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Common variants near *CAV1* and *CAV2* are associated with primary open-angle glaucoma

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We conducted a genome-wide association study for primary open-angle glaucoma (POAG) in 1,263 affected individuals (cases) and 34,877 controls from Iceland. We identified a common sequence variant at 7q31 (rs4236601[A], odds ratio (OR) = 1.36, $P = 5.0 \times 10^{-10}$). We then replicated the association in sample sets of 2,175 POAG cases and 2,064 controls from Sweden, the UK and Australia (combined OR = 1.18, $P = 0.0015$) and in 299 POAG cases and 580 unaffected controls from Hong Kong and Shantou, China (combined OR = 5.42, $P = 0.0021$). The risk variant identified here is located close to *CAV1* and *CAV2*, both of which are expressed in the trabecular meshwork and retinal ganglion cells that are involved in the pathogenesis of POAG.

Glaucoma is the leading cause of irreversible blindness worldwide, affecting approximately 70 million people[1]. It is a chronic degenerative optic neuropathy with progressive loss of retinal ganglion cells and axons resulting in a corresponding thinning of the neuroretinal rim of the optic nerve and a characteristic visual field defect. It is distinct from other forms of optic neuropathy in that the neuroretinal rim of the optic nerve retains its normal pink color as it becomes progressively thinner, leading to an enlarged optic-nerve cup. POAG is the most common form of glaucoma. Excluding rare primary juvenile glaucoma with age of onset between 10 and 35 years, POAG is arbitrarily divided into high-pressure glaucoma (defined as ≥22 mmHg) and normal-pressure glaucoma. POAG is thought to have a multifactorial etiology, with the main risk factors being age, elevated intraocular (IOP) pressure, family history, race, central corneal thickness (CCT), hypertension, diabetes and myopia. The familiality of POAG has been known for decades, and studies have revealed three- to ninefold greater risk of POAG in first-degree relatives of POAG cases than in the population in general[2].

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Table 1 Association of rs4236601[A] with POAG and XFG

Phenotype			Frequency			
Cohort (n_c/n_a)[a]	n_c[a]	n_a[a]	Controls	Cases	OR (95% CI)	*P*
POAG—Europeans						
Discovery Samples						
Iceland[b]	34,877	1,263	0.228	0.287	1.36 (1.23–1.50)	5.0×10^{-10}
Replication Samples						
Sweden	198	200	0.207	0.258	1.33 (0.95–1.85)	0.092
Leicester, UK	661	404	0.267	0.293	1.14 (1.93–1.38)	0.2
Southampton, UK	204	467	0.281	0.290	1.04 (0.81–1.35)	0.75
Australia	1,001	1,104			1.23 (1.06–1.43)	0.0063
GIST	147	457	0.262	0.293	1.17 (0.87–1.57)	0.29
ANZRAG	361	517	0.254	0.300	1.25 (1.01–1.55)	0.038
BMES	493	130	0.260	0.307	1.26 (0.93–1.71)	0.13
Replication combined[c]	2,064	2,175			1.18 (1.06–1.31)	0.0015
European combined[c]	36,941	3,438			1.27 (1.18–1.36)	2.2×10^{-11}
POAG—Asians						
Hong Kong Chinese	248	176	0.004	0.020	5.01 (1.04–24.27)	0.038
Shantou Chinese	332	123	0.003	0.016	5.47 (1.0–30.06)	0.049
Chinese combined I[c]	580	299	0.003	0.018	5.42 (1.72–17.08)	0.0021
Chinese combined II[c,d]	1,607	299	0.006	0.018	3.33 (1.56–7.08)	0.003
XFG						
Iceland[e]	34,839	190	0.228	0.232	1.02 (0.79–1.31)	0.87
Sweden	198	198	0.207	0.237	1.19 (0.85–1.67)	0.30
Combined[c]	35,037	388			1.08 (0.88–1.32)	0.47

[a]Number of controls n_c and cases n_a. [b]*P* value and CI for the Icelandic sample set were adjusted by dividing the χ^2 statistic by the genomic control inflation factor (λ_g) = 1.182. [c]Results for the different sample sets were combined using a Mantel-Haenszel model. [d]Additional 1,027 population controls were included in the analysis of the Hong Kong sample set. [e]*P* value and CI for the Icelandic sample set were adjusted by dividing the χ^2 statistic by λ_g = 1.056.

POAG is a genetically heterogeneous disease that shows linkage to at least 20 genetic loci[3]. Three genes predisposing to glaucoma have been isolated from these loci: *MYOC* (encoding myocilin)[4], *OPTN* (encoding optineurin)[5] and *WDR36* (encoding WD repeat domain 36)[6], although the association with *WDR36* does not replicate in all populations. The variants in these genes are rare and may together contribute to 5–6% of all POAG cases[2]. More recently, rare mutations in *NTF4* have been found in individuals with POAG[7], and a genome-wide association study (GWAS) yielded two common exonic variants in *LOXL1* that explain over 99% of the cases with exfoliation glaucoma (XFG) in individuals of European ancestry[8]. This association with XFG has been replicated in several other populations of European, African and Asian ancestry, although the variants do not associate with POAG in these populations[8,9]. A recent GWAS conducted in a Japanese population identified three loci with suggestive evidence for association with POAG[10], although this association was not replicated in an independent study in an Indian population[11].

To search for genomic variants that confer risk of POAG, we conducted a GWAS on 1,263 POAG cases diagnosed by Icelandic ophthalmologists using established glaucoma criteria[12] and 34,877 population controls from Iceland (**Supplementary Note**). After quality filtering, 303,117 SNPs typed with the Illumina HumanHap300 or HumanHapCNV370 BeadChips were tested for association with POAG. The results were adjusted for relatedness using the method of genomic controls[13] by dividing the χ^2 statistic by the genomic inflation factor 1.182.

Two highly correlated SNPs, rs4236601[A] and rs1052990[G] (r^2 = 0.64 based on the Utah (CEU) HapMap(r22) samples), reached genome-wide significance of $P < 1.6 \times 10^{-7}$ (**Supplementary Fig. 1** and **Supplementary Table 1**). These variants, with OR = 1.36 ($P = 5.0 \times 10^{-10}$) and OR = 1.32 ($P = 1.1 \times 10^{-9}$), respectively, are located within the same linkage disequilibrium (LD) block between *CAV1* and *CAV2* (encoding caveolin 1 and 2) on 7q31 (**Table 1** and **Fig. 1**). After adjusting for the observed association with rs4236601[A], neither rs1052990[G] nor any other variant in the 7q31 region showed significant association with POAG (**Supplementary Table 2**). None of the variants described in a previous study[10] or any other highly correlated variants associated with POAG in the Icelandic samples (**Supplementary Table 3**).

We typed rs4236601 in 200 POAG cases and 194 controls from Sweden, in 871 POAG cases and 865 controls from Leicester and Southampton, UK, and in 1,104 POAG cases and 1,001 controls from Australia. In the Swedish set, rs4236601[A] conferred similar risk of POAG as that observed in the Icelandic dataset (OR = 1.33, *P* = 0.092), whereas the estimated risk was less in the two UK sets (OR = 1.14, *P* = 0.2 and OR = 1.04, *P* = 0.75) (**Table 1**). The Australian sample consisted of three studies—a study from Tasmania (GIST), a study from South Australia (ANZRAG) and the Blue Mountains Eye Study (BMES)—that individually have estimated OR = 1.17 (*P* = 0.29), OR = 1.25 (*P* = 0.038) and OR = 1.26 (*P* = 0.13), respectively. Combined, the replication sets gave OR = 1.18 (95% CI 1.06–1.31, *P* = 0.0015), and including the discovery set gave OR = 1.27 (95% CI 1.18–1.36, $P = 2.2 \times 10^{-11}$). There was heterogeneity in the effect estimates among the study populations (P_{het} = 0.048); in particular, the estimated effect in the samples from Southampton was low (**Table 1**). POAG is a heterogeneous disease and therefore this heterogeneity in the estimated effect sizes is not surprising. In the Southampton samples, the risk was confined to a subset of normal-pressure glaucoma cases, whereas we observed no risk for the majority of the cases diagnosed with high-pressure glaucoma (**Supplementary Table 4**). Higher risk in normal-pressure cases was also observed, although not consistently, in the POAG cases from Iceland and Australia. rs4236601[A] did not associate with XFG in samples from Iceland and Sweden (**Table 1**).

The estimated population frequency of rs4236601[A] ranges from 20.7% to 28.1% in the four populations studied, and the corresponding population attributed risk percentage was 12%, calculated using the mean of the population frequencies and the estimated OR of 1.27. About 6% of the individuals in the four populations carry two copies of the risk allele, and their risk of developing POAG is 1.6 times greater than those carrying no risk variant.

The frequency of the risk variant rs4236601[A] differs between ethnicities. In the HapMap populations, the estimated frequency ranges from 45% in the Yoruba population and 28% in the Utah CEPH population to 2% in the Han Chinese population. We did not detect the variant in the 60 HapMap individuals from Japan. We tested the variant for association with POAG in 299 cases and 580 unaffected controls of Chinese origin from Hong Kong and Shantou (**Table 1**). Although the variant is rare, with a frequency of about 1.8% in cases and less than 0.4% in controls, the association was significant and yielded an OR of 5.42 (*P* = 0.0021). We also tested the variant in 1,027 population controls from Hong Kong, where its frequency is slightly higher than in the unaffected control population and has a frequency

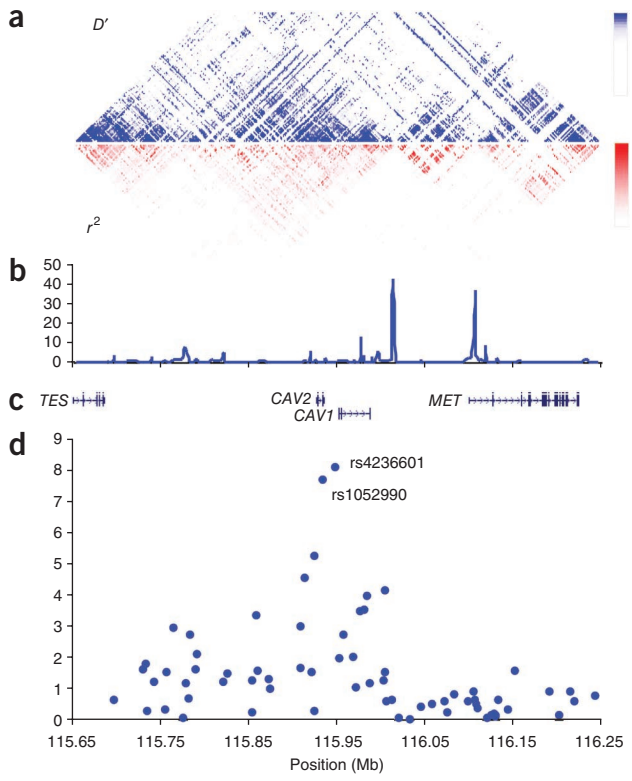

Figure 1 The 7q31 locus. (**a**) The pairwise correlation structure in a 600-kb interval (115.65–116.25 Mb, NCBI B36) on chromosome 7. The upper plot shows pairwise *D′* for 533 common SNPs (defined as those having minor allele frequency >5%) from the Utah CEU HapMap (r22) samples. The lower plot shows the corresponding r^2 values. (**b**) Estimated sex-averaged recombination rates (saRR) in cM/Mb from the HapMap Phase II data[31]. (**c**) Location of known genes in the region. (**d**) Schematic view of the association with POAG for all 70 markers tested in the GWAS in the region. All panels use the same horizontal scale shown in **d**.

of 0.7%. The greater risk and lower frequency of rs4236601[A] in the Chinese population as compared to European populations raises the possibility that it tags some rare unknown causative variant through LD that is stronger in the Chinese population than in European populations. We note that in the Chinese (CHB) HapMap (r22) samples, 32 SNP alleles, spread across 174 kb, are perfect surrogates ($r^2 = 1$) of rs4236601, whereas in the Utah CEU HapMap sample, there are only five such SNP alleles covering 12.6 kb (**Supplementary Fig. 2**). Of the 32 CHB surrogate SNPs, we tested 31 for association in the Icelandic sample set but observed no association independent of rs4236601 (**Supplementary Table 2**). Thus, either the risk attributable to this locus differs in European and Chinese populations or there remains an undetected rare causative variant that is not well tagged by existing SNPs in the Utah CEU HapMap samples.

To search for protein-coding mutations responsible for the association, we sequenced the promoter region, exons and exon-intron boundaries of *CAV1* and *CAV2* in 280 POAG cases and 358 controls from Iceland (**Supplementary Note**). SNPs identified through this effort were imputed into the remaining Icelandic POAG case and control samples using recently developed methods of long-range phasing of haplotypes in sets of related individuals[14]. Two of the identified SNPs, the nonsynonymous coding variant rs8940 and rs1052990 located in the 3′ untranslated region end of *CAV2*, were also genotyped in the samples from Australia and Sweden. Although several of the identified variants showed significant association with POAG, none of the tested SNPs remained significant after adjusting for the effect of rs4236601[A] and none of them account for the association of rs4236601[A] with POAG (**Supplementary Table 5** and **Supplementary Note**). This indicates that rs4236601 is unlikely to tag mutations within the coding region of *CAV1* or *CAV2*.

To evaluate whether the 7q31 variant predisposes to POAG through known risk factors, we tested for association of rs4236601[A] with IOP, CCT, hypertension, type 2 diabetes (T2D) and myopia in 1,713 samples from the Twins Eye Study in Tasmania (TEST)[15]; in 691 Australian POAG cases and 439 controls with IOP measurements; in 316 samples with IOP and CCT measurements without glaucoma from the Reykjavik Eye Study; in 883 individuals from Iceland with spherical equivalent refraction error of −3 diopters or higher and in 2,251 T2D cases and in 34,647 controls and 7,007 hypertension cases and 31,521 controls from Iceland. Of the six traits tested, nominally significant association was only observed for increased IOP ($P = 0.034$; **Supplementary Table 6**).

The LD block containing rs4236601 contains two known genes, *CAV1* and *CAV2*, and few uncharacterized expressed sequence tags. *CAV1* and *CAV2* are members of the caveolin gene family that also includes the muscle-specific *CAV3* gene. *CAV1* and *CAV2* are expressed in most human cell types, including tissues such as the scleral spur cells[16], trabecular meshwork[17] and retinal ganglion cells[18] of the eye, but alterations in these tissues are thought to play a role in the pathology of POAG, leading to loss of retinal ganglion cell axons, along with supportive glia and vasculature. Notably, under experimental conditions, *CAV1* showed consistent upregulation in the trabecular meshwork after one hour of increased IOP[19].

CAV1 and CAV2 are involved in the formation of caveolae which are specialized invaginations of the plasma membrane that are rich in cholesterol and other lipids, and they take part in transcytosis. However, it is the role of caveolae in signal transduction through interaction with signaling molecules that has been most extensively studied. Caveolae recruit and compartmentalize various signaling molecules through direct physical interaction mediated by the caveolin scaffolding domain (CSD) in CAV1. This interaction generally results in inhibition of signaling[20–23]. Caveolins have been suggested as regulators of adult neural stem cell proliferation, as evidenced by increased proliferation of adult neural stem cells in Cav1, Cav2 and Cav3 knockout mice[24]. The regulation by CAV1 of the endothelial nitric oxide synthase (eNOS), an enzyme that produces nitric oxide, is well documented, but the interaction of CAV1 and eNOS leads to eNOS inactivation[25,26] and reduced nitric oxide production. Nitric oxide plays an important role in the regulation of many physiological functions in the cardiovascular system and the central and peripheral nervous systems. Nitric oxide produced in excessive amounts causes cytotoxicity, neurodegeneration, apoptotic cell death and circulatory failure. In addition to nitric oxide signaling, CAV1 has been shown to be an important regulator of TGF-β signaling through interaction with the TGF-β type 1 receptor. Both nitric oxide and TGF-β signaling have been implicated as culprits in the pathogenesis of POAG[27,28].

We tested the effect of rs4236601 on *CAV1* and *CAV2* mRNA expression measured in 747 blood samples and 606 adipose tissue samples[29]. No correlation between the POAG variant and *CAV1* or *CAV2* expression was observed (data not shown); however, as gene regulation can be highly tissue specific, the effect of rs4236601 on *CAV1* and *CAV2* expression in ocular tissue, which is more relevant for glaucoma than blood and adipose tissue and where the expression of *CAV1* or *CAV2* is more likely to influence or lead to disease, can not be excluded.

It is of interest to note that there was a recent report of an association of a SNP, rs3807989[A], within the same LD block as rs4236601

with the PR interval (an electrocardiogram measurement) and atrial fibrillation[30]. rs3807989 is weakly correlated with rs4236601 ($r^2 < 0.01$) and does not associate with POAG, nor does rs4236601[A] associate with PR interval or atrial fibrillation. The *CAV1-CAV2* locus thus adds to the growing list of loci where closely spaced signals show distinct associations with diverse traits.

We have identified a sequence variant, rs4236601[A], that is associated with POAG susceptibility in populations of European and east Asian ancestry. The variant does not have a major effect on known risk factors for POAG such as IOP and central corneal thickness, and it has not been associated with susceptibility to diseases such as T2D, hypertension or myopia that are all risk factors of POAG. This sequence variant is in the same LD block as *CAV1* and *CAV2*. The frequency of the POAG variant differs between ethnicities; in particular, the frequency of the variant is much lower in east Asian populations than in individuals of European descent. These data highlight the importance of considering the genetic component in the risk of common complex diseases in the context of geographic ancestry.

METHODS

Methods and any associated references are available in the online version of the paper at http://www.nature.com/naturegenetics/.

Note: Supplementary information is available on the Nature Genetics website.

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AUTHOR CONTRIBUTIONS

The study was designed, the results interpreted and the first draft written by G.T., U.T., F.J. and K.S. The statistical analysis was performed by G.T., A.W.H. and A.K. A. Jonasdottir, A.S. and S.A.G. did the bioinformatic analysis of the 7q31 region, A.H. did the phylogenetic analysis and G.M. did the imputation. Genotyping at deCODE genetics was supervised by G.B.W. and U.T. Those responsible for case and control ascertainment, recruitment and phenotype information were F.J., G.J.G., H.S., K.P.M., M.S.G. and O.S. (Icelandic POAG cases and controls); K.P.M. and K.T. (Icelandic myopia cases); L.S., M.A.A., R.C.T. and W.S.S.K. (Leicester POAG cases); D.A.C. and D.St.C. (controls used for Leicester cases); A. Jacob, A.J.C., A.J.L., A.M. J.G. and S.E. (Southampton POAG cases and controls); C.W. (Swedish POAG cases and controls); P.M., C.J.H., N.G.M., S.M., T.L.Y., A.W.H., J.E.C., K.P.B. and D.A.M. (Australian POAG cases and controls and collection, genotyping and analysis of the Australian Twin study); C.P.P., D.S.C.L., P.O.S.T., A.D.W., J.H. and M.Z. (collection and genotyping of Hong Kong and Shantou POAG cases and unaffected controls); and J.C.N.C. and N.T. (population controls from Hong Kong). All authors contributed to the final version of the paper.

COMPETING FINANCIAL INTERESTS

The authors declare competing financial interests: details accompany the full-text HTML version of the paper at http://www.nature.com/naturegenetics/.

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ONLINE METHODS

Subjects and genotyping. Detailed information on all case-control sample sets and the genotyping methods are found in the **Supplementary Note**.

Association analysis. For case-control association analysis, we used a standard likelihood ratio statistic implemented in the NEMO software[32] to calculate two-sided *P* values and ORs for each individual allele, assuming a multiplicative model for risk[33]. Allele frequencies were estimated by maximum likelihood statistics and tests of differences between cases and controls were done using a generalized likelihood ratio test. This method is particularly useful in situations where there are some missing genotypes for the marker of interest, and genotypes of another marker in strong LD with the marker of interest are used to provide some partial information. This was used in the association tests presented in **Supplementary Tables 2** and **5** to ensure that the comparison of the highly correlated markers was done using the same number of individuals. To handle uncertainties with phase and missing genotypes, maximum likelihood estimates, likelihood ratios and *P* values were computed directly for the observed data, and hence the loss of information due to uncertainty in phase and missing genotypes was automatically captured by the likelihood ratios. Results from multiple case-control groups were combined using a Mantel-Haenszel model[34] in which the groups were allowed to have different population frequencies for alleles, haplotypes and genotypes but were assumed to have common relative risks. Heterogeneity in the effect estimate was tested assuming that the estimated ORs for different groups follow a log-normal distribution and using a likelihood ratio χ^2 test with degrees of freedom equal to the number of groups compared minus one. The correlation between variations in IOP, CCT and spherical equivalent refraction error (SEq), calculated as the average over both eyes, and the number of copies of rs4236601[A] an individual carries was tested using multiple regression, including the age at exam and sex of the individual as explanatory variables. The spherical equivalent refraction error was calculated as:

$$\text{SEq} = \text{spherical error} + \text{cylinder error}/2$$

and myopia was defined when SEq was −3 diopters or more.

Correction for relatedness. Some of the individuals in both the Icelandic control and control groups are related to each other, causing the χ^2 statistic to have a mean >1 and median >0.455. We estimated the inflation factor for the genome-wide association by calculating the average of the 303,117 χ^2 statistics, which was a method of genomic control[13] to adjust for both relatedness and potential population stratification. The inflation factor was estimated as 1.182, and the results presented from the genome-wide association and in **Table 1** and **Supplementary Tables 1**, **2**, **3** and **5** are based on adjusting the χ^2 statistics by dividing each of them by 1.182. For the case-control association study on T2D and hypertension, presented in **Supplementary Table 6b**, the method of genomic control was also used to estimate the inflation factors to adjust the corresponding *P* values. For T2D, the adjustment factor was 1.320, and for hypertension it was 1.354. For the case-control association of myopia and the regression of variation in IOP, CCT and SEq with rs4236601[A], presented in **Supplementary Tables 6a**,**b**, we used simulations to determine the adjustment factors for relatedness[35]. This was done both for the Icelandic sample sets and for the Twin Eye Study. 50,000 sets of genotypes were simulated for a SNP with the same frequency as rs4236601[A] conditional on the known relatedness of the individuals in the sample sets, and the association tests were repeated for each of the genotype sets. The resulting *P* values were converted to χ^2-values, and the inflation factors estimated as described above. For the Twin Eye Study, the adjustment factors were 1.189, 1.209, 1.344 and 1.264 for myopia, IOP, CCT and SEq, respectively. For the Icelandic sample set, the adjustment factors were 1.065, 1.000, 1.054 and 1.063 for myopia, IOP, CCT and SEq, respectively.

Sequencing of *CAV1* and *CAV2*. The exons of *CAV1* and *CAV2* and the sequences flanking the exons were sequenced in 280 Icelandic POAG cases and 358 Icelandic controls. Further details on the sequencing are provided in the **Supplementary Note**. The SNPs identified through the sequencing were subsequently imputed into the remaining Icelandic POAG cases and controls using methods of long-range phasing of related individuals[14] and were then tested for association with POAG.

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