



# THE UNIVERSITY *of* EDINBURGH

## Edinburgh Research Explorer

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

**Citation for published version:**

Springelkamp, H, Iglesias, AI, Mishra, A, Höhn, R, Wojciechowski, R, Khawaja, AP, Nag, A, Wang, YX, Wang, JJ, Cuellar-partida, G, Gibson, J, Cooke Bailey, JN, Vithana, EN, Gharahkhani, P, Boutin, T, Ramdas, WD, Zeller, T, Luben, RN, Yonova-doing, E, Viswanathan, AC, Yazar, S, Cree, AJ, Haines, JL, Koh, JY, Souzeau, E, Wilson, JF, Amin, N, Müller, C, Venturini, C, Kearns, LS, Hee Kang, J, Consortium, N, Tham, YC, Zhou, T, Van Leeuwen, EM, Nickels, S, Sanfilippo, P, Liao, J, Linde, HVD, Zhao, W, Van Koolwijk, LME, Zheng, L, Rivadeneira, F, Baskaran, M, Van Der Lee, SJ, Perera, S, De Jong, PTVM, Oostra, BA, Uitterlinden, AG, Fan, Q, Hofman, A, Shyong Tai, E, Vingerling, JR, Sim, X, Wolfs, RCW, Teo, YY, Lemij, HG, Khor, CC, Willemsen, R, Lackner, KJ, Aung, T, Jansonius, NM, Montgomery, G, Wild, PS, Young, TL, Burdon, KP, Hysi, PG, Pasquale, LR, Wong, TY, Klaver, CCW, Hewitt, AW, Jonas, JB, Mitchell, P, Lotery, AJ, Foster, PJ, Vitart, V, Pfeiffer, N, Craig, JE, Mackey, DA, Hammond, CJ, Wiggs, JL, Cheng, C, Van Duijn, CM & Macgregor, S 2017, 'New insights into the genetics of primary open-angle glaucoma based on meta-analyses of intraocular pressure and optic disc characteristics' *Human Molecular Genetics*, pp. ddw399. DOI: 10.1093/hmg/ddw399

**Digital Object Identifier (DOI):**

[10.1093/hmg/ddw399](https://doi.org/10.1093/hmg/ddw399)

**Link:**

[Link to publication record in Edinburgh Research Explorer](#)

**Document Version:**

Peer reviewed version

**Published In:**

Human Molecular Genetics

**Publisher Rights Statement:**

Author's final peer-reviewed manuscript as accepted for publication.

**General rights**

Copyright for the publications made accessible via the Edinburgh Research Explorer is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

**Take down policy**

The University of Edinburgh has made every reasonable effort to ensure that Edinburgh Research Explorer content complies with UK legislation. If you believe that the public display of this file breaches copyright please contact [openaccess@ed.ac.uk](mailto:openaccess@ed.ac.uk) providing details, and we will remove access to the work immediately and investigate your claim.



## 1 Title

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

4

## 5 Authors

6 Henriët Springelkamp(1, 2)\*, Adriana I. Iglesias(1-3)\*, Aniket Mishra(4, 5)\*, René Höhn(6, 7)\*, Robert  
7 Wojciechowski(8-10)\*, Anthony P. Khawaja(11), Abhishek Nag(12), Ya Xing Wang(13, 14), Jie Jin  
8 Wang(15), Gabriel Cuellar-Partida(4), Jane Gibson(16), Jessica N. Cooke Bailey(17), Eranga N. Vithana  
9 (18-20), Puya Gharakhani(4), Thibaud Boutin(21), Wishal D. Ramdas(2), Tanja Zeller(22), Robert N.  
10 Luben(23), Ekaterina Yonova-Doing(12), Ananth C. Viswanathan(11), Seyhan Yazar(24), Angela J.  
11 Cree(25), Jonathan L. Haines(17), Jia Yu Koh(18), Emmanuelle Souzeau(26), James F. Wilson(21, 27),  
12 Najaf Amin(1), Christian Müller(22), Cristina Venturini(12), Lisa S. Kearns(28), Jae Hee Kang(29),  
13 NEIGHBORHOOD Consortium\*\*, Yih Chung Tham(18, 20), Tiger Zhou(26), Elisabeth M. van  
14 Leeuwen(1), Stefan Nickels(7), Paul Sanfilippo(24, 28), Jiemin Liao(18, 20), Herma van der Linde(3),  
15 Wanting Zhao(18), Leonieke M.E. van Koolwijk(1), Li Zheng(18, 30), Fernando Rivadeneira(1, 31, 32),  
16 Mani Baskaran(18-20), Sven J. van der Lee(1), Shamira Perera(18, 19), Paulus T.V.M. de Jong(33-35),  
17 Ben A. Oostra(3), André G. Uitterlinden(1, 31, 32), Qiao Fan(18), Albert Hofman(1, 32), E-Shyong  
18 Tai(19, 36, 37), Johannes R. Vingerling(2), Xueling Sim(37), Roger C.W. Wolfs(2), Yik Ying Teo(37, 38),  
19 Hans G. Lemij(39), Chiea Chuen Khor(18, 30), Rob Willemsen(3), Karl J Lackner(6), Tin Aung(18, 20),  
20 Nomdo M. Jansonius(40), Grant Montgomery(41), Philipp S Wild(42-44), Terri L. Young(45), Kathryn  
21 P. Burdon(46), Pirro G. Hysi(12), Louis R. Pasquale(29, 47), Tien Yin Wong(18-20), Caroline C.W.  
22 Klaver(1, 2), Alex W. Hewitt(28, 46), Jost B. Jonas(48), Paul Mitchell(15), Andrew J Lotery(25), Paul J.  
23 Foster(11), Veronique Vitart(21), Norbert Pfeiffer(7), Jamie E. Craig(26), David A. Mackey(24, 46) ±,  
24 Christopher J. Hammond(12) ±, Janey L. Wiggs(47) ±, Ching-Yu Cheng(18-20) ±, Cornelia M. van  
25 Duijn(1) ±, Stuart MacGregor(4) ±.

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

- 46 15. Centre for Vision Research, Department of Ophthalmology and Westmead Institute for  
47 Medical Research, University of Sydney, Sydney, New South Wales, Australia.
- 48 16. Centre for Biological Sciences, Faculty of Natural and Environmental Sciences, University of  
49 Southampton, Southampton, UK.
- 50 17. Department of Epidemiology and Biostatistics, Case Western Reserve University, Cleveland,  
51 Ohio, USA.
- 52 18. Singapore Eye Research Institute, Singapore National Eye Centre, Singapore, Singapore.
- 53 19. Duke-National University of Singapore Graduate Medical School, Singapore, Singapore.
- 54 20. Department of Ophthalmology, Yong Loo Lin School of Medicine, National University of  
55 Singapore, Singapore.
- 56 21. Medical Research Council Human Genetics Unit, Institute of Genetics and Molecular  
57 Medicine, University of Edinburgh, Edinburgh, UK.
- 58 22. Clinic for General and Interventional Cardiology, University Heart Center Hamburg, Hamburg,  
59 Germany.
- 60 23. Department of Public Health and Primary Care, Institute of Public Health, University of  
61 Cambridge School of Clinical Medicine, Cambridge, UK.
- 62 24. Centre for Ophthalmology and Visual Science, Lions Eye Institute, University of Western  
63 Australia, Perth, Australia.
- 64 25. Clinical and Experimental Sciences, Faculty of Medicine, University of Southampton,  
65 Southampton, UK.
- 66 26. Department of Ophthalmology, Flinders University, Adelaide, Australia.
- 67 27. Centre for Global Health Research, The Usher Institute for Population Health Sciences and  
68 Informatics, University of Edinburgh, Scotland, UK.
- 69 28. Centre for Eye Research Australia (CERA), University of Melbourne, Royal Victorian Eye and  
70 Ear Hospital, Melbourne, Victoria, Australia.
- 71 29. Division of Network Medicine, Department of Medicine, Brigham and Women's Hospital,  
72 Boston, Massachusetts, USA.
- 73 30. Division of Human Genetics, Genome Institute of Singapore, Singapore, Singapore.
- 74 31. Department of Internal Medicine, Erasmus Medical Center, Rotterdam, the Netherlands.
- 75 32. Netherlands Consortium for Healthy Ageing, Netherlands Genomics Initiative, the Hague, the  
76 Netherlands.
- 77 33. Department of Ophthalmology, Academic Medical Center, Amsterdam, the Netherlands.
- 78 34. Department of Ophthalmology, Leiden University Medical Center, Leiden, the Netherlands.
- 79 35. The Netherlands Institute of Neuroscience KNAW, Amsterdam, the Netherlands.
- 80 36. Department of Medicine, National University of Singapore and National University Health  
81 System, Singapore, Singapore.
- 82 37. Saw Swee Hock School of Public Health, National University of Singapore and National  
83 University Health System, Singapore, Singapore.
- 84 38. Department of Statistics and Applied Probability, National University of Singapore, Singapore,  
85 Singapore.
- 86 39. Glaucoma Service, The Rotterdam Eye Hospital, Rotterdam, the Netherlands.
- 87 40. Department of Ophthalmology, University of Groningen, University Medical Center  
88 Groningen, Groningen, the Netherlands.
- 89 41. Department of Molecular Epidemiology, Queensland Institute of Medical Research, Herston,  
90 Brisbane, Queensland, Australia.
- 91 42. Preventive Cardiology and Preventive Medicine / Center for Cardiology, University Medical  
92 Center Mainz, Mainz, Germany.
- 93 43. Center for Thrombosis and Hemostasis, University Medical Center Mainz, Mainz, Germany.
- 94 44. German Center for Cardiovascular Research (DZHK), partner site RhineMain, Mainz,  
95 Germany.

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

104

105 \* These authors contributed equally to this work.

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

107 ± These authors jointly supervised this work.

108

109 **Corresponding author:** Stuart MacGregor Telephone number: + 61 7 3845 3563; email :

110 [Stuart.MacGregor@qimrberghofer.edu.au](mailto:Stuart.MacGregor@qimrberghofer.edu.au)

111

112 **Abstract**

113 Primary open-angle glaucoma (POAG), the most common optic neuropathy, is a heritable disease.  
114 Siblings of POAG cases have a ten-fold increase risk of developing the disease. Intraocular pressure  
115 (IOP) and optic nerve head characteristics are used clinically to predict POAG risk. We conducted a  
116 genome-wide association meta-analysis of IOP and optic disc parameters and validated our findings  
117 in multiple sets of POAG cases and controls. Using imputation to the 1000 genomes (1000G)  
118 reference set, we identified 9 new genomic regions associated with vertical cup disc ratio (VCDR) and  
119 1 new region associated with IOP. Additionally, we found 5 novel loci for optic nerve cup area and 6  
120 for disc area. Previously it was assumed that genetic variation influenced POAG either through IOP or  
121 via changes to the optic nerve head; here we present evidence that some genomic regions affect  
122 both IOP and the disc parameters. We characterized the effect of the novel loci through pathway  
123 analysis and found that pathways involved are not entirely distinct as assumed so far. Further, we  
124 identified a novel association between *CDKN1A* and POAG. Using a zebrafish model we show that  
125 *six6b* (associated with POAG and optic nerve head variation) alters the expression of *cdkn1a*. In  
126 summary, we have identified several novel genes influencing the major clinical risk predictors of  
127 POAG and showed that genetic variation in *CDKN1A* is important in POAG risk.

128

129

130

131

132

133

## 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  $10.4 \pm 2.5$   
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  $0.42 \pm 0.09$ (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-analysis 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 ( $n = 37,930$ ,  $\lambda = 1.05$ ; **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 ( $n = 23,899$ ,  $\lambda = 1.08$ ), 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 =  $4.13 \times 10^{-3}$ ) was nominally  
186 significant in the analysis of individuals of Asian descent ( $n = 8,373$ ,  $\lambda = 1.01$ ). In the combined analysis  
187 ( $n = 32,272$ ,  $\lambda = 1.06$ ), 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  $\beta$  (p-value) decreased from -0.007 ( $2.15 \times 10^{-9}$ )  
193 to -0.002 ( $5.60 \times 10^{-2}$ ) 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 ( $n = 22,489$ ,  $\lambda = 1.06$ ) 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 ( $n = 29,828$ ,  $\lambda = 1.06$ , **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 ( $n = 22,504$ ,  $\lambda = 1.06$ ) 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 ( $n = 7,307$ ,  $\lambda$   
215  $= 1.02$ ). An additional four SNPs reached genome-wide significance in the combined meta-analysis ( $n$   
216  $= 29,811$ ,  $\lambda = 1.07$ ): *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 ( $p\text{-value} < 1.00 \times 10^{-05}$ ) 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  $P_{\text{gene-based}} <$   
245  $0.05/24,769 = 2.02 \times 10^{-6}$  (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  $1.61 \times 10^{-6}$ );  
247 *RARB* (p-value  $1.86 \times 10^{-6}$ ) and *HORMAD2-AS1* (p-value  $1.04 \times 10^{-6}$ ) 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  $1.20 \times 10^{-6}$ ) and *REM2* (p-value  $1.55 \times 10^{-6}$ ), and *THSD4* (p-value  $5.44 \times 10^{-8}$ ) 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  $8.42 \times 10^{-7}$ ) and *LOC149950* (p-value  $3.87 \times 10^{-7}$ ).

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 ( $r^2=0.03$  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  $1.0 \times 10^{-308}$ ), followed by VCDR and disc area (RhoG = 0.62, p-value =  $7.36 \times 10^{-23}$ ). Genetic correlation  
271 between cup and disc area was 0.31 (p-value =  $1.00 \times 10^{-04}$ ). 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  $0.05/75 = 6.67 \times 10^{-4}$ ) (**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 =  $7.4 \times 10^{-7}$ ). In our previous  
314 paper, the SNP rs6054374 near to *BMP2* was already associated with POAG (OR = 0.92, p-value  $3.74 \times$   
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 =  $8.52 \times 10^{-6}$ ). *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 ( $r^2 > 0.85$ ) 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  $3.42 \times 10^{-6}$  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 ( $p\text{-value} < 1.0 \times 10^{-5}$ ) 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 TNF $\alpha$ -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 x 10<sup>-8</sup> (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  $= 6.67 \times 10^{-4}$  ( $0.05 / 75$   
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](http://getfiles.qimr.edu.au/puyaGharahkhani/1000G_imputed_IGGC_eye_quantitative_traits/)  
533 [http://getfiles.qimr.edu.au/puyaGharahkhani/1000G\\_imputed\\_IGGC\\_eye\\_quantitative\\_traits/](http://getfiles.qimr.edu.au/puyaGharahkhani/1000G_imputed_IGGC_eye_quantitative_traits/)

534

535 **Acknowledgments**

536 We gratefully acknowledge the contributions of all participants who volunteered within each cohort  
537 and the personnel responsible for the recruitment and administration of each study. We also thank  
538 the various funding sources that made this work possible. The funders had no role in study design,  
539 data collection and analysis, decision to publish, or preparation of the manuscript. Complete funding  
540 information and acknowledgments can be found in the **Supplementary Material**.

541

542 **Conflict of interest**

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

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

549

550

551

552

553

554



555 **References**

- 556 1 Weinreb, R.N., Aung, T. and Medeiros, F.A. (2014) The pathophysiology and treatment of  
557 glaucoma: a review. *JAMA*, **311**, 1901-1911.
- 558 2 Ernest, P.J., Busch, M.J., Webers, C.A., Beckers, H.J., Hendrikse, F., Prins, M.H. and Schouten,  
559 J.S. (2013) Prevalence of end-of-life visual impairment in patients followed for glaucoma. *Acta*  
560 *Ophthalmol*, **91**, 738-743.
- 561 3 Peters, D., Bengtsson, B. and Heijl, A. (2014) Factors associated with lifetime risk of open-  
562 angle glaucoma blindness. *Acta Ophthalmol*, **92**, 421-425.
- 563 4 Ramdas, W.D., Rizopoulos, D., Wolfs, R.C., Hofman, A., de Jong, P.T., Vingerling, J.R. and  
564 Jansonius, N.M. (2011) Defining glaucomatous optic neuropathy from a continuous measure of optic  
565 nerve damage - the optimal cut-off point for risk-factor analysis in population-based epidemiology.  
566 *Ophthalmic Epidemiol*, **18**, 211-216.
- 567 5 Wolfs, R.C., Klaver, C.C., Ramrattan, R.S., van Duijn, C.M., Hofman, A. and de Jong, P.T. (1998)  
568 Genetic risk of primary open-angle glaucoma. Population-based familial aggregation study. *Arch*  
569 *Ophthalmol*, **116**, 1640-1645.
- 570 6 Cuellar-Partida, G., Craig, J.E., Burdon, K.P., Wang, J.J., Vote, B.J., Souzeau, E., McAllister, I.L.,  
571 Isaacs, T., Lake, S., Mackey, D.A. *et al.* (2016) Assessment of polygenic effects links primary open-  
572 angle glaucoma and age-related macular degeneration. *Sci Rep*, **6**, 26885.
- 573 7 Burdon, K.P., Macgregor, S., Hewitt, A.W., Sharma, S., Chidlow, G., Mills, R.A., Danoy, P.,  
574 Casson, R., Viswanathan, A.C., Liu, J.Z. *et al.* (2011) Genome-wide association study identifies  
575 susceptibility loci for open angle glaucoma at TMCO1 and CDKN2B-AS1. *Nat Genet*, **43**, 574-578.
- 576 8 Gharahkhani, P., Burdon, K.P., Fogarty, R., Sharma, S., Hewitt, A.W., Martin, S., Law, M.H.,  
577 Cremin, K., Bailey, J.N., Loomis, S.J. *et al.* (2014) Common variants near ABCA1, AFAP1 and GMDS  
578 confer risk of primary open-angle glaucoma. *Nat Genet*, **46**, 1120-1125.
- 579 9 Hysi, P.G., Cheng, C.Y., Springelkamp, H., Macgregor, S., Bailey, J.N., Wojciechowski, R., Vitart,  
580 V., Nag, A., Hewitt, A.W., Hohn, R. *et al.* (2014) Genome-wide analysis of multi-ancestry cohorts  
581 identifies new loci influencing intraocular pressure and susceptibility to glaucoma. *Nat Genet*, **46**,  
582 1126-1130.
- 583 10 Thorleifsson, G., Walters, G.B., Hewitt, A.W., Masson, G., Helgason, A., DeWan, A.,  
584 Sigurdsson, A., Jonasdottir, A., Gudjonsson, S.A., Magnusson, K.P. *et al.* (2010) Common variants near  
585 CAV1 and CAV2 are associated with primary open-angle glaucoma. *Nat Genet*, **42**, 906-909.
- 586 11 van Koolwijk, L.M., Ramdas, W.D., Ikram, M.K., Jansonius, N.M., Pasutto, F., Hysi, P.G.,  
587 Macgregor, S., Janssen, S.F., Hewitt, A.W., Viswanathan, A.C. *et al.* (2012) Common genetic  
588 determinants of intraocular pressure and primary open-angle glaucoma. *PLoS Genet*, **8**, e1002611.
- 589 12 Ramdas, W.D., van Koolwijk, L.M., Lemij, H.G., Pasutto, F., Cree, A.J., Thorleifsson, G.,  
590 Janssen, S.F., Jacoline, T.B., Amin, N., Rivadeneira, F. *et al.* (2011) Common genetic variants  
591 associated with open-angle glaucoma. *Hum Mol Genet*, **20**, 2464-2471.
- 592 13 Springelkamp, H., Iglesias, A.I., Cuellar-Partida, G., Amin, N., Burdon, K.P., van Leeuwen, E.M.,  
593 Gharahkhani, P., Mishra, A., van der Lee, S.J., Hewitt, A.W. *et al.* (2015) ARHGEF12 influences the risk  
594 of glaucoma by increasing intraocular pressure. *Hum Mol Genet*, **24**, 2689-2699.
- 595 14 Wiggs, J.L., Kang, J.H., Yaspan, B.L., Mirel, D.B., Laurie, C., Crenshaw, A., Brodeur, W.,  
596 Gogarten, S., Olson, L.M., Abdrabou, W. *et al.* (2011) Common variants near CAV1 and CAV2 are  
597 associated with primary open-angle glaucoma in Caucasians from the USA. *Hum Mol Genet*, **20**, 4707-  
598 4713.
- 599 15 Wiggs, J.L., Yaspan, B.L., Hauser, M.A., Kang, J.H., Allingham, R.R., Olson, L.M., Abdrabou, W.,  
600 Fan, B.J., Wang, D.Y., Brodeur, W. *et al.* (2012) Common variants at 9p21 and 8q22 are associated  
601 with increased susceptibility to optic nerve degeneration in glaucoma. *PLoS Genet*, **8**, e1002654.
- 602 16 Chen, Y., Lin, Y., Vithana, E.N., Jia, L., Zuo, X., Wong, T.Y., Chen, L.J., Zhu, X., Tam, P.O., Gong,  
603 B. *et al.* (2014) Common variants near ABCA1 and in PMM2 are associated with primary open-angle  
604 glaucoma. *Nat Genet*, **46**, 1115-1119.

605 17 Charlesworth, J., Kramer, P.L., Dyer, T., Diego, V., Samples, J.R., Craig, J.E., Mackey, D.A.,  
606 Hewitt, A.W., Blangero, J. and Wirtz, M.K. (2010) The path to open-angle glaucoma gene discovery:  
607 endophenotypic status of intraocular pressure, cup-to-disc ratio, and central corneal thickness. *Invest*  
608 *Ophthalmol Vis Sci*, **51**, 3509-3514.

609 18 Bulik-Sullivan, B.K., Loh, P.R., Finucane, H.K., Ripke, S., Yang, J., Schizophrenia Working Group  
610 of the Psychiatric Genomics, C., Patterson, N., Daly, M.J., Price, A.L. and Neale, B.M. (2015) LD Score  
611 regression distinguishes confounding from polygenicity in genome-wide association studies. *Nat*  
612 *Genet*, **47**, 291-295.

613 19 Ramdas, W.D., van Koolwijk, L.M., Ikram, M.K., Jansonius, N.M., de Jong, P.T., Bergen, A.A.,  
614 Isaacs, A., Amin, N., Aulchenko, Y.S., Wolfs, R.C. *et al.* (2010) A genome-wide association study of  
615 optic disc parameters. *PLoS Genet*, **6**, e1000978.

616 20 Springelkamp, H., Hohn, R., Mishra, A., Hysi, P.G., Khor, C.C., Loomis, S.J., Bailey, J.N., Gibson,  
617 J., Thorleifsson, G., Janssen, S.F. *et al.* (2014) Meta-analysis of genome-wide association studies  
618 identifies novel loci that influence cupping and the glaucomatous process. *Nat Commun*, **5**, 4883.

619 21 Springelkamp, H., Mishra, A., Hysi, P.G., Gharahkhani, P., Hohn, R., Khor, C.C., Cooke Bailey,  
620 J.N., Luo, X., Ramdas, W.D., Vithana, E. *et al.* (2015) Meta-analysis of Genome-Wide Association  
621 Studies Identifies Novel Loci Associated With Optic Disc Morphology. *Genet Epidemiol*, **39**, 207-216.

622 22 Ramdas, W.D., Wolfs, R.C., Hofman, A., de Jong, P.T., Vingerling, J.R. and Jansonius, N.M.  
623 (2011) Heidelberg Retina Tomograph (HRT3) in population-based epidemiology: normative values  
624 and criteria for glaucomatous optic neuropathy. *Ophthalmic Epidemiol*, **18**, 198-210.

625 23 Coordinators, N.R. (2015) Database resources of the National Center for Biotechnology  
626 Information. *Nucleic Acids Res*, **43**, D6-17.

627 24 Wagner, A.H., Anand, V.N., Wang, W.H., Chatterton, J.E., Sun, D., Shepard, A.R., Jacobson, N.,  
628 Pang, I.H., Deluca, A.P., Casavant, T.L. *et al.* (2013) Exon-level expression profiling of ocular tissues.  
629 *Exp Eye Res*, **111**, 105-111.

630 25 Pers, T.H., Karjalainen, J.M., Chan, Y., Westra, H.J., Wood, A.R., Yang, J., Lui, J.C., Vedantam,  
631 S., Gustafsson, S., Esko, T. *et al.* (2015) Biological interpretation of genome-wide association studies  
632 using predicted gene functions. *Nat Commun*, **6**, 5890.

633 26 Skowronska-Krawczyk, D., Zhao, L., Zhu, J., Weinreb, R.N., Cao, G., Luo, J., Flagg, K., Patel, S.,  
634 Wen, C., Krupa, M. *et al.* (2015) P16INK4a Upregulation Mediated by SIX6 Defines Retinal Ganglion  
635 Cell Pathogenesis in Glaucoma. *Mol Cell*, **59**, 931-940.

636 27 Iglesias, A.I., Springelkamp, H., van der Linde, H., Severijnen, L.A., Amin, N., Oostra, B., Kockx,  
637 C.E., van den Hout, M.C., van Ijcken, W.F., Hofman, A. *et al.* (2014) Exome sequencing and functional  
638 analyses suggest that SIX6 is a gene involved in an altered proliferation-differentiation balance early  
639 in life and optic nerve degeneration at old age. *Hum Mol Genet*, **23**, 1320-1332.

640 28 Carnes, M.U., Liu, Y.P., Allingham, R.R., Whigham, B.T., Havens, S., Garrett, M.E., Qiao, C.,  
641 Investigators, N.C., Katsanis, N., Wiggs, J.L. *et al.* (2014) Discovery and functional annotation of SIX6  
642 variants in primary open-angle glaucoma. *PLoS Genet*, **10**, e1004372.

643 29 Black, G.C., Perveen, R., Wiszniewski, W., Dodd, C.L., Donnai, D. and McLeod, D. (1999) A  
644 novel hereditary developmental vitreoretinopathy with multiple ocular abnormalities localizing to a  
645 5-cM region of chromosome 5q13-q14. *Ophthalmology*, **106**, 2074-2081.

646 30 Hung, F.C., Cheng, Y.C., Sun, N.K. and Chao, C.C. (2013) Identification and functional  
647 characterization of zebrafish Gas7 gene in early development. *J Neurosci Res*, **91**, 51-61.

648 31 Coulson-Thomas, V.J., Gesteira, T.F., Coulson-Thomas, Y.M., Vicente, C.M., Tersariol, I.L.,  
649 Nader, H.B. and Toma, L. (2010) Fibroblast and prostate tumor cell cross-talk: fibroblast  
650 differentiation, TGF-beta, and extracellular matrix down-regulation. *Exp Cell Res*, **316**, 3207-3226.

651 32 Nakayama, K., Moriwaki, K., Imai, T., Shinzaki, S., Kamada, Y., Murata, K. and Miyoshi, E.  
652 (2013) Mutation of GDP-mannose-4,6-dehydratase in colorectal cancer metastasis. *PLoS One*, **8**,  
653 e70298.

654 33 Tsai, F.J., Lin, H.J., Chen, W.C., Tsai, C.H. and Tsai, S.W. (2004) A codon 31ser-arg  
655 polymorphism of the WAF-1/CIP-1/p21/tumour suppressor gene in Chinese primary open-angle  
656 glaucoma. *Acta Ophthalmol Scand*, **82**, 76-80.

657 34 Saglar, E., Yucel, D., Bozkurt, B., Ozgul, R.K., Irkec, M. and Ogun, A. (2009) Association of  
658 polymorphisms in APOE, p53, and p21 with primary open-angle glaucoma in Turkish patients. *Mol*  
659 *Vis*, **15**, 1270-1276.

660 35 Sotoodehnia, N., Isaacs, A., de Bakker, P.I., Dorr, M., Newton-Cheh, C., Nolte, I.M., van der  
661 Harst, P., Muller, M., Eijgelsheim, M., Alonso, A. *et al.* (2010) Common variants in 22 loci are  
662 associated with QRS duration and cardiac ventricular conduction. *Nat Genet*, **42**, 1068-1076.

663 36 Dunlop, M.G., Dobbins, S.E., Farrington, S.M., Jones, A.M., Palles, C., Whiffin, N., Tenesa, A.,  
664 Spain, S., Broderick, P., Ooi, L.Y. *et al.* (2012) Common variation near CDKN1A, POLD3 and SHROOM2  
665 influences colorectal cancer risk. *Nat Genet*, **44**, 770-776.

666 37 Scott, L.J., Mohlke, K.L., Bonnycastle, L.L., Willer, C.J., Li, Y., Duren, W.L., Erdos, M.R.,  
667 Stringham, H.M., Chines, P.S., Jackson, A.U. *et al.* (2007) A genome-wide association study of type 2  
668 diabetes in Finns detects multiple susceptibility variants. *Science*, **316**, 1341-1345.

669 38 Manning, A.K., Hivert, M.F., Scott, R.A., Grimsby, J.L., Bouatia-Naji, N., Chen, H., Rybin, D., Liu,  
670 C.T., Bielak, L.F., Prokopenko, I. *et al.* (2012) A genome-wide approach accounting for body mass  
671 index identifies genetic variants influencing fasting glycemic traits and insulin resistance. *Nat Genet*,  
672 **44**, 659-669.

673 39 Foroud, T., Koller, D.L., Lai, D., Sauerbeck, L., Anderson, C., Ko, N., Deka, R., Mosley, T.H.,  
674 Fornage, M., Woo, D. *et al.* (2012) Genome-wide association study of intracranial aneurysms  
675 confirms role of Anril and SOX17 in disease risk. *Stroke*, **43**, 2846-2852.

676 40 Myocardial Infarction Genetics, C., Kathiresan, S., Voight, B.F., Purcell, S., Musunuru, K.,  
677 Ardissino, D., Mannucci, P.M., Anand, S., Engert, J.C., Samani, N.J. *et al.* (2009) Genome-wide  
678 association of early-onset myocardial infarction with single nucleotide polymorphisms and copy  
679 number variants. *Nat Genet*, **41**, 334-341.

680 41 Lu, X., Wang, L., Chen, S., He, L., Yang, X., Shi, Y., Cheng, J., Zhang, L., Gu, C.C., Huang, J. *et al.*  
681 (2012) Genome-wide association study in Han Chinese identifies four new susceptibility loci for  
682 coronary artery disease. *Nat Genet*, **44**, 890-894.

683 42 Stacey, S.N., Helgason, H., Gudjonsson, S.A., Thorleifsson, G., Zink, F., Sigurdsson, A., Kehr, B.,  
684 Gudmundsson, J., Sulem, P., Sigurgeirsson, B. *et al.* (2015) New basal cell carcinoma susceptibility  
685 loci. *Nat Commun*, **6**, 6825.

686 43 Turnbull, C., Ahmed, S., Morrison, J., Pernet, D., Renwick, A., Maranian, M., Seal, S.,  
687 Ghousaini, M., Hines, S., Healey, C.S. *et al.* (2010) Genome-wide association study identifies five new  
688 breast cancer susceptibility loci. *Nat Genet*, **42**, 504-507.

689 44 Al Olama, A.A., Kote-Jarai, Z., Berndt, S.I., Conti, D.V., Schumacher, F., Han, Y., Benlloch, S.,  
690 Hazelett, D.J., Wang, Z., Saunders, E. *et al.* (2014) A meta-analysis of 87,040 individuals identifies 23  
691 new susceptibility loci for prostate cancer. *Nat Genet*, **46**, 1103-1109.

692 45 Shete, S., Hosking, F.J., Robertson, L.B., Dobbins, S.E., Sanson, M., Malmer, B., Simon, M.,  
693 Marie, Y., Boisselier, B., Delattre, J.Y. *et al.* (2009) Genome-wide association study identifies five  
694 susceptibility loci for glioma. *Nat Genet*, **41**, 899-904.

695 46 Wiggs, J.L., Hewitt, A.W., Fan, B.J., Wang, D.Y., Figueiredo Sena, D.R., O'Brien, C., Realini, A.,  
696 Craig, J.E., Dimasi, D.P., Mackey, D.A. *et al.* (2012) The p53 codon 72 PRO/PRO genotype may be  
697 associated with initial central visual field defects in caucasians with primary open angle glaucoma.  
698 *PLoS One*, **7**, e45613.

699 47 Smith, M.L., Chen, I.T., Zhan, Q., Bae, I., Chen, C.Y., Gilmer, T.M., Kastan, M.B., O'Connor,  
700 P.M. and Fornace, A.J., Jr. (1994) Interaction of the p53-regulated protein Gadd45 with proliferating  
701 cell nuclear antigen. *Science*, **266**, 1376-1380.

702 48 Kastan, M.B., Zhan, Q., el-Deiry, W.S., Carrier, F., Jacks, T., Walsh, W.V., Plunkett, B.S.,  
703 Vogelstein, B. and Fornace, A.J., Jr. (1992) A mammalian cell cycle checkpoint pathway utilizing p53  
704 and GADD45 is defective in ataxia-telangiectasia. *Cell*, **71**, 587-597.

705 49 Kearsey, J.M., Coates, P.J., Prescott, A.R., Warbrick, E. and Hall, P.A. (1995) Gadd45 is a  
706 nuclear cell cycle regulated protein which interacts with p21Cip1. *Oncogene*, **11**, 1675-1683.

707 50 Tam, C.W., Liu, V.W., Leung, W.Y., Yao, K.M. and Shiu, S.Y. (2008) The autocrine human  
708 secreted PDZ domain-containing protein 2 (sPDZD2) induces senescence or quiescence of prostate,  
709 breast and liver cancer cells via transcriptional activation of p53. *Cancer Lett*, **271**, 64-80.

710 51 Liu, H., Hew, H.C., Lu, Z.G., Yamaguchi, T., Miki, Y. and Yoshida, K. (2009) DNA damage  
711 signalling recruits RREB-1 to the p53 tumour suppressor promoter. *Biochem J*, **422**, 543-551.

712 52 Feng, H.C., Tsao, S.W., Ngan, H.Y., Xue, W.C., Kwan, H.S., Siu, M.K., Liao, X.Y., Wong, E. and  
713 Cheung, A.N. (2008) Overexpression of prostate stem cell antigen is associated with gestational  
714 trophoblastic neoplasia. *Histopathology*, **52**, 167-174.

715 53 Jin, H.S., Park, H.S., Shin, J.H., Kim, D.H., Jun, S.H., Lee, C.J. and Lee, T.H. (2011) A novel  
716 inhibitor of apoptosis protein (IAP)-interacting protein, Vestigial-like (Vgl)-4, counteracts apoptosis-  
717 inhibitory function of IAPs by nuclear sequestration. *Biochem Biophys Res Commun*, **412**, 454-459.

718 54 Gomez-Munoz, A., Kong, J.Y., Salh, B. and Steinbrecher, U.P. (2004) Ceramide-1-phosphate  
719 blocks apoptosis through inhibition of acid sphingomyelinase in macrophages. *J Lipid Res*, **45**, 99-105.

720 55 Flicek, P., Amode, M.R., Barrell, D., Beal, K., Billis, K., Brent, S., Carvalho-Silva, D., Clapham, P.,  
721 Coates, G., Fitzgerald, S. *et al.* (2014) Ensembl 2014. *Nucleic Acids Res*, **42**, D749-755.

722 56 Gu, S.M., Thompson, D.A., Srikumari, C.R., Lorenz, B., Finckh, U., Nicoletti, A., Murthy, K.R.,  
723 Rathmann, M., Kumaramanickavel, G., Denton, M.J. *et al.* (1997) Mutations in RPE65 cause  
724 autosomal recessive childhood-onset severe retinal dystrophy. *Nat Genet*, **17**, 194-197.

725 57 Marlhens, F., Bareil, C., Griffoin, J.M., Zrenner, E., Amalric, P., Eliaou, C., Liu, S.Y., Harris, E.,  
726 Redmond, T.M., Arnaud, B. *et al.* (1997) Mutations in RPE65 cause Leber's congenital amaurosis. *Nat*  
727 *Genet*, **17**, 139-141.

728 58 Hamel, C.P., Tsilou, E., Pfeffer, B.A., Hooks, J.J., Detrick, B. and Redmond, T.M. (1993)  
729 Molecular cloning and expression of RPE65, a novel retinal pigment epithelium-specific microsomal  
730 protein that is post-transcriptionally regulated in vitro. *J Biol Chem*, **268**, 15751-15757.

731 59 Moskovich, O., Herzog, L.O., Ehrlich, M. and Fishelson, Z. (2012) Caveolin-1 and dynamin-2  
732 are essential for removal of the complement C5b-9 complex via endocytosis. *J Biol Chem*, **287**, 19904-  
733 19915.

734 60 Ramdas, W.D., Amin, N., van Koolwijk, L.M., Janssens, A.C., Demirkan, A., de Jong, P.T.,  
735 Aulchenko, Y.S., Wolfs, R.C., Hofman, A., Rivadeneira, F. *et al.* (2011) Genetic architecture of open  
736 angle glaucoma and related determinants. *J Med Genet*, **48**, 190-196.

737 61 Wiggs, J.L., Hauser, M.A., Abdrabou, W., Allingham, R.R., Budenz, D.L., Delbono, E., Friedman,  
738 D.S., Kang, J.H., Gaasterland, D., Gaasterland, T. *et al.* (2013) The NEIGHBOR consortium primary  
739 open-angle glaucoma genome-wide association study: rationale, study design, and clinical variables. *J*  
740 *Glaucoma*, **22**, 517-525.

741 62 Gibson, J., Griffiths, H., De Salvo, G., Cole, M., Jacob, A., Macleod, A., Yang, Y., Menon, G.,  
742 Cree, A., Ennis, S. *et al.* (2012) Genome-wide association study of primary open angle glaucoma risk  
743 and quantitative traits. *Mol Vis*, **18**, 1083-1092.

744 63 Lavanya, R., Jeganathan, V.S., Zheng, Y., Raju, P., Cheung, N., Tai, E.S., Wang, J.J., Lamoureux,  
745 E., Mitchell, P., Young, T.L. *et al.* (2009) Methodology of the Singapore Indian Chinese Cohort (SICC)  
746 eye study: quantifying ethnic variations in the epidemiology of eye diseases in Asians. *Ophthalmic*  
747 *Epidemiol*, **16**, 325-336.

748 64 van der Valk, R., Webers, C.A., Schouten, J.S., Zeegers, M.P., Hendrikse, F. and Prins, M.H.  
749 (2005) Intraocular pressure-lowering effects of all commonly used glaucoma drugs: a meta-analysis  
750 of randomized clinical trials. *Ophthalmology*, **112**, 1177-1185.

751 65 Willer, C.J., Li, Y. and Abecasis, G.R. (2010) METAL: fast and efficient meta-analysis of  
752 genomewide association scans. *Bioinformatics*, **26**, 2190-2191.

753 66 Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M.A., Bender, D., Maller, J., Sklar,  
754 P., de Bakker, P.I., Daly, M.J. *et al.* (2007) PLINK: a tool set for whole-genome association and  
755 population-based linkage analyses. *American journal of human genetics*, **81**, 559-575.

756 67 Team., R.C. (2014) R: a language and environment for statistical computing,  
757 <http://www.R-project.org>, in press.  
758 68 Pruim, R.J., Welch, R.P., Sanna, S., Teslovich, T.M., Chines, P.S., Gliedt, T.P., Boehnke, M.,  
759 Abecasis, G.R. and Willer, C.J. (2010) LocusZoom: regional visualization of genome-wide association  
760 scan results. *Bioinformatics*, **26**, 2336-2337.  
761 69 Bulik-Sullivan, B., Finucane, H.K., Anttila, V., Gusev, A., Day, F.R., Loh, P.R., ReproGen, C.,  
762 Psychiatric Genomics, C., Genetic Consortium for Anorexia Nervosa of the Wellcome Trust Case  
763 Control, C., Duncan, L. *et al.* (2015) An atlas of genetic correlations across human diseases and traits.  
764 *Nat Genet*, **47**, 1236-1241.  
765 70 Mishra, A. and Macgregor, S. (2015) VEGAS2: Software for More Flexible Gene-Based Testing.  
766 *Twin Res Hum Genet*, **18**, 86-91.  
767 71 Ward, L.D. and Kellis, M. (2012) HaploReg: a resource for exploring chromatin states,  
768 conservation, and regulatory motif alterations within sets of genetically linked variants. *Nucleic Acids*  
769 *Res*, **40**, D930-934.  
770 72 Boyle, A.P., Hong, E.L., Hariharan, M., Cheng, Y., Schaub, M.A., Kasowski, M., Karczewski, K.J.,  
771 Park, J., Hitz, B.C., Weng, S. *et al.* (2012) Annotation of functional variation in personal genomes using  
772 RegulomeDB. *Genome Res*, **22**, 1790-1797.

773

## 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  $<7.0 \times 10^{-6}$  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  $<1.0 \times 10^{-5}$ ). 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  $\pm$  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 an association with vertical cup-disc ratio.

SNP	Nearest gene	A1/A2	IOP			VCDR			Cup area			Disc area		
			$\beta$	SE	P	$\beta$	SE	P	$\beta$	SE	P	B	SE	P
rs10918274	<i>TMCO1</i>	t/c	0.26	0.04	<b>5.64E-12</b>	0.005	0.002	8.38E-03	0.010	0.003	2.47E-03	0.000	0.006	9.49E-01
rs7635832	<i>FNDC3B</i>	g/t	-0.22	0.03	<b>6.61E-13</b>	-0.001	0.001	3.35E-01	-0.004	0.003	1.27E-01	0.002	0.005	7.08E-01
rs10281637	<i>CAV1/CAV2</i>	c/t	0.20	0.03	<b>3.96E-13</b>	0.004	0.001	5.28E-03	0.006	0.003	1.23E-02	-0.002	0.005	6.01E-01
8:78380944*	<i>PKIA</i>	i/r	1.00	0.17	<b>7.54E-09</b>	0.000	0.010	9.74E-01	-0.018	0.017	3.00E-01	0.018	0.031	5.61E-01
rs7815043*	<i>PKIA</i>	c/t	-0.10	0.03	<b>4.41E-05</b>	-0.001	0.001	3.13E-01	-0.001	0.002	8.32E-01	-0.002	0.004	5.66E-01
rs7944735	<i>Many genes</i>	c/g	0.19	0.03	<b>6.00E-11</b>	0.001	0.001	4.37E-01	0.006	0.003	3.33E-02	0.000	0.005	9.68E-01
11:120357425	<i>ARHGEF12</i>	d/r	0.18	0.03	<b>2.02E-09</b>	0.001	0.001	6.12E-01	0.001	0.003	6.45E-01	0.001	0.005	8.38E-01
rs12794618	<i>ARHGEF12</i>	c/t	0.17	0.03	<b>7.86E-09</b>	0.001	0.001	4.14E-01	0.002	0.003	4.84E-01	0.004	0.005	4.53E-01
rs55796939	<i>ADAMTS8</i>	t/c	0.36	0.06	<b>2.31E-08</b>	0.003	0.003	3.61E-01	0.006	0.006	3.19E-01	-0.003	0.010	7.95E-01
rs2472496	<i>ABCA1</i>	g/a	0.17	0.02	<b>1.93E-13</b>	0.004	0.001	<b>6.83E-05</b>	0.010	0.002	<b>9.63E-07</b>	0.003	0.004	4.75E-01
rs8176741	<i>ABO</i>	a/g	0.24	0.04	<b>3.47E-10</b>	0.007	0.002	<b>4.51E-05</b>	0.019	0.003	<b>7.12E-08</b>	0.004	0.006	5.42E-01
rs9913911	<i>GAS7</i>	g/a	-0.17	0.02	<b>7.01E-12</b>	-0.006	0.001	<b>1.84E-07</b>	-0.008	0.002	<b>2.48E-04</b>	-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 <  $5.37 \times 10^{-4}$ ; 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;  $\beta$ , 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.

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



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

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  $< 5.37 \times 10^{-4}$ ; 0.05/93). Nearest gene, reference NCBI build37; A1, reference allele; A2, other allele;  $\beta$ , 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**

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

SNP	Nearest gene	A1/A	IOP			VCDR			Cup area			Disc area		
			$\beta$	SE	P	$\beta$	SE	P	$\beta$	SE	P	B	SE	P
rs13016883	<i>TRIB2</i>	c/g	0.01	0.03	5.64E-01	0.006	0.001	<b>3.44E-06</b>	0.016	0.002	<b>1.83E-11</b>	0.001	0.004	8.30E-01
rs35084382	<i>DUSP1</i>	c/t	-0.10	0.07	1.32E-01	-0.018	0.003	<b>2.05E-08</b>	-0.033	0.006	<b>2.17E-08</b>	-0.031	0.011	5.51E-03
rs11759831	<i>CRISPLD1*</i>	t/g	-0.05	0.05	3.10E-01	0.009	0.002	<b>1.07E-04</b>	0.021	0.004	<b>1.66E-06</b>	0.022	0.008	5.47E-03
rs1360589	<i>CDKN2B-AS1</i>	c/t	-0.04	0.02	8.42E-02	-0.013	0.001	<b>1.43E-34</b>	-0.024	0.002	<b>2.90E-28</b>	-0.008	0.004	4.45E-02
rs11613189	<i>FAM101A</i>	t/c	-0.03	0.03	2.27E-01	-0.005	0.001	<b>6.04E-06</b>	-0.016	0.002	<b>2.01E-12</b>	0.002	0.004	6.42E-01
rs2251069	<i>DDHD1</i>	c/t	0.01	0.02	7.29E-01	-0.006	0.001	<b>7.41E-08</b>	-0.013	0.002	<b>1.20E-09</b>	0.001	0.004	7.11E-01
rs6598351	<i>FAM169B*</i>	t/c	-0.02	0.03	5.26E-01	0.006	0.001	<b>2.80E-05</b>	0.012	0.003	<b>1.77E-05</b>	-0.004	0.005	3.90E-01
rs11646917	<i>SALL1</i>	t/g	-0.01	0.03	6.65E-01	-0.009	0.001	<b>4.83E-10</b>	-0.015	0.003	<b>4.76E-09</b>	-0.015	0.005	1.30E-03
rs11867840	<i>BCAS3</i>	g/a	0.04	0.03	1.04E-01	-0.006	0.001	<b>4.86E-06</b>	-0.018	0.002	<b>2.35E-13</b>	0.011	0.004	1.00E-02
rs6054375	<i>BMP2</i>	t/g	0.03	0.03	2.45E-01	-0.010	0.001	<b>6.92E-15</b>	-0.018	0.002	<b>1.83E-15</b>	-0.003	0.004	4.74E-01
rs3791679	<i>EFEMP1/PNPT</i>	g/a	0.04	0.03	1.72E-01	-0.005	0.001	<b>1.17E-04</b>	-0.013	0.002	<b>4.92E-08</b>	0.003	0.004	5.14E-01
rs12494328	<i>FLNB</i>	a/g	0.04	0.03	1.52E-01	0.006	0.001	<b>1.56E-06</b>	0.016	0.002	<b>6.03E-11</b>	0.009	0.004	4.50E-02
6:36592986	<i>CDKN1A</i>	d/r	-0.02	0.03	5.32E-01	0.006	0.001	<b>1.92E-05</b>	0.015	0.003	<b>1.12E-08</b>	-0.006	0.005	2.09E-01
rs72852338	<i>CDKN1A</i>	c/a	-0.02	0.03	5.46E-01	0.006	0.001	<b>3.29E-05</b>	0.014	0.003	<b>3.17E-08</b>	-0.005	0.005	2.97E-01
rs1074407	<i>TRIOBP</i>	t/a	0.11	0.02	<b>4.00E-06</b>	0.006	0.001	<b>3.32E-07</b>	0.012	0.002	<b>1.90E-08</b>	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 <  $5.37 \times 10^{-4}$ ; 0.05/93). Nearest gene, reference NCBI build37; A1, reference allele; A2, other allele;  $\beta$ , 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**

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

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  $< 5.37 \times 10^{-4}$ ; 0.05/93). Nearest gene, reference NCBI build37;  $\beta$ , 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)**

SNP	Nearest gene	A1/A2	IOP			VCDR			Cup area			Disc area		
			$\beta$	SE	P	$\beta$	SE	P	$\beta$	SE	P	B	SE	P
1:227562773	<i>CDC42BPA</i>	d/r	-0.10	0.05	3.01E-02	0.003	0.002	2.37E-01	0.024	0.004	<b>8.05E-09</b>	-0.055	0.008	<b>3.65E-13</b>
rs73102394	<i>CDC42BPA</i>	t/c	-0.09	0.05	4.34E-02	0.003	0.002	1.62E-01	0.022	0.004	<b>4.16E-08</b>	-0.053	0.007	<b>5.01E-13</b>
rs11811982	<i>CDC42BPA</i>	a/c	-0.12	0.05	1.35E-02	0.004	0.002	5.54E-02	0.027	0.004	<b>2.31E-10</b>	-0.062	0.008	<b>2.02E-15</b>
rs10021731	<i>UGT8</i>	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	<b>7.48E-07</b>
rs12220165	<i>CTNNA3*</i>	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	<b>2.51E-05</b>
rs787541	<i>U6, GADD45A</i>	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	<b>6.66E-08</b>
rs1367187	<i>DIRC3</i>	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	<b>1.03E-08</b>
rs2443724	<i>VGLL4</i>	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	<b>4.72E-08</b>
rs1013830	<i>CTNNA3</i>	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	<b>5.45E-08</b>

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 <  $5.37 \times 10^{-4}$ ; 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;  $\beta$ , 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
<b>IOP SNPs</b>									
rs10918274	<i>TMCO1</i>	t/c	1.39 (1.30-1.50)	1.39	<b>2.75E-19</b>	<b>1.37E-09</b>	++++	38.4	1.82E-01
rs7635832	<i>FNDC3B</i>	g/t	0.89 (0.83-0.95)	0.91	1.41E-03	3.65E-02	---?	33.9	2.20E-01
rs10281637	<i>CAV1/CAV2</i>	c/t	1.13 (1.07-1.20)	1.13	<b>2.32E-05</b>	<b>2.32E-05</b>	++++	0	4.89E-01
rs2487048	<i>ABCA1</i>	a/g	1.26 (1.19-1.33)	1.26	<b>2.65E-15</b>	3.82E-03	++++	82.9	5.53E-04
rs8176741	<i>ABO</i>	a/g	1.07 (0.99-1.17)	1.04	7.36E-02	5.25E-01	+++	58.5	6.51E-02
rs7944735	Many genes ( <i>NUP160, PTPRJ</i> )	c/g	1.06 (1.01-1.13)	1.07	2.99E-02	2.99E-02	++++	0	8.99E-01
11:120357425	<i>ARHGEF12</i>	d/r	1.16 (1.09-1.23)	1.19	<b>1.52E-06</b>	3.02E-02	++++	83.2	4.65E-04
rs55796939	<i>ADAMTS8</i>	t/c	1.07 (0.94-1.24)	1.17	2.72E-01	4.46E-01	+?--	78.6	9.35E-03
rs9913911	<i>GAS7</i>	g/a	0.80 (0.76-0.84)	0.80	<b>1.08E-17</b>	<b>1.08E-17</b>	----	0	7.50E-01
<b>VCDR SNPs</b>									
rs1925953	<i>RPE65</i>	t/a	1.07 (1.02-1.13)	1.10	4.21E-03	2.01E-02	++++	46.7	1.31E-01
rs1192414	<i>CDC7/TGFBR3</i>	a/g	1.08 (1.02-1.16)	1.08	9.26E-03	9.26E-03	++++	0	7.27E-01
rs10753787	<i>F5</i>	t/c	0.97 (0.93-1.03)	0.97	3.67E-01	3.67E-01	----	0	9.92E-01
rs6804624	<i>COL8A1</i>	c/t	0.99 (0.94-1.05)	0.99	8.14E-01	8.14E-01	---+	0	8.42E-01
rs72759609	<i>PDZD2</i>	c/t	0.90 (0.83-0.99)	0.91	3.20E-02	3.20E-02	----	0	9.53E-01
rs114503346	<i>DUSP1</i>	t/c	1.00 (0.80-1.25)	1.00	9.99E-01	8.80E-01	+?+-	42	1.78E-01
rs4960295	<i>RREB1</i>	a/g	0.99 (0.95-1.05)	1.00	9.50E-01	9.09E-01	+++	4.6	3.70E-01
rs10274998	<i>DGKB</i>	t/c	1.03 (0.98-1.10)	1.04	2.16E-01	2.16E-01	+++	0	5.38E-01
rs2157719	<i>CDKN2B-AS1</i>	c/t	0.69 (0.66-0.74)	0.69	<b>1.29E-40</b>	<b>1.29E-40</b>	----	0	5.67E-01
rs1900005	<i>ATOH7</i>	a/c	1.01 (0.96-1.07)	1.01	6.98E-01	6.77E-01	+++	5.1	3.67E-01
10:96008348	<i>PLCE1</i>	d/r	1.02 (0.97-1.09)	1.04	3.38E-01	3.15E-01	++?	35.3	2.13E-01
rs1346	<i>SSSCA1</i>	t/a	0.90 (0.85-0.97)	0.91	2.41E-03	2.41E-03	----	0	9.04E-01
rs4936099	<i>ADAMTS8</i>	c/a	0.94 (0.9-1.00)	0.94	5.75E-02	5.75E-02	----	0	9.63E-01
rs324780	<i>TMTC2</i>	g/a	0.93 (0.89-0.99)	0.93	1.35E-02	1.35E-02	----	0	7.69E-01
13:36629905	<i>DCLK1</i>	d/r	0.99 (0.94-1.05)	0.99	7.53E-01	8.00E-01	---+	6.2	3.62E-01
rs8015152	<i>SIX6</i>	t/c	1.21 (1.16-1.28)	1.19	<b>3.90E-15</b>	<b>7.08E-05</b>	++++	62.4	4.62E-02
rs4299136	<i>ASB7</i>	c/g	1.03 (0.97-1.10)	1.03	3.55E-01	3.55E-01	+++	0	8.29E-01
16:51461915	<i>SALL1</i>	i/r	0.94 (0.89-1.00)	0.94	3.85E-02	3.85E-02	----	0	7.82E-01
rs6107845	<i>BMP2</i>	a/g	0.89 (0.85-0.94)	0.91	1.02E-05	6.94E-03	----	43.1	1.53E-01
rs5752773	<i>CHEK2</i>	g/c	0.92 (0.88-0.98)	0.92	4.63E-03	4.63E-03	----	0	9.12E-01
rs2092172	<i>CARD10</i>	a/g	0.97 (0.92-1.04)	0.98	4.35E-01	4.35E-01	---+	0	7.76E-01
rs6764184	<i>FLNB</i>	t/g	1.07 (1.02-1.13)	1.02	5.73E-03	7.66E-01	+++	86.1	8.14E-05
rs7717697	<i>VCAN</i>	c/t	0.98 (0.93-1.04)	0.98	5.26E-01	5.26E-01	---?	0	7.30E-01
rs2920293	<i>PSCA</i>	g/c	1.03 (0.98-1.09)	1.03	2.25E-01	2.25E-01	+++?	0	3.79E-01
rs1681739	<i>ENO4</i>	t/c	1.02 (0.97-1.08)	1.03	3.92E-01	3.99E-01	+++	49.2	1.16E-01
rs7311936	<i>FAM101A</i>	c/g	0.99 (0.95-1.04)	1.00	8.12E-01	8.59E-01	+++	11	3.38E-01
14:23388793	<i>RBM23</i>	r/d	1.03 (0.98-1.10)	1.03	1.83E-01	1.83E-01	+++?	0	4.61E-01
<b>Cup area SNPs</b>									
rs2252865	<i>RERE</i>	t/c	1.11 (1.06-1.18)	1.11	5.76E-05	2.87E-02	+++	59.3	6.10E-02
rs4846112	<i>DHRS3</i>	a/g	0.95 (0.91-1.01)	0.96	1.18E-01	1.18E-01	----	0	5.53E-01
1:227562773	<i>CDC42BPA</i>	d/r	0.87 (0.79-0.97)	0.90	1.14E-02	2.11E-01	---?	48.6	1.43E-01
rs13016883	<i>TRIB2</i>	c/g	1.08 (1.03-1.14)	1.08	4.25E-03	4.25E-03	+++?	0	8.63E-01
rs35084382	<i>DUSP1</i>	c/t	1.04 (0.85-1.29)	1.05	6.72E-01	6.72E-01	+?+-	0	3.91E-01
rs117598310	<i>CRISPLD1</i>	t/g	1.08 (1.00-1.19)	1.09	5.39E-02	5.39E-02	+++	0	8.01E-01
rs1360589	<i>CDKN2B-AS1</i>	c/t	0.69 (0.66-0.73)	0.69	<b>1.90E-42</b>	<b>1.90E-42</b>	----	0	6.47E-01
rs10998036	<i>ATOH7</i>	c/g	1.01 (0.96-1.08)	1.02	5.42E-01	5.72E-01	+++	26	2.55E-01
10:96008348	<i>PLCE1</i>	d/r	1.02 (0.97-1.09)	1.04	3.38E-01	3.15E-01	++?	35.3	2.13E-01
rs1346	<i>SSSCA1</i>	t/a	0.90 (0.85-0.97)	0.91	2.41E-03	2.41E-03	----	0	9.04E-01
rs482507	<i>TMTC2</i>	c/t	0.94 (0.89-0.99)	0.94	2.03E-02	2.03E-02	----	0	7.46E-01
rs11613189	<i>FAM101A</i>	t/c	0.99 (0.95-1.05)	0.99	8.25E-01	7.77E-01	+++	18.5	2.98E-01
rs7323428	<i>DCLK1</i>	t/g	0.99 (0.94-1.05)	1.00	7.83E-01	8.87E-01	++-	13.6	3.25E-01
rs2251069	<i>DDHD1</i>	c/t	0.95 (0.91-1.00)	0.96	7.62E-02	7.62E-02	---+	0	4.08E-01
rs4436712	<i>SIX6</i>	t/g	1.24 (1.19-1.31)	1.23	<b>5.77E-18</b>	<b>1.52E-07</b>	++++	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
<b>Cup area SNPs</b>									
rs6598351	<i>FAM169B</i>	t/c	0.99 (0.93-1.06)	0.99	8.06E-01	8.06E-01	-+--	0	7.11E-01
rs11646917	<i>SALL1</i>	t/3g	0.98 (0.93-1.04)	0.98	5.49E-01	5.49E-01	---+	0	5.97E-01
rs11867840	<i>BCAS3</i>	g/a	1.06 (1.01-1.13)	1.06	1.83E-02	2.12E-02	++++	8.3	3.51E-01
rs6054375	<i>BMP2</i>	t/g	0.89 (0.85-0.94)	0.91	8.52E-06	9.93E-03	----	47.1	1.29E-01
rs738722	<i>CHEK2</i>	t/c	0.93 (0.89-0.99)	0.93	1.26E-02	1.26E-02	----	0	9.05E-01
rs3791679	<i>EFEMP1/PNPT1</i>	a/g	0.96 (0.92-1.02)	0.96	2.23E-01	2.23E-01	----	0	5.51E-01
rs12494328	<i>FLNB</i>	a/g	1.13 (1.07-1.20)	1.13	<b>1.28E-05</b>	<b>5.89E-04</b>	++++	26.9	2.50E-01
rs6804624	<i>COL8A1</i>	c/t	0.99 (0.94-1.05)	0.99	8.14E-01	8.14E-01	---+	0	8.42E-01
6:36592986	<i>CDKN1A</i>	d/r	1.14 (1.09-1.21)	1.15	<b>7.74E-07</b>	<b>1.04E-04</b>	++++	36.6	1.93E-01
rs2684249	<i>HSF2</i>	c/t	0.92 (0.88-0.97)	0.94	1.08E-03	1.66E-01	---+	63.3	4.25E-02
rs8176672	<i>ABO</i>	t/c	1.00 (0.91-1.11)	1.00	9.49E-01	9.49E-01	-+??	0	3.69E-01
rs4936099	<i>ADAMTS8</i>	c/a	0.94 (0.90-1.00)	0.94	5.75E-02	5.75E-02	----	0	9.63E-01
rs34222435	<i>ASB7</i>	t/c	1.03 (0.97-1.10)	1.03	3.66E-01	3.66E-01	++++	0	8.74E-01
rs1074407	<i>TRIOBP</i>	t/a	1.04 (1.00-1.10)	1.04	4.92E-02	8.66E-02	++++	32.9	2.15E-01
<b>Disc area SNPs</b>									
rs4658101	<i>CDC7/TGFB3</i>	a/g	1.08 (1.02-1.16)	1.08	7.81E-03	7.81E-03	++++	0	7.22E-01
1:169530520	<i>F5/SELP</i>	i/r	1.01 (0.96-1.08)	1.02	5.40E-01	5.40E-01	++??	0	7.14E-01
rs11811982	<i>CDC42BPA</i>	a/c	0.87 (0.80-0.97)	0.90	1.19E-02	8.28E-02	---+	20.5	2.87E-01
rs9843102	<i>ABI3BP</i>	a/g	0.92 (0.86-0.98)	0.92	1.37E-02	1.37E-02	----	0	6.24E-01
rs10021731	<i>UGT8</i>	c/t	1.01 (0.96-1.06)	1.01	6.82E-01	6.82E-01	---+	0	6.50E-01
8:88744441	<i>DCAF4L2</i>	d/r	1.03 (0.99-1.09)	1.04	0.1225	1.39E-01	++++	4.9	3.68E-01
rs12220165	<i>CTNNA3</i>	g/c	1.08 (1.01-1.16)	1.09	1.14E-02	1.14E-02	++++	0	9.04E-01
rs7916410	<i>ATOH7</i>	t/c	1.00 (0.96-1.06)	1.00	7.63E-01	7.45E-01	+++	3.9	3.73E-01
rs61101201	<i>ELP4/PAX6</i>	g/t	1.00 (0.94-1.06)	1.00	9.77E-01	9.77E-01	-+??	0	9.63E-01
rs442376	<i>TMTC2</i>	c/t	1.04 (0.99-1.10)	1.05	7.94E-02	7.94E-02	++++	0	6.82E-01
rs1345467	<i>SALL1</i>	g/a	1.07 (1.01-1.14)	1.07	1.86E-02	1.86E-02	++++	0	8.73E-01
rs5762752	<i>CHEK2</i>	c/g	0.92 (0.88-0.98)	0.92	4.90E-03	4.90E-03	----	0	8.29E-01
rs56385951	<i>CARD10</i>	a/g	0.99 (0.92-1.07)	1.00	9.15E-01	9.15E-01	++-	0	9.88E-01
1:3046430	<i>PRDM16</i>	i/r	0.97 (0.87-1.10)	0.98	7.13E-01	8.72E-01	++??	63.9	6.28E-02
rs787541	<i>U6, GADD45A</i>	c/g	0.98 (0.94-1.04)	0.98	6.10E-01	9.06E-01	---+	50.7	1.08E-01
rs1367187	<i>DIRC3</i>	c/t	0.95 (0.90-1.01)	0.96	1.11E-01	4.12E-01	++-	46.1	1.35E-01
rs2443724	<i>VGLL4</i>	c/g	0.91 (0.87-0.97)	0.91	1.04E-03	2.61E-02	---+	38	1.84E-01
rs11129176	<i>RARB</i>	a/g	0.99 (0.94-1.05)	1.00	8.85E-01	9.93E-01	----	40.4	1.69E-01
rs1997404	<i>COL8A1</i>	g/t	1.00 (0.95-1.06)	1.00	9.60E-01	9.60E-01	++++	0	6.18E-01
rs34935520	<i>SIX6</i>	g/a	1.26 (1.20-1.33)	1.26	<b>2.82E-20</b>	<b>6.73E-14</b>	++++	21.5	2.81E-01
rs60779155	<i>ASB7</i>	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<sup>2</sup> statistic measuring heterogeneity on a scale of 0% to 100%; i, insertion; d, deletion; r, reference.

## Abbreviations

<b>Abbreviation</b>	<b>Explanation</b>
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
$\beta$	Effect size