Genome-wide analysis of multi-ancestry cohorts identifies new loci influencing intraocular pressure and susceptibility to glaucoma

Elevated intraocular pressure (IOP) is an important risk factor in developing glaucoma, and variability in IOP might herald glaucomatous development or progression. We report the results of a genome-wide association study meta-analysis of 18 population cohorts from the International Glaucoma Genetics Consortium (IGGC), comprising 35,296 multi-ancestry participants for IOP. We confirm genetic association of known loci for IOP and primary open-angle glaucoma (POAG) and identify four new IOP-associated loci located on chromosome 3q25.31 within the FNDC3B gene ($P = 4.19 \times 10^{-8}$ for rs6445055), two on chromosome 9 ($P = 2.80 \times 10^{-11}$ for rs2472493 near ABCA1 and $P = 6.39 \times 10^{-11}$ for rs8176693 within ABO) and one on chromosome 11p11.2 (best $P = 1.04 \times 10^{-11}$ for rs747782). Separate meta-analyses of 4 independent POAG cohorts, totaling 4,284 cases and 95,560 controls, showed that 3 of these loci for IOP were also associated with POAG.

POAG is the leading cause of irreversible blindness in the world \(^1\). The only modifiable risk factor for the development and progression of glaucoma is high IOP \(^2\), and lowering IOP is currently the only therapy that can reduce glaucomatous progression, even in forms of glaucoma that have IOP close to the statistical norm for the population (normal-tension glaucoma, or NTG) \(^3\)\(^4\). POAG and IOP are highly heritable: the lifetime risk of developing POAG is 22% among first-degree relatives of cases \(^5\), which is approximately ten times higher than the risk for the rest of the population \(^1\). Heritability for IOP is estimated to be approximately 55% (ref. \(6\)). Genetic studies have shown that the genetic risks for POAG and IOP are partly shared; polymorphisms within the TMCO1 gene are associated with both POAG risk \(^7\) and IOP \(^8\). Studying genetic determinants of IOP is therefore likely to provide critical insights into the genetic architecture of POAG and open new avenues for therapeutic intervention.

In this study, we present the results from a meta-analysis of genome-wide association studies (GWAS) of IOP from 18 studies participating in IGGC and an assessment of the importance of the genetic findings for susceptibility to POAG (Fig. 1). The IOP meta-analysis included 35,296 subjects (7,738 Asians and 27,558 individuals of European descent) drawn from the general populations of 7 countries. The demographic characteristics of these population-based cohorts are given in Supplementary Table 1. Genotyping assays and imputation to HapMap 2 haplotypes were performed at individual sites. Association analyses were performed using an additive model with IOP as the outcome and the number of alleles at each polymorphic site as the predictor, adjusting for age and sex. IOP levels for participants who were receiving IOP-lowering therapy at the time of the study and for whom data on baseline, pretreatment levels were not available were imputed as previously described \(^8\). Subjects who had undergone surgery or had other eye diseases that could affect IOP were excluded (Supplementary Note). Secondary analyses were carried out adjusting for central corneal thickness (CCT), which is known to influence IOP measurements \(^9\).

After applying conventional quality control filters, we performed a fixed-effects meta-analysis of the 22 autosomes across the cohorts with approximately 2.5 million markers. Within-study genomic inflation factors \(^10\) ranged between 0.992 and 1.043 (Supplementary Fig. 1 and Supplementary Table 2), indicating a lack of major population stratification bias within each study. SNPs available in fewer than 16 cohorts or showing large effect heterogeneity (defined as $P > 75\%$) \(^11\) were removed. We found 145 SNPs (Supplementary Table 3) whose associations crossed the conventional genome-wide significance threshold for association ($P < 5 \times 10^{-8}$) \(^12\). All of these SNPs clustered around seven separate regions of the genome (Fig. 2 and Supplementary Figs. 2 and 3). Two of the regions associated with IOP in our meta-analysis had previously been implicated in IOP variability: the regions near

A full list of authors and affiliations appears at the end of the paper.

Received 26 September 2013; accepted 7 August 2014; published online 31 August 2014; doi:10.1038/ng.3087
the TMCO1 locus (P = 2.19 × 10⁻⁹ for rs7555523) and near the GAS7 gene (P = 1.03 × 10⁻¹¹ for rs9913911). A third associated locus, new for IOP, was near the CAV1 and CAV2 genes (P = 6.27 × 10⁻⁹ for rs10258482) and GAS7 (P = 5.22 × 10⁻¹³ for rs12150284). All alleles associated with higher IOP levels also increased glaucoma risk (Table 1).

We then examined whether the effect sizes of SNPs on IOP levels (βIOP) were linearly related to their effect sizes on POAG (βPOAG) using a causal inference framework as previously described. In a linear regression analysis, we observed a significant association between βIOP and βPOAG (P = 0.03; Supplementary Table 4), suggesting that the strength of a SNP’s effect on IOP levels is correlated with its effect on risk for POAG.

We subsequently investigated the relationship between variants within the seven regions associated with IOP and cis regulation of mRNA expression in 3 tissues (adipose, lymphoblastoid cell lines (LCLs) and skin) from a sample of 856 UK subjects. The most significant expression quantitative trait locus (eQTL) associations were generally observed in LCLs for most loci, except for CAV1, where effects were strongest in adipose and skin tissues (Table 2). Significant eQTL association was observed for rs4656461 and rs7555523 (P = 0.003 and 0.0001 with TMCO1 and ALDH9A1 transcript levels in skin and LCLs, respectively), rs2024211 (P = 5.43 × 10⁻¹⁰ and 3.84 × 10⁻¹³ with CAV1 transcript levels in adipose and skin tissues, respectively), rs2472493 (P = 3.67 × 10⁻⁵ with ABCA1 transcript levels in LCLs) and rs1681630 on chromosome 11 (P = 2.72 × 10⁻¹⁰ with SPI1 transcript levels in LCLs), among others (Table 2 and Supplementary Table 5a). These SNPs also had the strongest eQTL effects for their respective transcripts (Supplementary Table 5b).

We measured the mRNA expression levels of the identified genes in adult ocular tissues using RT-PCR. We found that most of the identified genes, including TMCO1, FNDC3B, CAV1-CAV2, ABCA1 and GAS7, were expressed in most ocular tissues (Supplementary Table 6). The genes within the chromosome 11 locus showed varied expression levels across ocular tissues. Gene-based tests or enrichment for Gene Ontology terms did not identify any new genes or pathways after correction for multiple testing (Supplementary Tables 7 and 8).

### Table 1 Results for association with IOP from the general-population cohorts for SNPs significant at a multiple-testing correction level (P < 5 × 10⁻⁸) and their association with POAG in case-control validation meta-analyses

| Chr. | Position (bp) | rsID | A1/A2 | Nearest gene | Association with IOP in the discovery cohort | Association in POAG case-control cohorts |
|------|---------------|------|-------|--------------|---------------------------------------------|------------------------------------------|
|      |               |      |       |              | β | SE | P | Heterogeneity | P² | OR (95% CI) | P |
| 1    | 165,687,205   | rs4656461 | G/A | TMCO1 | 0.228 | 0.039 | 6.51 × 10⁻⁹ | 0.46 | 0.00 | 1.38 (1.28–1.50) | 2.55 × 10⁻¹⁵ |
| 1    | 165,718,979   | rs7555523 | G/A | TMCO1 | 0.235 | 0.039 | 2.19 × 10⁻⁹ | 0.55 | 0.00 | 1.40 (1.30–1.52) | 1.34 × 10⁻¹⁶ |
| 1    | 171,992,387   | rs6445055 | A/G | FNDC3B | -0.177 | 0.030 | 4.19 × 10⁻⁸ | 0.17 | 0.24 | 0.92 (0.85–0.99) | 0.03 |
| 7    | 116,150,095   | rs10258482 | A/C | CAV1 | 0.196 | 0.029 | 1.87 × 10⁻¹¹ | 0.81 | 0.00 | 1.20 (1.13–1.28) | 6.27 × 10⁻⁹ |
| 7    | 116,150,952   | rs10262524 | A/C | CAV1 | 0.186 | 0.029 | 9.69 × 10⁻¹¹ | 0.67 | 0.00 | 1.20 (1.13–1.28) | 1.99 × 10⁻⁸ |
| 10   | 107,695,848   | rs2472493 | G/A | ABCA1 | 0.159 | 0.024 | 2.80 × 10⁻¹⁵ | 0.00 | 0.00 | 1.21 (1.16–1.34) | 1.45 × 10⁻⁹ |
| 11   | 136,131,415   | rs8176743 | T/C | ABO | 0.261 | 0.039 | 3.08 × 10⁻¹¹ | 0.53 | 0.00 | 1.07 (0.96–1.19) | 0.2 |
| 11   | 47,468,545    | rs12419342 | C/T | RAPSN | 0.153 | 0.026 | 4.77 × 10⁻⁷ | 0.75 | 0.00 | 1.09 (1.02–1.16) | 0.008 |
| 11   | 47,940,925    | rs747782 | C/T | NUP150, PTPRJ | 0.203 | 0.030 | 1.04 × 10⁻¹¹ | 0.95 | 0.00 | 1.03 (0.96–1.11) | 0.36 |
| 11   | 47,969,152    | rs1681630 | T/C | PTPRJ | 0.144 | 0.026 | 1.69 × 10⁻⁸ | 0.60 | 0.00 | 1.06 (0.99–1.12) | 0.08 |
| 11   | 48,004,369    | rs7946766 | T/C | PTPRJ | 0.230 | 0.035 | 2.71 × 10⁻¹¹ | 0.35 | 0.09 | 1.03 (0.95–1.12) | 0.43 |
| 17   | 10,031,183    | rs9913911 | G/A | GAS7 | -0.179 | 0.026 | 1.03 × 10⁻¹¹ | 4.00 | 0.61 | 0.80 (0.75–0.85) | 2.98 × 10⁻¹³ |

Chr., chromosome; A1/A2, reference/alternative alleles; β, linear regression coefficient (mm Hg); SE, standard error of the regression coefficient; OR, odds ratio; 95% CI, 95% confidence interval for OR.
Altogether, these SNPs explained approximately 1.2% of the heritability for IOP in the TwinsUK cohort, 1.5% of the phenotypic variability in IOP in the Rotterdam Study and between 0.6 and 1.2% of the phenotypic variability in IOP in Asians. *FNDC3B* has been associated with CCT, and, as CCT has a significant effect on IOP measurements, we performed an additional meta-analysis of IOP adjusted for age, sex and CCT in a smaller subsample that had CCT measures (19,563 subjects from 13 population cohorts). The association for rs6445055 remained nominally significant although it was weaker (P = 9.87 × 10^{-4}, β = −0.121 in comparison to −0.177 before adjustment for CCT). This finding suggests that this locus has at least some CCT-independent effect over IOP levels. The association evidence remained consistent, although slightly weaker, for the other loci (Supplementary Table 9).

We report association of variants within the *ABCA1* gene with IOP and POAG. A strong eQTL effect was observed in LCLs (P = 3.67 × 10^{-5}) for the most highly associated SNP (rs2472493) in our analyses. *ABCA1* is expressed in many tissues and its expression in leukocytes is significantly upregulated in individuals with glaucoma.

Associations for a number of SNPs within the *ABO* blood group gene and IOP, although statistically significant and homogeneous across the participating cohorts, were not observed in the glaucoma case-control meta-analysis. This might be owing to type I error in the initial meta-analysis or insufficient power to detect a primarily IOP-led effect in cases that included individuals with NTG, resulting in a type II error in the latter analysis. Four of the nine GWAS polymorphisms associated at genome-wide significance in the *ABO* locus were nonsynonymous variants, determining the B blood group.

This finding might be relevant, given previous observations that the B blood group is epidemiologically associated with glaucoma, of which four are newly discovered, is a key step toward better understanding the mechanisms of IOP regulation, currently the only modifiable risk factor for POAG.

### METHODS

Methods and any associated references are available in the online version of the paper.

**Note:** Any Supplementary Information and Source Data files are available in the online version of the paper.

### ACKNOWLEDGMENTS

We gratefully acknowledge the contributions of all participants who volunteered within each cohort and the personnel responsible for the recruitment and administration of each study. We also thank the various funding sources that made this work possible. Complete funding information and acknowledgments can be found in the Supplementary Note.

### AUTHOR CONTRIBUTIONS

P.G.H., C.-Y.C., H.S., S.M., J.N.C.B. and R.W. performed analyses and drafted the manuscript. S.M., A.J.L., J.E.B.-W., V.V., L.R.P., N.P., C.D., A.V., D.A.M., J.E.C., S.H., M.D., C.H. and T.A. jointly conceived the project and supervised the work. P.G.H., H.S., R.W., A.N., A.W.H., A.M., C.V., R.H., G.T., B.A.O., S.-M.S.,
1. Quigley, H.A. & Broman, A.T. The number of people with glaucoma worldwide in 2010 and 2020. Br. J. Ophthalmol. 90, 262–267 (2006).

2. Heijl, A., Leske, M.C., Bengtsson, B., Hyman, L. & Hussein, M. Reduction of intraocular pressure and glaucoma progression: results from the Early Manifest Glaucoma Trial. Arch. Ophthalmol. 120, 1268–1279 (2002).

3. Collaborative Normal-Tension Glaucoma Study Group. The effectiveness of intraocular pressure reduction in the treatment of normal-tension glaucoma. Collaborative Normal-Tension Glaucoma Study Group. Am. J. Ophthalmol. 126, 498–505 (1998).

4. Kass, M.A. et al. The Ocular Hypertension Treatment Study: a randomized trial designed to test the effect of降低眼内压 on the risk of primary open-angle glaucoma. Arch. Ophthalmol. 120, 701–713 (2002).

5. Wolfs, R.C. et al. Genetic risk of primary open-angle glaucoma. Population-based familial aggregation study. Arch. Ophthalmol. 116, 1640–1645 (1998).

6. Sanfilippo, P.G., Hewitt, A.W., Hammond, C.J. & Mackey, D.A. The heritability of ocular traits. Surv. Ophthalmol. 55, 561–583 (2010).

7. Burdon, K.P. et al. Genome-wide association study identifies susceptibility loci for open-angle glaucoma at TMC01 and CDKN2B-AS1. Nat. Genet. 43, 574–578 (2011).

8. van Koolwijk, L.M. et al. Common genetic determinants of intraocular pressure and primary-open-angle glaucoma. PLoS Genet. 8, e1002611 (2012).

9. Shah, S. et al. Relationship between corneal thickness and measured intraocular pressure in a general ophthalmology clinic. Ophthalmology 106, 2154–2160 (1999).

10. Devlin, B. & Roeder, K. Genomic control for association studies. Stat. Med. 21, 1539–1558 (2002).

11. Higgins, J.P. & Thompson, S.G. Quantifying heterogeneity in a meta-analysis. Stat. Med. 21, 1539–1558 (2002).

12. Dudbridge, F. & Gusnanto, A. Estimation of significance thresholds for genomewide association scans. Genet. Epidemiol. 32, 227–234 (2008).

13. Thorleifsson, G. et al. Common variants near CAV1 and CAV2 are associated with primary open-angle glaucoma. Nat. Genet. 42, 906–909 (2010).

14. Ozeki, A.B. et al. Genome-wide association study and meta-analysis of intraocular pressure. Hum. Genet. 133, 41–57 (2014).

15. Do, R. et al. Common variants associated with plasma triglycerides and risk for coronary artery disease. Nat. Genet. 45, 1345–1352 (2013).

16. Grundberg, E. et al. Mapping cis- and trans-regulatory effects across multiple tissues in twins. Nat. Genet. 44, 1084–1089 (2012).

17. Moayyeri, A., Hammond, C.J., Hart, D.J. & Spector, T.D. The UK Adult Twin Registry. Twin Res. UK Resour. Cent. Hum. Genet. 16, 144–149 (2013).

18. Hofman, A. et al. The Rotterdam Study: 2012 objectives and design update. Eur. J. Epidemiol. 26, 657–686 (2011).

19. Lu, Y. et al. Genome-wide association analyses identify multiple loci associated with central corneal thickness and keratocornea. Nat. Genet. 45, 155–163 (2013).

20. Tonru, P.A. et al. The influence of central corneal thickness and age on intraocular pressure measured by pneumotonometry, non-contact tonometry, the Tono-Pen XL, and Goldmann applanation tonometry. Br. J. Ophthalmol. 89, 851–854 (2005).

21. Denis, M. et al. Expression, regulation, and activity of ABCA1 in human cell lines. Mol. Genet. Metab. 78, 265–274 (2003).

22. Teghizyan, K. et al. An enhanced expression of ABC1 transporter in circulating leukocytes as a potential molecular marker for the diagnostics of glaucoma. Amino Acids 28, 207–211 (2005).

23. Denomme, G.A. et al. Consortium for Blood Group Genes (CBGG): 2009 report. Immunohematology 26, 47–50 (2010).

24. Khan, M.I. et al. Association of ABO blood groups with glaucoma in the Pakistani population. Can. J. Ophthalmol. 44, 582–586 (2009).

25. Janssen, S.F. et al. Gene expression and functional annotation of the human ciliary body epithelia. PLoS ONE 7, e44973 (2012).

26. Charlesworth, J. et al. The path to open-angle glaucoma gene discovery: endophenotypic status of intraocular pressure, cup-to-disc ratio, and central corneal thickness. Invest. Ophthalmol. Vis. Sci. 51, 3509–3514 (2010).

27. Purcell, S.M. et al. Common polygenic variation contributes to risk of schizophrenia and bipolar disorder. Nature 460, 719–725 (2009).

28. Carbonaro, P., Andrew, T., Mackey, D.A., Spector, T.D. & Hammond, C.J. Comparison of three methods of intraocular pressure measurement and their relation to central corneal thickness. Eye (Lond.) 24, 1165–1170 (2010).
ONLINE METHODS

IGGC participants. All studies participating in this meta-analysis are part of the International Glaucoma Genetics Consortium. The discovery cohorts included 27,558 individuals of European ancestry from 14 studies (AILIENOR, BATS, BMES2,3, EF5,11,12, Framingham Family Study31,43, GH5, GHSS, ORCADES3, RAINE32,33, RS-1, RS-2, RS-3 (ref. 38), TESS39 and TwinsUK40). In addition, 7,738 individuals of Asian ancestry from 4 cohorts (BES41, SCES42, SMES43 and SIND42) were included. In addition, four case-control population panels were used, all of European ancestry: ANZRAG, MEEI, NEIGHBOR and deCODE. General methods, demographics and phenotyping of the study cohorts have previously been described extensively, and details are provided in the Supplementary Note and Supplementary Table 1. All studies were performed with the approval of their local medical ethics committees, and written informed consent was obtained from all participants in accordance with the Declaration of Helsinki.

Phenotype measurements. Eligible participants underwent an ophthalmologic examination including measurements of IOP and, for most but not all studies, measurements of CCT. Each participating cohort was phenotyped separately, and the IOP measurement methods used by each are described in Supplementary Table 1.

Genotyping and imputation. Study samples were genotyped on either Illumina or Affymetrix platforms. Each study performed SNP imputation using the genotype data, together with HapMap Phase 2 ancestry-matched reference panels (CEU (Utah residents of Northern and Western European ancestry), JPT + CHB (Japanese in Tokyo and Han Chinese in Beijing) or the four HapMap populations) on the basis of the Build 36 databases (release 22 or 24). Markov Chain Haplotyping software, IMPUTE44,45 or MACH46, were adopted for imputation. A detailed description regarding genotyping platforms and imputation procedures for each study is provided in Supplementary Table 1.

Stringent quality control of genotype data was applied in each cohort. Samples with low call rates (<95%) or with sex discrepancies were excluded. CRYPTICALLY related samples and outliers in population structure from principal component analyses were also excluded. SNPs flagged with missingness of >5%, gross departure from Hardy-Weinberg equilibrium (P < 1 x 10^-8) or minor allele frequency (MAF) of <1% were removed from further analyses.

Statistical analysis. For each study, an allele dosage regression model at each directly genotyped or imputed SNP was conducted to determine its association with IOP. Data for eyes with previous glaucoma surgery or laser treatment were excluded. For subjects receiving IOP-lowering medication, we added 25% to the measured IOP levels to estimate pretreatment IOP, on the basis of a reported average of a 17–33% reduction in IOP caused by IOP-lowering medication in a meta-analysis of clinical trials47. The mean of the right and left IOP measurements was used. When data from only one eye were available, the IOP measurement from the available eye was used.

For the analyses, we assumed an additive genetic model where the dosage of each SNP was a continuous variable ranging from 0 to 2 for the minor alleles carried. Primary analysis for IOP was adjusted for age and sex. Additional adjustment for principal components was carried out for increased statistical weights inverse variance approach, assuming fixed effects, as for initial adjustment for principal components was carried out by a few participating carried. Primary analysis for IOP was adjusted for age and sex. Additional left IOP measurements was used. When data from only one eye were available, directly genotyped or imputed SNP was conducted to determine its association with IOP from the meta-analysis of (i.e., P < 0.000001, P < 0.00001, P < 0.001, P < 0.005, P < 0.01, P < 0.5). Profiles derived from IOP SNP effects were tested for association with the phenotype (here, POAG) using logistic regression. Variance explained was assessed using Nagelkerke’s pseudo R^2 measure.

To assess whether and to what degree IOP levels confer POAG risk, we performed a causal inference analysis using an instrumental variable framework as previously described. In brief, we obtained estimates of effect size (β_{IP}) for the association of a given SNP with IOP from the meta-analysis of the 18 discovery cohorts. For the association of a given SNP with POAG, we obtained estimates of the effect size (β_{IP}) from the four case-control panels as described above. We selected the SNP with the strongest association from each of the loci with genome-wide significant association with IOP that we identified. To assess whether the strength of a SNP’s association with POAG predicted risk of POAG, we conducted linear regression analysis using the effect size of each SNP for IOP (β_{IP}) as an independent variable and the effect size of POAG (β_{POAG}) as a dependent outcome variable. A total of seven independent IOP-associated SNPs were used for this analysis, including rs7555523 (TMCO1), rs6445055 (FND3CB), rs10258482 (CAV1), rs2472493 (ABCA1), rs8176743 (ABO), rs747782 (NUP160-PTPRB) and rs9913911 (GAS5).

Gene expression in human tissues. Adult ocular samples were obtained from the normal eyes of an 82-year-old European-ancestry female from the North Carolina Eye Bank. All adult ocular samples were stored in RNAlater (Qiagen) within 6.5 h of collection and shipped on dry ice overnight to the laboratory. Isolated tissues were snap frozen and stored at −80 °C until RNA extraction. RNA was extracted from each tissue sample independently using the Ambion mirVana total RNA extraction kit. Tissue samples were homogenized in Ambion lysis buffer using an Omni Bead Ruptor Tissue Homogenizer according to the provided protocol. Reverse-transcription reactions were performed with the Invitrogen SuperScript III First-Strand Synthesis kit. Expression of the identified genes was assessed by running 10-μl reactions with Qiagen PCR products consisting of 1.26 μl of water, 1.0 μl of 10x buffer, 1.0 μl of dNTPs, 0.3 μl of MgCl2, 2.0 μl of Q-Solution, 0.06 μl of Taq polymerase, 1.0 μl of forward primer, 1.0 μl of reverse primer and 1.5 μl of cDNA. Reactions were run an Eppendorf MasterCycler Pro S thermocycler with touchdown PCR decreasing the annealing temperature by 1 °C per cycle from 72 °C to
55 °C followed by 50 cycles of 94 °C for 30 s, 55 °C for 30 s and 72 °C for 30 s with a final elongation of 7 min at 72 °C. All primer sets were designed using Primer3 (ref. 52). Products were run on a 2% agarose gel at 70 V for 35 min. Primer sets were run on a custom tissue panel including Human MTC Panel I and Fetal MTC Panel I (Clontech) and an ocular tissue panel.

29. Mitchell, P., Smith, W., Attebo, K. & Wang, J.J. Prevalence of age-related maculopathy in Australia. The Blue Mountains Eye Study. *Ophtalmology* **102**, 1450–1460 (1995).
30. Foran, S., Wang, J.J. & Mitchell, P. Causes of visual impairment in two older population cross-sections: the Blue Mountains Eye Study. *Ophthalmic Epidemiol.* **10**, 215–225 (2003).
31. Aulchenko, Y.S. *et al.* Linkage disequilibrium in young genetically isolated Dutch population. *Eur. J. Hum. Genet.* **12**, 527–534 (2004).
32. Pardo, L.M., MacKay, I., Oostra, B., van Duijn, C.M. & Aulchenko, Y.S. The effect of genetic drift in a young genetically isolated population. *Ann. Hum. Genet.* **69**, 288–295 (2005).
33. Leibowitz, H.M. *et al.* The Framingham Eye Study monograph: an ophthalmological and epidemiological study of cataract, glaucoma, diabetic retinopathy, macular degeneration, and visual acuity in a general population of 2631 adults, 1973–1975. *Surv. Ophthalmol.* **24**, 335–610 (1980).
34. Vitart, V. *et al.* New loci associated with central cornea thickness include COL5A1, AKAP13 and AVGR8. *Hum. Mol. Genet.* **19**, 4304–4311 (2010).
35. Evans, S., Newnham, J., MacDonald, W. & Hall, C. Characterisation of the possible effect on birthweight following frequent prenatal ultrasound examinations. *Early Hum. Dev.* **45**, 203–214 (1996).
36. Newnham, J.P., Evans, S.F., Michael, C.A., Stanley, F.J. & Landau, I. Effects of frequent ultrasound during pregnancy: a randomised controlled trial. *Lancet* **342**, 887–891 (1993).
37. Williams, L.A., Evans, S.F. & Newnham, J.P. Prospective cohort study of factors influencing the relative weights of the placenta and the newborn infant. *Br. Med. J.* **314**, 1864–1868 (1997).
38. Hofman, A. *et al.* The Rotterdam Study: 2014 objectives and design update. *Eur. J. Epidemiol.* **28**, 889–926 (2013).
39. Mackey, D.A. *et al.* Twins eye study in Tasmania (TEST): rationale and methodology to recruit and examine twins. *Twin Res. Hum. Genet.* **12**, 441–454 (2009).
40. Spector, T.D. & Williams, F.M. The UK Adult Twin Registry (TwinsUK). *Twin Res. Hum. Genet.* **9**, 899–906 (2006).
41. Wang, Y.X., Xu, L., Yang, H. & Jonas, J.B. Prevalence of glaucoma in North China: the Beijing Eye Study. *Am. J. Ophthalmol.* **150**, 917–924 (2010).
42. Lavanya, R. *et al.* Methodology of the Singapore Indian Chinese Cohort (SICCI) eye study: quantifying ethnic variations in the epidemiology of eye diseases in Asians. *Ophthalmic Epidemiol.* **16**, 325–336 (2009).
43. Fong, A.W. *et al.* Rationale and methodology for a population-based study of eye diseases in Malay people: the Singapore Malay eye study (SiMES). *Ophthalmic Epidemiol.* **14**, 25–35 (2007).
44. Marchini, J., Howie, B., Myers, S., McVean, G. & Donnelly, P. A new multipoint method for genome-wide association studies by imputation of genotypes. *Nat. Genet.* **39**, 906–913 (2007).
45. Howie, B.N., Donnelly, P. & Marchini, J. A flexible and accurate genotype imputation method for the next generation of genome-wide association studies. *PLoS Genet.* **5**, e1000529 (2009).
46. Li, Y., Willer, C.J., Ding, J., Scheet, P. & Abecasis, G.R. MaCH: using sequence and genotype data to estimate haplotypes and unobserved genotypes. *Genet. Epidemiol.* **34**, 816–834 (2010).
47. van der Valk, R. *et al.* Intraocular pressure–lowering effects of all commonly used glaucoma drugs: a meta-analysis of randomized clinical trials. *Ophthalmology* **112**, 1177–1185 (2005).
48. Stephens, M. & Balding, D.J. Bayesian statistical methods for genetic association studies. *Nat. Rev. Genet.* **10**, 681–690 (2009).
49. Higgins, J.P., Thompson, S.G., Deeks, J.J. & Altman, D.G. Measuring inconsistency in meta-analyses. *Br. Med. J.* **327**, 557–560 (2003).
50. Liu, J.Z. *et al.* A versatile gene-based test for genome-wide association studies. *Am. J. Hum. Genet.* **87**, 139–145 (2010).
51. Nagelkerke, N.J.D. A note on a general definition of the coefficient of determination. *Biometrika* **78**, 691–692 (1991).
52. Rozen, S. & Skaletsky, H. Primer3 on the WWW for general users and for biologist programmers. *Methods Mol. Biol.* **132**, 365–386 (2000).