasmus Medical Center, Rotterdam, the Netherlands.
3. Department of Clinical Genetics, Erasmus Medical Center, Rotterdam, the Netherlands.
4. Statistical Genetics, QIMR Berghofer Medical Research Institute, Royal Brisbane Hospital, Brisbane, Australia.
5. Department of Complex Trait Genetics, VU University, Center for Neurogenomics and Cognitive Research, Amsterdam, the Netherlands.
6. Department of Ophthalmology, Inselspital, Bern, Switzerland.
7. Department of Ophthalmology, University Medical Center Mainz, Mainz, Germany.
8. Computational and Statistical Genomics Branch, National Human Genome Research Institute (NIH), Baltimore, MD, USA.
9. Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA.
10. Wilmer Eye Institute, Johns Hopkins School of Medicine, Baltimore, MD, USA.
11. NIHR Biomedical Research Centre, Moorfields Eye Hospital NHS Foundation Trust and UCL Institute of Ophthalmology, London, UK.
12. Department of Twin Research and Genetic Epidemiology, King's College London, London, UK.
13. Beijing Institute of Ophthalmology, Beijing Tongren Eye Center, Beijing Tongren Hospital, Capital Medical University, Beijing, China.
14. Beijing Ophthalmology and Visual Science Key Lab, Beijing, China.

15. Centre for Vision Research, Department of Ophthalmology and Westmead Institute for Medical Research, University of Sydney, Sydney, New South Wales, Australia.
16. Centre for Biological Sciences, Faculty of Natural and Environmental Sciences, University of Southampton, Southampton, UK.
17. Department of Epidemiology and Biostatistics, Case Western Reserve University, Cleveland, Ohio, USA.
18. Singapore Eye Research Institute, Singapore National Eye Centre, Singapore, Singapore.
19. Duke-National University of Singapore Graduate Medical School, Singapore, Singapore.
20. Department of Ophthalmology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore.
21. Medical Research Council Human Genetics Unit, Institute of Genetics and Molecular Medicine, University of Edinburgh, Edinburgh, UK.
22. Clinic for General and Interventional Cardiology, University Heart Center Hamburg, Hamburg, Germany.
23. Department of Public Health and Primary Care, Institute of Public Health, University of Cambridge School of Clinical Medicine, Cambridge, UK.
24. Centre for Ophthalmology and Visual Science, Lions Eye Institute, University of Western Australia, Perth, Australia.
25. Clinical and Experimental Sciences, Faculty of Medicine, University of Southampton, Southampton, UK.
26. Department of Ophthalmology, Flinders University, Adelaide, Australia.
27. Centre for Global Health Research, The Usher Institute for Population Health Sciences and Informatics, University of Edinburgh, Scotland, UK.
28. Centre for Eye Research Australia (CERA), University of Melbourne, Royal Victorian Eye and Ear Hospital, Melbourne, Victoria, Australia.
29. Division of Network Medicine, Department of Medicine, Brigham and Women's Hospital, Boston, Massachusetts, USA.
30. Division of Human Genetics, Genome Institute of Singapore, Singapore, Singapore.
31. Department of Internal Medicine, Erasmus Medical Center, Rotterdam, the Netherlands.
32. Netherlands Consortium for Healthy Ageing, Netherlands Genomics Initiative, the Hague, the Netherlands.
33. Department of Ophthalmology, Academic Medical Center, Amsterdam, the Netherlands.
34. Department of Ophthalmology, Leiden University Medical Center, Leiden, the Netherlands.
35. The Netherlands Institute of Neuroscience KNAW, Amsterdam, the Netherlands.
36. Department of Medicine, National University of Singapore and National University Health System, Singapore, Singapore.
37. Saw Swee Hock School of Public Health, National University of Singapore and National University Health System, Singapore, Singapore.
38. Department of Statistics and Applied Probability, National University of Singapore, Singapore, Singapore.
39. Glaucoma Service, The Rotterdam Eye Hospital, Rotterdam, the Netherlands.
40. Department of Ophthalmology, University of Groningen, University Medical Center Groningen, Groningen, the Netherlands.
41. Department of Molecular Epidemiology, Queensland Institute of Medical Research, Herston, Brisbane, Queensland, Australia.
42. Preventive Cardiology and Preventive Medicine / Center for Cardiology, University Medical Center Mainz, Mainz, Germany.
43. Center for Thrombosis and Hemostasis, University Medical Center Mainz, Mainz, Germany.
44. German Center for Cardiovascular Research (DZHK), partner site RhineMain, Mainz, Germany.

45. Department of Ophthalmology and Visual Sciences, University of Wisconsin School of Medicine and Public Health, Madison, Wisconsin, USA.
46. School of Medicine, Menzies Research Institute Tasmania, University of Tasmania, Hobart, Australia.
47. Department of Ophthalmology, Harvard Medical School and Massachusetts Eye and Ear Infirmary, Boston, Massachusetts, USA.
48. Department of Ophthalmology, Medical Faculty Mannheim of the Ruprecht-Karls-University of Heidelberg, Heidelberg, Germany.

* These authors contributed equally to this work.

Membership of the NEIGHBORHOOD Consortium is listed in the **Supplementary Material

± These authors jointly supervised this work.

Corresponding author: Stuart MacGregor Telephone number: + 61 7 3845 3563; email : Stuart.MacGregor@qimrberghofer.edu.au

Abstract

Primary open-angle glaucoma (POAG), the most common optic neuropathy, is a heritable disease. Siblings of POAG cases have a ten-fold increase risk of developing the disease. Intraocular pressure (IOP) and optic nerve head characteristics are used clinically to predict POAG risk. We conducted a genome-wide association meta-analysis of IOP and optic disc parameters and validated our findings in multiple sets of POAG cases and controls. Using imputation to the 1000 genomes (1000G) reference set, we identified 9 new genomic regions associated with vertical cup disc ratio (VCDR) and 1 new region associated with IOP. Additionally, we found 5 novel loci for optic nerve cup area and 6 for disc area. Previously it was assumed that genetic variation influenced POAG either through IOP or via changes to the optic nerve head; here we present evidence that some genomic regions affect both IOP and the disc parameters. We characterized the effect of the novel loci through pathway analysis and found that pathways involved are not entirely distinct as assumed so far. Further, we identified a novel association between *CDKN1A* and POAG. Using a zebrafish model we show that *six6b* (associated with POAG and optic nerve head variation) alters the expression of *cdkn1a*. In summary, we have identified several novel genes influencing the major clinical risk predictors of POAG and showed that genetic variation in *CDKN1A* is important in POAG risk.

134 ## Introduction

135 In primary open-angle glaucoma (POAG), loss of retinal ganglion cells and nerve fibers manifests itself
136 clinically as optic nerve damage, which leads to visual field loss and, eventually in 15% of these with
137 visual loss to visual impairment and blindness (1-3). The optic nerve damage is characterized by an
138 increase in cup area, the central portion of the optic nerve head (or optic disc), and/or a decrease in
139 rim area (the area of the disc occupied by the retinal nerve fiber axons). This damage can be
140 quantified by the vertical cup-disc ratio (VCDR), comparing the vertical diameter of the cup with the
141 vertical diameter of the total optic disc. The VCDR ranges from 0 to 1. In the clinical setting, optic
142 nerve heads with a VCDR above 0.7 or an asymmetry between eyes above 0.2 are considered to be
143 suspect for glaucoma. The interpretation of VCDR depends on the disc area, i.e. discs with larger area
144 have on average a higher VCDR and the cut-off point between a normal and abnormal VCDR might be
145 higher (4).

146 Elevated intraocular pressure (IOP) is a well-recognized risk factor for POAG and current therapies
147 lower IOP by various mechanisms; eye pressure is considered to be normal between 8 and 21 mmHg,
148 although many eyes with eye pressure in this range can exhibit glaucomatous optic nerve features.
149 Analyses of first-degree relatives of POAG patients, have shown that the sib relative risk is 10.4 ± 2.5
150 (5).Classic twin studies in POAG are lacking and there is no consensus on heritability for POAG, but
151 work by Cuellar-Partida et al estimated the heritability of POAG based on genome-wide array data at
152 0.42 ± 0.09(6). Several genome-wide association studies (GWAS) have identified new POAG genes by
153 examining POAG directly or studying endophenotypes like VCDR and IOP(7-16). Several genes
154 associated with VCDR and IOP - *CDKN2B-AS1*, *SIX6* (VCDR); and *CAV1/CAV2*, *TMCO1*, *ABCA1* and
155 *ARHGEF12* (IOP) - are associated with POAG. Notably, no genes have been genome-wide associated
156 with both VCDR and IOP. Charlesworth et al. previously found a genetic correlation between VCDR
157 and IOP (RhoG = 0.45, P = 0.0012), however, genes underlying this relationship have not yet been
158 identified(17).

159 The aims of this study were to (1) identify new genes associated with the POAG endophenotypes IOP,
160 VCDR, cup area, and disc area, and ultimately POAG, using the 1000 Genomes imputations reference
161 panel, and (2) investigate the genetic overlap between the different endophenotypes. To accomplish
162 these aims we performed a meta-analysis of GWAS of these four traits within the International
163 Glaucoma Genetics Consortium (IGGC).

164

165 # Results

166 ## Intraocular pressure

167 We first conducted a meta-analysis of GWAS in the European cohorts, top hit variants were
168 replicated in the Asians meta-analysis. Next, we performed a meta-analyses of the European and
169 Asians cohorts. After removal of single nucleotide polymorphisms (SNPs) with minor allele frequency
170 (MAF) < 0.01 and low imputation quality, approximately 8 million SNPs were included. Whilst the
171 meta-analysis of individuals of European descent yielded no novel associations, combined meta-
172 analysis of individuals of European and Asian descent (n = 37,930, λ = 1.05; **Supplementary Material,**
173 **Figs S1a, S1b** and **S2b**), yielded nine genomic regions reaching genome-wide significance, of which
174 eight genomic regions were already known (**Supplementary Material, Figs S1a, S1b, S2b**, and **Table**
175 **S3**)(9, 11, 13). The peak SNP in the new genomic region was rs55796939 on chromosome 11q25 near
176 *ADAMTS8* (**Supplementary Material, Figs S3** and **S4**).The estimated heritability (h^2) of IOP using the
177 LD score regression method(18) and the summary statistics of European-only meta-analysis was 0.13.

178

179 ## Vertical cup-disc ratio

180 In the meta-analysis of individuals of European descent (n = 23,899, λ = 1.08), 21 genomic regions
181 were genome-wide significant (**Supplementary Material, Figs S5a, S6a** and **Table S4**). Five genomic
182 regions were novel (near to the genes *RPE65* on chr. 1p31, *F5* on chr. 1q23, *PDZD2* on chr. 5p13.3,
183 *RREB1* on chr. 6p25, and *DGKB* on chr. 7p21.2) (**Supplementary Material, Figs S7** and **S8**); the other
184 genomic regions have been previously associated with VCDR or cup area, two highly correlated
185 traits(19-21). Of the five novel genomic regions, *RREB1* (p-value = 4.13×10^{-3}) was nominally
186 significant in the analysis of individuals of Asian descent (n= 8,373, λ = 1.01). In the combined analysis
187 (n = 32,272, λ = 1.06), another four novel genomic regions, near to the genes *VCAN* on chr. 5q14.3,
188 *PSCA* on chr. 8q24.2, *ENO4* on chr. 10q25.3, and *RBM23* on chr. 14q11.2 (**Supplementary Material,**

189 **Figs S5b** and **S6b**), were genome-wide significant leading to a total of nine (5+4) novel genomic
190 regions associated with VCDR. Of these novel genomic regions, *F5* has been associated with disc area
191 previously(21). Disc area influences the VCDR(22), and therefore we corrected VCDR for disc area in a
192 secondary analysis. After correction for disc area, the β (p-value) decreased from -0.007 (2.15×10^{-9})
193 to -0.002 (5.60×10^{-2}) in the subset with disc area available, suggesting that *F5* acts primarily on disc
194 area and secondary to VCDR through its relation to disc area. The calculated h^2 of VCDR using the
195 European -only meta-analysis was 0.31.

196

197 **Cup area**

198 The meta-analysis of individuals of European descent (n = 22,489, λ = 1.06) yielded 17 genome-wide
199 significant regions of which 14 regions were already implicated for cup area or VCDR (**Supplementary**
200 **Material, Figs S9a**, **S10a** and **Table S5**)(20, 21). There were three novel associations on chr. 1q42.11
201 near *CDC42BPA*, chr. 8q21.11 near *CRISPLD1*, and on chr. 15q26.3 near *FAM169B* (**Figs S11** and **S12**).
202 *CDC42BPA* has previously been associated with disc area and the fact that the association with cup
203 area adjusting for disc area is genome wide significant suggests an independent effect on cup area. In
204 the combined analysis of European and Asian individuals (n = 29,828, λ = 1.06, **Supplementary**
205 **Material, Figs S9b** and **S10b**) all loci except *FAM169B* and *CRISPLD1* remained genome-wide
206 significant, and there was one additional genome-wide significant SNPs at chr. 6p21.2 (*CDKN1A*) and
207 one highly suggestive significant SNP at chr. 9q34.2 (*ABO;* previously associated to IOP). For cup area
208 the estimated h^2 was 0.27.

209

210 **Disc area**

211 The meta-analysis of individuals of European descent (n = 22,504, λ = 1.06) resulted in 13 genome-
212 wide significant regions, of which two were not previously associated with disc area: *UGT8* on chr.

213 4q26 and *CTNNA3* on chr. 10q22.2 (**Supplementary Material, Figs S13a**, **S14a**, **S15**, **S16**, and **Table**
214 **S6**). These SNPs were not significant in the meta-analysis of individuals of Asian descent (n = 7,307, λ
215 = 1.02). An additional four SNPs reached genome-wide significance in the combined meta-analysis (n
216 = 29,811, λ = 1.07): *PRDM16* on chr. 1p36.23-p33, *GADD45A* on chr. 1p31.2, *VGLL4* on chr. 3p25.3,
217 and *ASB7* on chr. 15q26.3 (**Supplementary Material, Figs S13b** and **S14b**). The estimated h^2 for disc
218 area was 0.27.

219

220 **Characterization of the lead association signals**

221 In total, 82 SNPs were associated with one or more of the above endophenotypes. Functional
222 characterization of the 82 SNPs was performed using a range of bioinformatics tools (see **Methods**).
223 In total, 650 variants in linkage disequilibrium (LD) with the 82 lead SNPs (R^2 >0.8) were examined for
224 functional annotation. Overall, 61% (50/82) of the associated loci are in LD with variants located in
225 regulatory regions according to the ENCODE data (e.g. DNase I hypersensitive sites, transcription
226 factor binding sites and motifs; see **Supplementary Material, Table S7**). We investigated the
227 expression levels of the identified candidate genes using the UniGene database(23). Of all reviewed
228 genes, *CDKN1A, PAX6* and *DUSP1* showed the highest number of transcripts per million in the eye
229 (**Supplementary Material, Table S8**). According to the Ocular Tissue database(24), *CDKN1A* is highly
230 expressed in the optic nerve head, as well as *DUSP1,* which also shows high expression in the
231 trabecular meshwork. Both genes were associated with optic nerve head parameters. *PAX6* is highly
232 expressed in the ciliary body and retina, in this study we found it associated with disc area. Other
233 highly expressed genes in the optic nerve include *EFEMP1* and *ABI3BP*, which are associated with cup
234 area and disc area, respectively (**Supplementary Material, Table S9**). To evaluate whether associated
235 genes are highly expressed in a particular tissue, we performed tissue enrichment analyses for each
236 trait using suggestive SNPs (p-value < 1.00×10^{-05}) in DEPICT(25). No FDR significant tissue enrichment
237 was found for IOP, VCDR or disc area. However, for cup area we found an enrichment for

238 membranes, joints, stem cells and other related connective tissues. Similar results were found when
239 suggestive SNPs associated with VCDR, cup and disc area (the optic nerve parameters) were analyzed
240 together (**Table S10**).

241

242 **Gene-based test**

243 To identify new loci not found through per-SNP tests, we performed gene-based testing using
244 VEGAS2. Reflecting the smaller number of tests, our gene-based significance threshold is $P_{gene\text{-}based}$ <
245 0.05/24,769 = 2.02 x 10^{-6} (24,769 genes tested). Using the gene-based test we found several novel
246 loci (**Supplementary Material, Table S11**). *C9* was significantly associated with IOP (p-value 1.61 x 10^{-}
247 ^{6}); *RARB* (p-value 1.86 x 10^{-6}) and *HORMAD2-AS1* (p-value 1.04 x 10^{-6}) were associated with VCDR.
248 These genes were previously associated with disc area, so the novel associations with VCDR could
249 possibly be driven by the influence of disc area on VCDR(21). In the cup area analysis, the genes
250 *LRP10* (p-value 1.20 x 10^{-6}) and *REM2* (p-value 1.55 x 10^{-6}), and *THSD4* (p-value 5.44 x 10^{8}) were
251 significantly associated. The first two genes are located near to *RBM23*, which was significant in the
252 per-SNP test. *THSD4* is located near to *KPNB1*, which was associated with VCDR in our previous meta-
253 analysis(20). In the disc area analysis we found two genes that were significantly associated with disc
254 area: *ANKRA2* (p-value 8.42 x 10^{-7}) and *LOC149950* (p-value 3.87 x 10^{-7}).

255

256 **Characterizing the overlap in biological pathways involved in glaucoma endophenotypes**

257 In total, 86 SNPs were associated with one or more of the above endophenotypes. The effect
258 estimates and p-values of these SNPs for all four endophenotypes are shown in **Table 1-3**. *ADAMTS8*
259 (IOP and VCDR, **Table 1** and **Table2b**) and *ABO* (IOP and cup area, **Table 1**) were genome-wide
260 significantly associated with two traits. Of note is that there were different variants involved in
261 *ADAMTS8*: rs55796939 for IOP and rs4936099 for VCDR (r2=0.03 between these SNPs in 1000G

262 European samples). **Figure 1** shows the overlap in associations across endophenotypes – we depict
263 annotated genes for which at least one SNP was genome-wide significant in at least one trait.
264 Overlap is defined as nominal significance or stronger for the second trait. The figure shows as
265 expected a strong overlap in variants associated to disc area, cup area and VCDR. Further, overlap is
266 noted in genes associated to IOP, cup area and VCDR. We next explored the genetic overlap between
267 optic disc parameters and IOP using our GWAS results from the European-only meta-analyses, which
268 comprised a larger sample size (n=22,489-29,578). As expected genetic correlation between optic
269 disc parameters was high, VCDR and cup area showed the highest correlation (RhoG = 0.83, p-value <
270 1.0×10^{-308}), followed by VCDR and disc area (RhoG = 0.62, p-value = 7.36×10^{-23}). Genetic correlation
271 between cup and disc area was 0.31 (p-value = 1.00×10^{-04}). No significant genetic correlation was
272 found between IOP and optic disc parameters, the highest correlation was between IOP and VCDR
273 (RhoG = 0.06, p-value 0.3) (**Table S12**).

274 To further characterize the overlap in biological functions, gene set enrichment of loci associated
275 with IOP and optic disc parameters was performed using DEPICT(25). We first investigated enriched
276 pathways or gene sets using only genome-wide associated SNPs. No significant pathways were found
277 after FDR correction. However, pathways involved in metabolic processes such as "increased
278 circulating leptin level", "abnormal fat cell morphology" and "increased insulin sensitivity" were
279 suggestive when we analyzed the list of SNPs associated with VCDR, cup area and disc area (FDR<0.2,
280 see **Supplementary Material, Table S13**). We next searched for enriched pathways using suggestive
281 SNPs (p-value $<1.0 \times 10^{-5}$). We further investigated potential overlap in pathways across the
282 endophenotypes, and found 57 significant pathways when using VCDR, cup area and IOP variants;
283 and 100 pathways when analysing suggestive VCDR, cup area and disc area variants. Note that in the
284 first analysis we investigated pathways enriched when IOP genes are taken into account, while in the
285 second one we analysed genes influencing the optic nerve head characteristics. Due to a high degree
286 of redundancy between pathways, we clustered the significant pathways into meta-pathways,

287 resulting in 11 meta-pathways for VCDR, cup area and IOP (**Figure 2a**, **Supplementary Material,**
288 **Table S14**); and 17 for VCDR, cup area, and disc area (**Figure 2b**, **Supplementary Material, Table**
289 **S15**). Most of the gene sets found in both analyses highlighted pathways involved in cell
290 differentiation, notch signaling, regulatory DNA binding and embryonic development, which reflects
291 the pathways found when VCDR and CA variants are analyzed (**Supplementary Material, Fig S17**).
292 Furthermore, we found "abnormal fat cell morphology" and "abnormal liver morphology"
293 significantly enriched; a key gene in these pathways is *ABCA1*. When IOP genes are included the
294 elongation factor, RNA Polymerase II (ELL2) protein complex" shows an enrichment. When disc area
295 genes are included, pathways such as "blood vessel development", "protein import into nucleus",
296 "Thrombospondin 1 (THBS1) and SMAD3 protein complex", and "abnormal eye morphology" were
297 significant. Key genes in the latter include: *CDKN2B, FAT4, LRIG3, SIX6, COL8A1, SOX11, RND3, BOC,*
298 *WNT2B* and *CYP26A1.*

299

300 **From endophenotypes to primary open-angle glaucoma**

301 To evaluate the implications of our findings in the context of glaucoma, we examined the association
302 between the genome-wide significant SNPs found in this study and 4 independent POAG studies (n=
303 6,429 cases and 41,404 controls). In total, 75 independent (i.e. $R^2 < 0.8$) SNPs associated with one or
304 more of the endophenotypes were assessed in the case/control studies. Of these, 32 were nominal
305 significantly associated with POAG (p-value <0.05; the chance that 32 SNPs of 75 SNPs have a p-value
306 <0.05 is $< 2.2 \times 10^{-16}$), and 11 independent SNPs were Bonferroni significantly associated with POAG
307 (p-value 0.05/75 = 6.67×10^{-4}) (**Table 4**). Two out of the 11 Bonferroni significant SNPs, the
308 rs2487048 in the *ABCA1* gene and the 11:120357425 in the *ARHGEF12* showed high heterogeneity
309 (I^2). To estimate the common effect size we performed a random effect meta-analysis. The odds ratio
310 (OR) remained almost the same for both variants, although p-values were not significant after
311 adjusting for multiple testing, which is in line with the heterogeneity observed. All other nine SNPs

312 surpassed the Bonferroni threshold for significance in both fixed and random-effect models. The
313 association between *CDKN1A* and POAG is novel (OR = 1.14, p-value = 7.4 x 10^{-7}). In our previous
314 paper, the SNP rs6054374 near to *BMP2* was already associated with POAG (OR = 0.92, p-value 3.74 x
315 10^{-3}), but the most significantly associated SNP in the current meta-analysis rs6107845 near to *BMP2*
316 shows a slightly larger effect on POAG (OR = 0.89, p-value = 8.52 x 10^{-6}). *CDKN1A*, the novel
317 associated POAG candidate gene belongs to the cyclin dependent kinase inhibitor (CDKN) gene family
318 as well as *CDKN2A*, *CDKN2B/CDKN2B-AS1*, which all lie in a well-known glaucoma associated locus on
319 chr.9p21.

320

321 **Expression of cdkn1a after knockdown of six6b in zebrafish**

322 Previous studies have shown that the transcription factor *SIX6* alters the expression of *CDKN2A* and
323 *CDKN2B* (26, 27). All three genes (*SIX6, CDKN2A* and *CDKN2B*) are in loci associated with POAG,
324 suggesting a possible functional link between these POAG-loci. Given that *CDKN1A* is part of the
325 same gene family as *CDKN2A* and *CDKN2B* we tested whether SIX6 also regulates *CDKN1A*. To assess
326 the potential for functional regulation of *CDKN1A* through *SIX6*, we first performed *in silico* analyses
327 and observed that *SIX6* binds to *CDKN1A* (core score = 0.812). Then we used a previously studied
328 zebrafish model(27), in which we tested whether knockdown of *six6b* alters the expression of *cdkn1a*
329 *in vivo*. Knockdown of *six6b* was achieved using morpholino technology(27). 85% of the knockdown
330 embryos showed a small eye phenotype, reduced optic nerve thickness and an up-regulation of the
331 expression levels of *cdkn2a/cdkn2b*, as observed in previous studies (n=220)(27, 28). In zebrafish,
332 there is only one gene which is analogous to the human *CDKN2A* and *CDKN2B* and it is referred to in
333 this paper as *cdkn2a/cdkn2b*. We evaluated the expression levels of *cdkn1a* in eyes of *six6b* deficient
334 embryos by RT-qPCR. A 41-fold overexpression of *cdkn1a* in the eye of *six6b* knockdown embryos
335 was found (p-value = 0.001) (**Figure 3**), showing that *in vivo* downregulation of *six6b* affects the

336 expression levels not only of *cdkn2a/cdkn2b* but also of *cdkn1a*, likely by binding to their sequence,
337 repressing their expression.
338

339 ## Discussion

340 This meta-analysis within the IGGC identified a novel genomic region associated with IOP, nine
341 genomic regions associated with VCDR, five with cup area, and six with disc area. Eleven genomic
342 regions were associated with POAG. Of these regions, the association between *CDKN1A* and POAG is
343 novel.

344 We identify some specific loci that underlie the genetic correlation between IOP and VCDR described
345 earlier(17). *ADAMTS8* and *ABO* were genome-wide significant for both IOP and VCDR or cup area.
346 Variants found close to *ABO* (rs8176672 for cup area and rs8176741 for IOP) are in LD (r2 >0.85) with
347 rs12216891, which lies in an enhancer and promoter histone mark, suggesting a potential regulatory
348 mechanism in that region. Furthermore, *TRIOBP* is genome-wide significant for cup area, and reached
349 a p-value of 3.42×10^{-6} for IOP. Interestingly, *TRIOBP* is approximately 180 kb away from *CARD10*
350 which is associated with disc area. There is a large overlap between VCDR/cup area and disc area.
351 Since VCDR is related to disc area, it might be that the effect found for VCDR is due to the effect of
352 disc area. Most of these overlapping genes are still Bonferroni significant in the cup area analysis in
353 which we corrected for disc area. Only *CDC7/TGFBR3* and *F5* are genome-wide significant for VCDR as
354 well as for disc area, but the effect is negligible after correction for disc area, suggesting that these
355 two genes play primarily a role in disc area.

356 When suggestive SNPs (p-value $<1.0 \times 10^{-5}$) for VCDR and cup area are analyzed together using
357 DEPICT, we found an enrichment of pathways involved in cell differentiation, development,
358 regulatory DNA binding and Notch signaling. Including disc area SNPs to the VCDR and cup area
359 analysis reveals additional joint pathways: 1) eye and blood vessel development, 2) cancer, 3) protein

360 import into nucleus, and 4) thrombospondin 1 and SMAD3 complexes, related to the extracellular
361 matrix. The extracellular matrix pathway has been previously implicated in optic nerve
362 degeneration(20). *ADAMTS8* , *COL8A1* and the novel identified gene *VCAN* (versican) are involved in
363 the metabolism and composition of the extracellular matrix. Interestingly, mutations in *VCAN* have
364 been implicated in several ophthalmologic disorders(29).Of interest, known POAG genes also fit in
365 the pathways identified in this study, for example *GAS7* and *SIX6* play a role during development(27,
366 30), *TGFBR3* has been implicated in extracellular matrix regulation(31) and in cancer as well as
367 *GMDS*(32).

368 Common variants in *CDKN2B* and its antisense *CDKN2B-AS1* have been robustly found associated
369 with POAG. The gene *CDKN1A*, also known as *p21*, *CIP-1* or *WAF-1*, belongs to the same family as
370 *CDKN2B* and also encodes a cyclin-dependent kinase inhibitor. Upregulation of *CDKN1A* causes G1
371 arrest and inhibits proliferation of the cell. Herein, for the first time, we provide genome-wide
372 significant evidence for association of *CDKN1A* variants with cup area. Two prior small cohort studies
373 suggested a possible role of *CDKN1A* in POAG. Tsai et al.(33) found an association between a codon
374 31 polymorphism in *CDKN1A* and POAG in 58 patients and 59 controls from China (OR = 2.39 [1.14-
375 5.01]). Saglar et al. found no statistically significant association between the codon 31 polymorphism
376 and POAG in 75 patients and 119 controls from Turkey (OR = 1.70, p-value = 0.25)(34). Our study
377 provides strong evidence for the role of *CDKN1A* in POAG risk in a large sample consisting of 6,429
378 cases and 41,404 controls and shows the first convincing evidence for association of *CDKN1A* and
379 POAG in individuals of European descent. A potential functional interaction between POAG-loci (i.e.
380 *SIX6* and CDKN genes) has been shown previously (26, 27). We performed *in vivo* studies in
381 embryonic zebrafish eye and found that knockdown of *six6b* upregulates both *cdkn2a/cdkn2b* and
382 *cdkn1a*. The mechanism behind the genetic variation in *SIX6*, and the CDKN genes remains obscure.
383 However, in a recent study, Skowronska-Krawczyk et al. showed that *SIX6* regulates the expression of
384 *CDKN2A* in the context of IOP elevation (26). More comprehensive studies at the individual tissue

385 level e.g. retinal ganglion cell layer or optic nerve should be performed to evaluate the consequences
386 of the genetic variation associated with POAG; for example, if associated variants in *SIX6* lead to up-
387 regulation of CDKN genes.

388 Genetic variation in CDK inhibitor genes has been found associated with various diseases and traits.
389 SNPs close to *CDKN1A*, for example, have been associated with electrocardiographic measures (35)
390 and colorectal cancer(36). While the *CDKN2A/CDKN2B* and *CDKN2B-AS1* locus has been found in
391 GWAS of Type 2 Diabetes(37), fasting glucose(38), intracranial aneurysm(39), myocardia
392 infraction(40), coronary heart disease(41) and various cancer, including basal cell carcinoma(42),
393 breast(43) and prostate(44) cancer, and glioma(45). Interestingly, the same allele in *CDKN2B* that
394 decrease risk of POAG, was found associated with increased risk of glioma. The functional
395 significance of the genetic variation in the CDK genes remains to be determined, possible mechanism
396 in POAG might be related with the disruption of a delicate balance between proliferation and
397 apoptosis.

398 It has been suggested that p53 plays a role in POAG, especially in POAG with paracentral visual field
399 loss(46). Intriguingly, p53 has been also related to the CDK-inhibitors and to four of the new genes
400 pointed out by this study (*GADD45A*, *PDZD2*, *RREB1* and *PSCA*). *GADD45A* is involved in growth arrest
401 through p53-dependent and independent mechanisms(47, 48) and can interact via *CDKN1A*(49).
402 *PDZD2* is a tumor suppressor gene, reported to activate p53 by transcriptional regulation (50). *RREB1*
403 has an effect on p53 by binding to its promotor and transactivates its expression(51). While *PSCA*
404 expression correlates with the expression of p53 in choriocarcinoma, suggesting a role of *PSCA* in cell
405 growth through p53-related pathways(52). Other genes play a role in apoptosis or cell growth via
406 other mechanisms than p53: *VGLL4* inhibits Bax- and TNFa-induced apoptosis(53) and *DGKB* is a
407 regulator of diacylglycerol, which is important for cell growth and differentiation. *UGT8* plays a role in
408 the biosynthesis of the sphingolipids of myelin membranes of the central and peripheral nervous
409 system; sphingolipids are also implicated in apoptosis(54).

410 Another interesting novel gene is *RPE65* (retinal pigment epithelium -specific protein 65kDa). This
411 gene has been associated with retinitis pigmentosa (RP) (55, 56) and Leber congenital amaurosis type
412 2 (LCA2)(57). As the name implies, the encoded protein is located in the retinal pigment
413 epithelium(58). Both diseases (RP and LCA2) are not characterized by an excavation of the optic
414 nerve head. However, we have checked several online databases for expression in different tissues.
415 In the eye, it is also highly expressed in the optic nerve head (**S8 and S9 Tables**) suggesting that this
416 gene could be involved in other ocular processes. Little expression is found in the brain, with no
417 expression in other tissues or organs in the body. Future studies are necessary to confirm our finding.

418 Of the genes identified by gene-based testing, *C9* (complement component 9) is especially
419 interesting. Its protein is part of the membrane attack complex (MAC), together with the proteins
420 C5b, C6, C7, and C8. This complex activates several steps that lead to cell death, and cells protect
421 themselves by removing the complex through endocytosis. Caveolin is one of the proteins involved in
422 endocytosis and the *CAV1/CAV2* genes are associated with IOP and POAG. It has been shown that
423 inhibition of caveolin-1 inhibits the endocytosis of MAC(59).

424 To our best knowledge, this meta-analysis is the largest study of IOP and optic nerve head
425 parameters to date, using well-characterized datasets from populations world-wide. A limitation of
426 our study is the lack of an available dataset for replication of the novel associations detected by
427 combined European and Asian ancestry samples. However, the heterogeneity of these novel genomic
428 regions is generally low in the meta-analysis. For VCDR, cup area, and disc area we have identified
429 novel SNPs in the analysis of individuals with European ancestry. Of the nine novel associations found
430 in these populations (*RPE65*, *PDZD2*, *RREB1*, *DGKB* for VCDR; *CDC42BPA, CRISPLD1* and *FAM169B* for
431 cup area; and *CTNNA3* and *UGT8* for disc area), only *RREB1* was nominally significant in the
432 individuals with Asian ancestry. Five of the seven non-significant SNPs in the individuals with Asian
433 ancestry had an effect estimate in the same direction. As the analysis in individuals with Asian
434 ancestry contains a smaller number of individuals, this could be due to lack of power.

435 We have identified 21 genetic variants associated with POAG endophenotypes: IOP, VCDR, cup area,
436 and disc area. The effect estimates of single SNP associations are small. We expected small effects of
437 many SNPs, since glaucoma endophenotypes are highly polygenic traits(60). Although individual SNP
438 effects are small, the joint effect of the various genetic variants could yield a clinical relevant
439 parameter. These association results do not imply that the variants described here have a causal
440 effect. Fine-mapping and functional studies are required to identify the causal variants tagged by our
441 findings and the exact molecular mechanisms involved in POAG. Although the overlap between IOP-
442 loci and the optic disc parameters-loci is not large , this is the first study showing a genome-wide
443 significant evidence of the genetic correlation between IOP and VCDR; we expect that larger sample
444 sizes and improved imputation accuracy may help to find more of the loci underlying the genetic
445 correlation between these two endophenotypes. Of the novel associations, *CDKN1A* is strongly
446 associated with POAG, This finding is in line with other studies(26), pointing to the CDK-inhibitor
447 genes as key players in the development of POAG. The p53 pathway has been implicated in POAG
448 and interact in different cellular contexts with four of the new genes pointed out by this study
449 (*GADD45A*, *PDZD2*, *RREB1* and *PSCA*). Functional studies need to be performed to assess the role of
450 *p53* and CDK-inhibitors in the pathophysiology of POAG. A more comprehensive study of these
451 mechanisms may inform the development of new therapies for POAG.

452

453 # Materials and methods

454 ## Study design

455 We performed a meta-analysis on directly genotyped and imputed SNPs to the 1000 Genomes
456 reference panel. We analyzed four outcomes: IOP, VCDR, cup area, and disc area; the same approach
457 was used for all four outcomes. In the first stage, we conducted a meta-analysis of GWAS including
458 22,489-29,578 individuals of European ancestry. Subsequently, we evaluated the genome-wide
459 significant SNPs from the first stage in a meta-analysis of GWAS including 7,307-8,373 individuals of
460 Asian ancestry. Finally, we performed a mega/meta-analysis of GWAS from all individual studies
461 including individuals of European and Asian ancestry. We subsequently tested the effect of all
462 genome-wide significant SNPs on POAG in four independent case-control studies (n = 6,429/41,404).
463 These cases are not part of our primary meta-analyses which focused on quantitative traits in the
464 general population (IOP, VCDR, cup area and disc area). Three of the case-control studies were of
465 European ancestry(7, 61, 62) and one of Asian ancestry(63).

466

467 ## Subjects, phenotyping and genotyping

468 All 19 studies included in this meta-analysis are part of the IGGC (**S1a Table**).Details for each
469 individual study can be found in **Supplementary Material** and **Tables S1b**, **S1c** and **S2**. The
470 ophthalmological examinations included measurements of IOP and optic nerve head assessment. All
471 19 studies contributed to the IOP mega/meta-analysis, 18 to the VCDR and 16 to the cup area and
472 disc area mega/meta-analysis .Validation of mega/meta-analysis results was performed in four
473 independent POAG case-control studies. Studies performed genomic imputation using 1000
474 Genomes phase 1 reference samples . Study-specific quality control can be found in the
475 **Supplementary Material**. All studies were performed with the approval of their local medical ethics

476 committee, and written informed consent was obtained from all participants in accordance with the
477 Declaration of Helsinki.

478

479 **Statistical analysis**

480 In the IOP analysis, individuals who underwent IOP-lowering laser or surgery were removed from the
481 analysis; in individuals receiving IOP-lowering medication, the IOP value was multiplied by 1.3 to
482 estimate a pre-medication IOP value(64). The mean IOP, VCDR, cup area, and disc area of both eyes
483 was used for the analyses. SNPs with MAF < 0.01 and imputation quality scores <0.3 (proper-info of
484 IMPUTE) or R2<0.3 (MACH) were removed from the analyses. Each individual study performed a
485 linear regression between each endophenotype (IOP, VCDR, cup area, and disc area) and the SNPs,
486 under the assumption of an additive model for the effect of the risk allele. Analyses were adjusted
487 for age, sex and the first five principal components (for population-based studies) or family structure
488 (for family-based studies).

489 We performed an inverse variance weighted fixed-effect meta-analysis with METAL software(65). P
490 values for heterogeneity were calculated by using the Cochran's Q-test for heterogeneity. SNPs with
491 a p-value for heterogeneity <0.001 were removed from the results, as well as SNPs only present in
492 three studies. We used the 'genomic control' option in METAL to correct the standard error of each
493 individual study for estimated genomic inflation. To evaluate if our results exhibit signs of population
494 stratification we estimated the intercept using the LD score regression method(18) in the European-
495 only meta-analyses. The intercept, which estimates inflation after removing polygenic signals, was:
496 1.0142 for IOP, 1.0199 for VCDR, 1.0074 for cup area and 1.0167 for disc area (**Table S16**).

497 In the meta-analyses of individuals with European ancestry, a p-value $<5.0 \times 10^{-8}$ (the genome-wide
498 threshold of association) was considered significant. In the second stage, these genome-wide
499 significant SNPs were validated in individuals with Asian ancestry, and in this look-up a p value <0.05

500 was considered significant. Finally, in the mega/meta-analysis of individuals with European and Asian
501 ancestry a p-value of $<5.0 \times 10^{-8}$ was considered significant. In total, we identified 75 independent
502 SNPs across different genomic regions for all the traits together. Therefore, the significance level
503 after Bonferroni correction in the meta-analysis of POAG cohorts was = 6.67×10^{-4} (0.05 / 75
504 independent SNPs). To estimate the common effect size of the top SNPs associated with IOP, optic
505 disc parameters and their effect in the look-up in the POAG cohorts a random-effect meta-analysis
506 was performed using plink(66) http://pngu.mgh.harvard.edu/purcell/plink/ parameter *--meta-*
507 *analysis*. Manhattan, regional and forest plots were made using R(67) and LocusZoom(68).

508 Genetic correlation between optic disc parameters (VCDR, cup and disc area) and IOP was estimated
509 using the LD score regression method(18, 69). GWAS summary statistics from the Europeans-only
510 meta-analyses were used to calculate the genetic overlap. To restrict the analyses to well imputed
511 SNPs, we included only SNPs with MAF > 0.01 that were present in HapMap3. GWAS data were
512 harmonized using the "munge_sumstats.py" function. For analyses we used the pre-computed LD
513 scores for Europeans "eur_w_ld_ch" available at https://github.com/bulik/ldsc/wiki. The ldsc.py
514 function (with all default settings) was used to calculate the genetic correlation between traits. In
515 addition, we calculated the heritability (h^2) estimates from the GWAS meta-analysis using the same
516 function.

517 **Gene-based test using VEGAS**

518 A gene-based test was performed using the VEGAS2 software(70), with a 50kb gene boundary. We
519 used the parameter '-top 100' (default) to perform gene-based tests. This parameter considers
520 association test statistics of all variants mapped to a gene to compute gene-based test statistics. The
521 1000 Genomes European and Asian populations were used as a reference to calculate LD for
522 European and Asian ancestry data respectively. After calculation of gene-based test statistics for
523 Asian and European ancestry populations separately, meta-analyses were conducted using Fisher's
524 method for combining p-values.

525 **Functional characterization, expression data, zebrafish and gene-set enrichment**

526 We investigated for evidence of regulatory functions of associated loci HaploReg version 2(71) and
527 Regulomedb version 1.1(72). We investigated the expression of the associated genes using NCBI's
528 UniGene(23) and The Ocular Tissue Database(24). We also investigated the expression of *cdkn1a* in a
529 *six6b* knockdown zebrafish and used DEPICT to investigate gene-set enrichment. More information
530 about these analyses can be found in the **Supplementary Material**.

531 **Data access**

532 Access to the summary association statistics for IOP and optic disc parameters is available at
533 http://getfiles.qimr.edu.au/puyaGharahkhani/1000G_imputed_IGGC_eye_quantitative_traits/

534

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Conflict of interest

Dr. Pasquale has been a paid speaker for Allergan. He also served as a nonpaid consultant to Novartis and a paid consultant to Bausch + Lomb. He has received support to travel to the Exfoliation Glaucoma Think Tank Meeting in NYC by the Glaucoma Foundation.

Dr. Jonas: Consultant for MundiPharma Co.; Allergan Inc.; Merck Sharp & Dohme Co., Inc.; Alimera Co.; Boehringer Ingelheim Co., Sanofi Co., Pfizer Co.; Patent holder with CellMed AG, Alzenau, Germany and with University of Heidelberg / Germany

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Legends to Figures

Figure 1. Overlap between the genes associated with one or more endophenotypes. Genes with a genome-wide significant association for at least one trait are shown. These genes are counted as overlapping genes if they are Bonferroni significantly associated with the other trait(s). Chr 11p11.2 (see intraocular pressure circle) means a region on chromosome 11p11.2 that is associated with IOP and has many genes in it; the likely causative gene in this region is not identified yet. Genes in bold are genes associated with primary open-angle glaucoma (POAG) in our meta-analysis of four case-control studies.*Genes associated with familial forms of POAG (e.g. *MYOC* and *OPTN*) or found in case-control association studies which did not show an association with the endophenotypes explored in this study.

Figure 2. Pathways significantly enriched for: A) Loci associated with the vertical cup-disc ratio, cup area and intraocular pressure (p-value <7.0 x 10-6 in the GWAS). In total 11 meta-pathways were identified after clustering the 57 pathways identified by DEPICT. B) Loci associated with vertical cup-disc ratio, cup area and disc area (p-value <1.0 x 10-5). In total 17 meta-pathways were identified after clustering the 100 pathways identified by DEPICT. In both figures, meta-pathways are represented by nodes coloured according to statistical significance, and edges are scaled according to the correlation between meta-pathways. *The pathway "Abnormal eye morphology" clustered with the meta-pathway "Chordate embryonic development". ELL2=Elongation Factor, RNA Polymerase II, DVL3= Dishevelled Segment Polarity Protein 3, THBS1=Thrombospondin 1, RFX2= Regulatory Factor X, 2. MDFI=MyoD Family Inhibitor.

Figure 3. ***cdkn1a* mRNA expression change**

Overexpression of *cdkn1a* and *cdkn2a/cdkn2b* in response to six6b depletion is shown. All samples expression were normalized to the control gene sdha. Relative expression was calculated by setting the wild-type expression level at 1. Values represent mean ± standard error of the mean. *P<0.05; **P<0.005.

Table 1. Single nucleotide polymorphisms (SNPs) that are genome-wide significantly associated with IOP and show and association with vertical cup-disc ratio.

			IOP			VCDR			Cup area			Disc area		
SNP	**Nearest gene**	**A1/A2**	**β**	**SE**	**P**	**β**	**SE**	**P**	**β**	**SE**	**P**	**B**	**SE**	**P**
rs10918274	*TMCO1*	t/c	0.26	0.04	**5.64E-12**	0.005	0.002	8.38E-03	0.010	0.003	2.47E-03	0.000	0.006	9.49E-01
rs7635832	*FNDC3B*	g/t	-0.22	0.03	**6.61E-13**	-0.001	0.001	3.35E-01	-0.004	0.003	1.27E-01	0.002	0.005	7.08E-01
rs10281637	*CAV1/CAV2*	c/t	0.20	0.03	**3.96E-13**	0.004	0.001	5.28E-03	0.006	0.003	1.23E-02	-0.002	0.005	6.01E-01
8:78380944*	*PKIA*	i/r	1.00	0.17	**7.54E-09**	0.000	0.010	9.74E-01	-0.018	0.017	3.00E-01	0.018	0.031	5.61E-01
rs7815043*	*PKIA*	c/t	-0.10	0.03	**4.41E-05**	-0.001	0.001	3.13E-01	-0.001	0.002	8.32E-01	-0.002	0.004	5.66E-01
rs7944735	*Many genes*	c/g	0.19	0.03	**6.00E-11**	0.001	0.001	4.37E-01	0.006	0.003	3.33E-02	0.000	0.005	9.68E-01
11:120357425	*ARHGEF12*	d/r	0.18	0.03	**2.02E-09**	0.001	0.001	6.12E-01	0.001	0.003	6.45E-01	0.001	0.005	8.38E-01
rs12794618	*ARHGEF12*	c/t	0.17	0.03	**7.86E-09**	0.001	0.001	4.14E-01	0.002	0.003	4.84E-01	0.004	0.005	4.53E-01
rs55796939	*ADAMTS8*	t/c	0.36	0.06	**2.31E-08**	0.003	0.003	3.61E-01	0.006	0.006	3.19E-01	-0.003	0.010	7.95E-01
rs2472496	*ABCA1*	g/a	0.17	0.02	**1.93E-13**	0.004	0.001	**6.83E-05**	0.010	0.002	**9.63E-07**	0.003	0.004	4.75E-01
rs8176741	*ABO*	a/g	0.24	0.04	**3.47E-10**	0.007	0.002	**4.51E-05**	0.019	0.003	**7.12E-08**	0.004	0.006	5.42E-01
rs9913911	*GAS7*	g/a	-0.17	0.02	**7.01E-12**	-0.006	0.001	**1.84E-07**	-0.008	0.002	**2.48E-04**	-0.001	0.004	8.41E-01

For these SNPs, the associations with the other traits are also included. SNPs that are Bonferroni significantly associated with other traits are shown in bold (p-value < 5.37×10^{-4}; 0.05/93). In the first rows, the SNPs genome-wide significantly associated with intraocular pressure (IOP) are shown. Next, the SNPs associated with IOP, vertical cup-disc ratio (VCDR), and cup area are shown. Nearest gene, reference NCBI build37; A1, reference allele; A2, other allele; β, effect size on the endophenotype (IOP, VCDR, cup area or disc area) based on allele A1; SE, standard error of the effect size; i, insertion; d, deletion; r, reference. *This locus was not considered as new because the signal came from only one INDEL (8:78380944). *Genome-wide associated in the European-only meta-analysis

Table 2a. Single nucleotide polymorphisms (SNPs) that are genome-wide significantly associated with at least one of the optic disc parameters.

			IOP			VCDR			Cup area			Disc area		
SNP	**Nearest**	**A1/A2**	**β**	**SE**	**P**	**β**	**SE**	**P**	**β**	**SE**	**P**	**B**	**SE**	**P**
rs6804624	*COL8A1*	c/t	-0.01	0.03	6.54E-01	0.008	0.001	**8.63E-12**	0.013	0.002	**1.99E-08**	0.020	0.004	**9.67E-07**
rs7916697	*ATOH7*	a/g	0.01	0.03	7.43E-01	-0.018	0.001	**2.46E-45**	-0.017	0.002	**1.32E-12**	-0.094	0.004	**1.34E-102**
10:96008348	*PLCE1*	d/r	0.01	0.03	5.73E-01	0.007	0.001	**4.57E-08**	0.013	0.002	**1.72E-08**	0.015	0.004	**2.22E-04**
rs324780	*TMTC2*	g/a	0.03	0.02	2.79E-01	-0.011	0.001	**7.16E-23**	-0.016	0.002	**1.57E-13**	-0.029	0.004	**8.58E-13**
rs4299136	*ASB7*	c/g	-0.03	0.03	4.22E-01	0.010	0.002	**2.68E-12**	0.018	0.003	**4.09E-10**	0.024	0.005	**4.02E-06**
16:51461915	*SALL1*	r/i	0.02	0.03	4.34E-01	0.010	0.001	**2.62E-13**	0.013	0.003	**6.78E-07**	0.032	0.005	**2.38E-12**
rs4784295	*SALL1*	c/g	0.02	0.03	5.63E-01	0.009	0.001	**3.93E-13**	0.013	0.003	**1.63E-07**	0.031	0.005	**1.12E-11**
rs5752773	*CHEK2*	g/c	0.01	0.03	6.91E-01	-0.012	0.001	**1.49E-20**	-0.024	0.003	**4.12E-21**	-0.024	0.005	**1.48E-07**
rs2092172	*CARD10*	a/g	0.00	0.03	8.86E-01	0.009	0.001	**3.08E-12**	0.011	0.003	**3.34E-05**	0.032	0.005	**1.44E-11**
rs7717697	*VCAN*	c/t	0.01	0.02	7.21E-01	-0.007	0.001	**6.66E-09**	-0.009	0.002	**1.19E-05**	-0.018	0.004	**4.84E-06**
rs1681739	*ENO4*	t/c	0.03	0.02	2.23E-01	0.006	0.001	**2.44E-08**	0.011	0.002	**3.70E-07**	0.019	0.004	**1.85E-06**
rs60779155	*ASB7*	a/g	-0.02	0.04	6.61E-01	0.010	0.002	**3.76E-10**	0.019	0.003	**3.75E-09**	0.030	0.006	**8.26E-08**
rs1830890	*PLCE1*	g/a	0.01	0.02	8.14E-01	0.006	0.001	**3.02E-08**	0.012	0.002	**1.06E-07**	0.013	0.004	5.51E-04
rs482507	*TMTC2*	c/t	0.02	0.02	3.48E-01	-0.011	0.001	**2.19E-19**	-0.017	0.002	**2.56E-14**	-0.030	0.004	**4.49E-13**
rs4436712	*SIX6*	t/g	-0.04	0.02	1.47E-01	0.009	0.001	**5.48E-14**	0.025	0.002	**1.50E-29**	-0.018	0.004	**6.59E-06**
rs738722	*CHEK2*	t/c	0.02	0.03	3.57E-01	-0.012	0.001	**4.94E-20**	-0.024	0.003	**7.81E-22**	-0.021	0.005	**2.63E-06**
rs2684249	*HSF2*	c/t	0.03	0.02	2.08E-01	-0.006	0.001	**1.64E-07**	-0.012	0.002	**3.04E-08**	-0.015	0.004	**1.49E-04**
rs34222435	*ASB7*	t/c	-0.03	0.03	3.86E-01	0.010	0.002	**3.07E-12**	0.019	0.003	**1.07E-10**	0.025	0.005	**2.98E-06**

			IOP			VCDR			Cup area			Disc area		
SNP	**Nearest**	**A1/A2**	**β**	**SE**	**P**	**β**	**SE**	**P**	**β**	**SE**	**P**	B	SE	P
rs7916410	*ATOH7*	t/c	0.00	0.03	9.76E-01	-0.018	0.001	**1.14E-45**	-0.017	0.002	**6.11E-12**	-0.097	0.004	**7.06E-109**
rs442376	*TMTC2*	c/t	-0.03	0.03	3.09E-01	0.011	0.001	**1.50E-17**	0.017	0.002	**3.18E-12**	0.032	0.004	**4.92E-14**
rs1345467	*SALL1*	g/a	0.01	0.03	6.53E-01	0.009	0.001	**4.96E-12**	0.012	0.003	**1.07E-06**	0.032	0.005	**6.41E-13**
rs5762752	*CHEK2*	c/g	0.01	0.03	6.61E-01	-0.011	0.001	**4.83E-18**	-0.021	0.002	**6.72E-19**	-0.023	0.004	**2.26E-08**
rs11129176	*RARB*	a/g	0.02	0.03	4.17E-01	0.005	0.001	**3.17E-05**	0.010	0.002	**1.01E-05**	0.023	0.004	**3.40E-08**
rs1997404	*COL8A1*	g/t	-0.03	0.03	3.24E-01	0.008	0.001	**2.39E-11**	0.013	0.002	**7.71E-08**	0.024	0.004	**1.90E-08**
rs34935520	*SIX6*	g/a	-0.04	0.02	1.13E-01	0.009	0.001	**7.95E-14**	0.025	0.002	**6.96E-29**	-0.023	0.004	**7.61E-08**

For these SNPs, the associations with the other traits are also included. Here the SNPs genome-wide significantly associated with at least one of the optic disc parameters and that are Bonferroni significantly associated with the other disc parameters are shown in bold (p-value < 5.37×10^{-4}; 0.05/93).Nearest gene, reference NCBI build37; A1, reference allele; A2, other allele; β, effect size on the effect size on the endophenotype (IOP, VCDR, cup area or disc area) based on allele A1; SE, standard error of the effect size; i, insertion; d, deletion; r, reference.

Table2b. Single nucleotide polymorphisms (SNPs) that are genome-wide significantly associated with vertical cup-disc ratio or with cup area

			IOP			VCDR			Cup area			Disc area		
SNP	**Nearest gene**	**A1/A**	**β**	**SE**	**P**	**β**	**SE**	**P**	**β**	**SE**	**P**	**B**	**SE**	**P**
rs1925953	*RPE65**	t/a	-0.02	0.02	3.26E-01	0.006	0.001	**1.55E-07**	0.010	0.002	**1.50E-05**	0.006	0.004	1.08E-01
rs72759609	*PDZD2*	c/t	-0.04	0.05	3.50E-01	-0.012	0.002	**7.10E-09**	-0.020	0.004	**1.98E-06**	-0.021	0.008	5.62E-03
rs11450334	*DUSP1*	t/c	-0.12	0.08	1.27E-01	-0.021	0.004	**1.31E-08**	-0.035	0.007	**2.90E-07**	-0.035	0.013	5.83E-03
rs4960295	*RREB1*	a/g	0.02	0.02	4.75E-01	0.007	0.001	**2.49E-10**	0.009	0.002	**3.73E-05**	0.012	0.004	3.29E-03
rs10274998	*DGKB*	t/c	0.02	0.03	4.38E-01	0.008	0.001	**4.68E-08**	0.012	0.003	**8.08E-06**	0.011	0.005	2.65E-02
rs2157719	*CDKN2B-AS1*	c/t	-0.04	0.02	9.81E-02	-0.013	0.001	**3.75E-35**	-0.024	0.002	**3.31E-28**	-0.008	0.004	3.03E-02
rs3891783	*PLCE1*	g/c	0.04	0.02	1.01E-01	0.007	0.001	**1.06E-10**	0.011	0.002	**3.28E-07**	0.012	0.004	1.52E-03
rs1346	*SSSCA1*	t/a	-0.05	0.03	1.20E-01	-0.013	0.002	**7.51E-18**	-0.019	0.003	**9.31E-11**	-0.016	0.005	2.10E-03
rs4936099	*ADAMTS8*	c/a	-0.03	0.03	2.38E-01	-0.007	0.001	**6.70E-09**	-0.013	0.002	**4.96E-08**	-0.006	0.004	1.72E-01
13:3662990	*DCLK1*	d/r	-0.02	0.03	5.70E-01	0.007	0.001	**2.98E-08**	0.018	0.002	**2.20E-14**	-0.005	0.004	2.36E-01
rs7323428	*DCLK1*	t/g	-0.02	0.03	4.13E-01	0.007	0.001	**1.86E-08**	0.019	0.002	**1.67E-15**	-0.005	0.004	2.23E-01
rs8015152	*SIX6*	t/c	-0.06	0.02	2.27E-02	0.010	0.001	**2.86E-18**	0.024	0.002	**8.15E-26**	-0.011	0.004	6.18E-03
rs6107845	*BMP2*	a/g	0.03	0.02	2.80E-01	-0.009	0.001	**3.44E-17**	-0.017	0.002	**2.90E-15**	-0.004	0.004	3.27E-01
rs6764184	*FLNB*	t/g	0.05	0.03	5.03E-02	0.007	0.001	**1.89E-08**	0.015	0.002	**1.30E-10**	0.010	0.004	1.92E-02
rs7311936	*FAM101A*	c/g	-0.03	0.02	1.69E-01	-0.006	0.001	**2.48E-09**	-0.013	0.002	**4.52E-09**	0.003	0.004	5.14E-01
14:2338879	*RBM23*	r/d	0.02	0.03	3.99E-01	0.007	0.001	**2.56E-08**	0.013	0.003	**2.01E-07**	0.009	0.005	4.29E-02
rs3794453	*RBM23*	a/t	0.01	0.02	7.22E-01	0.007	0.001	**7.25E-08**	0.011	0.002	**2.88E-07**	0.009	0.004	3.11E-02
rs2252865	*RERE*	t/c	0.05	0.03	4.11E-02	0.005	0.001	**2.66E-05**	0.014	0.002	**1.33E-09**	0.003	0.004	5.08E-01

			IOP			VCDR			Cup area			Disc area		
SNP	**Nearest gene**	**A1/A**	**β**	**SE**	**P**	**β**	**SE**	**P**	**β**	**SE**	**P**	**B**	**SE**	**P**
rs13016883	*TRIB2*	c/g	0.01	0.03	5.64E-01	0.006	0.001	**3.44E-06**	0.016	0.002	**1.83E-11**	0.001	0.004	8.30E-01
rs35084382	*DUSP1*	c/t	-0.10	0.07	1.32E-01	-0.018	0.003	**2.05E-08**	-0.033	0.006	**2.17E-08**	-0.031	0.011	5.51E-03
rs11759831	*CRISPLD1**	t/g	-0.05	0.05	3.10E-01	0.009	0.002	**1.07E-04**	0.021	0.004	**1.66E-06**	0.022	0.008	5.47E-03
rs1360589	*CDKN2B-AS1*	c/t	-0.04	0.02	8.42E-02	-0.013	0.001	**1.43E-34**	-0.024	0.002	**2.90E-28**	-0.008	0.004	4.45E-02
rs11613189	*FAM101A*	t/c	-0.03	0.03	2.27E-01	-0.005	0.001	**6.04E-06**	-0.016	0.002	**2.01E-12**	0.002	0.004	6.42E-01
rs2251069	*DDHD1*	c/t	0.01	0.02	7.29E-01	-0.006	0.001	**7.41E-08**	-0.013	0.002	**1.20E-09**	0.001	0.004	7.11E-01
rs6598351	*FAM169B**	t/c	-0.02	0.03	5.26E-01	0.006	0.001	**2.80E-05**	0.012	0.003	**1.77E-05**	-0.004	0.005	3.90E-01
rs11646917	*SALL1*	t/g	-0.01	0.03	6.65E-01	-0.009	0.001	**4.83E-10**	-0.015	0.003	**4.76E-09**	-0.015	0.005	1.30E-03
rs11867840	*BCAS3*	g/a	0.04	0.03	1.04E-01	-0.006	0.001	**4.86E-06**	-0.018	0.002	**2.35E-13**	0.011	0.004	1.00E-02
rs6054375	*BMP2*	t/g	0.03	0.03	2.45E-01	-0.010	0.001	**6.92E-15**	-0.018	0.002	**1.83E-15**	-0.003	0.004	4.74E-01
rs3791679	*EFEMP1/PNPT*	g/a	0.04	0.03	1.72E-01	-0.005	0.001	**1.17E-04**	-0.013	0.002	**4.92E-08**	0.003	0.004	5.14E-01
rs12494328	*FLNB*	a/g	0.04	0.03	1.52E-01	0.006	0.001	**1.56E-06**	0.016	0.002	**6.03E-11**	0.009	0.004	4.50E-02
6:36592986	*CDKN1A*	d/r	-0.02	0.03	5.32E-01	0.006	0.001	**1.92E-05**	0.015	0.003	**1.12E-08**	-0.006	0.005	2.09E-01
rs72852338	*CDKN1A*	c/a	-0.02	0.03	5.46E-01	0.006	0.001	**3.29E-05**	0.014	0.003	**3.17E-08**	-0.005	0.005	2.97E-01
rs1074407	*TRIOBP*	t/a	0.11	0.02	**4.00E-06**	0.006	0.001	**3.32E-07**	0.012	0.002	**1.90E-08**	0.008	0.004	3.92E-02

For these SNPs, the associations with the other traits are also included. Here the SNPs genome-wide significantly associated with vertical cup-disc ratio or cup area and that are Bonferroni significantly associated with VCDR or cup area are shown in bold (p-value < 5.37×10^{-4}; 0.05/93).Nearest gene, reference NCBI build37; A1, reference allele; A2, other allele; β, effect size on effect size on the endophenotype (IOP, VCDR, cup area or disc area) based on allele A1; SE, standard error of the effect size; i, insertion; d, deletion; r, reference.*Genome-wide associated in the European-only meta-analysis

Table2c. **Single nucleotide polymorphisms (SNPs) that are genome-wide significantly associated with vertical cup-disc ratio or with disc area**

			IOP			VCDR			Cup area			Disc area		
SNP	**Nearest gene**	**A1/A**	**β**	**SE**	**P**	**β**	**SE**	**P**	**β**	**SE**	**P**	**B**	**SE**	**P**
rs1192414	*CDC7/TGFBR*	a/g	0.06	0.03	5.66E-02	0.014	0.001	**1.78E-23**	0.007	0.003	1.12E-02	0.087	0.005	**7.44E-71**
rs10753787	*F5*	t/c	-0.03	0.02	1.69E-01	-0.007	0.001	**2.48E-09**	-0.005	0.002	2.14E-02	-0.019	0.004	**1.60E-06**
rs2920293	*PSCA*	g/c	0.00	0.02	8.57E-01	-0.006	0.001	**5.04E-09**	-0.007	0.002	9.17E-04	-0.015	0.004	**9.94E-05**
rs4658101	*CDC7/TGFBR*	a/g	0.06	0.03	4.46E-02	0.013	0.001	**5.19E-23**	0.007	0.003	1.13E-02	0.089	0.005	**8.01E-77**
1:16953052	*F5/SELP*	i/r	0.02	0.03	4.22E-01	0.007	0.001	**7.20E-07**	0.005	0.003	5.44E-02	0.033	0.005	**1.49E-12**
rs2239854	*F5/SELP*	a/g	0.03	0.03	2.64E-01	0.006	0.001	**8.37E-07**	0.005	0.002	5.04E-02	0.030	0.004	**7.60E-13**
rs9843102	*ABI3BP*	a/g	0.00	0.03	9.84E-01	-0.006	0.002	**2.18E-04**	-0.002	0.003	5.88E-01	-0.036	0.005	**1.35E-11**
8:88744441	*DCAF4L2*	d/r	-0.01	0.02	6.98E-01	0.006	0.001	**6.66E-07**	0.006	0.002	4.53E-03	0.026	0.004	**2.04E-11**
rs6468996	*DCAF4L2*	t/c	0.00	0.02	9.12E-01	0.005	0.001	**2.52E-07**	0.006	0.002	2.14E-03	0.025	0.004	**5.16E-11**
rs61101201	*ELP4/PAX6*	g/t	0.02	0.03	5.51E-01	0.006	0.001	**2.27E-06**	0.005	0.002	4.51E-02	0.028	0.004	**1.53E-10**
rs56385951	*CARD10*	a/g	-0.06	0.04	9.08E-02	0.011	0.002	**1.87E-11**	0.008	0.003	8.83E-03	0.047	0.006	**1.49E-16**
1:3046430	*PRDM16*	i/r	-0.04	0.04	4.14E-01	0.007	0.002	5.35E-04	-0.002	0.004	7.15E-01	0.044	0.007	**1.79E-09**
rs12028027	*PRDM16*	c/t	-0.03	0.04	4.97E-01	0.007	0.002	**2.15E-04**	-0.001	0.004	8.58E-01	0.043	0.007	**1.46E-09**

For these SNPs, the associations with the other traits are also included. Here the SNPs genome-wide significantly associated with vertical cup-disc ratio or with disc area that are Bonferroni significantly associated with the VCDR or disc area are shown in bold (p-value < 5.37×10^{-4}; 0.05/93).Nearest gene, reference NCBI build37; β, effect size on effect size on the endophenotype (IOP, VCDR, cup area or disc area) based on allele A1; SE, standard error of the effect size; i, insertion; d, deletion; r, reference.

Table 3 Single nucleotide polymorphisms (SNPs) that are genome-wide significantly associated with optic nerve head parameters (cup area and disc area)

			IOP			VCDR			Cup area			Disc area		
SNP	**Nearest gene**	**A1/A2**	**β**	**SE**	**P**	**β**	**SE**	**P**	**β**	**SE**	**P**	**B**	**SE**	**P**
1:227562773	*CDC42BPA*	d/r	-0.10	0.05	3.01E-02	0.003	0.002	2.37E-01	0.024	0.004	**8.05E-09**	-0.055	0.008	**3.65E-13**
rs73102394	*CDC42BPA*	t/c	-0.09	0.05	4.34E-02	0.003	0.002	1.62E-01	0.022	0.004	**4.16E-08**	-0.053	0.007	**5.01E-13**
rs11811982	*CDC42BPA*	a/c	-0.12	0.05	1.35E-02	0.004	0.002	5.54E-02	0.027	0.004	**2.31E-10**	-0.062	0.008	**2.02E-15**
rs10021731	*UGT8*	c/t	0.01	0.02	8.23E-01	-0.002	0.001	5.56E-02	-0.002	0.002	2.68E-01	-0.020	0.004	**7.48E-07**
rs12220165	*CTNNA3**	g/c	0.02	0.03	5.88E-01	-0.004	0.002	1.47E-02	-0.004	0.003	1.92E-01	-0.023	0.005	**2.51E-05**
rs787541	*U6, GADD45A*	c/g	0.07	0.03	7.08E-03	0.002	0.001	7.47E-02	0.002	0.002	4.82E-01	0.023	0.004	**6.66E-08**
rs1367187	*DIRC3*	c/t	-0.07	0.03	9.74E-03	0.002	0.001	2.46E-01	-0.002	0.003	4.87E-01	0.026	0.005	**1.03E-08**
rs2443724	*VGLL4*	c/g	0.00	0.02	8.62E-01	-0.003	0.001	1.53E-02	0.000	0.002	9.15E-01	-0.022	0.004	**4.72E-08**
rs1013830	*CTNNA3*	t/c	0.00	0.05	9.49E-01	-0.007	0.002	4.80E-03	-0.004	0.005	4.10E-01	-0.046	0.008	**5.45E-08**

For these SNPs, the associations with the other traits are also included. SNPs that are Bonferroni significantly associated with other traits are shown in bold (p-value < 5.37×10^{-4}; 0.05/93). In the first rows, the SNPs genome-wide significantly associated with cup area are shown. Next, SNPs associated with only disc area, are shown. Nearest gene, reference NCBI build37; A1, reference allele; A2, other allele; β, effect size on the endophenotype (IOP, VCDR, cup area or disc area) based on allele A1; SE, standard error of the effect size; i, insertion; d, deletion; r, reference. *Genome-wide associated in the European-only meta-analysis.

Table 4. **Association with primary open-angle glaucoma in a meta-analysis of four independent glaucoma case-control studies (ANZRAG, NEIGHBORHOOD, Singapore, and Southampton).**

	Nearest gene	A1/A2	OR (95% CI)	OR (R)	P-value	P-value (R)	Direction	I2	P-value of heterogeneity
IOP SNPs									
rs10918274	*TMCO1*	t/c	1.39 (1.30-1.50)	1.39	**2.75E-19**	**1.37E-09**	++++	38.4	1.82E-01
rs7635832	*FNDC3B*	g/t	0.89 (0.83-0.95)	0.91	1.41E-03	3.65E-02	---?	33.9	2.20E-01
rs10281637	*CAV1/CAV2*	c/t	1.13 (1.07-1.20)	1.13	**2.32E-05**	**2.32E-05**	++++	0	4.89E-01
rs2487048	*ABCA1*	a/g	1.26 (1.19-1.33)	1.26	**2.65E-15**	3.82E-03	++++	82.9	5.53E-04
rs8176741	*ABO*	a/g	1.07 (0.99-1.17)	1.04	7.36E-02	5.25E-01	-+-+	58.5	6.51E-02
rs7944735	Many genes *(NUP160, PTPRJ)*	c/g	1.06 (1.01-1.13)	1.07	2.99E-02	2.99E-02	++++	0	8.99E-01
11:120357425	*ARHGEF12*	d/r	1.16 (1.09-1.23)	1.19	**1.52E-06**	3.02E-02	++++	83.2	4.65E-04
rs55796939	*ADAMTS8*	t/c	1.07 (0.94-1.24)	1.17	2.72E-01	4.46E-01	+?--	78.6	9.35E-03
rs9913911	*GAS7*	g/a	0.80 (0.76-0.84)	0.80	**1.08E-17**	**1.08E-17**	----	0	7.50E-01
VCDR SNPs									
rs1925953	*RPE65*	t/a	1.07 (1.02-1.13)	1.10	4.21E-03	2.01E-02	++++	46.7	1.31E-01
rs1192414	*CDC7/TGFBR3*	a/g	1.08 (1.02-1.16)	1.08	9.26E-03	9.26E-03	++++	0	7.27E-01
rs10753787	*F5*	t/c	0.97 (0.93-1.03)	0.97	3.67E-01	3.67E-01	----	0	9.92E-01
rs6804624	*COL8A1*	c/t	0.99 (0.94-1.05)	0.99	8.14E-01	8.14E-01	---+	0	8.42E-01
rs72759609	*PDZD2*	c/t	0.90 (0.83-0.99)	0.91	3.20E-02	3.20E-02	----	0	9.53E-01
rs114503346	*DUSP1*	t/c	1.00 (0.80-1.25)	1.00	9.99E-01	8.80E-01	+?-+	42	1.78E-01
rs4960295	*RREB1*	a/g	0.99 (0.95-1.05)	1.00	9.50E-01	9.09E-01	-+-+	4.6	3.70E-01
rs10274998	*DGKB*	t/c	1.03 (0.98-1.10)	1.04	2.16E-01	2.16E-01	++-+	0	5.38E-01
rs2157719	*CDKN2B-AS1*	c/t	0.69 (0.66-0.74)	0.69	**1.29E-40**	**1.29E-40**	----	0	5.67E-01
rs1900005	*ATOH7*	a/c	1.01 (0.96-1.07)	1.01	6.98E-01	6.77E-01	+-++	5.1	3.67E-01
10:96008348	*PLCE1*	d/r	1.02 (0.97-1.09)	1.04	3.38E-01	3.15E-01	+-+?	35.3	2.13E-01
rs1346	*SSSCA1*	t/a	0.90 (0.85-0.97)	0.91	2.41E-03	2.41E-03	----	0	9.04E-01
rs4936099	*ADAMTS8*	c/a	0.94 (0.9-1.00)	0.94	5.75E-02	5.75E-02	----	0	9.63E-01
rs324780	*TMTC2*	g/a	0.93 (0.89-0.99)	0.93	1.35E-02	1.35E-02	----	0	7.69E-01
13:36629905	*DCLK1*	d/r	0.99 (0.94-1.05)	0.99	7.53E-01	8.00E-01	--+-	6.2	3.62E-01
rs8015152	*SIX6*	t/c	1.21 (1.16-1.28)	1.19	**3.90E-15**	**7.08E-05**	++++	62.4	4.62E-02
rs4299136	*ASB7*	c/g	1.03 (0.97-1.10)	1.03	3.55E-01	3.55E-01	++-+	0	8.29E-01
16:51461915	*SALL1*	i/r	0.94 (0.89-1.00)	0.94	3.85E-02	3.85E-02	----	0	7.82E-01
rs6107845	*BMP2*	a/g	0.89 (0.85-0.94)	0.91	1.02E-05	6.94E-03	----	43.1	1.53E-01
rs5752773	*CHEK2*	g/c	0.92 (0.88-0.98)	0.92	4.63E-03	4.63E-03	----	0	9.12E-01
rs2092172	*CARD10*	a/g	0.97 (0.92-1.04)	0.98	4.35E-01	4.35E-01	--+-	0	7.76E-01
rs6764184	*FLNB*	t/g	1.07 (1.02-1.13)	1.02	5.73E-03	7.66E-01	++-+	86.1	8.14E-05
rs7717697	*VCAN*	c/t	0.98 (0.93-1.04)	0.98	5.26E-01	5.26E-01	---?	0	7.30E-01
rs2920293	*PSCA*	g/c	1.03 (0.98-1.09)	1.03	2.25E-01	2.25E-01	++-?	0	3.79E-01
rs1681739	*ENO4*	t/c	1.02 (0.97-1.08)	1.03	3.92E-01	3.99E-01	+--+	49.2	1.16E-01
rs7311936	*FAM101A*	c/g	0.99 (0.95-1.04)	1.00	8.12E-01	8.59E-01	+---	11	3.38E-01
14:23388793	*RBM23*	r/d	1.03 (0.98-1.10)	1.03	1.83E-01	1.83E-01	+++?	0	4.61E-01
Cup area SNPs									
rs2252865	*RERE*	t/c	1.11 (1.06-1.18)	1.11	5.76E-05	2.87E-02	++-+	59.3	6.10E-02
rs4846112	*DHRS3*	a/g	0.95 (0.91-1.01)	0.96	1.18E-01	1.18E-01	----	0	5.53E-01
1:227562773	*CDC42BPA*	d/r	0.87 (0.79-0.97)	0.90	1.14E-02	2.11E-01	--+?	48.6	1.43E-01
rs13016883	*TRIB2*	c/g	1.08 (1.03-1.14)	1.08	4.25E-03	4.25E-03	+++?	0	8.63E-01
rs35084382	*DUSP1*	c/t	1.04 (0.85-1.29)	1.05	6.72E-01	6.72E-01	+?-+	0	3.91E-01
rs117598310	*CRISPLD1*	t/g	1.08 (1.00-1.19)	1.09	5.39E-02	5.39E-02	+++	0	8.01E-01
rs1360589	*CDKN2B-AS1*	c/t	0.69 (0.66-0.73)	0.69	**1.90E-42**	**1.90E-42**	----	0	6.47E-01
rs10998036	*ATOH7*	c/g	1.01 (0.96-1.08)	1.02	5.42E-01	5.72E-01	+---	26	2.55E-01
10:96008348	*PLCE1*	d/r	1.02 (0.97-1.09)	1.04	3.38E-01	3.15E-01	+-+?	35.3	2.13E-01
rs1346	*SSSCA1*	t/a	0.90 (0.85-0.97)	0.91	2.41E-03	2.41E-03	----	0	9.04E-01
rs482507	*TMTC2*	c/t	0.94 (0.89-0.99)	0.94	2.03E-02	2.03E-02	----	0	7.46E-01
rs11613189	*FAM101A*	t/c	0.99 (0.95-1.05)	0.99	8.25E-01	7.77E-01	++--	18.5	2.98E-01
rs7323428	*DCLK1*	t/g	0.99 (0.94-1.05)	1.00	7.83E-01	8.87E-01	+-+-	13.6	3.25E-01
rs2251069	*DDHD1*	c/t	0.95 (0.91-1.00)	0.96	7.62E-02	7.62E-02	--+-	0	4.08E-01
rs4436712	*SIX6*	t/g	1.24 (1.19-1.31)	1.23	**5.77E-18**	**1.52E-07**	++++	48.8	1.19E-01

	Nearest gene	A1/A2	OR (95% CI)	OR (R)	P-value	P-value (R)	Direction	I2	P-value of heterogeneity
Cup area SNPs									
rs6598351	*FAM169B*	t/c	0.99 (0.93-1.06)	0.99	8.06E-01	8.06E-01	-+--	0	7.11E-01
rs11646917	*SALL1*	t/3g	0.98 (0.93-1.04)	0.98	5.49E-01	5.49E-01	--++	0	5.97E-01
rs11867840	*BCAS3*	g/a	1.06 (1.01-1.13)	1.06	1.83E-02	2.12E-02	++++	8.3	3.51E-01
rs6054375	*BMP2*	t/g	0.89 (0.85-0.94)	0.91	8.52E-06	9.93E-03	----	47.1	1.29E-01
rs738722	*CHEK2*	t/c	0.93 (0.89-0.99)	0.93	1.26E-02	1.26E-02	----	0	9.05E-01
rs3791679	*EFEMP1/PNPT1*	a/g	0.96 (0.92-1.02)	0.96	2.23E-01	2.23E-01	----	0	5.51E-01
rs12494328	*FLNB*	a/g	1.13 (1.07-1.20)	1.13	**1.28E-05**	**5.89E-04**	++-+	26.9	2.50E-01
rs6804624	*COL8A1*	c/t	0.99 (0.94-1.05)	0.99	8.14E-01	8.14E-01	---+	0	8.42E-01
6:36592986	*CDKN1A*	d/r	1.14 (1.09-1.21)	1.15	**7.74E-07**	**1.04E-04**	++++	36.6	1.93E-01
rs2684249	*HSF2*	c/t	0.92 (0.88-0.97)	0.94	1.08E-03	1.66E-01	---+	63.3	4.25E-02
rs8176672	*ABO*	t/c	1.00 (0.91-1.11)	1.00	9.49E-01	9.49E-01	-+-?	0	3.69E-01
rs4936099	*ADAMTS8*	c/a	0.94 (0.90-1.00)	0.94	5.75E-02	5.75E-02	----	0	9.63E-01
rs34222435	*ASB7*	t/c	1.03 (0.97-1.10)	1.03	3.66E-01	3.66E-01	++-+	0	8.74E-01
rs1074407	*TRIOBP*	t/a	1.04 (1.00-1.10)	1.04	4.92E-02	8.66E-02	++++	32.9	2.15E-01
Disc area SNPs									
rs4658101	*CDC7/TGFBR3*	a/g	1.08 (1.02-1.16)	1.08	7.81E-03	7.81E-03	++++	0	7.22E-01
1:169530520	*F5/SELP*	i/r	1.01 (0.96-1.08)	1.02	5.40E-01	5.40E-01	++-?	0	7.14E-01
rs11811982	*CDC42BPA*	a/c	0.87 (0.80-0.97)	0.90	1.19E-02	8.28E-02	--++	20.5	2.87E-01
rs9843102	*ABI3BP*	a/g	0.92 (0.86-0.98)	0.92	1.37E-02	1.37E-02	----	0	6.24E-01
rs10021731	*UGT8*	c/t	1.01 (0.96-1.06)	1.01	6.82E-01	6.82E-01	--++	0	6.50E-01
8:88744441	*DCAF4L2*	d/r	1.03 (0.99-1.09)	1.04	0.1225	1.39E-01	++-+	4.9	3.68E-01
rs12220165	*CTNNA3*	g/c	1.08 (1.01-1.16)	1.09	1.14E-02	1.14E-02	++++	0	9.04E-01
rs7916410	*ATOH7*	t/c	1.00 (0.96-1.06)	1.00	7.63E-01	7.45E-01	+-++	3.9	3.73E-01
rs61101201	*ELP4/PAX6*	g/t	1.00 (0.94-1.06)	1.00	9.77E-01	9.77E-01	-+-?	0	9.63E-01
rs442376	*TMTC2*	c/t	1.04 (0.99-1.10)	1.05	7.94E-02	7.94E-02	-+++	0	6.82E-01
rs1345467	*SALL1*	g/a	1.07 (1.01-1.14)	1.07	1.86E-02	1.86E-02	++++	0	8.73E-01
rs5762752	*CHEK2*	c/g	0.92 (0.88-0.98)	0.92	4.90E-03	4.90E-03	----	0	8.29E-01
rs56385951	*CARD10*	a/g	0.99 (0.92-1.07)	1.00	9.15E-01	9.15E-01	+-+-	0	9.88E-01
1:3046430	*PRDM16*	i/r	0.97 (0.87-1.10)	0.98	7.13E-01	8.72E-01	+--?	63.9	6.28E-02
rs787541	*U6, GADD45A*	c/g	0.98 (0.94-1.04)	0.98	6.10E-01	9.06E-01	--++	50.7	1.08E-01
rs1367187	*DIRC3*	c/t	0.95 (0.90-1.01)	0.96	1.11E-01	4.12E-01	+-+-	46.1	1.35E-01
rs2443724	*VGLL4*	c/g	0.91 (0.87-0.97)	0.91	1.04E-03	2.61E-02	--+-	38	1.84E-01
rs11129176	*RARB*	a/g	0.99 (0.94-1.05)	1.00	8.85E-01	9.93E-01	+---	40.4	1.69E-01
rs1997404	*COL8A1*	g/t	1.00 (0.95-1.06)	1.00	9.60E-01	9.60E-01	-+++	0	6.18E-01
rs34935520	*SIX6*	g/a	1.26 (1.20-1.33)	1.26	**2.82E-20**	**6.73E-14**	++++	21.5	2.81E-01
rs60779155	*ASB7*	a/g	1.02 (0.96-1.10)	1.03	4.52E-01	4.52E-01	+--+	0	5.02E-01

Results are shown for the most significantly associated single nucleotide polymorphisms from the endophenotype analyses. Nearest gene, reference NCBI build37; A1, reference allele; A2, other allele; OR, estimated odds ratio for allele A1; 95% CI, confidence interval; OR (R), estimated odds ratio for allele A1 in random effect meta-analysis; P-value (R), p-value in random effect meta-analysis; I^2 statistic measuring heterogeneity on a scale of 0% to 100%; i, insertion; d, deletion; r, reference.

Abbreviations

Abbreviation	Explanation
A1	Reference allele
A2	Other allele
Chr	Chromosome
CI	confidence interval
d	Deletion
FDR	False Discovery Rate
GWAS	Genome-wide association studies
h^2	Heritability
i	Insertion
I^2	Statistic measuring heterogeneity on a scale of 0% to 100%
IGGC	International Glaucoma Genetics Consortium
IOP	Intraocular pressure
LCA2	Leber Congenital Amaurosis type 2
LD	Linkage disequilibrium
MAF	Minor allele frequency
OR	Estimated odds ratio for allele A1
OR (R)	Estimated odds ratio for allele A1 in random effect meta-analysis
POAG	Primary open-angle glaucoma
r	Reference
RP	Retinitis Pigmentosa
SE	Standard error
SNPs	Single nucleotide polymorphisms
VCDR	Vertical cup-disc ratio
β	Effect size