OXFORD

Human Molecular Genetics, 2015, Vol. 24, No. 9 2689–2699

doi: 10.1093/hmg/ddv027
Advance Access Publication Date: 30 January 2015
Original Article

ORIGINAL ARTICLE

ARHGEF12 influences the risk of glaucoma by increasing intraocular pressure

Henriët Springelkamp[1,2,†], Adriana I. Iglesias[2,†], Gabriel Cuellar-Partida[4], Najaf Amin[2], Kathryn P. Burdon[6], Elisabeth M. van Leeuwen[2], Puya Gharahkhani[4], Aniket Mishra[4], Sven J. van der Lee[2], Alex W. Hewitt[6,7], Fernando Rivadeneira[2,3,8], Ananth C. Viswanathan[9], Roger C.W. Wolfs[1], Nicholas G. Martin[5], Wishal D. Ramdas[1], Leonieke M. van Koolwijk[2], Craig E. Pennell[10], Johannes R. Vingerling[1,2], Jenny E. Mountain[11], André G. Uitterlinden[2,3,8], Albert Hofman[2,8], Paul Mitchell[12], Hans G. Lemij[13], Jie Jin Wang[12], Caroline C.W. Klaver[1,2], David A. Mackey[6,14], Jamie E. Craig[15], Cornelia M. van Duijn[2,‡] and Stuart MacGregor[4,*‡]

[1]Department of Ophthalmology, [2]Department of Epidemiology and [3]Department of Internal Medicine, Erasmus Medical Center, 3000 CA Rotterdam, The Netherlands, [4]Statistical and [5]Genetic Epidemiology, QIMR Berghofer Medical Research Institute, Royal Brisbane Hospital, Brisbane, QLD 4006, Australia, [6]School of Medicine, Menzies Research Institute Tasmania, University of Tasmania, Hobart, TAS 7000, Australia, [7]Centre for Eye Research Australia (CERA), University of Melbourne, Royal Victorian Eye and Ear Hospital, Melbourne, VIC 3002, Australia, [8]Netherlands Consortium for Healthy Ageing, Netherlands Genomics Initiative, 2593 CE The Hague, The Netherlands, [9]NIHR Biomedical Research Centre, Moorfields Eye Hospital NHS Foundation Trust and UCL Institute of Ophthalmology, London EC1V 2PD, UK, [10]School of Women's and Infants' Health, University of Western Australia, Crawley, WA 6009, Australia, [11]Telethon Kids Institute, Subiaco, WA 6008, Australia, [12]Centre for Vision Research, Department of Ophthalmology and Westmead Millennium Institute, University of Sydney, Sydney, NSW 2006, Australia, [13]Glaucoma Service, The Rotterdam Eye Hospital, 3011 BH Rotterdam, The Netherlands, [14]Centre for Ophthalmology and Visual Science, Lions Eye Institute, University of Western Australia, Perth, WA 6009, Australia and [15]Department of Ophthalmology, Flinders University, Adelaide, SA 5042, Australia

*To whom correspondence should be addressed at: 300 Herston Road, Herston, Brisbane, QLD 4006, Australia. Tel: +61 7 3845 3563; Fax: +61 7 3362 0101; Email: stuart.macgregor@qimrberghofer.edu.au

† H.S. and A.I.I. contributed equally to this work.
‡ C.M.v.D. and S.M. jointly supervised this work.

Received: November 4, 2014. **Revised:** December 12, 2014. **Accepted:** January 21, 2015

Abstract

Primary open-angle glaucoma (POAG) is a blinding disease. Two important risk factors for this disease are a positive family history and elevated intraocular pressure (IOP), which is also highly heritable. Genes found to date associated with IOP and POAG are *ABCA1*, *CAV1/CAV2*, *GAS7* and *TMCO1*. However, these genes explain only a small part of the heritability of IOP and POAG. We performed a genome-wide association study of IOP in the population-based Rotterdam Study I and Rotterdam Study II using single nucleotide polymorphisms (SNPs) imputed to 1000 Genomes. In this discovery cohort ($n = 8105$), we identified a new locus associated with IOP. The most significantly associated SNP was rs58073046 ($\beta = 0.44$, *P*-value = 1.87×10^{-8}, minor allele frequency = 0.12), within the gene *ARHGEF12*. Independent replication in five population-based studies ($n = 7471$) resulted in an effect size in the same direction that was significantly associated ($\beta = 0.16$, *P*-value = 0.04). The SNP was also significantly associated with POAG in two independent case–control studies [$n = 1225$ cases and $n = 4117$ controls; odds ratio (OR) = 1.53, *P*-value = 1.99×10^{-8}], especially with high-tension glaucoma (OR = 1.66, *P*-value = 2.81×10^{-9}; for normal-tension glaucoma OR = 1.29, *P*-value = 4.23×10^{-2}). *ARHGEF12* plays an important role in the RhoA/RhoA kinase pathway, which has been implicated in IOP regulation. Furthermore, it binds to *ABCA1* and links the *ABCA1*, *CAV1/CAV2* and *GAS7* pathway to Mendelian POAG genes (*MYOC*, *OPTN*, *WDR36*). In conclusion, this study identified a novel association between IOP and *ARHGEF12*.

Introduction

Glaucoma is a heritable eye disease affecting the optic nerve, which leads to irreversible visual field loss and eventually to blindness. Primary open-angle glaucoma (POAG) is the most common form of glaucoma. Individuals with a first-degree family member affected with POAG have a 10-fold increased risk of developing the disease (1). Variants in *MYOC*, *OPTN* and *WDR36* explain some familial forms of POAG (2–7). However, disease-causing mutations in these genes are rare in POAG patients and therefore explain only a small part of the overall heritability. Genome-wide association studies (GWAS) have identified *CAV1/CAV2*, *TMCO1*, *SIX6* and *CDKN2B-AS1* as POAG genes, and recently *ABCA1*, *AFAP1* and *GMDS* were added to the list (8–12).

Elevated intraocular pressure (IOP) is an important risk factor for glaucoma and the target of glaucoma therapy is lowering the IOP. IOP is highly heritable with heritability estimates ranging between 0.29 and 0.67 (13,14). *TMCO1*, *GAS7* and *FAM125B* were implicated in IOP, as well as the *CAV1/CAV2* region (12,15). The International Glaucoma Genetics Consortium (IGGC) recently published a meta-analysis of IOP, reporting four new genes for IOP (*FNDC3B*, *ABCA1*, *ABO* and a region on chromosome 11. p11.2 with many genes in it), and showed that one of the new genes (*ABCA1*) also influences the risk of developing POAG (16). This has shown that investigating the genetics of IOP is a fruitful approach to discover genes related to POAG.

The IGGC meta-analysis utilized data imputed to the HapMap 2 reference panel. In this study, we aimed to identify new genetic variants associated with IOP using 1000 Genomes reference panel to increase the number of variants analysed in the population-based Rotterdam Study.

Results

After exclusion of 95 subjects with a history of IOP-lowering laser or surgery, 8105 subjects were included in the meta-analysis of the discovery cohorts [Rotterdam Study I (RS-I) and Rotterdam Study II (RS-II)]. The demographics of all individual studies are shown in Table 1. The inflation factor (λ) was 1.03 for RS-I and 1.01 for RS-II, indicating good control of population substructures. The λ of the meta-analysis was 1.04 (Supplementary Material, Fig. S1). In the meta-analysis, three single nucleotide polymorphisms (SNPs) reached genome-wide significance (Fig. 1). These three SNPs were located on chromosome 11q23.3 in the *ARHGEF12* gene. The most significantly associated SNP was the intronic variant rs58073046 [$\beta = 0.44$, *P*-value = 1.87×10^{-8}, minor allele frequency (MAF) = 0.12; Fig. 2 and Table 2]. Since IOP can be influenced by the central corneal thickness (CCT), we adjusted for CCT. Adjustment for CCT was possible in only 25% of the data set. In this small subset, by chance the effect of rs58073046 on IOP without adjustment for CCT was smaller ($\beta = 0.34$, *P*-value = 4.44×10^{-2}, $n = 2036$). After adjustment for CCT, the effect estimate was 0.36 and remained marginally significant despite the small sample size (*P*-value for effect of rs58073046 on IOP corrected for CCT = 3.35×10^{-2}, $n = 2036$).

When combining the results of the validation cohort, rs58073046 was replicated ($\beta = 0.16$, *P*-value = 4.13×10^{-2}, $n = 7471$; Table 2). The effect estimates of each individual study are shown in Figure 3. Figure 2 shows that 93 SNPs in the chromosome 11q23.3 region reached a *P*-value below 5.0×10^{-5} in the discovery cohort. All 93 SNPs were included in the combined meta-analysis of the discovery and replication cohorts (see Supplementary Material, Table S1). The two most significant associations are two SNPs in linkage disequilibrium (pairwise correlation r^2 is 1 in 1000 Genomes Pilot 1 in Northern Europeans) which has thus similar effect sizes and *P*-values (rs58073046: $\beta = 0.30$, *P*-value = 6.12×10^{-8}; rs11217863: $\beta = 0.30$ for the minor allele, *P*-value = 6.22×10^{-8}; for all studies together). Twenty-four of the 93 SNPs included in the combined meta-analysis are located in regulatory elements, particularly at enhancers (16/24) and promoter flanking regions (7/24), and one at a CTCF-binding site, suggesting an effect on IOP by altering regulation of *ARHGEF12* or other genes.

Expression profile of human *ARHGEF12* was investigated using UniGene, an expressed sequence tag (EST) database from NCBI. Positive expression was found in various tissues, being particularly high in the eye, vascular tissue, ear, adipose tissue, mouth, uterus and skin (Supplementary Material, Table S2). Eye-specific expression of *ARHGEF12* was examined through the eye-centric genome browser, EyeBrowse, which showed that *ARHGEF12* is expressed in the cornea, lens, iris, trabecular meshwork, retina, optic nerve and human foetal eye (Supplementary Material, Table S3a). Compared with other genes in the neighbourhood (*POU2F3* and *TMEM136*), *ARHGEF12* presents the highest EST counts in the eye (Supplementary Material, Table S3b). This finding is consistent with microarray data from the Ocular Tissue Database in which the highest expression of *ARHGEF12* occurred in the trabecular meshwork (Supplementary Material, Table S4).

The most significantly associated SNP (rs58073046) explained 0.4% (RS-I) and 0.3% (RS-II) of the variance in IOP (Table 3). The

Table 1. Characteristics of the discovery and replication studies

	Discovery cohorts ($n = 8105$)		Replication cohorts ($n = 7471$)				
	RS-I	RS-II	RS-III	BATS	BMES	Raine	TEST
n included in analysis	6010	2095	2992	1152	1769	895	663
Mean age (SD)	69.2 (9.0)	64.8 (7.9)	57.2 (6.8)	20.1 (4.0)	64.0 (8.3)	20.0 (0.4)	25.6 (18.8)
% male	40	46	44	53	43	49	60
Mean IOP (SD)	14.7 (3.2)	14.2 (3.1)	13.6 (2.9)	15.8 (2.9)	16.1 (2.7)	14.9 (4.7)	15.8 (3.1)
n of participants with IOP-lowering medication	112	40	35	–	38	–	–
n of participants with IOP-lowering laser/surgery	59	36	12	–	18	–	–

BATS, Brisbane Adolescent Twins Study; BMES, Blue Mountains Eye Study; IOP, intraocular pressure; *n*, number of samples; RS, Rotterdam Study; SD, standard deviation; TEST, Twins Eye Study in Tasmania.

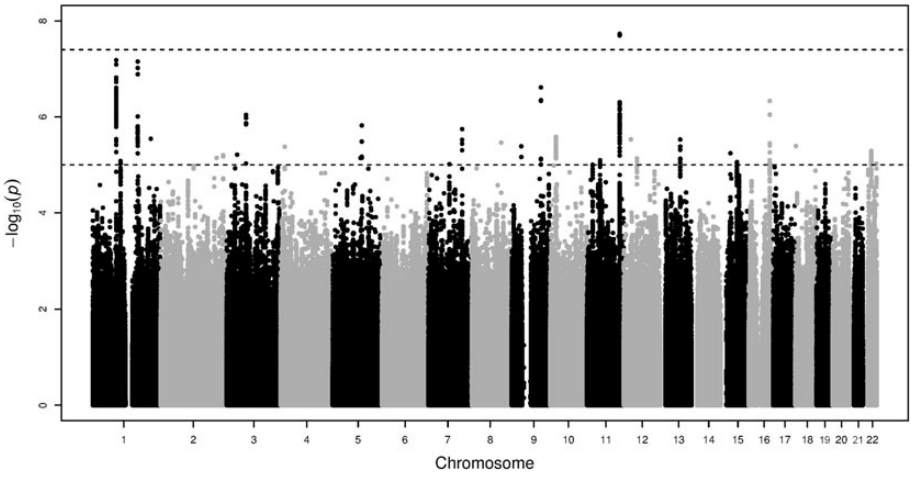

Figure 1. Manhattan plot of the meta-analysis of GWAS for IOP in the discovery phase ($n = 8105$). Each dot represents a SNP. The plot shows –log10-transformed *P*-values for all SNPs. The upper black-dotted horizontal line represents the threshold of genome-wide significance (*P*-value $< 5.0 \times 10^{-8}$); the lower black-dotted horizontal line represents a *P*-value of 1×10^{-5}.

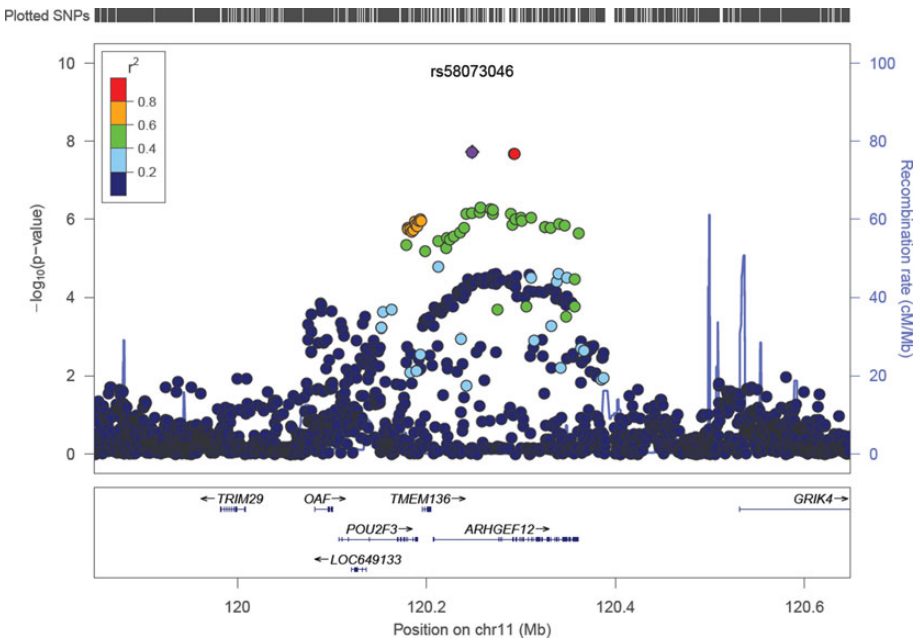

Figure 2. Regional association and recombination plot of the 11q23.3 region in the meta-analysis of the discovery cohorts. Plots are centred on rs58073046 (purple diamond), the most significantly associated single SNP in this region, and flanked by the meta-analysis results for SNPs in the 400-kb region surrounding it. SNPs are shaded according to their pairwise correlation (r^2) with rs58073046. The blue line represents the estimated recombination rates; the gene annotations are shown below the figure.

Table 2. Summary of the discovery and replication findings of the genome-wide search for IOP-related genes using data imputed to the 1000 Genomes reference

SNP	Chr/pos	A1/A2	MAF	Discovery stage ($n = 8105$)			Replication stage ($n = 7471$)			Meta-analysis ($n = 15\,576$)			
				β	SE	P-value	β	SE	P-value	β	SE	P-value	I^2
rs58073046	11/120248493	g/a	0.12	0.44	0.08	1.87×10^{-8}	0.16	0.08	4.13×10^{-2}	0.30	0.06	6.22×10^{-8}	41.6

A1, allele 1, the effect allele; A2, allele 2; β, effect size on IOP based on allele 1; Chr, chromosome; MAF, minor allele frequenty (=A1); I^2, I^2 for heterogeneity between all samples; pos, position; SE, standard error; SNP, single nucleotide polymorphism.

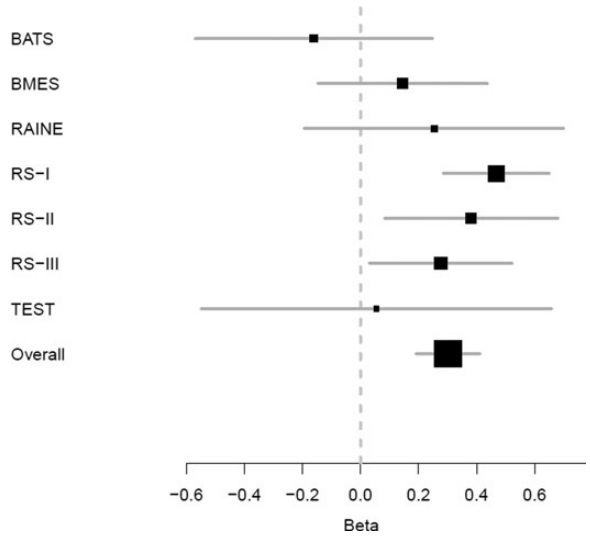

Figure 3. Forest plot for rs58073046 (chromosome 11q23.3). For each study, the square shows the β linear regression coefficient or the average difference in IOP for each additional copy of the minor allele (G) and the lines represent the standard error of the estimate. BATS, Brisbane Adolescent Twins Study; BMES, Blue Mountains Eye Study; RS, Rotterdam Study; TEST, Twins Eye Study in Tasmania.

explained phenotypic variance increased to 1.0% (RS-I) and 0.6% (RS-II) by adding the 24 regulatory variants at 11q23.3, and to 2.2% (RS-I) and 2.6% (RS-II) by adding all the other 11q23.3 variants which reached a *P*-value below 5.0×10^{-5} in the discovery cohort, but the differences in explained variance are not statistically significant between the models.

The SNP (rs58073046) was also genome-wide significantly associated with POAG in 1225 cases and 4117 controls [odds ratio (OR) = 1.53, *P*-value = 1.99×10^{-8}; Table 4]. The association of rs58073046 was stronger for high-tension glaucoma (HTG) (OR = 1.66, *P*-value = 2.81×10^{-9}) than for normal-tension glaucoma (NTG) (OR = 1.29, *P*-value = 4.23×10^{-2}).

Figure 4 shows a network map of protein interactions created using the Ingenuity Pathway Analysis (IPA) software. *ARHGEF12* binds directly to ABCA1 and RhoA proteins, and interacts through other proteins with genes implicated in POAG by GWAS (*CAV1/CAV2*, *GAS7*) or linkage analysis (*MYOC*, *OPTN* and *WDR36*). No evidence was found for interactions with protein products of other known IOP genes such as *ABO*, *TMCO1* or *FNDC3B*.

Discussion

The aim of this study was to identify new genetic variants that influence IOP using GWAS data sets imputed to the 1000 Genomes reference panel. We have identified a new region, chromosome 11q23.3, associated with IOP. The SNP rs58073046 is located in *ARHGEF12*. This gene was previously associated with POAG, but the findings did not replicate (8). The association of the region with IOP is new.

Gharahkhani *et al.* (8) previously reported an association between POAG and rs11827818 (OR 1.52, *P*-value = 9.2×10^{-9}), an intronic SNP located within the *TMEM136* gene near to *ARHGEF12*, in 1155 cases and 1992 controls from the ANZRAG study. We checked the association between the variant found by Gharahkhani *et al.* and POAG in the Genetic Research in Isolated Populations (GRIP)/Erasmus Rucphen Family (ERF) study consisting of 110 POAG cases. The magnitude for rs11827818 was smaller (OR 1.15 for overall glaucoma and OR 1.36 for HTG) than the magnitude of the most associated SNP rs58073046 observed in our study (OR 1.46 for overall POAG and OR 1.79 for HTG). These two SNPs (rs11827818 and rs58073046) are in partial linkage disequilibrium (pairwise correlation r2 is 0.51 in 1000 Genomes Pilot 1 in Northern Europeans). Gharahkhani *et al.* used the genotyped SNP rs2276035 within *ARHGEF12* for replication in other POAG case–control studies; however, this SNP did not clearly replicate. In our GRIP/ERF study, rs2276035 was not associated with POAG (OR 0.99 for overall POAG and OR 1.22 for those with HTG).

In our analysis of IOP, the SNP found by Gharahkhani *et al.* (rs11827818) was associated with an increased mean IOP level in our discovery cohorts but did not reach genome-wide significance ($\beta = 0.30$, *P*-value = 6.41×10^{-6}). In our replication cohorts, the effect of rs58073046 on IOP was heterogeneous between studies, particularly in one small study (BATS) in which the effect was in the opposite direction. The I^2 for heterogeneity was 41.5 in the combined analysis of all studies. However, after removal of BATS, the heterogeneity I^2 was 0.0 and the *P*-value became 5.04×10^{-9}. BATS is a relatively small and younger sample, which might explain the failure to replicate the findings. The effect estimates from all other replication cohorts were in the same direction as that from our discovery cohorts, although smaller in magnitude.

ARHGEF12 [Rho guanine nucleotide exchange factor (GEF) 12; previously known as Leukemia-Associated Rho Guanine Nucleotide Exchange Factor or *LARG*] may regulate RhoA GTPases (17). Rho proteins are important for numerous cellular processes. Activation of RhoA protein will lead to the activation of ROCK, a RhoA kinase. It has been shown that RhoA/RhoA kinase signalling plays a role in regulation of trabecular meshwork plasticity, fibrogen activity and myofibroblast activation (18). Activation of RhoA proteins can also decrease the permeability of Schlemm's canal cells (19). This links *ARHGEF12* to POAG as the regulation of IOP is a balance between the production of aqueous humour by the ciliary body and the outflow through the trabecular meshwork and Schlemm's canal cells. The changes in trabecular meshwork and Schlemm's canal cells lead to an increased resistance for the aqueous humour outflow and subsequently an elevated IOP. ROCK-inhibitors can decrease IOP by inducing

Table 3. The explained variance of IOP in the Rotterdam Study I (RS-I) and Rotterdam Study II (RS-II)

	RS-I Explained variance (%)	P-value	RS-II Explained variance (%)	P-value
Model 1 = rs58073046	0.4		0.3	
Model 2 = model 1 + promotor flanking region SNPs	0.6	0.28	0.4	0.89
Model 3 = model 2 + enhancers + CTCF binding site	1.0	0.19	0.6	0.82
Model 4 = model 3 + all other SNPs (93 in total)	2.2	0.06	2.6	0.16

Models with different predictors were tested and the P-value shows the P-value of the difference in explained variance for models 2–4 compared with model 1.

Table 4. Result of the association of rs58073046 with POAG

	Controls (*n*)	All POAG Cases (*n*)	OR	95% CI	P-value	HTG Cases (*n*)	OR	95% CI	P-value	NTG Cases (*n*)	OR	95% CI	P-value
ANZRAG	1992	1115	1.54	1.32–1.80	3.14×10^{-7}	709	1.65	1.38–1.97	2.12×10^{-7}	330	1.33	1.03–1.72	3.01×10^{-2}
ERF/GRIP	2125	110	1.46	0.91–2.35	1.27×10^{-1}	68	1.79	1.04–3.09	4.10×10^{-2}	42	0.92	0.41–2.06	8.36×10^{-1}
Meta-analysis	4117	1225	1.53	1.32–1.78	1.99×10^{-8}	777	1.66	1.41–1.97	2.81×10^{-9}	372	1.29	1.01–1.64	4.23×10^{-2}

ANZRAG, Australian and New Zealand Registry of Advanced Glaucoma; CI, confidence interval; ERF/GRIP, Erasmus Rucphen Family study and Genetic Research in Isolated Populations; HTG, high-tension glaucoma; NTG, normal-tension glaucoma; OR, odds ratio; POAG, primary open-angle glaucoma.
Note that the sum of HTG and NTG is not equal to the total number of cases in the ANZRAG cohort, since peak IOP measures were only available for 1039 of the 1155 cases. The table shows the association result for all POAG, as well as for the subtypes HTG (IOP >21 mmHg) and NTG (IOP ≤21 mmHg).

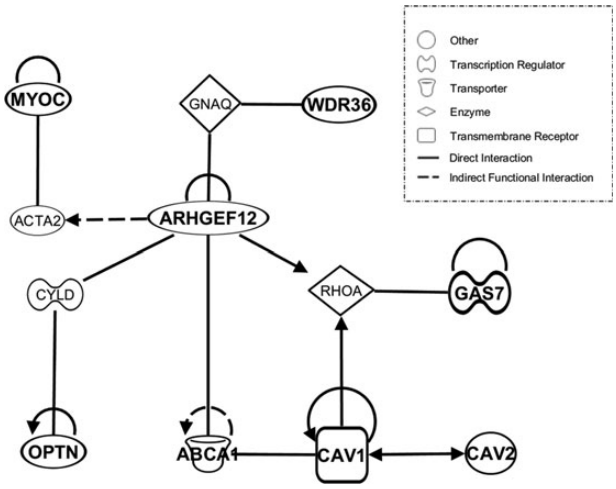

Figure 4. Network map of protein–protein interactions between *ARHGEF12* with (**A**) previously known genes associated with IOP and glaucoma (*ABCA1*, *CAV1*/*CAV2*, *GAS7*), and (**B**) known genes involved in familial forms of glaucoma (*MYOC*, *OPTN*, *WDR36*). Map was built using Ingenuity Pathway Analysis. Solid lines imply direct relationships between proteins (e.g. physical protein–protein interaction or enzyme-substrate); dotted lines imply indirect functional relationships, such as co-expression, phosphorylation/dephosphorylation, activation/deactivation, transcription or inhibition. Proteins in bold correspond to known glaucoma genes. Meaning of symbols is shown on the right side of the figure.

relaxation of trabecular meshwork and ciliary body muscles and seems to be a good new target for IOP-lowering therapy (20).

Interestingly, *ARHGEF12* links the *ABCA1*, *CAV1*/*CAV2* and *GAS7* genes, which has been previously associated with IOP as well as with POAG, to Mendelian POAG genes (*MYOC*, *OPTN* and *WDR36*). The *ARHGEF12* gene interacts with *ABCA1*. *ARHGEF12* can extend the half-life of the ABCA1 protein, by binding to its C terminus and subsequently activating RhoA, which in turn prevents ABCA1 degradation (21). *ABCA1* plays a role in the transport of different molecules across extra- and intra-cellular membranes and the interference of *ARHGEF12* in the degradation of ABCA1 protein might extend the transportation of molecules. *ABCA1* is not the only glaucoma gene that has a role in the transport of vesicles. *CAV1*, *CAV2* and *FAM125B* have been also implicated in vesicle transport (15).

In flies, *RhoGEF2* is the single homologue of mammalian *ARHGEF1*, *ARHGEF11* and *ARHGEF12*, and has been extensively studied in the context of tumorigenesis (22). Flies lacking *RhoGEF2* showed an early embryonic lethality (23,24), while overexpression of this gene in the eye resulted in small eyes, ablation of eye tissue, aberrant proliferation patterns, tissue morphology and partially blocked differentiation (22). Overexpression of *Rho1* GTPase results in a rough eye phenotype with reduced retinal thickness (25), but in the presence of *RhoGEF2*, the retina thickness is recovered (23), supporting the role of *RhoGEF2* as upstream activator of *Rho1* in the developing eye. No data about eye morphology or histology have been described in either knockout flies or mice. The absence of *arhgef12* in mice leads to embryonic lethality with incomplete penetrance, which might be explained by redundancy of *arhgef11* and *arhgef12* (26). These findings suggest that *arhgef12* expression is required during eye and general development and that its absence may impact animal viability.

POU2F3 is another gene in the region on chromosome 11. It is a member of the POU domain family of transcription factors, which regulate cell type-specific differentiation pathways. *POU2F3* specifically regulates differentiation of keratinocytes (27). *POU2AF1* is a POU class-associating factor and is associated with CCT (28). Because IOP is related to CCT, we performed an additional analysis with extra adjustment for CCT in the discovery cohorts. Only a small subset of the discovery cohorts had CCT data available; therefore, the association did not reach genome-wide significance after this additional adjustment. However, the β was similar, suggesting that the signal of association on chromosome 11 is independent of CCT.

TMEM136 (transmembrane protein 136) is the gene between *ARHGEF12* and *POU2F3*. Compared with *TMEM136* and *POU2F3*, *ARHGEF12* showed the highest expression in the eye and particularly in the trabecular meshwork and ciliary body

(Supplementary Material, Tables S3b and S4). These findings, besides its interaction with known POAG and IOP genes, are compatible with the view that *ARHGEF12* is most likely the gene causing the association signal. Nonetheless, further functional studies focusing on eye phenotypes are needed to clarify the role of chromosome 11q23.3 in the regulation of IOP and its influence on the risk of glaucoma.

In summary, our meta-analysis of GWAS has identified a new locus that may be important for the regulation of IOP and the risk of glaucoma. *ARHGEF12* is the most likely gene causing the association signal. It plays a role in the RhoA/RhoA kinase signalling which has been proven to be an important new target for glaucoma therapy. Our study shows that investigating the genetics of IOP is a fruitful way to elucidate the genetics of glaucoma.

Materials and Methods

We performed a meta-analysis of GWAS in two discovery cohorts—RS-I and RS-II—which are identical in population structure. Our replication cohorts include the Brisbane Adolescent Twins Study (BATS), Blue Mountains Eye Study (BMES), the Western Australian Pregnancy Cohort (Raine) Study, the Rotterdam Study III (RS-III) and Twins Eye Study in Tasmania (TEST). Next, we validated our findings in the Australian and New Zealand Registry of Advanced Glaucoma (ANZRAG) and GRIP/ERF POAG case–control studies. All studies adhered to the tenets of the Declaration of Helsinki and written, informed consent was obtained from all participants.

The Rotterdam Study

The Rotterdam Study is a population-based study established in Rotterdam, The Netherlands (29). It consists of three cohorts. The original cohort, RS-I, started in 1990 and includes 7983 subjects aged 55 years and older. The second cohort, RS-II, was added in 2000 and includes 3011 subjects aged 55 years and older. The last cohort, RS-III, includes 3932 subjects of 45 years of age and older and started in 2006. In all three cohorts, IOP was measured for both eyes with Goldmann applanation tonometry (Haag-Streit, Bern, Switzerland). The measurement was done twice. If the second measurement was different from the first measurement, a third measurement was performed and the median of all three values was taken. A subset of participants from RS-I underwent CCT measurements at baseline using ultrasound pachymetry (Allergan Humphrey 850, Carl Zeiss Meditec, Dublin, CA, USA). Another subset of participants from RS-I, RS-II and RS-III underwent CCT measurements at follow-up using a non-contact biometer (Lenstar LS900, Haag-Streit, Köniz, Switzerland). Other ophthalmic baseline and follow-up examinations, which are still ongoing, were described previously (30). DNA was isolated from whole blood according to standard procedures. Genotyping of SNPs was performed using the Illumina Infinium II HumanHap550 array (RS-I), the Illumina Infinium HumanHap 550-Duo array (RS-I, RS-II) and the Illumina Infinium Human 610-Quad array (RS-I, RS-III). Samples with low call rate (<97.5%), with excess autosomal heterozygosity (>0.336) or with sex-mismatch were excluded, as were outliers identified by the identity-by-state clustering analysis (outliers were defined as being >3 standard deviation (SD) from population mean or having identity-by-state probabilities >97%). A set of genotyped input SNPs with call rate >98%, MAF > 0.001 and Hardy–Weinberg equilibrium (HWE) P-value > 10^{-6} was used for imputation. The Markov Chain Haplotyping (MACH) package version 1.0 software (Rotterdam, The Netherlands; imputed to plus strand of NCBI build 37, 1000 Genomes phase I version 3) and minimac version 2012.8.6 were used for the analysis. GWAS analyses were performed using the ProbABEL package (31). The analyses were adjusted for age, sex and the first five principal components. The Rotterdam Study has been approved by the institutional review board (Medical Ethics Committee) of the Erasmus Medical Center and by the review board of The Netherlands Ministry of Health, Welfare and Sports.

Brisbane Adolescent Twins Study and Twins Eye Study in Tasmania

The Australian Twin Eye Study comprises participants examined as part of TEST or BATS. In most participants, the IOP was measured with the TONO-PEN XL (Reichert, Inc., New York, NY, USA) (32). The Australian twin cohorts were genotyped on the Illumina Human Hap610W Quad array. The inclusion criteria for the SNPs were a MAF > 0.01, HWE P-value ≥ 10^{-6} and a SNP call rate >95% or Illumina Beadstudio Gencall Score ≥0.7, resulting in 543 862 SNPs. Imputation was done with reference to the August 4, 2010 version of the publicly released 1000 Genomes Project European genotyping using MACH. For BATS data, 1152 people from 517 families were included in the analyses. For TEST data, 663 individuals from 350 families were included. Association analyses were performed in Merlin (http://www.sph.umich.edu/csg/abecasis/merlin/) by using the –fastassoc option. Ancestry, initially determined through self-reporting, was verified through Principal Component decomposition. The analyses were adjusted for age, sex, the technique of IOP measurement and the first five principal components. The studies were approved by the human ethics committees of the University of Tasmania, Royal Victorian Eye and Ear Hospital, and Queensland Institute of Medical Research.

Blue Mountains Eye Study

The Blue Mountains Eye Study is a population-based cohort study of common eye diseases in older Australians living in the Blue Mountains region, west of Sydney, Australia. IOP was measured using Goldmann applanation tonometry (Haag-Streit, Bern, Switzerland) (33). DNA was extracted from whole blood and quality was validated by Sequenom iPLEX assay. Genotyping was performed on the Illumina Infinium platform using the Human660W-Quad, a Wellcome Trust Case Control Consortium 2 designed custom chip containing Human550 probes with 60 000 additional probes to capture common copy-number variations from the Structural Variation Consortium (34). Genotyped data were filtered to include SNPs with genotyping rate ≥0.97, MAF ≥ 1%, HWE P-value ≥ 10^{-6}. Samples with call rates <95% were excluded from analysis. Relatedness filtering based on estimated identity by descent was performed, so that no pairs of individuals shared more than 20% of their genome. Ancestry outliers with >6 SD from 1000 Genomes northern European ancestry samples were removed. The IMPUTE2 software was used for imputation of data on 1000 Genomes phase 1 release version 3 (35,36). The association test was performed using SNPTEST _v2.5-beta4 (37,38). The analyses were adjusted for age, sex and the first five principal components. The study was approved by the Human Research Ethics Committees of the University of Sydney and Sydney West Area Health Service.

Raine

The Western Australian Pregnancy Cohort (Raine) study is an ongoing prospective cohort study of pregnancy, childhood,

adolescence and young adulthood in Perth, Western Australia (39). At the initiation of the study, 2900 pregnant women were recruited at 16–18 weeks gestation from the state's largest public women's hospital and surrounding private practices for a randomized clinical trial investigating effects of intensive ultrasound and Doppler studies in pregnancy outcomes. Following this study, the offspring of the recruited individuals have been evaluated in detail during childhood and adolescence. At the 20-year review of the cohort, Raine participants underwent a comprehensive ocular examination for the first time (40). As part of this examination, IOP was measured using an Icare TAO1i Tonometer (Icare Finland Oy, Helsinki, Finland). DNA samples and consents for GWAS studies were available from the previous assessments. Genotype data were generated using the genome-wide Illumina 660 Quad Array at the Centre for Applied Genomics (Toronto, Ontario, Canada). Relatedness filtering based on estimated identity by descent was performed, so that no pairs of individuals shared more than 20% of their genome. We also excluded people who had a high degree of missing genotyping data (>3%). The data were filtered for a HWE P-value $> 1 \times 10^{-6}$, SNP call rate >95% and a MAF > 0.01. GWAS imputation was performed in the MACH v1.0.16 software using the 23 November 2010 version of the 1000 Genome Project European genotyping. The association analyses were adjusted for age, sex and the first two principal components. This study was approved by the Human Research Ethics Committee of the University of Western Australia.

Australian and New Zealand Registry of Advanced Glaucoma

ANZRAG recruits cases of advanced glaucoma Australia-wide through ophthalmologist referral. The cohort also included participants enrolled in the Glaucoma Inheritance Study in Tasmania (GIST) who met the criteria for ANZRAG. This cohort has been described previously (9). Advanced POAG was defined as best-corrected visual acuity worse than 6/60 due to POAG, or a reliable 24-2 Visual Field with a mean deviation of worse than −22 db or at least two out of four central fixation squares affected with a Pattern SD of <0.5%. The less severely affected eye was also required to have signs of glaucomatous disc damage. Clinical exclusion criteria for this advanced POAG study were: (i) pseudoexfoliation or pigmentary glaucoma, (ii) angle closure or mixed mechanism glaucoma; (iii) secondary glaucoma due to aphakia, rubella, rubeosis or inflammation; (iv) infantile glaucoma and (v) glaucoma in the presence of a known associated syndrome. The ANZRAG cohort included 1155 ANZRAG glaucoma cases and 1992 controls genotyped on Illumina Omni1M or OmniExpress arrays and imputed against 1000 Genomes Phase 1 Europeans. The case set included all samples from the previously published GWAS (9). Controls were drawn from the Australian Cancer Study (225 oesophageal cancer cases, 317 Barrett's oesophagus cases and 552 controls) or from a study of inflammatory bowel diseases (303 cases and 595 controls). The quality control methods were performed in PLINK by removing individuals with more than 3% missing genotypes, SNPs with call rate <97%, MAF <0.01 and HWE P-value < 0.0001 in controls and HWE P-value $< 5 \times 10^{-10}$ in cases (41). The same quality control protocol was used before merging the cases and controls to avoid mismatches between the merged data sets. After merging, the genotypes for 569 249 SNPs common to the arrays were taken forward for analysis. Relatedness filtering based on estimated identity by descent was performed, so that no pairs of individuals shared more than 20% of their genome. Principal components were computed for all participants and reference samples of known northern European ancestry (1000G British, CEU and Finland participants) using the smartpca package from EIGENSOFT software (42,43). Participants with principal component 1 or 2 values >6 SD from the known northern European ancestry group were excluded. Imputation was conducted using IMPUTE2 in 1-Mb sections, with the 1000 Genomes phase 1 Europeans (March 2012 release) used as the reference panel (35,36). SNPs with imputation quality score >0.8 and MAF > 0.01 were carried forward for analysis. Association testing on the imputed data was performed in SNPTEST _v2.5-beta3 using an additive model (-frequentist 1) and full dosage scores (-method expected) with sex and the first six principal components fitted as covariates (37,38). All were Australians of European ancestry. Approval was obtained from the Human Research Ethics Committees of Southern Adelaide Health Service/Flinders University, University of Tasmania, QIMR Berghofer Institute of Medical Research (Queensland Institute of Medical Research) and the Royal Victorian Eye and Ear Hospital.

Peak IOP measures were available for 1039 of the 1155 cases in the ANZRAG cohort. Of these cases, 330 (31.8%) had NTG (IOP ≤ 21 mmHg) and 709 (68.2%) had HTG (IOP >21 mmHg). Association testing for NTG and HTG was performed in SNPTEST _v2.5-beta3 as explained above, using 1992 shared population controls.

Erasmus Rucphen Family study and Genetic Research in Isolated Populations programme

The ERF study is a family-based cohort in a genetically isolated population in the southwest of the Netherlands with over 3000 participants aged between 18 and 86 years (44,45). In the region of the ERF population, a total of 110 patients with glaucoma who did not participate in the ERF study were recruited in three local hospitals. Their visual fields were tested with standard automated perimetry (Humphrey Field Analyzer c24-2 SITA Standard test programme) or the Octopus 101 (G2 program with TOP strategy) (Haag-Streit, Bern, Switzerland). The diagnosis of glaucoma was made by the patient's ophthalmologist and confirmed by a glaucoma specialist (HGL). It was based on a glaucomatous appearance of the optic disc (notching or thinning of the neuroretinal rim), combined with a matching glaucomatous visual field defect, and open-angles seen by gonioscopy. Classification of HTG (IOP > 21 mmHg) and NTG (IOP ≤ 21 mmHg) was based on IOP at the time of diagnosis. Participants from the ERF study were used as the control group ($n = 2125$). Genotyping was performed with the 318 K array of the Illumina Infinium II whole-genome genotyping assay (HumanHap300-2). Samples with low call rate (<97.5%), with excess autosomal heterozygosity (>0.336) or with sex-mismatch were excluded. A set of genotyped input SNPs with call rate >98%, with MAF > 0.01 and with HWE P-value $> 10^{-6}$ was used for imputation. We used the MACH package version 1.0.18.c software (Rotterdam, The Netherlands; imputed to plus strand of NCBI build 37, 1000 Genomes Phase I version 3) and minimac version 2012.8.15 for the analyses. Association tests were performed using the ProbABEL package (31). The analyses were adjusted for age and sex. All measurements in these studies were conducted after the Medical Ethics Committee of the Erasmus University had approved the study protocols.

Expression data

We investigated the expression profile of several genes using NCBI's UniGene (46), which is an organized view of the

transcriptome that evaluates semi-quantitatively the EST calculated as the number of transcripts per million (available online at http://www.ncbi.nlm.nih.gov/unigene/). The EST data for 'Breakdown by body site' that show the approximate gene expression pattern in different tissues were chosen.

Expression of genes in eye tissues was evaluated using two databases: the EyeBrowse and the Ocular Tissue Database. The EyeBrowse is a customized eye-centric version of the UCSC Genome Browser, which includes (A) eye-derived ESTs from the National Eye Institute (47) and (B) the EyeSage project (48,49). The EyeBrowse is available at http://eyebrowse.cit.nih.gov/. We only selected human data. In the Ocular Tissue Database, the gene expression is indicated as Affymetrix Probe Logarithmic Intensity Error (PLIER) normalized value. The PLIER normalization method was described by Wagner *et al.* (50). The Ocular Tissue Database is available at https://genome.uiowa.edu/otdb/.

Ensembl Genome Browser

The Ensembl Genome Browser release version 77 was used to investigate regulatory variants in genome-wide significant regions (51).

Ingenuity Pathway Analysis

Network map was created using the IPA software (Ingenuity Systems, http://www.ingenuity.com, Redwood City, CA, USA), where (i) *ARHGEF12*, (ii) known IOP-associated genes (*ABO* and *FNDC3B*), (iii) known genes associated with both IOP and POAG (*ABCA1, CAV1/CAV2, GAS7* and *TMCO1*) as well as (iv) known genes involved in familial forms of glaucoma (*OPTN, TMCO1, WDR36*) were selected. The 'Path explorer' function (shortest + 1) was used to map protein–protein interactions between *ARHGEF12* and the rest of included genes. All direct and indirect interactions are supported by at least one reference from the literature, a textbook or canonical information stored in the Ingenuity Pathways Knowledge Base.

Statistical analysis

We used the mean IOP of the right and left eye for the analysis. If IOP was missing for one eye, the IOP of the other eye was used. For participants receiving IOP-lowering medication, we added 30% to the IOP measurement to estimate a pre-medication IOP value (52). Participants who underwent IOP-lowering laser or surgery were excluded from the analysis. GWAS was performed on each individual study as described above under the assumption of an additive model for the effect of the risk allele. In a secondary analysis in the discovery phase, CCT was included as an extra covariate. We used METAL software to carry out an inverse variance weighted fixed-effect meta-analysis between RS-I and RS-II (53). SNPs with MAF <0.01 or with imputation quality score $R^2 < 0.5$ were excluded. For the meta-analysis of RS-I and RS-II, a *P*-value of $<5.0 \times 10^{-8}$ (threshold of genome-wide significance) was considered statistically significant. Next, we validated the association results of the SNPs that reached genome-wide significance in five other studies (BMES, BATS, Raine, RS-III and TEST). In the validation phase, a *P*-value of <0.05 was considered statistically significant. Furthermore, in the discovery and validation cohorts, we meta-analysed all the SNPs with *P*-value $< 5.0 \times 10^{-5}$ in the region that reached genome-wide significance in the discovery cohort. We calculated the explained variance (R^2) of IOP by the new SNPs in RS-I and RS-II. In the first model, we calculated the explained variance for the most significantly associated SNP. Next, we added SNPs located within a regulatory element or all SNPs with *P*-value $< 5.0 \times 10^{-5}$ to the model. The nested models were compared using an *F*-test. Finally, we investigated the effect of the genome-wide significant SNPs on POAG in ANZRAG and ERF. A Manhattan plot, regional plots and forest plots were made using *R* (54) and LocusZoom (55).

Supplementary Material

Supplementary Material is available at *HMG* online.

Acknowledgements

We would like to thank the contributions of all study participants and staff at the recruitment centers.

Conflict of Interest statement. None declared.

Funding

S.M. is supported by Australian Research Council (ARC) and Australian National Health & Medical Research Council (NHMRC) Fellowships.

Erasmus Rucphen Family (ERF) Study and Rotterdam Study

The Rotterdam Study and ERF were supported by the Netherlands Organisation of Scientific Research (NWO; 91111025); Erasmus Medical Center and Erasmus University, Rotterdam, The Netherlands; Netherlands Organization for Health Research and Development (ZonMw); UitZicht; the Research Institute for Diseases in the Elderly; the Ministry of Education, Culture and Science; the Ministry for Health, Welfare and Sports; the European Commission (DG XII); the Municipality of Rotterdam; the Netherlands Genomics Initiative/NWO; Center for Medical Systems Biology of NGI; Stichting Lijf en Leven; Stichting Oogfonds Nederland; Landelijke Stichting voor Blinden en Slechtzienden; Algemene Nederlandse Vereniging ter Voorkoming van Blindheid; Medical Workshop; Heidelberg Engineering; Topcon Europe BV. Henriët Springelkamp is supported by the NWO Graduate Programme 2010 BOO (022.002.023), by Prins Bernhard Cultuurfonds (Cultuurfondsbeurs from Jan de Ruijsscher/Pia Huisman fonds) and by Novartis Pharma B.V. We acknowledge the contribution of Ada Hooghart, Corina Brussee, Riet Bernaerts-Biskop, Patricia van Hilten, Pascal Arp, Jeanette Vergeer and Maarten Kooijman.

The generation and management of GWAS genotype data for the Rotterdam Study is supported by the Netherlands Organisation of Scientific Research NWO Investments (nr. 175.010.2005.011, 911-03-012). This study is funded by the Research Institute for Diseases in the Elderly (014-93-015; RIDE2), the Netherlands Genomics Initiative (NGI)/Netherlands Organisation for Scientific Research (NWO) project nr. 050-060-810. We thank Pascal Arp, Mila Jhamai, Marijn Verkerk, Lizbeth Herrera and Marjolein Peters for their help in creating the GWAS database, and Karol Estrada and Maksim V. Struchalin for their support in creation and analysis of imputed data.

The authors are grateful to the study participants, the staff from the Rotterdam Study and the participating general practitioners and pharmacists.

Australian and New Zealand Registry of Advanced Glaucoma (ANZRAG)

Support for recruitment of ANZRAG was provided by the Royal Australian and New Zealand College of Ophthalmology

(RANZCO) Eye Foundation. Genotyping was funded by the National Health and Medical Research Council of Australia (#535074 and #1023911). This work was also supported by funding from the BrightFocus Foundation and a Ramaciotti Establishment Grant. The authors acknowledge the support of Ms Bronwyn Usher-Ridge in patient recruitment and data collection, and Dr Patrick Danoy and Dr Johanna Hadler for genotyping.

Controls for the ANZRAG discovery cohort were drawn from the Australian Cancer Study, the Study of Digestive Health and from a study of inflammatory bowel diseases. The Australian Cancer Study was supported by the Queensland Cancer Fund and the National Health and Medical Research Council (NHMRC) of Australia (Program no. 199600, awarded to David C. Whiteman, Adele C. Green, Nicholas K. Hayward, Peter G. Parsons, David M. Purdie, and Penelope M. Webb). The Study of Digestive Health was supported by grant number 5 RO1 CA 001833 from the National Cancer Institute (awarded to David C. Whiteman). We acknowledge David C. Whiteman and Graham Radford-Smith for providing access to these control samples.

The Barrett's and Esophageal Adenocarcinoma Genetic Susceptibility Study (BEAGESS) sponsored the genotyping of oesophageal cancer and Barrett's oesophagus cases, which were used as unscreened controls in the ANZRAG discovery cohort. BEAGESS was funded by grant R01 CA136725 from the National Cancer Institute.

Blue Mountains Eye Study

BMES was supported by the Australian National Health & Medical Research Council (NH&MRC), Canberra Australia (974159, 211069, 457349, 512423, 475604, 529912); the Centre for Clinical Research Excellence (Translational Clinical Research in Major Eye Diseases, 519923); NH&MRC research fellowships (358702, 632909 to J.J.W); and the Wellcome Trust, UK as part of Wellcome Trust Case Control Consortium 2 (A. Viswanathan, P. McGuffin, P. Mitchell, F. Topouzis, P. Foster) for genotyping costs of the entire BMES population (085475/B/08/Z, 085475/Z/08/Z, 076113).

In addition to three co-authors (A.C.V., P.M. and J.J.W.), the BMES GWAS team includes Elena Rochtchina from the Centre for Vision Research, Department of Ophthalmology and Westmead Millennium Institute University of Sydney (NSW Australia); John Attia, Rodney Scott, Elizabeth G. Holliday from the University of Newcastle (Newcastle, NSW Australia); Paul N. Baird and Jing Xie from the Centre for Eye Research Australia, University of Melbourne; Michael T. Inouye, Medical Systems Biology, Department of Pathology and Department of Microbiology and Immunology, University of Melbourne (Victoria, Australia); and Tien Y. Wong and Xueling Sim from the National University of Singapore, Singapore.

The Centre for Eye Research Australia receives Operational Infrastructure Support from the Victorian government.

Western Australian Pregnancy Cohort (Raine) Study

Core management of the Raine Study is funded by the University of Western Australia, Telethon Kids Institute, Raine Medical Research Foundation, UWA Faculty of Medicine, Dentistry and Health Sciences, Women's and Infant's Research Foundation and Curtin University. Genotyping was funded by NHMRC project grant 572613 (CI-A Pennell). Support for the Raine Eye Health Study was provided by NHMRC Grant 1021105 (CI-A Mackey), Lions Eye Institute, the Australian Foundation for the Prevention of Blindness, Ophthalmic Research Institute of Australia and Alcon Research Institute. The Raine Eye Health Study authors thank the Raine Eye Health Study participants and their families. They also thank the Raine Study management team, Craig E. Pennell, and the teams at TKI and LEI for cohort coordination and data collection, particularly: Charlotte McKnight, Seyhan Yazar, Hannah Forward, Wei Ang, Alex Tan, Alla Soloshenko, Sandra Oates and Diane Wood.

Twins Eye Study in Tasmania (TEST) and Brisbane Adolescent Twin Study (BATS)

Genotyping for part of the Australian twin sample was funded by an NHMRC Medical Genomics Grant. Genotyping for the remainder of twin controls was performed by the National Institutes of Health (NIH) Center for Inherited Research (CIDR) as part of an NIH/National Eye Institute (NEI) grant 1RO1EY018246, and we are grateful to Dr Camilla Day and staff. We thank Sullivan Nicolaides and Queensland Medical Laboratory for pro bono collection and delivery of blood samples and other pathology services for assistance with blood collection. The QIMR twin studies were supported by grants from the National Health and Medical Research Council (NHMRC) of Australia (241944, 339462, 389927, 389875, 389891, 389892, 389938, 443036, 442915, 442981, 496610, 496739, 552485, 552498), the Cooperative Research Centre for Discovery of Genes for Common Human Diseases (CRC), Cerylid Biosciences (Melbourne) and donations from Neville and Shirley Hawkins. We thank Grant Montgomery, Margaret J. Wright, Megan J. Campbell, Anthony Caracella, Scott Gordon, Dale R. Nyholt, Anjali K. Henders, B. Haddon, D. Smyth, H. Beeby, O. Zheng, B. Chapman for their input into project management, databases, sample processing and genotyping. We are grateful to the many research assistants and interviewers for assistance with the studies contributing to the QIMR twin collection.

References

1. Wolfs, R.C., Klaver, C.C., Ramrattan, R.S., van Duijn, C.M., Hofman, A. and de Jong, P.T. (1998) Genetic risk of primary open-angle glaucoma. Population-based familial aggregation study. *Arch. Ophthalmol.*, **116**, 1640–1645.
2. Kubota, R., Noda, S., Wang, Y., Minoshima, S., Asakawa, S., Kudoh, J., Mashima, Y., Oguchi, Y. and Shimizu, N. (1997) A novel myosin-like protein (myocilin) expressed in the connecting cilium of the photoreceptor: molecular cloning, tissue expression, and chromosomal mapping. *Genomics*, **41**, 360–369.
3. Monemi, S., Spaeth, G., DaSilva, A., Popinchalk, S., Ilitchev, E., Liebmann, J., Ritch, R., Heon, E., Crick, R.P., Child, A. *et al.* (2005) Identification of a novel adult-onset primary open-angle glaucoma (POAG) gene on 5q22.1. *Hum. Mol. Genet.*, **14**, 725–733.
4. Rezaie, T., Child, A., Hitchings, R., Brice, G., Miller, L., Coca-Prados, M., Heon, E., Krupin, T., Ritch, R., Kreutzer, D. *et al.* (2002) Adult-onset primary open-angle glaucoma caused by mutations in optineurin. *Science*, **295**, 1077–1079.
5. Sarfarazi, M., Child, A., Stoilova, D., Brice, G., Desai, T., Trifan, O.C., Poinoosawmy, D. and Crick, R.P. (1998) Localization of the fourth locus (GLC1E) for adult-onset primary open-angle glaucoma to the 10p15-p14 region. *Am. J. Hum. Genet.*, **62**, 641–652.
6. Sheffield, V.C., Stone, E.M., Alward, W.L., Drack, A.V., Johnson, A.T., Streb, L.M. and Nichols, B.E. (1993) Genetic linkage of familial open angle glaucoma to chromosome 1q21-q31. *Nat. Genet.*, **4**, 47–50.
7. Stone, E.M., Fingert, J.H., Alward, W.L., Nguyen, T.D., Polansky, J.R., Sunden, S.L., Nishimura, D., Clark, A.F., Nystuen, A.,

Nichols, B.E. *et al.* (1997) Identification of a gene that causes primary open angle glaucoma. *Science*, **275**, 668–670.
8. Gharahkhani, P., Burdon, K.P., Fogarty, R., Sharma, S., Hewitt, A.W., Martin, S., Law, M.H., Cremin, K., Bailey, J.N., Loomis, S.J. *et al.* (2014) Common variants near ABCA1, AFAP1 and GMDS confer risk of primary open-angle glaucoma. *Nat. Genet.*, **46**, 1120–1125.
9. Burdon, K.P., Macgregor, S., Hewitt, A.W., Sharma, S., Chidlow, G., Mills, R.A., Danoy, P., Casson, R., Viswanathan, A.C., Liu, J. Z. *et al.* (2011) Genome-wide association study identifies susceptibility loci for open angle glaucoma at TMCO1 and CDKN2B-AS1. *Nat. Genet.*, **43**, 574–578.
10. Ramdas, W.D., van Koolwijk, L.M., Lemij, H.G., Pasutto, F., Cree, A.J., Thorleifsson, G., Janssen, S.F., Jacoline, T.B., Amin, N., Rivadeneira, F. *et al.* (2011) Common genetic variants associated with open-angle glaucoma. *Hum. Mol. Genet.*, **20**, 2464–2471.
11. Thorleifsson, G., Walters, G.B., Hewitt, A.W., Masson, G., Helgason, A., DeWan, A., Sigurdsson, A., Jonasdottir, A., Gudjonsson, S.A., Magnusson, K.P. *et al.* (2010) Common variants near CAV1 and CAV2 are associated with primary open-angle glaucoma. *Nat. Genet.*, **42**, 906–909.
12. van Koolwijk, L.M., Ramdas, W.D., Ikram, M.K., Jansonius, N. M., Pasutto, F., Hysi, P.G., Macgregor, S., Janssen, S.F., Hewitt, A.W., Viswanathan, A.C. *et al.* (2012) Common genetic determinants of intraocular pressure and primary open-angle glaucoma. *PLoS Genet.*, **8**, e1002611.
13. Sanfilippo, P.G., Hewitt, A.W., Hammond, C.J. and Mackey, D. A. (2010) The heritability of ocular traits. *Surv. Ophthalmol.*, **55**, 561–583.
14. van Koolwijk, L.M., Despriet, D.D., van Duijn, C.M., Pardo Cortes, L.M., Vingerling, J.R., Aulchenko, Y.S., Oostra, B.A., Klaver, C.C. and Lemij, H.G. (2007) Genetic contributions to glaucoma: heritability of intraocular pressure, retinal nerve fiber layer thickness, and optic disc morphology. *Invest. Ophthalmol. Vis. Sci.*, **48**, 3669–3676.
15. Nag, A., Venturini, C. and Small, K.S., International Glaucoma Genetics, C., Young, T.L., Viswanathan, A.C., Mackey, D.A., Hysi, P.G. and Hammond, C. (2014) A genome-wide association study of intra-ocular pressure suggests a novel association in the gene FAM125B in the TwinsUK cohort. *Hum. Mol. Genet.*, **23**, 3343–3348.
16. Hysi, P.G., Cheng, C.Y., Springelkamp, H., Macgregor, S., Bailey, J.N., Wojciechowski, R., Vitart, V., Nag, A., Hewitt, A.W., Hohn, R. *et al.* (2014) Genome-wide analysis of multi-ancestry cohorts identifies new loci influencing intraocular pressure and susceptibility to glaucoma. *Nat. Genet.*, **46**, 1126–1130.
17. Fukuhara, S., Chikumi, H. and Gutkind, J.S. (2000) Leukemia-associated Rho guanine nucleotide exchange factor (LARG) links heterotrimeric G proteins of the G(12) family to Rho. *FEBS Lett.*, **485**, 183–188.
18. Pattabiraman, P.P., Maddala, R. and Rao, P.V. (2014) Regulation of plasticity and fibrogenic activity of trabecular meshwork cells by Rho GTPase signaling. *J. Cell. Physiol.*, **229**, 927–942.
19. Kumar, J. and Epstein, D.L. (2011) Rho GTPase-mediated cytoskeletal organization in Schlemm's canal cells play a critical role in the regulation of aqueous humor outflow facility. *J. Cell. Biochem.*, **112**, 600–606.
20. Wang, J., Liu, X. and Zhong, Y. (2013) Rho/Rho-associated kinase pathway in glaucoma (Review). *Int. J. Oncol.*, **43**, 1357–1367.
21. Okuhira, K., Fitzgerald, M.L., Tamehiro, N., Ohoka, N., Suzuki, K., Sawada, J., Naito, M. and Nishimaki-Mogami, T. (2010) Binding of PDZ-RhoGEF to ATP-binding cassette transporter A1 (ABCA1) induces cholesterol efflux through RhoA activation and prevention of transporter degradation. *J. Biol. Chem.*, **285**, 16369–16377.
22. Brumby, A.M., Goulding, K.R., Schlosser, T., Loi, S., Galea, R., Khoo, P., Bolden, J.E., Aigaki, T., Humbert, P.O. and Richardson, H.E. (2011) Identification of novel Ras-cooperating oncogenes in *Drosophila melanogaster*: a RhoGEF/Rho-family/JNK pathway is a central driver of tumorigenesis. *Genetics*, **188**, 105–125.
23. Barrett, K., Leptin, M. and Settleman, J. (1997) The Rho GTPase and a putative RhoGEF mediate a signaling pathway for the cell shape changes in Drosophila gastrulation. *Cell*, **91**, 905–915.
24. Hacker, U. and Perrimon, N. (1998) DRhoGEF2 encodes a member of the Dbl family of oncogenes and controls cell shape changes during gastrulation in Drosophila. *Genes Dev.*, **12**, 274–284.
25. Hariharan, I.K., Hu, K.Q., Asha, H., Quintanilla, A., Ezzell, R.M. and Settleman, J. (1995) Characterization of rho GTPase family homologues in *Drosophila melanogaster*: overexpressing Rho1 in retinal cells causes a late developmental defect. *EMBO J.*, **14**, 292–302.
26. Mikelis, C.M., Palmby, T.R., Simaan, M., Li, W., Szabo, R., Lyons, R., Martin, D., Yagi, H., Fukuhara, S., Chikumi, H. *et al.* (2013) PDZ-RhoGEF and LARG are essential for embryonic development and provide a link between thrombin and LPA receptors and Rho activation. *J. Biol. Chem.*, **288**, 12232–12243.
27. Cabral, A., Fischer, D.F., Vermeij, W.P. and Backendorf, C. (2003) Distinct functional interactions of human Skn-1 isoforms with Ese-1 during keratinocyte terminal differentiation. *J. Biol. Chem.*, **278**, 17792–17799.
28. Lu, Y., Vitart, V., Burdon, K.P., Khor, C.C., Bykhovskaya, Y., Mirshahi, A., Hewitt, A.W., Koehn, D., Hysi, P.G., Ramdas, W. D. *et al.* (2013) Genome-wide association analyses identify multiple loci associated with central corneal thickness and keratoconus. *Nat. Genet.*, **45**, 155–163.
29. Hofman, A., Darwish Murad, S., van Duijn, C.M., Franco, O.H., Goedegebure, A., Ikram, M.A., Klaver, C.C., Nijsten, T.E., Peeters, R.P., Stricker, B.H. *et al.* (2013) The Rotterdam Study: 2014 objectives and design update. *Eur. J. Epidemiol.*, **28**, 889–926.
30. Wolfs, R.C., Borger, P.H., Ramrattan, R.S., Klaver, C.C., Hulsman, C.A., Hofman, A., Vingerling, J.R., Hitchings, R.A. and de Jong, P.T. (2000) Changing views on open-angle glaucoma: definitions and prevalences—the Rotterdam Study. *Invest. Ophthalmol .Vis. Sci.*, **41**, 3309–3321.
31. Aulchenko, Y.S., Struchalin, M.V. and van Duijn, C.M. (2010) ProbABEL package for genome-wide association analysis of imputed data. *BMC Bioinformatics*, **11**, 134.
32. Mackey, D.A., Mackinnon, J.R., Brown, S.A., Kearns, L.S., Ruddle, J.B., Sanfilippo, P.G., Sun, C., Hammond, C.J., Young, T.L., Martin, N.G. *et al.* (2009) Twins eye study in Tasmania (TEST): rationale and methodology to recruit and examine twins. *Twin. Res. Hum. Genet.*, **12**, 441–454.
33. Mitchell, P., Smith, W., Attebo, K. and Wang, J.J. (1995) Prevalence of age-related maculopathy in Australia. The Blue Mountains Eye Study. *Ophthalmology*, **102**, 1450–1460.
34. Conrad, D.F., Pinto, D., Redon, R., Feuk, L., Gokcumen, O., Zhang, Y., Aerts, J., Andrews, T.D., Barnes, C., Campbell, P. *et al.* (2010) Origins and functional impact of copy number variation in the human genome. *Nature*, **464**, 704–712.
35. Howie, B., Marchini, J. and Stephens, M. (2011) Genotype imputation with thousands of genomes. *G3 (Bethesda)*, **1**, 457–470.

36. Howie, B.N., Donnelly, P. and Marchini, J. (2009) A flexible and accurate genotype imputation method for the next generation of genome-wide association studies. *PLoS. Genet.*, **5**, e1000529.
37. Marchini, J. and Howie, B. (2010) Genotype imputation for genome-wide association studies. *Nat. Rev. Genet.*, **11**, 499–511.
38. Wellcome Trust Case Control, C. (2007) Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. *Nature*, **447**, 661–678.
39. McKnight, C.M., Newnham, J.P., Stanley, F.J., Mountain, J.A., Landau, L.I., Beilin, L.J., Puddey, I.B., Pennell, C.E. and Mackey, D.A. (2012) Birth of a cohort–the first 20 years of the Raine study. *Med. J. Aust.*, **197**, 608–610.
40. Yazar, S., Forward, H., McKnight, C.M., Tan, A., Soloshenko, A., Oates, S.K., Ang, W., Sherwin, J.C., Wood, D., Mountain, J. A. *et al.* (2013) Raine eye health study: design, methodology and baseline prevalence of ophthalmic disease in a birth-cohort study of young adults. *Ophthalmic. Genet.*, **34**, 199–208.
41. Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M.A., Bender, D., Maller, J., Sklar, P., de Bakker, P.I., Daly, M.J. *et al.* (2007) PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am. J. Hum. Genet.*, **81**, 559–575.
42. Patterson, N., Price, A.L. and Reich, D. (2006) Population structure and eigenanalysis. *PLoS. Genet.*, **2**, e190.
43. Price, A.L., Patterson, N.J., Plenge, R.M., Weinblatt, M.E., Shadick, N.A. and Reich, D. (2006) Principal components analysis corrects for stratification in genome-wide association studies. *Nat. Genet.*, **38**, 904–909.
44. Aulchenko, Y.S., Heutink, P., Mackay, I., Bertoli-Avella, A.M., Pullen, J., Vaessen, N., Rademaker, T.A., Sandkuijl, L.A., Cardon, L., Oostra, B. *et al.* (2004) Linkage disequilibrium in young genetically isolated Dutch population. *Eur. J. Hum. Genet.*, **12**, 527–534.
45. Pardo, L.M., MacKay, I., Oostra, B., van Duijn, C.M. and Aulchenko, Y.S. (2005) The effect of genetic drift in a young genetically isolated population. *Ann. Hum. Genet.*, **69**, 288–295.
46. NCBI Resource Coordinators. (2015) Database resources of the National Center for Biotechnology Information. *Nucleic Acids Res.*, **43**, D6–D17.
47. Wistow, G., Peterson, K., Gao, J., Buchoff, P., Jaworski, C., Bowes-Rickman, C., Ebright, J.N., Hauser, M.A. and Hoover, D. (2008) NEIBank: genomics and bioinformatics resources for vision research. *Mol. Vis.*, **14**, 1327–1337.
48. Bowes Rickman, C., Ebright, J.N., Zavodni, Z.J., Yu, L., Wang, T., Daiger, S.P., Wistow, G., Boon, K. and Hauser, M.A. (2006) Defining the human macula transcriptome and candidate retinal disease genes using EyeSAGE. *Invest. Ophthalmol. Vis. Sci.*, **47**, 2305–2316.
49. Liu, Y., Munro, D., Layfield, D., Dellinger, A., Walter, J., Peterson, K., Rickman, C.B., Allingham, R.R. and Hauser, M.A. (2011) Serial analysis of gene expression (SAGE) in normal human trabecular meshwork. *Mol. Vis.*, **17**, 885–893.
50. Wagner, A.H., Anand, V.N., Wang, W.H., Chatterton, J.E., Sun, D., Shepard, A.R., Jacobson, N., Pang, I.H., Deluca, A.P., Casavant, T.L. *et al.* (2013) Exon-level expression profiling of ocular tissues. *Exp. Eye. Res.*, **111**, 105–111.
51. Flicek, P., Amode, M.R., Barrell, D., Beal, K., Billis, K., Brent, S., Carvalho-Silva, D., Clapham, P., Coates, G., Fitzgerald, S. *et al.* (2014) Ensembl 2014. *Nucleic Acids Res.*, **42**, D749–D755.
52. van der Valk, R., Webers, C.A., Schouten, J.S., Zeegers, M.P., Hendrikse, F. and Prins, M.H. (2005) Intraocular pressure-lowering effects of all commonly used glaucoma drugs: a meta-analysis of randomized clinical trials. *Ophthalmology*, **112**, 1177–1185.
53. Willer, C.J., Li, Y. and Abecasis, G.R. (2010) METAL: fast and efficient meta-analysis of genomewide association scans. *Bioinformatics*, **26**, 2190–2191.
54. R Core Team. (2014) R: a language and environment for statistical computing. http://www.R-project.org.
55. Pruim, R.J., Welch, R.P., Sanna, S., Teslovich, T.M., Chines, P.S., Gliedt, T.P., Boehnke, M., Abecasis, G.R. and Willer, C.J. (2010) LocusZoom: regional visualization of genome-wide association scan results. *Bioinformatics*, **26**, 2336–2337.