Published in final edited form as:
Hum Genet. 2014 January ; 133(1): 41–57. doi:10.1007/s00439-013-1349-5.

Genome-wide association study and meta-analysis of intraocular pressure

A. Bilge Ozel,
Department of Human Genetics, University of Michigan, Ann Arbor MI 48109, USA

Sayoko E. Moroi,
Department of Ophthalmology and Visual Sciences, University of Michigan, Ann Arbor, MI, USA

David M. Reed,
Department of Ophthalmology and Visual Sciences, University of Michigan, Ann Arbor, MI, USA

Melisa Nika,
Department of Ophthalmology and Visual Sciences, University of Michigan, Ann Arbor, MI, USA

Caroline M. Schmidt,
Department of Ophthalmology and Visual Sciences, University of Michigan, Ann Arbor, MI, USA

Sara Akbari,
Department of Ophthalmology and Visual Sciences, University of Michigan, Ann Arbor, MI, USA

Kathleen Scott,
Department of Ophthalmology and Visual Sciences, University of Michigan, Ann Arbor, MI, USA

Frank Rozsa,
Department of Ophthalmology and Visual Sciences, University of Michigan, Ann Arbor, MI, USA

Hemant Pawar,
Department of Ophthalmology and Visual Sciences, University of Michigan, Ann Arbor, MI, USA

David C. Musch,
Department of Ophthalmology and Visual Sciences, University of Michigan, Ann Arbor, MI, USA;
Department of Epidemiology, University of Michigan, Ann Arbor, MI, USA

Paul R. Lichter,
Department of Ophthalmology and Visual Sciences, University of Michigan, Ann Arbor, MI, USA

Doug Gaasterland,
Eye Doctors of Washington DC, Washington, DC, USA

Kari Branham,
Department of Ophthalmology and Visual Sciences, University of Michigan, Ann Arbor, MI, USA

Jesse Gilbert,
Department of Ophthalmology and Visual Sciences, University of Michigan, Ann Arbor, MI, USA

Sarah J. Garnai,
Department of Ophthalmology and Visual Sciences, University of Michigan, Ann Arbor, MI, USA

Correspondence to: Sayoko E. Moroi, smoroi@med.umich.edu; Jun Z. Li, junzli@med.umich.edu.

NEIGHBOR Consortium: http://glaucomagenetics.org/.

Electronic supplementary material The online version of this article (doi: 10.1007/s00439-013-1349-5) contains supplementary material, which is available to authorized users.

Wei Chen,
Division of Pulmonary Medicine, Allergy and Immunology, Children's Hospital of Pittsburgh, University of Pittsburgh Medical School, Pittsburgh, PA, USA; Departments of Biostatistics and Human Genetics, University of Pittsburgh, Pittsburgh, PA, USA

Mohammad Othman,
Department of Ophthalmology and Visual Sciences, University of Michigan, Ann Arbor, MI, USA

John Heckenlively,
Department of Ophthalmology and Visual Sciences, University of Michigan, Ann Arbor, MI, USA

Anand Swaroop,
National Eye Institute, National Institutes of Health, Bethesda, MD, USA

Gonçalo Abecasis,
Department of Biostatistics, University of Michigan, Ann Arbor, MI, USA

David S. Friedman,
Department of Ophthalmology, Johns Hopkins University School of Medicine, Baltimore, MD, USA

Don Zack,
Department of Ophthalmology, Johns Hopkins University School of Medicine, Baltimore, MD, USA

Allison Ashley-Koch,
Center for Human Genetics, Duke University School of Medicine, Durham, NC, USA

Megan Ulmer,
Center for Human Genetics, Duke University School of Medicine, Durham, NC, USA

Jae H. Kang,
Channing Division of Network Medicine, Brigham and Women's Hospital, Boston, MA, USA

NEIGHBOR Consortium, **Yutao Liu**,
Center for Human Genetics, Duke University School of Medicine, Durham, NC, USA

Brian L. Yaspan,
Center for Human Genetics Research, Vanderbilt University School of Medicine, Nashville, TN, USA

Jonathan Haines,
Center for Human Genetics Research, Vanderbilt University School of Medicine, Nashville, TN, USA

R. Rand Allingham,
Center for Human Genetics, Duke University School of Medicine, Durham, NC, USA

Michael A. Hauser,
Center for Human Genetics, Duke University School of Medicine, Durham, NC, USA

Louis Pasquale,
Channing Division of Network Medicine, Brigham and Women's Hospital, Boston, MA, USA; Department of Ophthalmology, Harvard Medical School, Massachusetts Eye and Ear Infirmary, Boston, MA, USA

Janey Wiggs,
Department of Ophthalmology, Harvard Medical School, Massachusetts Eye and Ear Infirmary, Boston, MA, USA

Julia E. Richards, and

Department of Ophthalmology and Visual Sciences, University of Michigan, Ann Arbor, MI, USA; Department of Epidemiology, University of Michigan, Ann Arbor, MI, USA

Jun Z. Li
Department of Human Genetics, University of Michigan, Ann Arbor MI 48109, USA

Sayoko E. Moroi: smoroi@med.umich.edu; Jun Z. Li: junzli@med.umich.edu

Abstract

Elevated intraocular pressure (IOP) is a major risk factor for glaucoma and is influenced by genetic and environmental factors. Recent genome-wide association studies (GWAS) reported associations with IOP at *TMCO1* and *GAS7*, and with primary open-angle glaucoma (POAG) at *CDKN2B-AS1*, *CAV1/CAV2*, and *SIX1/SIX6*. To identify novel genetic variants and replicate the published findings, we performed GWAS and meta-analysis of IOP in >6,000 subjects of European ancestry collected in three datasets: the NEI Glaucoma Human genetics collaBORation, GLAUcoma Genes and ENvironment study, and a subset of the Age-related Macular Degeneration-Michigan, Mayo, AREDS and Pennsylvania study. While no signal achieved genome-wide significance in individual datasets, a meta-analysis identified significant associations with IOP at *TMCO1* (rs7518099-G, $p = 8.0 \times 10^{-8}$). Focused analyses of five loci previously reported for IOP and/or POAG, i.e., *TMCO1*, *CDKN2B-AS1*, *GAS7*, *CAV1/CAV2*, and *SIX1/SIX6*, revealed associations with IOP that were largely consistent across our three datasets, and replicated the previously reported associations in both effect size and direction. These results confirm the involvement of common variants in multiple genomic regions in regulating IOP and/or glaucoma risk.

Introduction

Glaucoma is a leading cause of blindness worldwide (Quigley and Broman 2006). It is characterized by a progressive optic neuropathy arising from apoptotic cell death of retinal ganglion cells (Nickells 2012). Genetic causes for several Mendelian forms of glaucoma have been reported [reviewed in Moroi et al. (2009) and Wiggs (2012)], but these genes do not play a major role in the common age-related forms of POAG. Recently, genome-wide association studies (GWAS) for POAG have successfully identified common variants near *CAV1* and *CAV2* in an Icelandic cohort (Thorleifsson et al. 2010), in *TMCO1* and *CDKN2B-AS1* in an Australian cohort (Burdon et al. 2011), and in *CDKN2B-AS* and *SIX1/SIX6* regions in individuals of European ancestry (Wiggs et al. 2012b). These variants are associated with structural glaucomatous optic neuropathy and the related functional visual field loss.

Given the complexity of POAG, identifying the genetic basis of endophenotypes is a rational strategy. Elevated IOP is the major risk factor for many forms of glaucoma, and it is heritable (Klein et al. 2004; Chang et al. 2005; Parssinen et al. 2007; van Koolwijk et al. 2007; Carbonaro et al. 2008; Zheng et al. 2009; Charlesworth et al. 2010; Lee et al. 2012; Freeman et al. 2013). Glaucoma clinical trials have shown that IOP is a major risk factor for glaucomatous optic nerve disease progression (The AGIS Investigators 2000; Kass et al. 2002; Anderson et al. 2003; Leske et al. 2007; Miglior et al. 2007; Musch et al. 2011). Even patients with normal tension glaucoma can benefit from IOP-lowering treatment (Anderson 2003). IOP, a quantifiable trait routinely measured in clinical care, represents the outcome of four complex physiological processes: aqueous humor formation, uveoscleral outflow, trabecular outflow, and episcleral venous pressure (Brubaker 2004). There is variance in IOP among healthy individuals and an even larger variance among patients with glaucoma (Liu et al. 2011; Pekmezci et al. 2011).

A recent GWAS of IOP in Caucasian cohorts identified common variants in the *GAS7* and *TMCO1* genes (van Koolwijk et al. 2012). Using a similar GWAS approach, we sought to identify DNA variants for IOP in three cohorts of European ancestry: NEIGHBOR (NEI Glaucoma Human genetics collaBORation), GLAUGEN (GLAUcoma Genes and ENvironment study), and a set of POAG cases and controls derived from the Michigan subset of AMD-MMAP (Age-related Macular Degeneration-Michigan, Mayo, AREDS and Pennsylvania) study. We performed GWAS for IOP in the three datasets and a meta-analysis of the combined datasets. We then selected genes previously reported for significant association with IOP and/or POAG, including *TMCO1*, *CDKN2B-AS1*, *GAS7*, *CAV1/CAV2* and *SIX1/SIX6*, and examined their association with IOP in our datasets. Finally, we analyzed subsets of the NEIGHBOR cohort to characterize potential heterogeneity in IOP association due to site, gender, or age effects.

Subjects and methods

Datasets

We analyzed IOP and genotype data in three datasets, as described below. All data were derived from subjects who provided informed consent according to protocols approved by the institutional review boards of the recruiting institutions, as previously described (Li et al. 2006; Wiggs et al. 2012a).

NEI Glaucoma Human genetics collaBORation (NEIGHBOR)—The NEIGHBOR study involves investigators from 12 institutions throughout the USA, and has been described before (Wiggs et al. 2012a; NEIGHBORHOOD Consortium 2013). Cases and controls were at least 35 years old and were of European ancestry. Refractive status was limited between eight diopters of myopia or eight diopters of hyperopia. Individuals with narrow angles, exfoliation syndrome, pigment dispersion syndrome, anterior segment dysgenesis, or chronic inflammatory changes were excluded. Additional exclusion criteria for controls included vertical cup-to-disc ratio greater than 0.6, or cup-to-disc ratio asymmetry of more than 0.2. All cases had evidence of reproducible visual field loss and glaucomatous optic neuropathy.

GLAUcoma Genes and ENvironment (GLAUGEN)—The GLAUGEN study includes POAG cases and controls that were ascertained from two epidemiologic studies: the Nurses' Health Study (NHS) and the Health Professionals Follow-up Study (HPFS), and from a third, clinic-based case–control study from the Massachusetts Eye and Ear Infirmary (MEEI). Clinical phenotype ascertainment was similar to the criteria described above for the NEIGHBOR cohort. The GLAUGEN study is part of the Gene Environment Association Studies Consortium (GENEVA) (Cornelis et al. 2010). More details regarding the GLAUGEN study can be found on dbGaP (www.ncbi.nlm.nih.gov/projects/gap).

Michigan subset of the Age-related Macular Degeneration-Michigan, Mayo, AREDS, Pennsylvania (AMD-MMAP)—The AMD-MMAP study was designed to identify the genetic basis of AMD (Li et al. 2006; Chen et al. 2010). This study population consisted of cases affected with AMD and controls unaffected with AMD. AMD cases were categorized based on the severity of the diagnosis in the worse eye, ranging from neovascularization to large drusen. Clinical research records of the Michigan subset of the study subjects were reviewed to select individuals who qualified as controls and POAG according to NEIGHBOR study design criteria (Wiggs et al. 2012a).

For all three datasets, POAG cases demonstrated characteristic glaucomatous visual field defects after a reproducible sequential visual field test, or one glaucomatous visual field test

plus a measurement of cup-to-disc ratio (CDR) of ≥0.7 in at least one eye. Controls had CDR ≤0.6 and no visual field loss.

IOP measurements

IOP data were obtained from the existing clinical research records of the NEIGHBOR, GLAUGEN, and AMD-MMAP MI studies. After applying topical anesthetic to the cornea surface, IOP was measured using either Goldmann applanation or Tonopen telemetry.

Genotyping, imputation and quality control

DNA samples in NEIGHBOR and GLAUGEN were genotyped at the Center for Inherited Disease Research (CIDR) (Baltimore, MD) and the Broad Institute (Cambridge, MA), respectively, using the Human 660W_Quad_v1 array from Illumina (San Diego, CA). Initial data processing and quality assessment have been previously described (Wiggs et al. 2012b). After the initial quality control processing, the genotype datasets contained 521,687 single nucleotide polymorphisms (SNPs) for 4,514 subjects in NEIGHBOR, and 495,161 SNPs for 2,116 subjects in GLAUGEN (dbGaP Study Accession: phs000308.v1.p1). Separately for the two datasets, we further filtered the genotype data by removing SNP markers meeting one of the following criteria: per-SNP missing call rate >10 %, minor allele frequency (MAF) of <2 %, or a Hardy–Weinberg equilibrium p value $<1.0 \times 10^{-4}$. These criteria yielded a final set of 506,679 SNPs and 484,176 SNPs for NEIGHBOR and GLAUGEN, respectively. The AMD-MMAP samples were genotyped at CIDR using the Illumina CNV370v1_C array. Initial data clean-up was described previously (Chen et al. 2010). The resulting dataset, containing 370,404 SNPs for 3,307 individuals, was subsequently imputed across ∼2.54 million SNP loci by using *MACH* (Willer et al. 2010) and HapMap2 CEU release 22 (60 unrelated individuals) (Frazer et al. 2007) as the reference population. From these, 1,774 subjects from the Michigan site were selected for this study. We then removed SNPs with low imputation quality (R-squared <0.3) or low allele frequency (MAF <2 %), resulting in ∼2.24 million SNPs. Across the three datasets 466,573 SNPs were shared in common.

Estimation of untreated IOP values

For subjects with IOP measurements available in both eyes, the higher IOP of the two eyes was defined as the individual's quantitative phenotype (Klein et al. 2004). The IOP values in the AMD-MMAP MI cohort were IOP measurements without treatment (referred to as “untreated IOP” hereafter). However, approximately 64 % of POAG cases in the other two datasets, 1,401 in NEIGHBOR and 477 in GLAUGEN, had only IOP measurements reported while under IOP-lowering treatment (referred to as “treated IOP” hereafter), and the information pertaining to the therapeutic modality used to lower IOP (medication, laser, or incisional surgery) was not available. We analyzed the quantitative relationship of untreated and treated IOP for subjects with both types of available measurements (n = 231) and implemented a method to estimate untreated IOP values for those with only treated IOP. This estimation was made by dividing the treated IOP values by a factor of 0.7 (see “Results”), which is similar to the IOP estimation method described by van der Valk et al. (2005) and van Koolwijk et al. (2012). The resulting IOP distributions were approximately normal for GLAUGEN and AMD-MMAP, but skewed for NEIGHBOR (Supplementary Fig. 1). We did not further transform the IOP values because (1) with large sample sizes (>1,000) the benefit of log transformation is small (Li et al. 2012); (2) we would maintain the original scale when reporting effect sizes (in mmHg/allele) and comparing with van Koolwijk et al. (2012), which did not transform the IOP values either; (3) the NEIGHBOR samples came from a case–control cohort, not from a single, inherently homogeneous population; (4) the ranked-based inverse normal transformation, conducted for each of the

three studies, did not change any of the meta-analysis conclusions reported below (not shown). Individuals with extreme IOP values (beyond three standard deviations from the overall mean of each study) were removed (n = 60 in NEIGHBOR, n = 12 in GLAUGEN, n = 3 in AMD-MMAP MI).

Population structure

We used multidimensional scaling (MDS) of the sample–sample identity-by-state matrix to assess potential population structures in each of the three datasets, involving 4,514 subjects in NEIGHBOR, 2,116 subjects in GLAUGEN, and 1,774 subjects in AMD-MMAP MI. This analysis relied on pruned sets of ∼15, ∼14, and ∼12 K autosomal SNPs for the three datasets, respectively, selected by a linkage disequilibrium (LD) r^2 threshold of 0.01 in windows of 50 SNPs and a moving step of five SNPs. By including 210 HapMap samples of European (CEU), East Asian, and African ancestries (Frazer et al. 2007), we confirmed that all the subjects in NEIGHBOR, 2,096 of 2,116 subjects in GLAUGEN, and 1,770 of 1,774 subjects in the AMD-MMAP MI dataset were of European ancestry (data not shown). We used these samples in downstream analysis and controlled for the residual, within-Europe population structure by using MDS scores as covariates (referred to as principal component (PC) scores below, as the MDS and PC scores are very similar in this situation).

IOP association analysis and meta-analysis

The association between IOP levels and genotypes at individual SNP markers was tested using linear regression analysis under an additive model in *PLINK* (Purcell et al. 2007). For the imputed data for AMD-MMAP, allele dosage scores were used in association tests. After single-SNP association tests, residual population stratification was assessed by using the genomic control factor (Devlin and Roeder 1999).

For association tests in the NEIGHBOR and GLAUGEN datasets, we incorporated age, gender, and the first five principal component scores (PC1–PC5) as covariates. No other clinical variable in NEIGHBOR and GLAUGEN was chosen as a covariate due to the uneven availability of clinical data across different recruiting sites. Site was not used as a covariate because the NEIGHBOR dataset contains both cases and controls from eight sites, with different case–control ratios, and only cases from four sites. For GLAUGEN, two of the three sites had only cases. For the MI dataset of AMD-MMAP, most subjects had data for most clinical variables; therefore, we screened for the relationship between IOP values and the available variables (plus the first ten principal component scores) by univariate regression analyses and selected as covariates those with regression p value < 0.05. Our analysis resulted in choosing the diabetes medication, spherical equivalent, AMD type, and the third principal component as covariates in the association tests in AMD-MMAP MI.

The association results of the three datasets were combined in a meta-analysis for the shared set of 466,573 SNPs, using the inverse sample size-weighted approach in *METAL* (Willer et al. 2010), implemented in a fixed-effects model, with study-specific genomic control correction. The genome-wide significance level for the meta-analysis was set at a p value of 1.1×10^{-7} to correct for multiple testing of this shared SNP set. Heterogeneity analyses of effect size differences across different age and gender groups, and across different study sites were carried out using *METAL*. For a given subject, if only the age at recruitment or the age at diagnosis was available, it was used in the analysis; if both were available, the recruitment age was used. Forest plots were made using custom R scripts to compare the effect size, direction, and standard error across various datasets, sample groups, or with published studies.

Results

Estimation of untreated IOP values

All POAG cases in NEIGHBOR and GLAUGEN were treated with glaucoma medication and/or surgery. However, 64 % of the cases did not have a reported untreated IOP measurement. The treatment effects on IOP, if unaccounted for, could obscure genetic association results. One solution is to analyze only the cases with untreated IOP values, but this would significantly reduce the sample size and subsequent power to find genetic associations. To increase statistical power, we sought to estimate untreated levels for these cases based on their recorded treated values. A previous study (van der Valk et al. 2005) reviewed IOP responses after medical treatment in 27 publications involving 28 randomized clinical trials and found that IOP was lowered by an average of ∼30 % in ∼14,000 individuals receiving different classes of medications. Such a quantitative relationship was subsequently used to estimate untreated IOP values for use in association tests (van Koolwijk et al. 2012). A similar IOP extrapolation, using a factor of 33 %, was adopted to study the heritability of IOP in a population-based study (Chang et al. 2005).

To re-examine this quantitative relationship, we took advantage of two subsets of subjects in the NEIGHBOR study that had both untreated and treated IOP values. In the Collaborative Initial Glaucoma Treatment Study (CIGTS) subset, 114 cases had untreated IOP values and those recorded at 3 months after treatment. Of these, 30 were treated surgically, allowing us to further compare the effects of medication and surgery. Linear regression of the untreated and treated IOP values yielded a slope of 0.69 (Fig. 1a). In the Michigan site of the NEIGHBOR study, 117 cases had untreated and treated values. The regression slope was 0.65 for 47 cases with medication, and 0.53 for 70 cases with surgery (Fig. 1b). The combined set of 117 cases had a slope of 0.65 (Fig. 1b). Taken together, these results confirmed the earlier findings and provided empirical justification for using 0.7 as the extrapolation factor in estimating untreated IOP values. Notably, this factor was similar between those who received surgery and those who received medication (Fig. 1b).

After estimating untreated IOP values, we obtained a final dataset of 4,088 individuals (1,903 cases, 2,185 controls) in NEIGHBOR, 1,153 individuals (816 cases, 337 controls) in GLAUGEN, and 995 individuals (128 cases, 867 controls) in AMD-MMAP MI. Extrapolation was not needed for AMD-MMAP MI since all cases had recorded untreated IOP values. The overall sample size was 6,236, with 2,847 cases and 3,389 controls. The demographic features and IOP summary values of the final dataset are presented in Table 1. In the NEIGHBOR and GLAUGEN datasets, the treated POAG cases had average treated IOP values of ∼15.7 mmHg, comparable to those in the controls (14.9 mmHg). After extrapolation by dividing with 0.7, the average estimated untreated values were ∼22.4 mmHg, comparable to the untreated values for cases with such values available (23.2 mmHg).

GWAS and meta-analysis of NEIGHBOR, GLAUGEN, AMD-MMAP MI datasets

We performed GWAS on the three datasets separately, using the recorded IOP for controls, untreated IOP for POAG cases with such values, and extrapolated IOP for those without, incorporating covariates as described in " Subjects and methods". None of the three datasets revealed genome-wide significant signals, at thresholds of $p < 9.9 \times 10^{-8}$ for NEIGHBOR, $p < 1.1 \times 10^{-7}$ for GLAUGEN, and $p < 2.1 \times 10^{-8}$ for AMD-MMAP MI, according to varying numbers of SNPs tested. The Manhattan plots for the three GWAS and the Q-Q plot of NEIGHBOR are shown in Supplementary Fig. 2.

We then performed a meta-analysis of the three datasets (n = 6,236) and identified a significant signal in 1p24.1 within intron 2 of the *TMCO1* gene (rs7518099 (G), beta = 0.76 mmHg/allele, $p = 8.0 \times 10^{-8}$) (Table 2; Fig. 2a). The top 20 SNPs in the meta-analysis, representing 14 associated regions, are shown in Table 2. The Q-Q plot for the meta-analysis showed little evidence of inflation, with a genomic control factor of 1.02 (Fig. 2b).

Replication of previously reported associated regions for IOP or POAG

Several GWAS have been published for either IOP or POAG. van Koolwijk et al. (2012) studied the genetic association of IOP in the Netherlands, involving a discovery phase (n = 11,972) and a replication phase (n = 7,482), and identified two significantly associated regions corresponding to the genes *GAS7* and *TMCO1*. Burdon et al. (2011) analyzed 590 glaucoma cases and 3,956 controls and identified two loci containing genes *TMCO1* and *CDKN2B-AS1* as significantly associated with open-angle glaucoma. Wiggs et al. (2012b) reported *CDKN2B-AS1* (referred to by the name *CDKN2B-AS* in that work) and *SIX1/SIX6* as highly significant for POAG in a dataset of 3,146 POAG cases and 3,487 controls. Thorleifsson et al. (2010) conducted a GWAS on 1,263 POAG cases and 34,877 controls from Iceland and identified one associated locus, *CAV1/CAV2*. Of the top 20 SNPs in our meta-analysis, one reached genome-wide significance, an SNP near *GAS7* (rs12150284) had a p value of 2.4×10^{-6}. This suggests that some of the previously reported association results, for either IOP or POAG, could have similar effect sizes in our cohorts, but did not reach genome-wide significance due to the limited sample size in our study. Further, our association results for IOP are partly driven by POAG case–control differences and could be signals for either IOP or POAG. We therefore focused on five loci previously reported for IOP or POAG and examined their association with IOP in our datasets.

We first selected the top SNPs from our meta-analysis in each of the five loci and used their beta values to assess if our findings replicated the effect size and effect direction from those previously reported. In our study, the top SNPs in the *TMCO1*, *CDKN2B-AS1*, *GAS7*, *CAV1/CAV2*, and *SIX1/SIX6* loci were rs7518099 ($p = 8.0 \times 10^{-8}$), rs1412829 ($p = 3.6 \times 10^{-4}$), rs12150284 ($p = 2.4 \times 10^{-6}$), rs4236601 ($p = 2.8 \times 10^{-4}$), and rs10483727 ($p = 9.1 \times 10^{-2}$), respectively (Fig. 2c–g). Their beta values, shown in Fig. 3, were mostly consistent across the three datasets we analyzed. A heterogeneity analysis for the top SNPs showed no significant difference among the three cohorts at *TMCO1* (I^2 = 0 %, p = 0.90), *GAS7* (I^2 = 0 %, p = 0.76), *CAV1/CAV2* (I^2 = 0 %, p = 0.80) and *SIX1/ SIX6* (I^2 = 28.7 %, p = 0.25), and a nominally significant difference at *CDKN2B-AS1* (I^2 = 72.5 %, p = 0.027). As expected, results for NEIGHBOR had tighter confidence intervals due to its larger sample size, and a prevailing influence on the overall effect size in the meta-analysis. Our top SNPs at *TMCO1* and *GAS7* were also analyzed by van Koolwijk et al. (2012) and ranked among the top 40 SNPs in that study, with beta values showing strong agreement with our meta-analysis results in both effect size and direction (Fig. 3a, c). Our top SNP at *CDKN2B-AS1*, rs1412829, was among the top 20 glaucoma-associated SNPs identified by Burdon et al. (2011), with a reported odds ratio (OR) of 1.45 for the T allele, and by Wiggs et al. (2012b), with a reported OR of 0.69 for the G allele. Since odds ratios higher than one indicate higher risk for glaucoma and correspond to positive beta values in association analysis with IOP, our observed negative beta value for the G allele was in agreement with its impact on glaucoma as reported previously. Similarly, our top SNP at *CAV1/CAV2* was one of the two top SNPs associated with glaucoma in Thorleifsson et al. (2010), with a reported OR of 1.36 for the A allele, and was the top SNP associated with IOP in this region in van Koolwijk et al. (2012), with positive beta for the A allele, consistent with our findings. Our top SNP at *SIX1/SIX6* was the top SNP in that locus associated with POAG in Wiggs et al. (2012b), with a reported OR of 1.32 for the A allele, consistent with its positive beta in our study. In

all, we conclude that our top SNPs in the five candidate regions replicated in effect size and direction for the association signals reported previously for POAG and/ or IOP.

We next examined the same top SNPs reported by van Koolwijk et al. (2012) to be associated with IOP: rs7555523 at *TMCO1*, and rs11656696 at *GAS7*. In our cohorts, rs7555523 information was available only in AMD-MMAP MI (among the imputed SNP sites). Its effect size and direction (beta = 0.55 mmHg/allele) were stronger than the previously published values (beta = 0.28 mmHg/allele) (Fig. 4a). The *GAS7* SNP, rs11656696 was genotyped in NEIGHBOR and imputed in AMD-MMAP MI. Its beta values, −0.40 mmHg/allele in NEIGHBOR and −0.06 mmHg/allele in AMD-MMAP MI, were also similar to those reported before (Fig. 4b). Thus, we could replicate the effect size and direction of association with *GAS7* and *TMCO1* at the same lead SNPs previously reported for IOP association.

In addition to *TMCO1* and *GAS7*, van Koolwijk et al. (2012) also reported five other regions with suggestive association with IOP: rs7894966 at the *BMP1RA* locus, rs216146 at *CSF1R*, rs1826598 at *ADAMTS18* and *NUDT7*, rs9841621 at *SATB1*, and rs21117760 at *FOXP1*. Of these five SNPs, the first two were not genotyped or imputed in our datasets and had relatively weak LD with genotyped SNPs in the HapMap data for European populations: at *BMP1RA*, the closest proxy SNP in our datasets was rs1124482, which has $r^2 = 0.61$ with rs7894966; at *CSF1R*, the closest proxy SNP was rs216137 ($r^2 = 0.67$ with rs216416). Given the lack of reliable proxies for these two SNPs, we focused on the other three SNPs which were present in our datasets. The effect size and the direction for rs1826598, rs9841621 and rs21117760 in our meta-analysis were in agreement with their reported values in van Koolwijk et al. (2012) (Fig. 4c–e), thus replicating the findings for these three regions as well.

We evaluated the lead SNPs reported for association with glaucoma by Burdon et al. (2011): rs4656461 and rs7518099 at *TMCO1*, and rs4977756 and rs10120688 at *CDKN2B-AS1*. Both SNPs at *TMCO1* showed a strong agreement in effect size and direction across the three datasets (Fig. 5). Of these, rs7518099 was the only SNP reaching genome-wide significance in our meta-analysis ($p = 8.0 \times 10^{-8}$), and rs4656461 showed suggestive association ($p = 8.3 \times 10^{-7}$). Burdon et al. (2011) reported ORs of 1.49 and 1.51 at these two loci, respectively, consistent with their positive beta values we observed for the same alleles. At *CDKN2B-AS1*, rs4977756 (G, beta = −0.26 mmHg/allele, $p = 4.8 \times 10^{-3}$) and rs10120688 (G, beta = −0.23 mmHg/allele, $p = 9.8 \times 10^{-3}$) showed suggestive significance in our meta-analysis. The ORs reported by Burdon et al. (2011) for these SNPs were 1.39 and 1.32, respectively, in agreement with the negative beta values for the opposing allele observed in our study. We conclude that we were able to replicate the findings of Burdon et al. (2011) for *TMCO1* and *CDKN2B-AS1* in effect size and direction.

Next, we investigated the two lead SNPs significantly associated with glaucoma in Thorleifsson et al. (2010): rs4236601 and rs1052990 at *CAV1/CAV2* (Fig. 6). SNP rs4236601 was found in all three datasets and was our top SNP in this region in the meta-analysis. The other SNP, rs1052990, was only present in NEIGHBOR and AMD-MMAP MI. Both SNPs showed strong agreement in effect size and direction across datasets. The ORs reported by Thorleifsson et al. (2010) were 1.36 and 1.32 for rs4236601 and rs1052990, respectively, consistent with their positive beta values for the same alleles in our datasets.

Finally, we examined the top SNPs in *CDKN2B-AS1* (rs2157719) and *SIX/SIX6* (rs10483727) associated with POAG status in Wiggs et al. (2012b). SNP rs2157719 was the second most significant SNP at *CDKN2B-AS1* in our meta-analysis ($p = 4.83 \times 10^{-4}$), and

rs10483727 was the most significant SNP at *SIX1/SIX2* ($p = 9.06 \times 10^{-2}$). Wiggs et al. (2012b) reported ORs of 0.69 and 1.32 at these two SNP loci, respectively, consistent with the negative and positive beta values we observed in the meta-analysis for the same alleles (Figs. 3e, 7a).

The NEIGHBOR study had the largest sample size ($n = 4,088$) among our three cohorts, with 7 of the 11 NEIGHBOR sites contributing both cases and controls, thus allowing us to examine the site-specific, gender-specific, and age-specific signals for both genome-wide and the top SNPs in the five loci examined above. To assess between-site heterogeneity, we ran GWAS for the seven NEIGHBOR sites separately, incorporating the extrapolated pre-treatment IOP values as described earlier, while controlling for age, gender, and PC1-5 calculated within each site. The analysis involved 1,215, 1,281, 384, 326, 301, 125, and 172 individuals for sites 1-6 and 8, respectively, for a total sample size of 3,804. None of the sites produced a signal of genome-wide significance, nor did the meta-analysis of the seven sites (Supplementary Fig. 3a). Compared with the original NEIGHBOR analysis using all samples combined, the seven-site meta-analysis resulted in an overall effect of slightly less significant *p* values (as shown by the Q-Q plot in Supplementary Fig. 3b). We next analyzed site-specific effects for the top SNPs at *TMCO1* (rs7518099), *CDKN2B-AS1* (rs1412829), *GAS7* (rs12150284), *CAV1/CAV2* (rs4236601), and *SIX1/SIX6* (rs10483727) identified in the meta-analysis (Supplementary Fig. 4). The effects of all five SNPs were in agreement across a majority of the sites. Heterogeneity analysis showed no significant difference among the seven sites at *TMCO1*, *GAS7*, or *SIX1/SIX6*, and a moderately significant difference at *CDKN2B-AS1* ($I^2 = 64.0$ %, $p = 0.011$) and *CAV1/CAV2* ($I^2 = 58.9$ %, p = 0.024), mostly driven by 1–2 “outlier” sites. In all, we conclude that the association effects were consistent across most of NEIGHBOR sites for the five regions examined. This result provided justification for the all-site combined analysis originally performed.

For between-gender differences, we ran association tests for males ($n = 1,905$) and females ($n = 2,183$) separately. IOP distributions were similar in the two genders, with a mean of 19.4 mmHg in males and 18.9 mmHg in females. An SNP of genome-wide significant association was observed among males at *TMCO1* (rs7518099-G, beta = 1.67 mmHg/allele, $p = 7.4 \times 10^{-8}$) (Supplementary Fig. 5). Gender-specific association results for the top SNPs at *TMCO1*, *CDKN2B-AS1*, *GAS7*, *CAV1/CAV2*, and *SIX1/SIX6* are shown in Supplementary Fig. 6. At *TMCO1*, the effect size of rs7518099 was significantly greater for males than for females ($I^2 = 88.9$ %, $p = 2.7 \times 10^{-3}$). There was no significant gender difference for the top SNPs at *CDKN2B-AS1* ($I^2 = 0$ %, $p = 0.68$), *GAS7* ($I^2 = 0$ %, $p = 0.32$), *CAV1/CAV2* locus ($I^2 = 0$ %, $p = 0.65$), or *SIX1/SIX6* ($I^2 = 18.5$ %, $p = 0.27$).

Finally, to examine age-dependent trends, we divided NEIGHBOR subjects using age cutoffs of 61, 69, and 77 years to obtain four groups of similar size ($n = 1,022$, 1,026, 1,027 and 1,017, respectively), with similar IOP distributions across groups (Supplementary Fig. 7a). The youngest group (Supplementary Fig. 7b) showed a genome-wide significant signal at 18q22 (rs1876486-A, beta = 3.43 mmHg/allele, $p = 3.4 \times 10^{-8}$). This intergenic SNP located between *ZNF407* and *ZADH2* has no known function and has minimal evidence of regulatory function in the ENCODE dataset (Boyle et al. 2012). Between-age group heterogeneity was significant for this SNP (Supplementary Fig. 7c, $I^2 = 83$ %, $p = 5.8 \times 10^{-4}$). No other SNPs were significantly associated in any of the other age groups (not shown). In the focused analysis of the five genes of interest, none showed significant heterogeneity among age groups (Supplementary Fig. 8). However, there is a consistent age trend for *CDKN2B-AS1* (rs1412829) and *CAV1/CAV2* (rs4236601), where associations were stronger in younger age groups showing stronger association (Supplementary Fig. 8).

Discussion

In this study we performed GWAS and meta-analysis of IOP across three datasets previously analyzed for case-control studies of primary open-angle glaucoma or age-related macular degeneration. While we found no signal of genome-wide significance in any of these three datasets, meta-analysis revealed that the *TMCO1* region is associated with IOP. *TMCO1* has been previously reported for association with IOP (van Koolwijk et al. 2012) and with glaucoma (Burdon et al. 2011; Sharma et al. 2012). In addition, we observed a near-threshold signal close to *GAS7* (rs12150284, $p = 2.4 \times 10^{-6}$), which was the second region reported in van Koolwijk et al. (2012). This motivated us to examine five regions previously reported for association with either IOP or glaucoma: *TMCO1*, *CDKN2B-AS1*, *GAS7*, *CAV1/CAV2*, and *SIX1/SIX6*. While the detailed results varied by datasets and by SNP marker, in general, we were able to replicate the effect size and direction of association with IOP for all five regions. At *TMCO1* and *GAS7*, we replicated the findings both for the top SNPs in our meta-analysis and for the exact lead SNPs previously reported.

While our results often did not reach genome-wide significance, the replication is supported by (1) similar effect size and same direction as previously reported; (2) consistency across our three datasets (except for *CDKN2B-AS1*, with $p = 0.027$ in heterogeneity test), which justifies the fixed-effect meta-analysis for most loci; (3) consistency across sites, gender, and age groups (with exceptions noted earlier); and (4) agreement with POAG association results in direction: higher risk alleles are positively associated with IOP. Given the comparable effect sizes as previously observed, the lack of statistical significance in our study can be primarily attributed to sample size differences. For example, our datasets had a smaller combined sample size ($n = 6{,}236$) when compared with the discovery ($n = 11{,}972$) and replication datasets ($n = 7{,}482$) by van Koolwijk et al. (2012).

In this study, we estimated untreated IOP values by applying linear extrapolation on the treated values. Given the tremendous heterogeneity in treatment methods and environmental factors, the procedure of dividing by 0.7 could be overly simplistic and may have introduced additional noise, for which we lack independent means to evaluate. When we analyzed controls and only the cases with recorded untreated IOP values, the meta-analysis results became weaker in each of the five candidate regions: when compared with the original analysis (including the extrapolated IOP values), all lead SNPs showed reduced beta values and less significant *p* values (not shown).

Since IOP is a risk factor for POAG, it remains an interesting question whether the five loci we examined are primarily associated with IOP or with POAG. This question is difficult to answer in a POAG case–control cohort such as NEIGHBOR, where many cases have only treated IOP values, precluding a standard regression of IOP with SNP genotype using case status as a covariate. In our study, if the real effect of a locus is on the risk of glaucoma but not IOP, but, because of the IOP-lowering treatment in some NEIGHBOR and GLAUGEN cases, we scaled their IOP by 1/0.7, this locus will show apparent association with IOP as a secondary effect. Future studies without the treatment confounder are needed to properly address this question.

IOP measurements are known to be influenced by central corneal thickness (CCT) (Kohlhaas et al. 2006). In our datasets, 823 of the 4,088 subjects in NEIGHBOR had available CCT values, including 158 controls and 665 cases. We investigated the impact of CCT on IOP association by adjusting the IOP values for the 823 subjects having an available CCT measurement. The analysis of the CCT-adjusted IOP did not yield any significant signals (at the threshold of $p < 9.9 \times 10^{-8}$) (Supplementary Fig. 9a) and the *p* values were similar to those from the original, unadjusted analysis (Supplementary Fig. 9b).

For gender-specific effects, the beta value of rs7518099 at *TMCO1* was significantly greater for males than for females. For age effects, we found a consistent age trend at *CDKN2B-AS1* (rs1412829) and *CAV1/CAV2* (rs4236601), with younger age groups showing stronger association. These results provide encouraging leads regarding the age-and gender-specific etiology of elevated IOP as an endophenotype for glaucoma. However, these findings are preliminary and need to be replicated.

We successfully replicated the reported association of *TMCO1* with IOP and POAG status. *TMCO1* is located ∼6 Mb upstream of *MYOC*, a known gene related to juvenile-onset OAG (Stone et al. 1997), and encodes a protein containing a transmembrane and coiled-coil domain (Zhang et al. 2010). It is highly conserved across species (Xin et al. 2010). Its function is currently unknown; however several studies have shown high expression levels in various ocular tissues, including the trabecular meshwork, the retina, and the secretory neuroepithelium of the ciliary body, which together or separately play a role in regulating IOP levels (Liton et al. 2006; Burdon et al. 2011; Sharma et al. 2012; van Koolwijk et al. 2012). *TMCO1* has been implicated in both ocular (Burdon et al. 2011) and developmental phenotypes (Xin et al. 2010), raising questions about its potential role in ocular development.

Another finding of interest is that rs1876486 on 18q22 is associated with IOP in the youngest group of the study population, with the largest beta values (3.4 mmHg/allele) ever reported for IOP, genome-wide significance, and decreasing effect with increasing age. This SNP is ∼15 kb from *AK096231* and ∼26 kb from *ZNF407*, but with minimal evidence for regulatory function except for ChIPSeq signals in three liver cell lines and a cancer cell line. Because the youngest age for study cases (35 years) falls within the age range of juvenile-onset open-angle glaucoma, it would be of interest to explore its potential role in juvenile-onset glaucoma.

In summary, our results contribute to the growing evidence that the complex physiological trait of IOP is associated with common DNA variants in multiple loci, in agreement with recent GWAS results (van Koolwijk et al. 2012) and past heritability estimates (Giuffre et al. 1995; Qureshi et al. 1996; Leske et al. 1997; Hashemi et al. 2005; Fukuoka et al. 2008; Wong et al. 2009; Freeman et al. 2013). Additional studies are needed to examine the effect of these biomarkers in other populations, especially those of African ancestry for whom POAG has higher prevalence and severity than in Caucasians. Understanding the effects of DNA variants on steady state IOP levels also provides important new knowledge that may allow us to study large fluctuations of IOP, a separate but postulated important clinical parameter in glaucoma management.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We acknowledge EY022124 (S.E.M.); 2RO1HL039693 and R01HL112642 (J.Z.L., A.B.O); EY011671, EY09580 (J.E.R.); EY007003 (Michigan Core Center for Vision Research); HG005259-01, 3R01EY015872-05S1, EY015872, EY010886, EY009847 (J.W.); HG004728, EY015473 (L.P.); EY016862, EY007758 (J.R.H.); HG002651 (G.A.); EY009149 (P.R.L.); EY006827 (D.G.); EY016862, EY016862, HL084729, HG002651 (G.R.A.); CA87969, CA49449, CA55075, EY09611 (J.H.K.); T32EY021453 (B.Y.); 3R01EY019126-02S1, EY13315 (M.A.H.); EY015543 (R.R.A.); U01-HG004424 (Broad Institute); HHSN268200782096C; RC1HG005334, U54HG004570 (ENCODE); HG004608 (C.M.M.); HG006389 (C.M.M., M.H.B); EY008208 (F.A.M.); EY015682 (A.R.); EY144428, EY144448, EY18660 (K.Z.); HL073389 (E.H.); U01 HG004446 (C.L.); HG004608 (C.M.); EY008208 (F.A.M.); EY012118, EY012118 (M.A.P–V.); EY015682 (A.R.); EY013178 (J.S.S.); RR015574; EY011008 (L.M.Z.);

Other supports included funding from University of Michigan Glaucoma Research Center (S.E.M., J.E.R., J.Z.L.); Ellison Medical Foundation (J.Z.L.); Harvard Glaucoma Center for Excellence, and the Margolis Fund (J.W. and L.P.); Research to Prevent Blindness (A.S., D.C.M., J.W., L.P., J.R.H., and J.E.R.); the Glaucoma Research Foundation (S.E.M., Y.L.); the Glaucoma Foundation (Y.L.); American Health Assistance Foundation (Y.L., J.E.R., J.R.H., A.S.); Elmer and Silvia Sramek Foundation (J.R.H., A.S.); Foundation Fighting Blindness (J.R.H., A.S.); the Macula Vision Research Foundation (J.R.H., A.S.); the Pew Charitable Trusts (J.R.H., A.S.); the Casey Macular Degeneration Center Fund (J.R.H., A.S.); the Marion W. and Edward F. Knight AMD Fund (J.R.H., A.S.); the Harold and Pauline Price Foundation, National Genotyping Centre of Spain (J.R.H., A.S.).

A list of the GLAUGEN collaborators is found at The Primary Open-Angle Glaucoma Genes and Environment (GLAUGEN) Study listing on dbGAP, study Accession: phs000308.v1.p1. www.ncbi.nlm.nih.gov/projects/gap, December 21, 2010. A list of the NEIGHBOR investigators is found at the NEIGHBORHOOD Consortium http://glaucomagenetics.org/members (accessed March 18, 2013).

References

Anderson DR. Collaborative normal tension glaucoma study. Curr Opin Ophthalmol. 2003; 14(2):86–90. [PubMed: 12698048]

Anderson DR, Drance SM, Schulzer M. Factors that predict the benefit of lowering intraocular pressure in normal tension glaucoma. Am J Ophthalmol. 2003; 136(5):820–829. [PubMed: 14597032]

Boyle AP, Hong EL, Hariharan M, Cheng Y, Schaub MA, Kasowski M, Karczewski KJ, Park J, Hitz BC, Weng S, Cherry JM, Snyder M. Annotation of functional variation in personal genomes using RegulomeDB. Genome Res. 2012; 22(9):1790–1797. [PubMed: 22955989]

Brubaker RF. Goldmann's equation and clinical measures of aqueous dynamics. Exp Eye Res. 2004; 78(3):633–637. [PubMed: 15106943]

Burdon KP, Macgregor S, Hewitt AW, Sharma S, Chidlow G, Mills RA, Danoy P, Casson R, Viswanathan AC, Liu JZ, Landers J, Henders AK, Wood J, Souzeau E, Crawford A, Leo P, Wang JJ, Rochtchina E, Nyholt DR, Martin NG, Montgomery GW, Mitchell P, Brown MA, Mackey DA, Craig JE. Genome-wide association study identifies susceptibility loci for open angle glaucoma at TMCO1 and CDKN2B-AS1. Nat Genet. 2011; 43(6):574–578. [PubMed: 21532571]

Carbonaro F, Andrew T, Mackey DA, Spector TD, Hammond CJ. Heritability of intraocular pressure: a classical twin study. Br J Ophthalmol. 2008; 92(8):1125–1128. [PubMed: 18653607]

Chang TC, Congdon NG, Wojciechowski R, Munoz B, Gilbert D, Chen P, Friedman DS, West SK. Determinants and heritability of intraocular pressure and cup-to-disc ratio in a defined older population. Ophthalmology. 2005; 112(7):1186–1191. [PubMed: 15939473]

Charlesworth J, Kramer PL, Dyer T, Diego V, Samples JR, Craig JE, Mackey DA, Hewitt AW, Blangero J, Wirtz MK. 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. 2010; 51(7):3509–3514. [PubMed: 20237253]

Chen W, Stambolian D, Edwards AO, Branham KE, Othman M, Jakobsdottir J, Tosakulwong N, Pericak-Vance MA, Campochiaro PA, Klein ML, Tan PL, Conley YP, Kanda A, Kopplin L, Li Y, Augustaitis KJ, Karoukis AJ, Scott WK, Agarwal A, Kovach JL, Schwartz SG, Postel EA, Brooks M, Baratz KH, Brown WL, et al. Genetic variants near TIMP3 and high-density lipoprotein-associated loci influence susceptibility to age-related macular degeneration. Proc Natl Acad Sci USA. 2010; 107(16):7401–7406. [PubMed: 20385819]

Cornelis MC, Agrawal A, Cole JW, Hansel NN, Barnes KC, Beaty TH, Bennett SN, Bierut LJ, Boerwinkle E, Doheny KF, Feenstra B, Feingold E, Fornage M, Haiman CA, Harris EL, Hayes MG, Heit JA, Hu FB, Kang JH, Laurie CC, Ling H, Manolio TA, Marazita ML, Mathias RA, Mirel DB, et al. The Gene, Environment Association Studies consortium (GENEVA): maximizing the knowledge obtained from GWAS by collaboration across studies of multiple conditions. Genet Epidemiol. 2010; 34(4):364–372. [PubMed: 20091798]

Devlin B, Roeder K. Genomic control for association studies. Biometrics. 1999; 55(4):997–1004. [PubMed: 11315092]

Frazer KA, Ballinger DG, Cox DR, Hinds DA, Stuve LL, Gibbs RA, Belmont JW, Boudreau A, Hardenbol P, Leal SM, Pasternak S, Wheeler DA, Willis TD, Yu F, Yang H, Zeng C, Gao Y, Hu

H, Hu W, Li C, Lin W, Liu S, Pan H, Tang X, Wang J, et al. A second generation human haplotype map of over 3.1 million SNPs. Nature. 2007; 449(7164):851–861. [PubMed: 17943122]

Freeman EE, Roy-Gagnon MH, Descovich D, Masse H, Lesk MR. The heritability of glaucoma-related traits corneal hysteresis, central corneal thickness, intraocular pressure, and choroidal blood flow pulsatility. PLoS One. 2013; 8(1):e55573. [PubMed: 23383229]

Fukuoka S, Aihara M, Iwase A, Araie M. Intraocular pressure in an ophthalmologically normal Japanese population. Acta Ophthalmol. 2008; 86(4):434–439. [PubMed: 18039350]

Giuffre G, Giammanco R, Dardanoni G, Ponte F. Prevalence of glaucoma and distribution of intraocular pressure in a population. The Casteldaccia Eye Study. Acta Ophthalmol Scand. 1995; 73(3):222–225. [PubMed: 7493232]

Hashemi H, Kashi AH, Fotouhi A, Mohammad K. Distribution of intraocular pressure in healthy Iranian individuals: the Tehran Eye Study. Br J Ophthalmol. 2005; 89(6):652–657. [PubMed: 15923494]

Kass MA, Heuer DK, Higginbotham EJ, Johnson CA, Keltner JL, Miller JP, Parrish RK 2nd, Wilson MR, Gordon MO. The Ocular Hypertension Treatment Study: a randomized trial determines that topical ocular hypotensive medication delays or prevents the onset of primary open-angle glaucoma. Arch Ophthalmol. 2002; 120(6):701–713. discussion 829–730. [PubMed: 12049574]

Klein BE, Klein R, Lee KE. Heritability of risk factors for primary open-angle glaucoma: the Beaver Dam Eye Study. Invest Ophthalmol Vis Sci. 2004; 45(1):59–62. [PubMed: 14691154]

Kohlhaas M, Boehm AG, Spoerl E, Pursten A, Grein HJ, Pillunat LE. Effect of central corneal thickness, corneal curvature, and axial length on applanation tonometry. Arch Ophthalmol. 2006; 124(4):471–476. [PubMed: 16606871]

Lee MK, Cho SI, Kim H, Song YM, Lee K, Kim JI, Kim DM, Chung TY, Kim YS, Seo JS, Ham DI, Sung J. Epidemiologic characteristics of intraocular pressure in the Korean and Mongolian populations: the Healthy Twin and the GENDISCAN study. Ophthalmology. 2012; 119(3):450–457. [PubMed: 22244945]

Leske MC, Connell AM, Wu SY, Hyman L, Schachat AP. Distribution of intraocular pressure. The Barbados Eye Study. Arch Ophthalmol. 1997; 115(8):1051–1057. [PubMed: 9258228]

Leske MC, Heijl A, Hyman L, Bengtsson B, Dong L, Yang Z. Predictors of long-term progression in the early manifest glaucoma trial. Ophthalmology. 2007; 114(11):1965–1972. [PubMed: 17628686]

Li M, Atmaca-Sonmez P, Othman M, Branham KE, Khanna R, Wade MS, Li Y, Liang L, Zareparsi S, Swaroop A, Abecasis GR. CFH haplotypes without the Y402H coding variant show strong association with susceptibility to age-related macular degeneration. Nat Genet. 2006; 38(9):1049–1054. [PubMed: 16936733]

Li X, Wong W, Lamoureux EL, Wong TY. Are linear regression techniques appropriate for analysis when the dependent (outcome) variable is not normally distributed? Invest Ophthalmol Vis Sci. 2012; 53(6):3082–3083. [PubMed: 22618757]

Liton PB, Luna C, Challa P, Epstein DL, Gonzalez P. Genomewide expression profile of human trabecular meshwork cultured cells, nonglaucomatous and primary open angle glaucoma tissue. Mol Vis. 2006; 12:774–790. [PubMed: 16862071]

Liu JH, Realini T, Weinreb RN. Asymmetry of 24-hour intraocular pressure reduction by topical ocular hypotensive medications in fellow eyes. Ophthalmology. 2011; 118(10):1995–2000. [PubMed: 21676464]

Miglior S, Torri V, Zeyen T, Pfeiffer N, Vaz JC, Adamsons I. Intercurrent factors associated with the development of open-angle glaucoma in the European glaucoma prevention study. Am J Ophthalmol. 2007; 144(2):266–275. [PubMed: 17543874]

Moroi SE, Raoof DA, Reed DM, Zöllner S, Qin Z, Richards JE. Progress toward personalized medicine for glaucoma. Expert Rev Ophthalmol. 2009; 4(2):145–161. [PubMed: 23914252]

Musch DC, Gillespie BW, Niziol LM, Lichter PR, Varma R. Intraocular pressure control and long-term visual field loss in the Collaborative Initial Glaucoma Treatment Study. Ophthalmology. 2011; 118(9):1766–1773. [PubMed: 21600658]

NEIGHBORHOOD Consortium. 2013. http://glaucomagenetics.org/

Nickells RW. The cell and molecular biology of glaucoma: mechanisms of retinal ganglion cell death. Invest Ophthalmol Vis Sci. 2012; 53(5):2476–2481. [PubMed: 22562845]

Parssinen O, Era P, Tolvanen A, Kaprio J, Koskenvuo M, Rantanen T. Heritability of intraocular pressure in older female twins. Ophthalmology. 2007; 114(12):2227–2231. [PubMed: 17640733]

Pekmezci M, Chang ST, Wilson BS, Gordon MO, Bhorade AM. Effect of measurement order between right and left eyes on intraocular pressure measurement. Arch Ophthalmol. 2011; 129(3):276–281. [PubMed: 21402981]

Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, Maller J, Sklar P, de Bakker PI, Daly MJ, Sham PC. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet. 2007; 81(3):559–575. [PubMed: 17701901]

Quigley HA, Broman AT. The number of people with glaucoma worldwide in 2010 and 2020. Br J Ophthalmol. 2006; 90(3):262–267. [PubMed: 16488940]

Qureshi IA, Xi XR, Huang YB, Lu HJ, Wu XD, Shiarkar E. Distribution of intraocular pressure among healthy Pakistani. Chin J Physiol. 1996; 39(3):183–188. [PubMed: 8955565]

Sharma S, Burdon KP, Chidlow G, Klebe S, Crawford A, Dimasi DP, Dave A, Martin S, Javadiyan S, Wood JP, Casson R, Danoy P, Griggs K, Hewitt AW, Landers J, Mitchell P, Mackey DA, Craig JE. Association of genetic variants in the TMCO1 gene with clinical parameters related to glaucoma and characterization of the protein in the eye. Invest Ophthalmol Vis Sci. 2012; 53(8): 4917–4925. [PubMed: 22714896]

Stone EM, Fingert JH, Alward WL, Nguyen TD, Polansky JR, Sunden SL, Nishimura D, Clark AF, Nystuen A, Nichols BE, Mackey DA, Ritch R, Kalenak JW, Craven ER, Sheffield VC. Identification of a gene that causes primary open angle glaucoma. Science. 1997; 275(5300):668–670. [PubMed: 9005853]

The AGIS Investigators. The Advanced Glaucoma Intervention Study (AGIS): 7. The relationship between control of intraocular pressure and visual field deterioration. Am J Ophthalmol. 2000; 130(4):429–440. [PubMed: 11024415]

Thorleifsson G, Walters GB, Hewitt AW, Masson G, Helgason A, DeWan A, Sigurdsson A, Jonasdottir A, Gudjonsson SA, Magnusson KP, Stefansson H, Lam DS, Tam PO, Gudmundsdottir GJ, Southgate L, Burdon KP, Gottfredsdottir MS, Aldred MA, Mitchell P, St Clair D, Collier DA, Tang N, Sveinsson O, Macgregor S, Martin NG, et al. Common variants near CAV1 and CAV2 are associated with primary open-angle glaucoma. Nat Genet. 2010; 42(10):906–909. [PubMed: 20835238]

van der Valk R, Webers CA, Schouten JS, Zeegers MP, Hendrikse F, Prins MH. Intraocular pressure-lowering effects of all commonly used glaucoma drugs: a meta-analysis of randomized clinical trials. Ophthalmology. 2005; 112(7):1177–1185. [PubMed: 15921747]

van Koolwijk LM, Despriet DD, van Duijn CM, Pardo Cortes LM, Vingerling JR, Aulchenko YS, Oostra BA, Klaver CC, Lemij HG. Genetic contributions to glaucoma: heritability of intraocular pressure, retinal nerve fiber layer thickness, and optic disc morphology. Invest Ophthalmol Vis Sci. 2007; 48(8):3669–3676. [PubMed: 17652737]

van Koolwijk LM, Ramdas WD, Ikram MK, Jansonius NM, Pasutto F, Hysi PG, Macgregor S, Janssen SF, Hewitt AW, Viswanathan AC, ten Brink JB, Hosseini SM, Amin N, Despriet DD, Willemse-Assink JJ, Kramer R, Rivadeneira F, Struchalin M, Aulchenko YS, Weisschuh N, Zenkel M, Mardin CY, Gramer E, Welge-Lussen U, Montgomery GW, et al. Common genetic determinants of intraocular pressure and primary open-angle glaucoma. PLoS Genet. 2012; 8(5):e1002611. [PubMed: 22570627]

Wiggs JL. The cell and molecular biology of complex forms of glaucoma: updates on genetic, environmental, and epigenetic risk factors. Invest Ophthalmol Vis Sci. 2012; 53(5):2467–2469. [PubMed: 22562842]

Wiggs JL, Hauser MA, Abdrabou W, Allingham RR, Budenz DL, Delbono E, Friedman DS, Kang JH, Gaasterland D, Gaasterland T, Lee RK, Lichter PR, Loomis S, Liu Y, McCarty C, Medeiros FA, Moroi SE, Olson LM, Realini A, Richards JE, Rozsa FW, Schuman JS, Singh K, Stein JD, Vollrath D, et al. The NEIGHBOR Consortium Primary Open-Angle Glaucoma Genome-wide Association Study: Rationale, Study Design, and Clinical Variables. J Glaucoma. 2012a

Wiggs JL, Yaspan BL, Hauser MA, Kang JH, Allingham RR, Olson LM, Abdrabou W, Fan BJ, Wang DY, Brodeur W, Budenz DL, Caprioli J, Crenshaw A, Crooks K, Delbono E, Doheny KF,

Friedman DS, Gaasterland D, Gaasterland T, Laurie C, Lee RK, Lichter PR, Loomis S, Liu Y, Medeiros FA, et al. Common variants at 9p21 and 8q22 are associated with increased susceptibility to optic nerve degeneration in glaucoma. PLoS Genet. 2012b; 8(4):e1002654. [PubMed: 22570617]

Willer CJ, Li Y, Abecasis GR. METAL: fast and efficient meta-analysis of genomewide association scans. Bioinformatics. 2010; 26(17):2190–2191. [PubMed: 20616382]

Wong TT, Wong TY, Foster PJ, Crowston JG, Fong CW, Aung T. The relationship of intraocular pressure with age, systolic blood pressure, and central corneal thickness in an Asian population. Invest Ophthalmol Vis Sci. 2009; 50(9):4097–4102. [PubMed: 19458324]

Xin B, Puffenberger EG, Turben S, Tan H, Zhou A, Wang H. Homozygous frameshift mutation in TMCO1 causes a syndrome with craniofacial dysmorphism, skeletal anomalies, and mental retardation. Proc Natl Acad Sci USA. 2010; 107(1):258–263. [PubMed: 20018682]

Zhang Z, Mo D, Cong P, He Z, Ling F, Li A, Niu Y, Zhao X, Zhou C, Chen Y. Molecular cloning, expression patterns and subcellular localization of porcine TMCO1 gene. Mol Biol Rep. 2010; 37(3):1611–1618. [PubMed: 19449125]

Zheng Y, Xiang F, Huang W, Huang G, Yin Q, He M. Distribution and heritability of intraocular pressure in Chinese children: the Guangzhou twin eye study. Invest Ophthalmol Vis Sci. 2009; 50(5):2040–2043. [PubMed: 19151388]

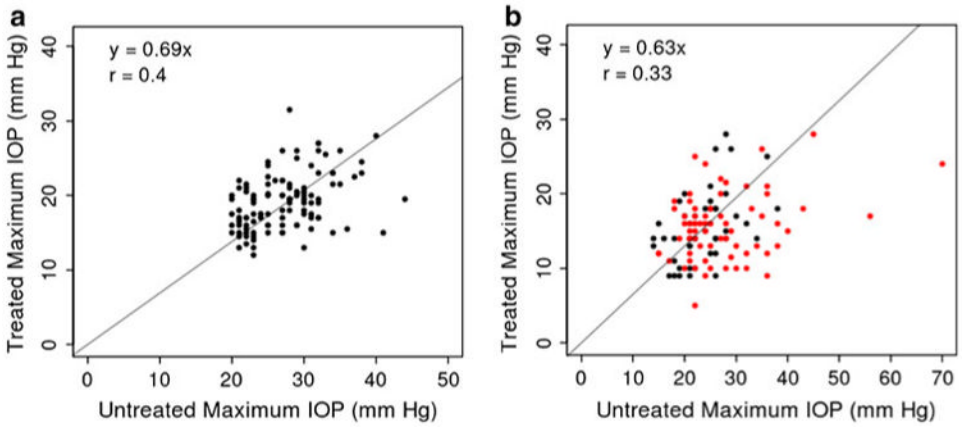

Fig. 1.

Relationship of the untreated and treated IOP values in two sample sets from NEIGHBOR. **a** The 114 cases from the CIGTS set, consisting of 30 cases treated surgically, with or without medication, and 84 cases treated with medication. **b** The 117 cases from the University of Michigan set: 70 cases treated surgically (shown in *red*, with regression line $y = 0.59x$; Pearson's correlation coefficient $r = 0.31$) and 47 cases treated with medication (shown in *black*, with $y = 0.69x$; $r = 0.46$). The observed relationship of the untreated and treated IOP values, with a slope of 0.67, justified the extrapolation procedure we adopted, and as reported previously (van der Valk et al. 2005). The regression equations and Pearson's correlation coefficients are shown

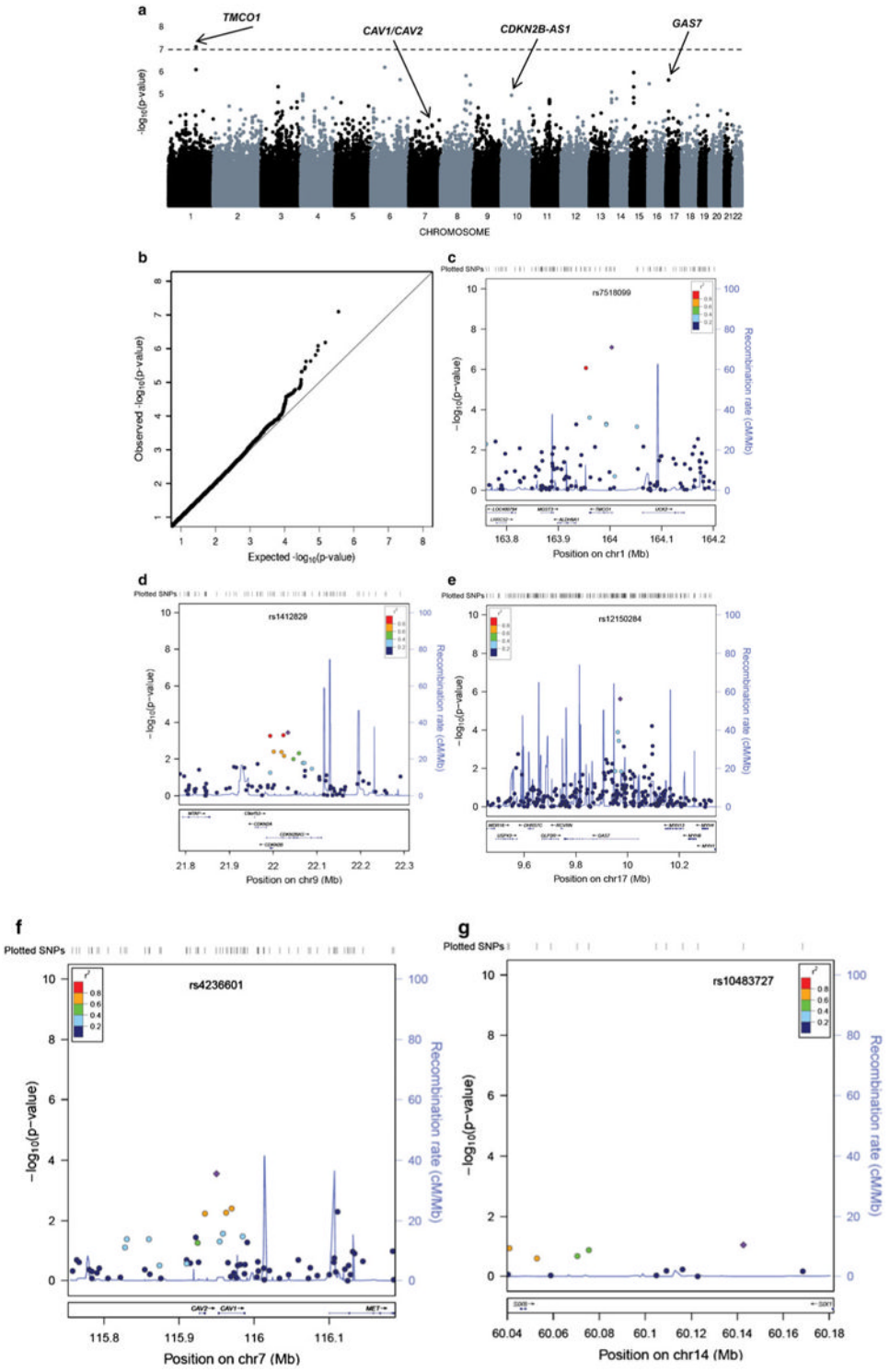

Fig. 2.

NEIGHBOR, GLAUGEN, and AMD-MMAP MI meta-analysis results over ∼467,000 SNPs. **a** Genome-wide $-\log_{10}$ (*p* value) plot. The horizontal line marks the 1.1×10^{-7} threshold of genome-wide significance. **b** Quantile–quantile plot of observed vs. expected $-\log_{10}$ (*p* value) of meta-analysis without correction by genomic control. **c–g** Regional plots

for *TMCO1*, *CDKN2B-AS1*, *GAS7*, *CAV1/CAV2 and SIX1/SIX6*, respectively. The lead SNP in each region is indicated by the *solid diamond*

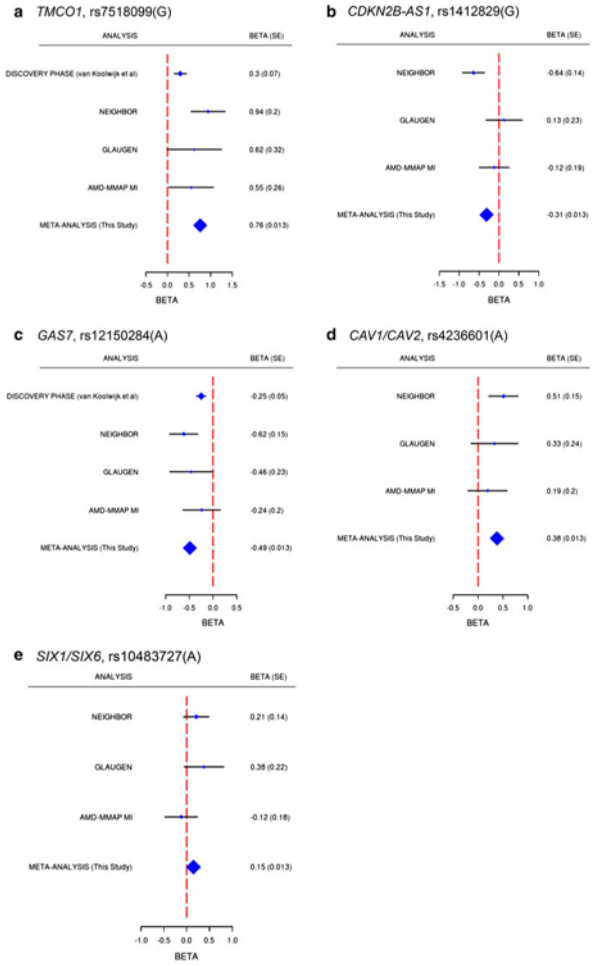

Fig. 3.

Comparison of the effect size and direction across datasets for the top SNPs in our meta-analysis in *TMCO1*, *CDKN2B-AS1*, *GAS7*, *CAV1/CAV2*, and *SIX1/SIX6*. In these "forest plots", point sizes are proportional to the weights (sample sizes) used in the meta-analysis; and the *horizontal lines* indicate the 95 % confidence interval of regression beta values. **a** *TMCO1* top SNP, rs7518099(G), **b** *CDKN2B-AS1* top SNP, rs1412829(G), **c** *GAS7* top SNP, rs12150284(A), **d** *CAV1/CAV2* top SNP, rs4236601(A), **e** *SIX1/SIX6* top SNP, rs10483727(A). In **a** and **c**, the beta values from van Koolwijk et al. (2012) are included

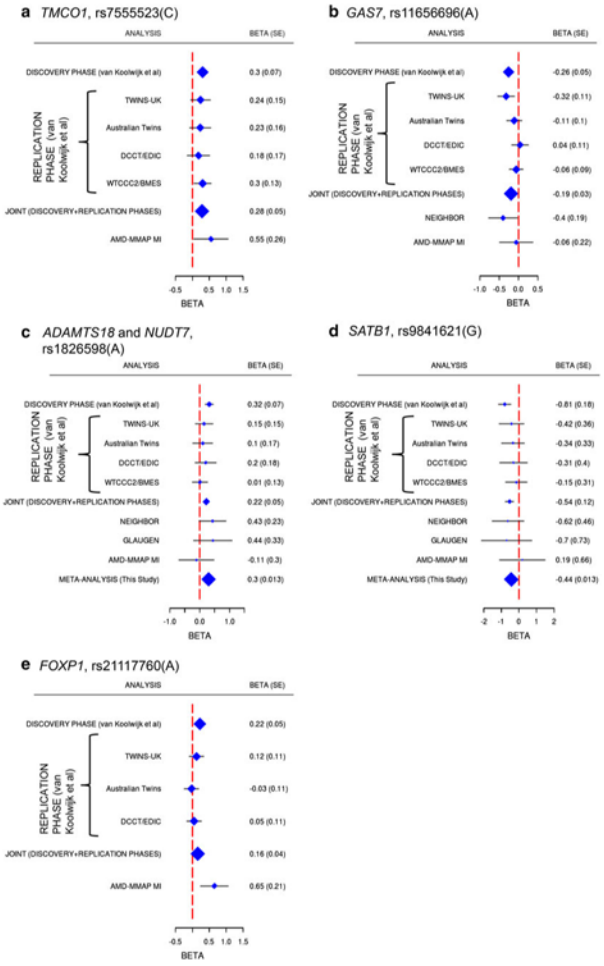

Fig. 4.

Comparison of the effect size and direction across datasets for SNPs significantly or suggestively associated with IOP in van Koolwijk et al. (2012). **a** rs7555523(C) at *TMCO1*, **b** rs11656696(A) at *GAS7*, **c** rs1826598(A) at the *ADAMTS18* and *NUDT7* locus, **d** rs9841621(G) at *SATB1*, **e** rs21117760(A) at *FOXP1*. Some of the SNPs were not genotyped in NEIGHBOR and/or GLAUGEN and are not shown for these datasets

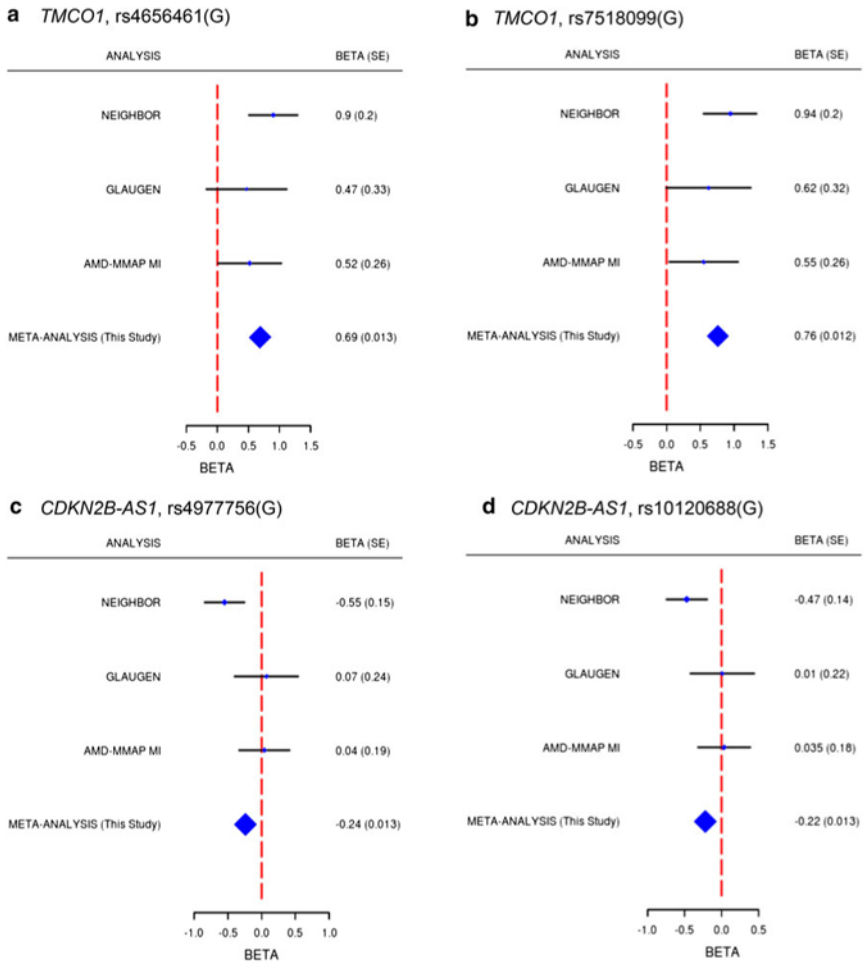

Fig. 5. Comparison of the effect size and direction across datasets for the top SNPs in two regions significantly associated with POAG in Burdon et al. (2011). a *TMCO1*, rs4656461(G), b *TMCO1*, rs7518099(G), c *CDKN2B-AS1*, rs4977756(G), d *CDKN2B-AS1*, rs10120688(G)

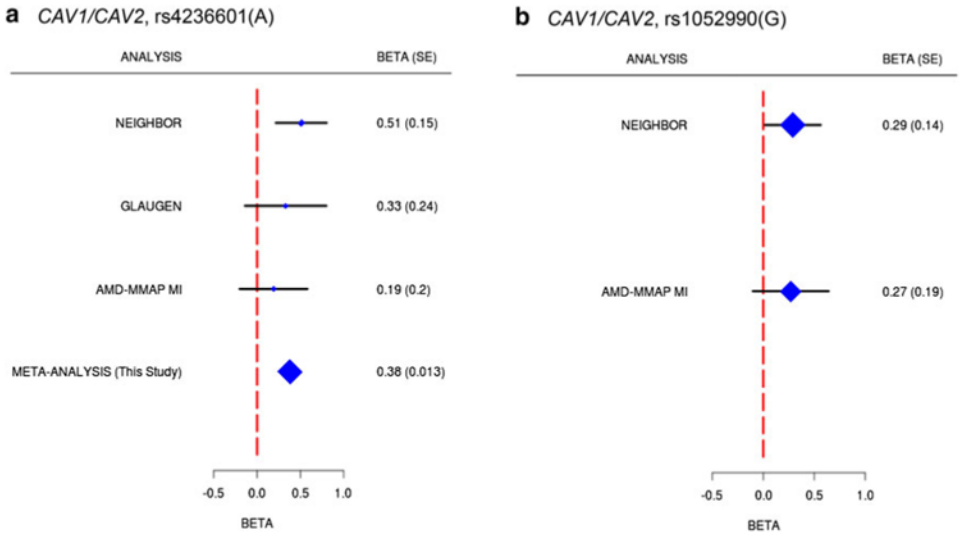

Fig. 6. Comparison of the effect size and directions across datasets for two lead SNPs in the *CAV1/CAV2* region significantly associated with POAG in Thorleifsson et al. (2010). a rs4236601(A), b rs1052990(G)

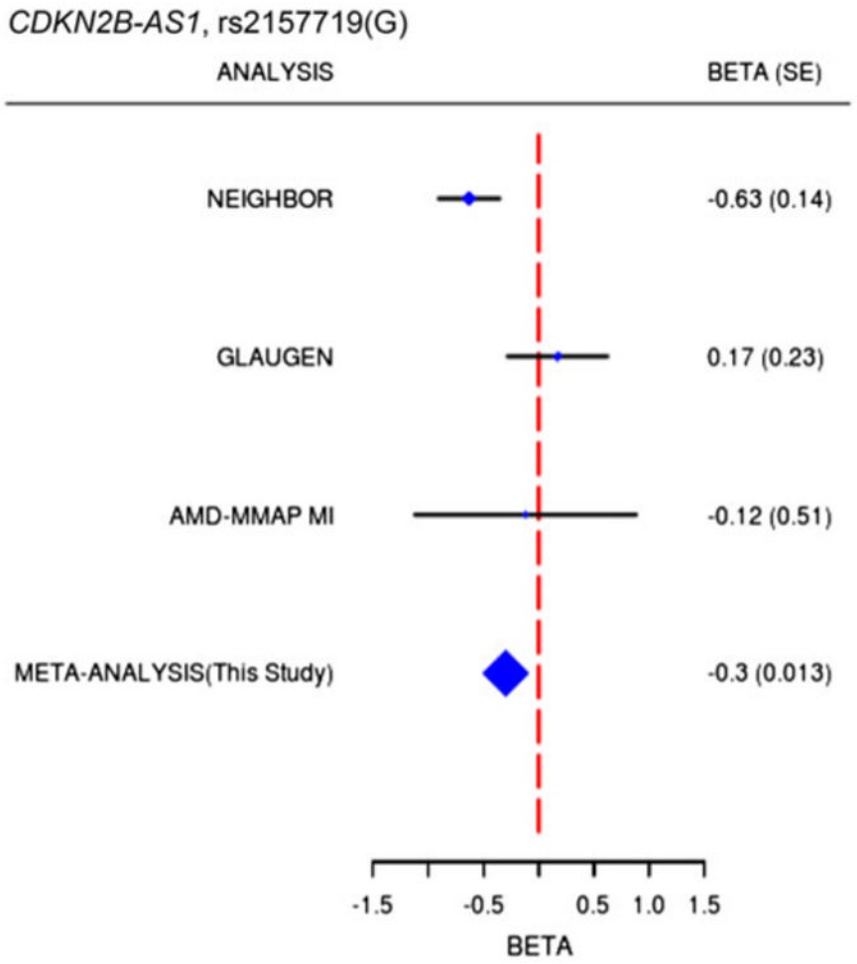

Fig. 7. Comparison of the effect size and directions across datasets for SNPs significantly associated with POAG in Wiggs et al. (2012b). *CDKN2B-AS1* SNP, rs2157719(G). The other signal, at *SIX1/SIX6*, rs10483727(A), is shown in Fig. 3e

Table 1
Characteristics of the three datasets included in the meta-analysis

Characteristic	NEIGHBOR	GLAUGEN	AMD-MMAP MI
Number of subjects[a] (*n*)	4,088	1,153	995
Age (years), mean ± SD (range)	68.4 ± 12.2 (35–101)	67.8 ± 10.4 (40–95)	76.6 ± 6.9 (48–95)
Female gender (%)	53.4	52.8	62.1
IOP (mmHg), mean ± SD (range), (*n*)			
Controls	15 ± 2.9 (1–28), (2,185)	14.7 ± 2.7 (5–21), (337)	17.9 ± 3.0 (9–30), (867)
Untreated cases	23.4 ± 6.6 (6–41), (502)	21.5 ± 3.9 (12–32), (339)	21.1 ± 4.0 (12–31), (128)
Treated cases	16.0 ± 4.7 (1–28), (1,401)	14.6 ± 3.7 (2–26), (477)	–

[a]Final counts of subjects, after removing those with IOP values beyond three SD's from the overall mean for each study. Cases in AMD-MMAP were POAG cases

Table 2

Description of the top 20 SNPs in 14 regions in the meta-analysis of NEIGHBOR (*n* = 4,088), GLAUGEN (*n* = 1,153) and AMD-MMAP MI (*n* = 995) cohorts, sorted by *p* value

SNP	CHR	Position[a]	A1	Closest gene/locus	NEIGHBOR				GLAUGEN			AMD-MMAP MI		
					p-value[b]	Freq	Beta (SE)[c]	*p*-value[d]	Freq	Beta (SE)[c]	*p*-value[d]	Freq	Beta (SE)[c]	*p*-value[d]
rs7518099	1q24.1	164003504	G	*TMCO1*	8.0E–08	0.14	0.94 (0.20)	3.9E–06	0.14	0.62 (0.32)	5.5E–02	0.14	0.55 (0.26)	3.7E–02
rs2025751	6p12.2	51730408	A	*PKHD1*	6.6E–07	0.46	0.60 (0.14)	2.1E–05	0.48	0.58 (0.22)	9.5E–03	0.45	0.21 (0.19)	2.7E–01
rs4656461	1q24.1	163953829	G	*TMCO1*	8.3E–07	0.14	0.90 (0.20)	1.1E–05	0.13	0.46 (0.33)	1.6E–01	0.14	0.52 (0.27)	4.9E–02
rs587847	15q14	35447341	C	*MEIS2*	1.1E–06	0.38	−0.69 (0.14)	1.6E–06	0.38	−0.19 (0.23)	4.1E–01	0.37	−0.31 (0.19)	9.9E–02
rs7815720	8q24.11	118158694	A	*SLC30A8*	1.6E–06	0.13	−1.02 (0.21)	7.3E–07	0.13	−0.44 (0.34)	1.9E–01	0.14	−0.18 (0.27)	5.0E–01
rs560713	6q23.3	136546729	A	*PDE7B*	2.3E–06	0.32	−0.53 (0.15)	4.2E–04	0.33	−0.53 (0.24)	2.8E–02	0.35	−0.45 (0.19)	1.8E–02
rs12150284	17p13.1	9971815	A	*GAS7*	2.4E–06	0.35	−0.62 (0.15)	2.7E–05	0.35	−0.46 (0.23)	4.6E–02	0.38	−0.24 (0.20)	2.2E–01
rs1906060	16p13.3	6048431	G	*A2BP1*	3.6E–06	0.20	0.59 (0.18)	7.9E–04	0.20	0.58 (0.28)	4.1E–02	0.20	0.71 (0.27)	7.9E–03
rs2945733	8q24.22	134684932	A	*ST3GAL1*	4.0E–06	0.13	0.51 (0.21)	1.4E–02	0.13	1.20 (0.33)	2.9E–04	0.12	0.77 (0.29)	6.9E–03
rs13093086	3p14.1	71137852	A	*FOXP1*	4.8E–06	0.49	−0.57 (0.14)	3.9E–05	0.51	−0.16 (0.23)	4.8E–01	0.51	−0.45 (0.19)	1.6E–02
rs4924148	15q14	35417657	G	*MEIS2*	4.9E–06	0.33	−0.73 (0.15)	1.1E–06	0.32	−0.18 (0.24)	4.4E–01	0.31	−0.15 (0.19)	4.2E–01
rs718433	14q11.2	21305730	G	*TRAJ17*	8.4E–06	0.39	−0.59 (0.14)	4.2E–05	0.38	−0.47 (0.23)	4.3E–02	0.39	−0.14 (0.19)	4.5E–01
rs6838291	4p16.1	7968706	A	*AFAP*	1.0E–05	0.19	0.59 (0.18)	8.8E–04	0.17	0.52 (0.29)	7.6E–02	0.18	0.59 (0.24)	1.4E–02
rs315644	10p11.21	36495901	C	*FZD8*	1.2E–05	0.21	0.59 (0.17)	4.3E–04	0.21	0.64 (0.27)	1.6E–02	0.15	0.35 (0.26)	1.9E–01
rs11930623	4p16.1	7982582	C	*AFAP*	1.3E–05	0.39	−0.63 (0.14)	1.1E–05	0.40	−0.16 (0.23)	5.0E–01	0.40	−0.25 (0.18)	1.7E–01
rs4383619	4p16.1	7914408	A	*AFAP*	1.3E–05	0.21	0.55 (0.17)	1.3E–03	0.20	0.46 (0.28)	9.2E–02	0.20	0.59 (0.23)	8.6E–03
rs603165	15q14	35462875	A	*MEIS2*	1.5E–05	0.46	0.62 (0.14)	1.0E–05	0.46	0.26 (0.22)	2.4E–01	0.48	0.13 (0.19)	4.9E–01
rs6848193	4q35.1	182875507	A	*AK056196*	1.5E–05	0.43	0.47 (0.14)	6.4E–04	0.43	0.35 (0.22)	1.1E–01	0.46	0.40 (0.18)	2.4E–02
rs807331	14q21.3	44239039	G	*AK123685*	1.6E–05	0.26	0.50 (0.16)	2.1E–03	0.25	0.47 (0.26)	6.8E–02	0.29	0.53 (0.20)	8.3E–03
rs10047948	14q11.2	21148369	G	*TRAJ17*	1.7E–05	0.12	−0.73 (0.22)	7.0E–04	0.12	−0.43 (0.36)	2.3E–01	0.12	−0.75 (0.28)	7.9E–03

[a] Positions are in NCBI build 36 coordinates

[b] Meta-analysis *p*-values

[c] mmHg/allele

[d] Study-specific *p*-values