Soo Khai Ng, Kathryn P. Burdon, Jude T. Fitzgerald, Tiger Zhou, Rhys Fogarty, Emmanuelle Souzeau, John Landers, Richard A. Mills, Robert J. Casson, Bronwyn Ridge, Stuart L. Graham, Alex W. Hewitt, David A. Mackey, Paul R. Healey, Jie Jin Wang, Paul Mitchell, Stuart MacGregor, and Jamie E. Craig
Genetic association at the 9p21 glaucoma locus contributes to sex bias in normal-tension glaucoma
Investigative Ophthalmology and Visual Science, 2016; 57(7):3416-3421

Originally published at:
http://doi.org/10.1167/iovs.16-19401

Genetic Association at the 9p21 Glaucoma Locus Contributes to Sex Bias in Normal-Tension Glaucoma

Soo Khai Ng,[1,2] Kathryn P. Burdon,[1,3] Jude T. Fitzgerald,[1] Tiger Zhou,[1] Rhys Fogarty,[1] Emmanuelle Souzeau,[1] John Landers,[1] Richard A. Mills,[1] Robert J. Casson,[2] Bronwyn Ridge,[1] Stuart L. Graham,[4] Alex W. Hewitt,[5] David A. Mackey,[6] Paul R. Healey,[7] Jie Jin Wang,[8] Paul Mitchell,[8] Stuart MacGregor,[9] and Jamie E. Craig[1]

[1]Department of Ophthalmology, Flinders University, Flinders Medical Centre, Adelaide, South Australia, Australia
[2]Ophthalmic Research Laboratories, South Australian Institute of Ophthalmology, University of Adelaide, Adelaide, South Australia, Australia
[3]Menzies Institute for Medical Research, University of Tasmania, Hobart, Tasmania, Australia
[4]Department of Clinical Medicine, Faculty of Medicine and Health Sciences, Macquarie University, Sydney, New South Wales, Australia
[5]Centre for Eye Research Australia, University of Melbourne, Royal Victorian Eye and Ear Hospital, East Melbourne, Victoria, Australia
[6]Centre for Ophthalmology and Visual Science, Lions Eye Institute, Perth, Western Australia, Australia
[7]Department of Ophthalmology, Westmead Hospital, Sydney, New South Wales, Australia
[8]Centre for Vision Research, Department of Ophthalmology and Westmead Millennium Institute, University of Sydney, Westmead, New South Wales, Australia
[9]Statistical Genetics, QIMR Berghofer Medical Research Institute, Brisbane, Queensland, Australia

Correspondence: Kathryn P. Burdon, Menzies Institute for Medical Research, University of Tasmania, Hobart, Tasmania, 7000, Australia; kathryn.burdon@utas.edu.au.
Jamie E. Craig, Department of Ophthalmology, Flinders Medical Centre, Adelaide, South Australia, Australia 5000; jamie.craig@flinders.edu.au.

Submitted: February 22, 2016
Accepted: May 10, 2016

Citation: Ng SK, Burdon KP, Fitzgerald JT, et al. Genetic association at the 9p21 glaucoma locus contributes to sex bias in normal-tension glaucoma. *Invest Ophthalmol Vis Sci*. 2016;57:3416–3421. DOI:10.1167/iovs.16-19401

Purpose. Many genome-wide association studies have identified common single nucleotide polymorphisms (SNPs) at the 9p21 glaucoma locus (*CDKN2B/CDKN2B-AS1*) to be significantly associated with primary open-angle glaucoma (POAG), with association being stronger in normal tension glaucoma (NTG) and advanced glaucoma. We aimed to determine whether any observed differences in genetic association at the 9p21 locus are influenced by sex.

Methods. Sex was assessed as a risk factor for POAG for 2241 glaucoma participants from the Australian and New Zealand Registry of Advanced Glaucoma, the Glaucoma Inheritance Study in Tasmania, and the Flinders Medical Centre. A total of 3176 controls were drawn from the Blue Mountains Eye Study and South Australia: 1523 advanced POAG and 718 nonadvanced POAG cases were genotyped along with 3176 controls. We selected 13 SNPs at the 9p21 locus, and association results were subanalyszd by sex for high-tension glaucoma (HTG) and NTG. Odds ratios (ORs) between sexes were compared.

Results. A sex bias was present within advanced NTG cases (57.1% female versus 42.9% male, $P = 0.0026$). In all POAG cases, the strongest associated SNP at 9p21 was rs1063192 (OR, 1.43; $P = 4 \times 10^{-18}$). This association was stronger in females (OR, 1.5; $P = 5 \times 10^{-13}$) than in males (OR, 1.35; $P = 7 \times 10^{-7}$), with a statistically significant difference in female to male OR comparison ($P = 1.0 \times 10^{-2}$). An NTG to HTG subanalysis yielded statistically significant results only in females (OR, 1.63; $P = 1.5 \times 10^{-4}$) but not in males (OR, 1.15; $P = 2.8 \times 10^{-1}$), with a statistically significant difference in female to male OR comparison ($P = 1.4 \times 10^{-4}$).

Conclusions. This study demonstrated that female sex is a risk factor for developing advanced NTG. The stronger genetic signals at the 9p21 locus among females may contribute at least in part to the observed sex bias for NTG.

Keywords: 9p21, primary open-angle glaucoma, normal-tension glaucoma, sex specific, sex bias

Primary open-angle glaucoma (POAG) is the most common type of glaucoma and is characterized pathologically by a progressive loss of retinal ganglion cells with corresponding loss of visual field. The prevalence of POAG in the age group over 40 years is estimated to be 2%–3% among Caucasian populations,[1–3] approximately 6%–7% among Black populations,[4,5] and 3.9% among the Japanese.[6]

Although demographic factors such as older age and black race are well known to be associated with an increased risk for POAG, there is no consensus with regards to sex as a risk factor. Results from previous large population-based studies have been inconsistent, with some studies reporting higher prevalence in females,[5,7] whereas others showed higher prevalence in males[8,9] or no association at all.[10–13] The Collaborative Normal-Tension Glaucoma Study Group reported female sex as an independent risk factor for disease progression in normal-tension glaucoma (NTG), a subtype of POAG with no recorded intraocular pressure (IOP) elevation (≤21 mm Hg).[14] A recent meta-analysis, however, reported greater POAG prevalence among males in comparison to age-matched females.[15]

There are obvious biological and physiological differences between males and females, and these differences are known to

Investigative Ophthalmology & Visual Science

affect the incidence and progression of various common diseases in human, notably cardiovascular and autoimmune diseases.[16] These sex differences have typically been attributed to the differences in the sex hormone levels between males and females and the genetic contribution of the sex chromosomes (chromosome X).[17] In glaucoma, there is evidence suggesting that the risk of POAG among females may be influenced by estrogen metabolism and estrogen exposure, both endogenously and exogenously.[18–20]

More recently, the autosomes, shared by both males and females, were also shown to contribute significantly to sex-specific disease differences due to sexual dimorphism in gene regulation and expression between sexes.[21] The dimorphism in the regulation and expression of genes is likely to explain part of the difference in a gene–environment interaction and also influence phenotypic traits in terms of sex-specific susceptibility to disease.[16]

With the advent of genome-wide association studies (GWASs), single nucleotide polymorphisms (SNPs) associated with a disease, particularly known to have sex-specific differences, can be systematically analyzed in depth to detect whether disease association is stronger in one sex than the other.[22] For instance, certain SNPs in the *RELN* gene were shown to have significant association for schizophrenia and bipolar disorder in females but not in males.[23,24] POAG is a genetically complex disease, and recent GWASs identified several SNPs within the *CDKN2B/CDKN2B-AS1* genes on chromosome 9p21 to have strong and reproducible association especially with the NTG subtype.[25–27] These previous studies did not specifically analyze the association of sex among the SNPs relevant to POAG. In this study, we aim to investigate this locus for the existence of sex effect and differences in association to POAG.

Patients and Methods

Participants

All participants provided written informed consent, and approval was obtained from the Human Research Ethics Committees of Southern Adelaide Health Service/Flinders University, University of Tasmania and University of Sydney. The study adhered to the tenets of the Declaration of Helsinki.

Participants were drawn from the Australian & New Zealand Registry of Advanced Glaucoma, the Glaucoma Inheritance Study in Tasmania, the Blue Mountains Eye Study (BMES, a population-based study of residents 49 years of age and older living in the Blue Mountains region west of Sydney), and patients attending eye clinics at Flinders Medical Centre, Adelaide, Australia. All participants were Australian of European descent. The cohorts and clinical definitions are as described in detail in earlier reports.[25,28,29]

Briefly, advanced glaucoma was defined by severe visual loss resulting from POAG. This included best-corrected visual acuity worse than 6/60 resulting from POAG or a reliable 24-2 Humphrey Visual Field with a mean deviation (MD) of worse than −22db or at least two of four central fixation squares affected with a pattern standard deviation of less than 0.5%. The field loss had to be the result of POAG, and the less severely affected eye also was also required to have signs of glaucomatous disc damage. Less severe or nonadvanced glaucoma was defined by concordant findings of typical glaucomatous visual field defects on the Humphrey 24-2 test, with corresponding optic disc rim thinning, including an enlarged vertical cup-to-disc ratio (VCDR) ($\geq$0.7) or VCDR asymmetry ($\geq$0.2) between the two eyes. The age at glaucoma diagnosis, highest recorded IOP, central corneal thickness (CCT), and MD in each eye were obtained from the medical records. For each variable (IOP, CCT, MD, and VCDR), the data from the worse eye were used. Any participants without sex or genotype data were excluded. Participants with any form of secondary glaucoma or mutations in the *myocilin* gene were also excluded.

Controls were drawn from the BMES and unaffected participants from South Australia.[28] All controls were examined and found to have no sign of glaucoma. A total of 2742 elderly participants from the BMES and 434 participants from South Australia were included. Parameters obtained from the controls included age, genotype data, IOP, CCT, and VCDR.

Genotyping and Association Analysis

Genotyping of the 13 SNPs at the 9p21 locus has been described previously.[25,28] The SNPs chosen were those from our previous GWASs and from targeted genotyping at the 9p21 locus. Briefly, samples used in the discovery phase of the reported GWAS were genotyped on the Human1M-Omni array (Illumina, Inc., San Diego, CA, USA), and samples used in the replication phase of the GWAS were typed on the MassArray platform (Sequenom, Inc., San Diego, CA, USA). The controls were genotyped on Illumina HumanHap 610W Quad and Illumina Human670Quad Bead arrays (BMES) or by MassArray (others).

Data including sex, age at diagnosis, highest recorded IOP, POAG subtype (NTG or HTG), and CCT were gathered from each participant where possible. Every SNP was analyzed for genetic association. The genetic association analyses were conducted using Plink (Plink version 1.07, 10 August 2009. Purcell S. Available at: http://pngu.mgh.harvard.edu/purcell/plink/. Accessed August 2015).[30] Initial analyses were conducted comparing POAG to controls in males, females, and both sexes combined. The same analyses were then conducted in advanced POAG cases only. We then ran separate association analyses in advanced cases of NTG (IOP $\leq$ 21 mm Hg) and HTG (IOP $>$ 21 mm Hg) for each sex and combined. To test for the effect of sex on the association at the 9p21 SNPs, the obtained odds ratios (ORs) were compared between the sexes by computing

$$\begin{aligned} T &= [\mathrm{Log}(\mathrm{OR}_{\mathrm{cases}}) - \log(\mathrm{OR}_{\mathrm{controls}})]/\mathrm{variance}[\mathrm{Log}(\mathrm{OR}_{\mathrm{cases}}) \\ &\quad -\log(\mathrm{OR}_{\mathrm{controls}})] \\ &= [\mathrm{Log}(\mathrm{OR}_{\mathrm{cases}}) - \log(\mathrm{OR}_{\mathrm{controls}})]/ \\ &\quad \{\mathrm{variance}[\mathrm{Log}(\mathrm{OR}_{\mathrm{cases}})] + \mathrm{variance}[\log(\mathrm{OR}_{\mathrm{controls}})]\}. \end{aligned}$$

P values were computed based on T following a χ_1^2 distribution. Using a Bonferroni correction, a P value of 0.004 was required to account for the multiple testing of the 13 SNPs (in practice, due to the correlation between these SNPs, this threshold may be overconservative).

Results

Overall, there were a total of 2241 cases of POAG and 3176 controls with sex and genotype data available. Among the POAG cases, 1180 (52.66%) were females and 1061 (47.34%) were males, whereas there were 1793 (56.5%) females to 1383 (43.5%) males among the controls (Table 1). The POAG cohort had a mean age of glaucoma diagnosis of 60.6 $\pm$ 14.3 years. The mean highest documented IOP was 27.1 $\pm$ 11.2 mm Hg, with 66.8% having the highest recorded IOP of $>$21 mm Hg. A total of 1523 (68%) were classified as having advanced disease, with 744 (48.85%) males and 779 (51.15%) females ($P = 0.37$). Details of the demographic data for both the POAG cohort and controls are shown in Table 1. A notable sex bias was present

Investigative Ophthalmology & Visual Science

Table 1. Demographics and Clinical Characteristics of the POAG Cases and Controls

	Mean ± SD or *N* (%)	
Variables	**POAG**	**Controls**
Number, *N*	2241	3176
Males	1061	1383
Females	1180	1793
Age, y ($P = 0.4$)	60.6 ± 14.3	55.3 ± 8.7
Males	59.5 ± 14.9	55.1 ± 8.5
Females	61.4 ± 13.7	55.5 ± 8.8
Female, *N* (%)	1180 (52.66%)	1793 (56.45%)
IOP, mm Hg	27.12 ± 11.25	17.29 ± 3.27
HTG, %	1084 (66.75%)	
NTG, %	540 (33.25%)	
VCDR	0.88 ± 0.12	0.47 ± 0.13
CCT, μm	518.0 ± 40.2	532.7 ± 84.4

F, female; M, male.

within advanced NTG cases (57.1% female versus 42.9% male, $P = 0.0026$), but not in HTG cases (48.1% female versus 51.9% male, $P = 0.24$; Table 2).

On association analysis conducted for all 13 SNPs of 9p21 among all POAG cases, 4 SNPs reached genome-wide significance ($P < 5 \times 10^{-8}$): rs1063192 ($P = 3.76 \times 10^{-18}$), rs4977756 ($P = 1.97 \times 10^{-16}$), rs10120688 ($P = 6.99 \times 10^{-11}$), and rs3731239 ($P = 5.63 \times 10^{-10}$) (Supplementary Table). Table 3 shows the association results stratified by sex for the top four SNPs. The top three SNPs, namely rs1063192, rs4977756, and rs10120688, reached genome-wide significance only in females but not in males. The OR difference between females and males was statistically significant for rs1063192 ($P = 1.04 \times 10^{-2}$), rs4977756 ($P = 1.37 \times 10^{-4}$), and rs3731239 ($P = 2.40 \times 10^{-3}$) (Table 3).

A strong association was observed when the analyses were conducted comparing only advanced cases to the controls (Table 4). In females, the observed association was stronger in the advanced cases than in overall POAG. This trend, however, was not observed among the males, which showed comparable ORs and significance levels in both advanced and overall POAG cases (Table 4).

The NTG subgroup was then compared directly to the HTG subgroup within advanced POAG, as this locus is known to be more strongly associated with NTG than HTG.[25,31] Three SNPs (rs1063192, rs4977756, and rs10120688) showed statistically significant association to NTG (when applying a conservative Bonferroni correction for 13 tests at 9p21) when both sexes were analyzed together. The risk allele A of SNP rs1063192 carries an OR of 1.40 ($P = 2.46 \times 10^{-4}$) for developing NTG (Table 5). Marked sex differences were again observed when the analyses were conducted separately for females and males (Table 5). Among the females, these SNPs were significantly associated with NTG, yielding ORs of 1.63 for rs1063192, 1.60 for rs4977756, and 1.62 for rs10120688 (Table 5). On the other hand, in males with NTG, these same SNPs carried weaker ORs of 1.15 for rs1063192 and 1.22 for both rs4977756 and rs10120688 and did not reach statistical significance. The OR difference between females and males was statistically significant (Tables 3 and 5).

Discussion

The association between SNPs at chromosome 9p21 and POAG has been widely established in multiple populations.[25–28,31] As previously noted, the association was significantly stronger among the NTG subgroup and also among the advanced blinding cases.[25,32,33] Our current study highlights that the strength of the association also varies markedly depending on sex. The data consistently showed that the association of these known glaucoma risk alleles at chromosome 9p21 with POAG is stronger in females than in males. These sex differences in the strength of association have not been previously reported among SNPs at the 9p21 locus.

The differences in the strength of association between sexes noticeably increased among the advanced POAG cases. It is well recognized that POAG progresses with increasing age, and therefore, the advanced cases are more frequently documented among the older age group.[15] Nevertheless, the underlying reason for the observed stronger association of the chromosome 9p21 risk alleles particularly among female advanced POAG cases in comparison to the male counterpart is unclear. Whether females with these risk alleles have higher risk of progression to advanced POAG remains to be elucidated. Also, the risk alleles of the three main SNPs, rs1063192, rs4977756, and rs10120688, conferred a statistically significant OR ranging from 1.60 to 1.63 with NTG among females only. The association with NTG did not reach statistical significance among males, and the ORs were substantially weaker (Table 5). The observation also suggests that this locus may confer a greater risk for NTG among females.

Table 2. Nonadvanced and Advanced POAG

Variables	**Male**	**Female**	***P* Value**	**Total**
Nonadvanced POAG				
N (%)	317 (44.15%)	401 (55.85%)	0.0017	718 (100%)
Age of diagnosis	67.3 ± 13.2	62.9 ± 12.5		
IOP	25.0 ± 9.5	24.8 ± 8.3		
VCDR	0.80 ± 0.14	0.75 ± 0.17		
CCT	526.7 ± 37.8	521.8 ± 44.5		
HTG, *N* (%)	120 (46.69%)	137 (53.31%)	0.29	257 (35.79%)
NTG, *N* (%)	34 (29.31%)	82 (70.69%)	8.2×10^{-6}	116 (16.16%)
Advanced POAG				
N (%)	744 (48.85%)	779 (51.15%)	0.37	1523 (100%)
Age of diagnosis	59.9 ± 14.6	62.5 ± 13.8		
IOP	28.1 ± 10.5	26.3 ± 10.3		
VCDR	0.92 ± 0.08	0.90 ± 0.09		
CCT	516.1 ± 41.4	516.3 ± 39.0		
HTG, *N* (%)	466 (51.95%)	431 (48.05%)	0.24	897 (58.90%)
NTG, *N* (%)	193 (42.89%)	257 (57.11%)	0.0026	450 (29.55%)

Investigative Ophthalmology & Visual Science

Table 3. Sex Comparison for Top Four SNPs in Association Analyses for 2232 POAG Cases

All POAG	Female					Male					
	Risk Allele Frequency					Risk Allele Frequency					*P* Value for Female–Male
SNP	Cases	Control	OR	*P*	95% CI	Cases	Control	OR	*P*	95% CI	Difference
rs1063192	0.655	0.558	1.50	4.73×10^{-13}	1.35–1.68	0.640	0.568	1.35	6.95×10^{-7}	1.20–1.52	1.04×10^{-2}
rs4977756	0.690	0.596	1.51	2.58×10^{-13}	1.35–1.69	0.667	0.608	1.29	2.72×10^{-5}	1.15–1.45	1.37×10^{-4}
rs10120688	0.564	0.481	1.40	5.86×10^{-9}	1.25–1.56	0.547	0.495	1.23	1.05×10^{-3}	1.09–1.39	2.40×10^{-3}
rs3731239	0.691	0.623	1.35	6.63×10^{-7}	1.20–1.53	0.694	0.639	1.28	2.29×10^{-4}	1.12–1.46	2.35×10^{-1}

Sex-specific differences in disease susceptibility, course, and severity have long been known, notably in cardiovascular and autoimmune diseases. Several SNPs within chromosome 9p21 namely rs2383207, rs4977574, rs10757274, rs10116277, and rs1333040 are also known to have significant association with coronary heart disease.[34,35] In our current analyses (Supplementary Table), the SNP rs2383207 conferred an OR of 1.39 ($P = 9.1 \times 10^{-7}$) among females and 1.19 ($P = 1.4 \times 10^{-2}$) among males for advanced POAG, almost reaching the level of genome-wide significance among females alone. The other four SNPs, however, did not show any suggestive association with POAG, consistent with previous studies.[25–27] The lack of association between relevant SNPs at chromosome 9p21 that confer risk for POAG and those for cardiovascular diseases is in accordance with the fact that there have been no clear association between POAG and cardiovascular diseases.[36,37]

In POAG overall, sex influences have not been strongly established previously. A recent meta-analysis by Tham et al. reported greater prevalence among males, with an OR of 1.36 (95% confidence interval [CI], 1.23–1.52).[15] Several studies have also highlighted the protective effect of estrogen and linked the risks of POAG among females with estrogen metabolism and exposure.[18–20] Lower IOP was shown to be associated with postmenopausal hormonal use and pregnancy, during which estrogen levels are elevated.[18,38,39] Early menopause (age less than 45 years)[19] was associated with increased risk of POAG, whereas later onset of menopause (age more than 54 years) was associated with reduced risk.[20] In the BMES, later age of menarche was also found to be associated with POAG.[40] Pasquale et al., however, reported that later age of menarche was associated with NTG only.[41] Overall evidence suggests that the risk of POAG is somehow inversely related to the cumulative exposure of estrogen. Meta-analyzed GWAS data also suggested an association between the estrogen SNPs pathways and POAG among females.[42] Although there are several proposed hypotheses, the exact pathophysiology of any putative protective effect of estrogen against POAG is still unknown.

There may be other biological mechanisms underlying the observed sex specificity such as epigenetic differences between the sexes. Sexual dimorphism in gene regulation and expression possibly mediates the differences in genotype–environmental interactions, which could subsequently lead to sex-specific susceptibility to POAG.[16,21] Sex-genetic specificity has been observed in several other diseases.[43,44] In hypertension, angiotensin-converting enzyme *DD* genotypes were significantly associated with hypertension in males only.[43,44] In schizophrenia and bipolar disorder, SNPs in the *RELN* gene were shown to have a significant association in females only.[23,24] The protein product of *RELN*, reelin is implicated in neuronal migration and has been shown to be essential for retinogeniculate targeting by retinal ganglion cells.[45,46] Also, the SNP rs7865618 on chromosome 9p21, known to have significant association with coronary artery disease, was recently shown to be male specific,[22] explaining at least in part the male bias in the incidence of coronary artery disease.[47]

One of the limitations of this study is that the result was obtained from a single population cohort (Australian of European descent). Our current study, however, comprises a large number of both POAG cases (2241) and controls (3176), and the analyses showed a notable sex difference in the strength of association of glaucoma risk alleles at chromosome 9p21, especially in the NTG and advanced disease. Future replication studies will confirm and strengthen these findings. Second, some phenotypic data were not available from the nonadvanced POAG group, and for a subset of the samples, we used different genotyping methods that could potentially introduce artifacts. However, the call rates (a good proxy for genotyping accuracy) for the SNPs were high irrespective of genotyping platform, and we believe our results to be robust. Our previous publications on POAG used a mixture of

Table 4. Association Analyses Comparing All POAG and Advanced POAG by Sex

	All POAG ($N = 2241$)		Advanced POAG ($N = 1523$)	
SNPs	OR (95% CI)	*P* Value	OR (95% CI)	*P* Value
Female, *N* (all): 1180, *N* (adv): 779				
rs1063192	1.50 (1.34–1.68)	4.73×10^{-13}	1.64 (1.44–1.87)	6.97×10^{-14}
rs4977756	1.51 (1.35–1.69)	2.58×10^{-13}	1.69 (1.48–1.93)	1.14×10^{-14}
rs10120688	1.40 (1.25–1.56)	5.86×10^{-9}	1.54 (1.35–1.75)	4.21×10^{-11}
rs3731239	1.35 (1.20–1.53)	6.63×10^{-7}	1.40 (1.22–1.60)	1.13×10^{-6}
Male, *N* (all): 1061, *N* (adv): 744				
rs1063192	1.35 (1.20–1.52)	6.95×10^{-7}	1.35 (1.18–1.54)	1.28×10^{-5}
rs4977756	1.29 (1.15–1.45)	2.72×10^{-5}	1.33 (1.16–1.53)	4.20×10^{-5}
rs10120688	1.23 (1.09–1.39)	1.05×10^{-3}	1.25 (1.09–1.43)	1.12×10^{-3}
rs3731239	1.28 (1.12–1.46)	2.29×10^{-4}	1.37 (1.19–1.59)	2.03×10^{-5}

adv, advanced POAG; all, all POAG.

TABLE 5. Association Analyses Comparing NTG With HTG by Sex Among Advanced POAG Cases Only.

Advanced POAG	Overall					Female				Male				
	Risk Allele Frequency					Risk Allele Frequency				Risk Allele Frequency				
SNP	NTG	HTG	OR	*P*	95% CI	NTG	HTG	OR	*P*	95% CI	NTG	HTG	OR	*P*	95% CI	*P* Value for Female–Male Difference
rs1063192	0.706	0.632	1.40	2.46×10^{-4}	1.17–1.69	0.740	0.636	1.63	1.46×10^{-4}	1.27–2.10	0.661	0.629	1.15	2.83×10^{-1}	0.89–1.50	1.38×10^{-4}
rs4977756	0.742	0.669	1.43	1.86×10^{-4}	1.18–1.72	0.767	0.673	1.60	3.45×10^{-4}	1.35–1.69	0.709	0.667	1.22	1.50×10^{-1}	0.93–1.60	4.39×10^{-3}
rs10120688	0.628	0.541	1.43	3.74×10^{-5}	1.21–1.70	0.657	0.542	1.62	6.23×10^{-5}	1.28–2.05	0.590	0.542	1.22	1.19×10^{-1}	0.95–1.57	1.21×10^{-3}
rs3731239	0.728	0.687	1.22	3.50×10^{-2}	1.01–1.47	0.745	0.666	1.46	3.42×10^{-3}	1.13–1.89	0.706	0.708	0.99	9.60×10^{-1}	0.76–1.30	3.49×10^{-4}

genotyping methods, and those findings have now been replicated by other groups,[48] giving us confidence that our findings here are robust. Third, the definition and diagnosis of NTG is often difficult in a cross-sectional population. Many of the diagnosed NTG patients would not have been subjected to phasing; hence, the highest recorded IOP in a clinical setting may not necessarily reflect the highest IOP.

In summary, the results of this study demonstrate a stronger association of the POAG relevant SNPs at chromosome 9p21 in females compared with males, particularly in the NTG and advanced disease. This genetic association would at least in part contribute to the observed significant sex bias for advanced NTG. Although the exact reason underlying such observations remains to be determined, we prompt other researchers performing GWAS to conduct additional analyses to specifically test for potential sex effects.

Acknowledgments

Supported by the National Health and Medical Research Council (NHMRC) of Australia (Grants 535074, 577074, 974159, 211069, and 457349); an NHMRC career development award (KPB); an NHMRC practitioner fellowship (JEC); and a Royal Australian and New Zealand College of Ophthalmology (RANZCO) Eye Foundation grant. The Blue Mountain Eye Study was supported by the NHMRC (Grants 974159, 211069, 457349, 512423, 475604, and 529912).

The sponsor or funding organization had no role in the design or conduct of this research.

Disclosure: **S.K. Ng**, None; **K.P. Burdon**, None; **J.T. Fitzgerald**, None; **T. Zhou**, None; **R. Fogarty**, None; **E. Souzeau**, None; **J. Landers**, None; **R.A. Mills**, None; **R.J. Casson**, None; **B. Ridge**, None; **S.L. Graham**, None; **A.W. Hewitt**, None; **D.A. Mackey**, None; **P.R. Healey**, None; **J.J. Wang**, None; **P. Mitchell**, None; **S. MacGregor**, None; **J.E. Craig**, None

References

1. Klein BE, Klein R, Sponsel WE, et al. Prevalence of glaucoma. The Beaver Dam Eye Study. *Ophthalmology*. 1992;99:1499–504.
2. Tielsch JM, Sommer A, Katz J, et al. Racial variations in the prevalence of primary open-angle glaucoma. The Baltimore Eye Survey. *JAMA*. 1991;266:369–374.
3. Mitchell P, Smith W, Attebo K, Healey PR. Prevalence of open-angle glaucoma in Australia. The Blue Mountains Eye Study. *Ophthalmology*. 1996;103:1661–1669.
4. Friedman DS, Jampel HD, Munoz B, West SK. The prevalence of open-angle glaucoma among blacks and whites 73 years and older: the Salisbury Eye Evaluation Glaucoma Study. *Arch Ophthalmol*. 2006;124:1625–1630.
5. Leske MC, Connell AM, Schachat AP, Hyman L. The Barbados Eye Study. Prevalence of open angle glaucoma. *Arch Ophthalmol*. 1994;112:821–829.
6. Iwase A, Suzuki Y, Araie M, et al. The prevalence of primary open-angle glaucoma in Japanese: the Tajimi Study. *Ophthalmology*. 2004;111:1641–1648.
7. Leibowitz HM, Krueger DE, Maunder LR, et al. The Framingham Eye Study monograph: an ophthalmological and epidemiological study of cataract, glaucoma, diabetic retinopathy, macular degeneration, and visual acuity in a general population of 2631 adults, 1973–1975. *Surv Ophthalmol*. 1980; 24(Suppl):335–610.
8. Bengtsson B. Aspects of the epidemiology of chronic glaucoma. *Acta Ophthalmol Suppl*. 1981;146:1–48.
9. Bengtsson B. The prevalence of glaucoma. *Br J Ophthalmol*. 1981;65:46–49.

10. Hollows FC, Graham PA. Intra-ocular pressure, glaucoma, and glaucoma suspects in a defined population. *Br J Ophthalmol*. 1966;50:570–586.
11. Armaly MF, Krueger DE, Maunder L, et al. Biostatistical analysis of the collaborative glaucoma study. I. Summary report of the risk factors for glaucomatous visual-field defects. *Arch Ophthalmol*. 1980;98:2163–2171.
12. Klein BE, Klein R, Jensen SC. Open-angle glaucoma and older-onset diabetes. The Beaver Dam Eye Study. *Ophthalmology*. 1994;101:1173–1177.
13. Tielsch JM. The epidemiology and control of open angle glaucoma: a population-based perspective. *Annu Rev Public Health*. 1996;17:121–136.
14. Drance S, Anderson DR, Schulzer M. Collaborative Normal-Tension Glaucoma Study Group. Risk factors for progression of visual field abnormalities in normal-tension glaucoma. *Am J Ophthalmol*. 2001;131:699–708.
15. Tham YC, Li X, Wong TY, et al. Global prevalence of glaucoma and projections of glaucoma burden through 2040: a systematic review and meta-analysis. *Ophthalmology*. 2014; 121:2081–2090.
16. Ober C, Loisel DA, Gilad Y. Sex-specific genetic architecture of human disease. *Nat Rev Genet*. 2008;9:911–922.
17. Alonso LC, Rosenfield RL. Oestrogens and puberty. *Best Pract Res Clin Endocrinol Metab*. 2002;16:13–30.
18. Affinito P, Di Spiezio Sardo A, Di Carlo C, et al. Effects of hormone replacement therapy on ocular function in post-menopause. *Menopause*. 2003;10:482–487.
19. Hulsman CA, Westendorp IC, Ramrattan RS, et al. Is open-angle glaucoma associated with early menopause? The Rotterdam Study. *Am J Epidemiol*. 2001;154:138–144.
20. Pasquale LR, Rosner BA, Hankinson SE, Kang JH. Attributes of female reproductive aging and their relation to primary open-angle glaucoma: a prospective study. *J Glaucoma*. 2007;16: 598–605.
21. Ellegren H, Parsch J. The evolution of sex-biased genes and sex-biased gene expression. *Nat Rev Genet*. 2007;8:689–698.
22. Liu LY, Schaub MA, Sirota M, Butte AJ. Sex differences in disease risk from reported genome-wide association study findings. *Hum Genet*. 2012;131:353–364.
23. Goes FS, Willour VL, Zandi PP, et al. Sex-specific association of the Reelin gene with bipolar disorder. *Am J Med Genet B Neuropsychiatr Genet*. 2010;153B:549–553.
24. Shifman S, Johannesson M, Bronstein M, et al. Genome-wide association identifies a common variant in the reelin gene that increases the risk of schizophrenia only in women. *PLoS Genet*. 2008;4:e28.
25. Burdon KP, Crawford A, Casson RJ, et al. Glaucoma risk alleles at CDKN2B-AS1 are associated with lower intraocular pressure, normal-tension glaucoma, and advanced glaucoma. *Ophthalmology*. 2012;119:1539–1545.
26. Wiggs JL, Yaspan BL, Hauser MA, et al. Common variants at 9p21 and 8q22 are associated with increased susceptibility to optic nerve degeneration in glaucoma. *PLoS Genet*. 2012;8:e1002654.
27. Osman W, Low SK, Takahashi A, et al. A genome-wide association study in the Japanese population confirms 9p21 and 14q23 as susceptibility loci for primary open angle glaucoma. *Hum Mol Genet*. 2012;21:2836–2842.
28. Burdon KP, Macgregor S, Hewitt AW, et al. Genome-wide association study identifies susceptibility loci for open angle glaucoma at TMCO1 and CDKN2B-AS1. *Nat Genet*. 2011;43: 574–578.
29. Souzeau E, Goldberg I, Healey PR, et al. Australian and New Zealand Registry of Advanced Glaucoma: methodology and recruitment. *Clin Experiment Ophthalmol*. 2012;40:569–575.
30. Purcell S, Neale B, Todd-Brown K, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet*. 2007;81:559–575.
31. Ng SK, Casson RJ, Burdon KP, Craig JE. Chromosome 9p21 primary open-angle glaucoma susceptibility locus: a review. *Clin Experiment Ophthalmol*. 2014;42:25–32.
32. Nakano M, Ikeda Y, Tokuda Y, et al. Common variants in CDKN2B-AS1 associated with optic-nerve vulnerability of glaucoma identified by genome-wide association studies in Japanese. *PLoS One*. 2012;7:e33389.
33. Takamoto M, Kaburaki T, Mabuchi A, et al. Common variants on chromosome 9p21 are associated with normal tension glaucoma. *PLoS One*. 2012;7:e40107.
34. Helgadottir A, Thorleifsson G, Manolescu A, et al. A common variant on chromosome 9p21 affects the risk of myocardial infarction. *Science*. 2007;316:1491–1493.
35. McPherson R, Pertsemlidis A, Kavaslar N, et al. A common allele on chromosome 9 associated with coronary heart disease. *Science*. 2007;316:1488–1491.
36. Dielemans I, Vingerling JR, Algra D, et al. Primary open-angle glaucoma, intraocular pressure, and systemic blood pressure in the general elderly population. The Rotterdam Study. *Ophthalmology*. 1995;102:54–60.
37. Orzalesi N, Rossetti L, Omboni S, et al. Vascular risk factors in glaucoma: the results of a national survey. *Graefes Arch Clin Exp Ophthalmol*. 2007;245:795–802.
38. Altintas O, Caglar Y, Yuksel N, et al. The effects of menopause and hormone replacement therapy on quality and quantity of tear, intraocular pressure and ocular blood flow. *Ophthalmologica*. 2004;218:120–129.
39. Phillips CI, Gore SM. Ocular hypotensive effect of late pregnancy with and without high blood pressure. *Br J Ophthalmol*. 1985;69:117–119.
40. Lee AJ, Mitchell P, Rochtchina E, et al. Female reproductive factors and open angle glaucoma: the Blue Mountains Eye Study. *Br J Ophthalmol*. 2003;87:1324–1328.
41. Pasquale LR, Kang JH. Female reproductive factors and primary open-angle glaucoma in the Nurses' Health Study. *Eye (Lond)*. 2011;25:633–641.
42. Pasquale LR, Loomis SJ, Weinreb RN, et al. Estrogen pathway polymorphisms in relation to primary open angle glaucoma: an analysis accounting for gender from the United States. *Mol Vis*. 2013;19:1471–1481.
43. Higaki J, Baba S, Katsuya T, et al. Deletion allele of angiotensin-converting enzyme gene increases risk of essential hypertension in Japanese men: the Suita Study. *Circulation*. 2000;101: 2060–2065.
44. Stankovic A, Zivkovic M, Alavantic D. Angiotensin I-converting enzyme gene polymorphism in a Serbian population: a gender-specific association with hypertension. *Scand J Clin Lab Invest*. 2002;62:469–475.
45. Rice DS, Nusinowitz S, Azimi AM, et al. The reelin pathway modulates the structure and function of retinal synaptic circuitry. *Neuron*. 2001;31:929–941.
46. Su J, Haner CV, Imbery TE, et al. Reelin is required for class-specific retinogeniculate targeting. *J Neurosci*. 2011;31:575–586.
47. Lerner DJ, Kannel WB. Patterns of coronary heart disease morbidity and mortality in the sexes: a 26-year follow-up of the Framingham population. *Am Heart J*. 1986;111:383–390.
48. Gharahkhani P, Burdon KP, Fogarty R, et al. Common variants near ABCA1, AFAP1 and GMDS confer risk of primary open-angle glaucoma. *Nat Genet*. 2014;46:1120–1125.