SCIENCE CHINA
Life Sciences

• RESEARCH PAPER •

July 2016 Vol.59 No.7: 694–699
doi: 10.1007/s11427-016-5073-y

Association of three single nucleotide polymorphisms at the *SIX1-SIX6* locus with primary open angle glaucoma in the Chinese population

Jinghong Sang[1,2], Liyun Jia[1,2], Bowen Zhao[1,2], Huaizhou Wang[1,2], Nihong Zhang[4] & Ningli Wang[1,2,3*]

[1]*Beijing Tongren Eye Center, Beijing Tongren Hospital, Capital Medical University, Beijing 100730, China;*
[2]*Beijing Ophthalmology & Visual Science Key Laboratory, Capital Medical University, Beijing 100730, China;*
[3]*Beijing Institute of Ophthalmology, Beijing Tongren Hospital, Capital Medical University, Beijing 100730, China;*
[4]*The First Affiliated Hospital of Nanyang Medical College, Nanyang 473000, China*

Received March 1, 2016; accepted March 16, 2016; published online June 3, 2016

This study investigated the genetic association of three single nucleotide polymorphisms (SNPs; rs10483727, rs33912345, and rs146737847) at the *SIX1-SIX6* locus with primary open angle glaucoma (POAG) in the Chinese population. A total of 866 subjects with POAG (685 high-tension glaucoma (HTG) and 181 normal-tension glaucoma (NTG)) and 266 control individuals were included. Significant genetic association was identified for rs10483727 in HTG (P=0.02; odds ratio (OR)=1.31), NTG (P=7.41×10^{-6}; OR=2.71), and POAG (i.e., HTG and NTG combined; P=0.001; OR=1.44). rs33912345 was also significantly associated with HTG (P=0.008; OR=1.36), NTG (P=2.72×10^{-6}; OR=2.27), and POAG (P=3.84×10^{-4}; OR=1.49). The rare SIX6 mutation, rs146737847, was not found in the subjects enrolled in this study. Stratification by patient age identified that both rs10483727 and rs33912345 were significantly associated with NTG in patients aged above 40 years (P=2.08×10^{-5}; OR=2.28), whereas in patients aged between 20–40 years, rs33912345 was significantly associated with NTG (P=0.017; OR=2.06). In HTG, the genetic associations for both rs10483727 and rs33912345 were significant in patients aged between 20–40 years (P=0.006; OR=1.56) but not in those aged above 40 years (P=0.118, OR=1.21 and P=0.042, OR=1.29, respectively). This study replicated the association of POAG with two SNPs at the *SIX1-SIX6* locus and demonstrated that SNPs, rs10483727 and rs33912345, are significantly associated with POAG, especially with NTG in patients aged above 40 years.

primary open angle glaucoma, *SIX1-SIX6*, Chinese population

Citation: Sang, J., Jia, L., Zhao, B., Wang, H., Zhang, N., and Wang, N. (2016). Association of three single nucleotide polymorphisms at the *SIX1-SIX6* locus with primary open angle glaucoma in the Chinese population. Sci China Life Sci 59, 694−699. doi: 10.1007/s11427-016-5073-y

INTRODUCTION

Glaucoma is the leading cause of irreversible blindness worldwide (Quigley and Broman, 2006). It is characterized by typical optic nerve atrophy owing to the death of retinal ganglion cells and visual field loss. The elevation of intraocular pressure (IOP) has long been considered as the major risk factor for glaucoma. However, patients with intraocular pressure within the normal range can also be affected with glaucoma, known as normal-tension glaucoma (NTG) (Anderson et al., 2001). In addition, epidemiological studies have shown that the prevalence of NTG is much higher among the Asian populations. In the Handan Eye study, NTG accounted for 90% of patients with primary open angle glaucoma (POAG); the proportion might be even higher in the Japanese population, in which 92% of the patients

*Corresponding author (email: wningli@vip.163.com)

with POAG were classified as NTG (Iwase et al., 2004; Liang et al., 2011).

POAG is a genetically complex disorder with a wide spectrum of phenotypes. Ocular quantitative traits such as central corneal thickness and optic nerve parameters contribute to increased risk of POAG and its progression (Wiggs et al., 2012). Variants in the *SIX1-SIX6* locus were first associated with the vertical cup-disc ratio (VCDR), which is one of the highly heritable endophenotypes used for diagnosing and evaluating disease progression (Macgregor et al., 2010; Ramdas et al., 2010). In addition, a genome-wide association study later identified that a single nucleotide polymorphism (SNP), rs10483727, in the *SIX1-SIX6* locus was significantly associated with POAG (Wiggs et al., 2012). Subsequent studies from different ethnic cohorts have also confirmed the association of rs10483727 with both VCDR and POAG (Fan et al., 2011; Osman et al., 2012). Moreover, a recent clinical and functional study of the *SIX6* gene identified a common variant (rs33912345) to be significantly associated with POAG and it was further found that patients homozygous for the rs33012345 "T" risk allele were predisposed to have a thinner retinal nerve fiber layer than patients homozygous for the non-risk allele "C" (Carnes et al., 2014).

The human *SIX* gene family comprises six members known as *SIX1-SIX6*, which encode homeobox protein transcription factors that are characterized by a divergent DNA-binding homeodomain and an upstream SIX domain and were reported to be involved in regulating the development of the visual system (Gallardo et al., 1999; Kawakami et al., 2000). Therefore, to address the genetic association of the SNPs at the *SIX1-SIX6* locus with POAG in Chinese northern population, we included the genome-wide associated SNP rs10483727, the common coding variant rs33912345, and a rare variant rs146737847 for this replication study.

RESULTS

The demographic features of all participants are shown in Table 1. This study included 685 patients with HTG with a mean age of 49.8±17.8 years and 181 patients with NTG with a mean age of 53.5±16.8 years. The control group consisted of 266 subjects with a mean age of 67.6±11.3 years. The POAG group was a combination of both patients with HTG and NTG, totaling 866 patients overall. During genotyping, the rare mutation rs146737847 was not identified in any of our patients or control subjects. For the other two SNPs, the genotype and allele frequencies were calculated in the patients and controls. Neither SNP deviated from Hardy-Weinberg equilibrium in either the patient or control groups (P>0.05). Each of the disease groups (HTG, NTG, and POAG) was then respectively compared with the controls.

In this study, rs10483727 demonstrated significant association with HTG, NTG, and POAG, in particular with NTG (OR=2.17, 95% CI: 1.04–1.64; P=7.41×10^{-6}). The frequency of the "T" risk allele was higher among the three patient groups than in the controls (77.1% in HTG, 84.8% in NTG, 78.7% in POAG, and 72.0% in controls; Table 2). The association for rs33912345 was also more significant with NTG (OR=2.27, 95% CI: 1.60–3.21, P=2.72×10^{-6}) than with HTG (OR=1.36, 95% CI: 1.08–1.70, P=0.008) or POAG (OR=1.49, 95% CI: 1.20–1.86, P=3.84×10^{-4}; Table 2). The frequency of the "T" risk allele was higher in the NTG group (85.4%) than in the HTG (77.7%) and control groups (72.0%).

In addition, we also stratified the patients with POAG by age. The genotype and allele frequencies of rs10483727 and rs33912345 were evaluated in different age groups (Tables 3 and 4). Among early-onset patients between 20–40 years old, the SNPrs10483727 showed a significant association with HTG (P=0.006) but not with NTG (P=0.031). The results from patients older than 40 years of age revealed no statistically significant association of rs10483727 with HTG (P=0.118) whereas a strong association was observed with NTG (OR=2.28, 95% CI: 1.55–3.35; P=2.08×10^{-5}). Similarly, SNP rs33912345 was associated with HTG in the younger but not in the older age subgroup. In contrast, rs33912345 showed a significant association with NTG in both subgroups (Tables 3 and 4). However, Breslow-Day tests showed that the *ORs* for both HTG and NTG between the early-onset and late-onset groups were not significantly different (P>0.05).

DISCUSSION

In our study, we investigated the association of two variants

Table 1 Demographic features of patients with POAG and controls[a)]

Variables	HTG (N=685)	NTG (N=181)	POAG (N=866)	Control (N=266)
Age, years (Mean±SD)	49.8±17.8	53.5±16.8	55.1±17.0	67.6±11.3
P value[†]	<0.001	<0.001	<0.001	
Male	475	104	579	114
Female	210	77	287	152
P value[†]	<0.001	<0.001	<0.001	

a) POAG, inclusive of both patients with HTG and NTG. N, number of subjects. †, Comparison between affected and unaffected subjects by unpaired t-tests for age and chi-square tests for gender.

Table 2 Genotype and allele frequency of rs10483727 and rs33912345 among all POAG patients and controls[a)]

Group	rs10483727					rs33912345				
	Genotype count (AA/AB/BB)[†]	*P* value	Allele count (A/B)	*P* value	*OR* (95% *CI*)	Genotype count (AA/AB/BB)[†]	*P* value	Allele count (A/B)	*P* value	*OR* (95% *CI*)
HTG	42 (6.1)/230(33.6) /413 (60.3)	0.076	314 (22.9) /1056 (77.1)	0.02	1.31 (1.04–1.64)	36 (5.3)/233 (34.0) /416 (60.7)	0.029	305 (22.3) /1065 (77.7)	0.008	1.36 (1.08–1.70)
NTG	5 (2.8)/45 (24.9) /131 (72.4)	7.02×10^{-5}	55 (15.2) /307 (84.8)	7.41×10^{-6}	2.17 (1.54–3.06)	4 (2.2)/45 (24.9) /132 (72.9)	3.29×10^{-5}	53 (14.6) /309 (85.4)	2.72×10^{-6}	2.27 (1.60–3.21)
POAG	47 (5.4)/275 (31.8) /544 (62.8)	0.007	369 (21.3) /1363 (78.7)	0.001	1.44 (1.15–1.79)	40 (4.6)/278 (32.1) /548 (63.3)	0.002	358 (20.7) /1374 (79.3)	3.84×10^{-4}	1.49 (1.20–1.86)
Control	23 (8.6)/103 (38.7) /140 (52.6)		149 (28.0) /383 (72.0)			24 (9.0)/101 (38.0) /141 (53.0)		149 (28.0) /383 (72.0)		

a) Genotype and allele counts are presented as number (%) for each SNP. OR, odds ratio. CI, confidence interval. †: A, minor allele; B, common allele. For rs10483727, the minor allele is C; for rs33912345, the minor allele is A.

Table 3 Genotype and allele frequencies of rs10483727 and rs33912345 among patients with POAG aged between 20–40 years and controls[a)]

Group	rs10483727					rs33912345				
	Genotype count (AA/AB/BB)[†]	*P* value	Allele count (A/B)	*P* value	*OR* (95% *CI*)	Genotype count (AA/AB/BB)[†]	*P* value	Allele count (A/B)	*P* value	*OR* (95% *CI*)
HTG	7 (3.8)/60 (32.4) /118 (63.8)	0.024	74 (20.0) /296 (80.0)	0.006	1.56 (1.13–2.14)	7 (3.8)/60 (32.4) /118 (63.8)	0.024	74 (20.0)/296 (80.0)	0.006	1.56 (1.13–2.14)
NTG	1 (2.3)/13 (29.5) /30 (68.2)	0.106	15 (17.0) /73 (83.0)	0.031	1.89 (1.05–3.41)	0/14 (31.8) /30 (68.2)	0.052	14 (15.9)/74 (84.1)	0.017	2.06 (1.13–3.75)
POAG	8 (3.5)/73 (31.9) /148 (64.6)	0.007	89 (19.4) /369 (80.6)	0.002	1.61 (1.20–2.18)	7 (3.1)/74 (32.3) /148 (64.6)	0.004	88 (19.2)/370 (80.8)	0.001	1.64 (1.21–2.21)
Control	23 (8.6)/103 (38.7) /140 (52.6)		149 (28.0) /383 (72.0)			24 (9.0)/101 (38.0) /141 (53.0)		149 (28.0)/383 (72.0)		

a) Genotype and allele counts are presented as number (%) for each SNP. †: A, minor allele; B, common allele. For rs10483727, the minor allele is C; for rs33912345, the minor allele is A.

Table 4 Genotype and allele frequencies of rs10483727 and rs33912345 among patients with POAG older than 40 years and controls[a)]

Group	rs10483727					rs33912345				
	Genotype count (AA/AB/BB)[†]	*P* value	Allele count (A/B)	*P* value	*OR* (95% *CI*)	Genotype count (AA/AB/BB)[†]	*P* value	Allele count (A/B)	*P* value	*OR* (95% *CI*)
HTG	35 (7.5)/157 (33.6) /275 (58.9)	0.259	227 (24.3)/707 (75.7)	0.118	1.21 (0.95–1.54)	29 (6.2)/159 (34.0) /279 (59.7)	0.14	217 (23.2)/717 (76.8)	0.042	1.29 (1.01–1.64)
NTG	4 (2.9)/32 (23.4) /101 (73.7)	1.56×10^{-4}	40 (14.6)/234 (85.4)	2.08×10^{-5}	2.28 (1.55–3.35)	4 (2.9)/32 (23.4) /101 (73.7)	1.88×10^{-4}	40 (14.6)/234 (85.4)	2.08×10^{-5}	2.28 (1.55–3.35)
POAG	39 (6.5)/189 (31.3) /376 (62.3)	0.028	267 (22.1)/941 (77.9)	0.008	1.37 (1.09–1.73)	33 (5.5)/191 (31.6) /380 (62.9)	0.012	257 (21.3)/951 (78.7)	0.002	1.44 (1.14–1.82)
Control	23 (8.6)/103 (38.7) /140 (52.6)		149 (28.0)/383 (72.0)			24 (9.0)/101 (38.0) /141 (53.0)		149 (28.0)/383 (72.0)		

a) Genotype and allele counts are presented as number (%) for each SNP. †: A, minor allele; B, common allele. For rs10483727, the minor allele is C; for rs33912345, the minor allele is A.

(rs10483727 and rs33912345) in the *SIX1-SIX6* locus with POAG and in particular evaluated the difference of risks of HTG and NTG in the Chinese population. The results suggested that rs10483727 and rs33912345 are significantly correlated with HTG, NTG, and overall POAG and especially with an increased incidence risk of NTG. When we divided the patients with POAG into two different subgroups based on age of diagnosis, it was found that the association for these two SNPs was significant in patients aged between 20–40 years but not in those aged above 40 years in the HTG group. However, in the NTG group, the genetic association was confirmed in the both younger and older subgroups for rs33912345. For rs10483727, the association was only detected in the subgroup of patients with NTG above 40 years.

The *SIX1-SIX6* locus was first identified as being associated with increased VCDR, which is considered as one of critical ocular biomarkers for the diagnosis of glaucoma and its progression to blindness (Ramdas et al., 2010). Several subsequent studies have also confirmed that the *SIX1/SIX6* locus was significantly correlated with POAG and VCDR in different independent ethnic cohorts (Burdon et al., 2015; Chen et al., 2015; Fan et al., 2011; Iglesias et al., 2014; Mabuchi et al., 2015; Osman et al., 2012b; Philomenadin et al., 2015; Ramdas et al., 2011; Wiggs et al., 2012), but the association has not yet been demonstrated in African cohorts owing to the high frequency of the risk allele both in patients and controls (Liu et al., 2013; Williams et al., 2015). Recently, two population-based studies using cohorts from Singapore and of European-descents showed that both glaucomatous and nonglaucomatous subjects with risk allele variants in the *SIX1/SIX6* locus (rs10483727 and rs33912345) were susceptible to thinner retinal nerve fiber layer thickness, which confirmed the results of previous studies (Carnes et al., 2014; Cheng et al., 2015; Kuo et al., 2015). Our results that both rs33912345 and rs10483727 were strongly associated with NTG and POAG were consistent with the findings of a previous association study also based on the Chinese population (Chen et al., 2015). Furthermore, we found that for both SNPs, the *ORs* in the NTG group (*OR*=2.17 for rs10483727 and *OR*=2.27 for rs33912345) were higher than those in the HTG and POAG groups; however, the results from the Chen et al. study did not show this trend.

Several experimental studies have indicated that SIX6 regulates early progenitor cell proliferation during mammalian retinogenesis and that the development of retinal ganglion cells was decreased in a *six6*$^{-/-}$ mouse model (Carnes et al., 2014; Li et al., 2002). Additionally, a recent study showed that the eye size and the volume of the optic nerve were reduced on morpholino knockdown of zebrafish six6a and that these phenotypes could be rescued with co-injection of the human *SIX6* non-risk allele. Given the results of several SIX6 functional studies, they speculated that the development of glaucomatous optic neuropathy and the progression of visual field loss might be hastened by a reduction in the initial number of retinal ganglion cells during the aging process (Carnes et al., 2014). These observations are consistent with the results from our cohort that the two tested risk variants were more significantly associated with NTG, especially with patients in the older age subgroup.

There are some limitations of our study. First, the number of patients with POAG and controls in this study was relatively small as was the sample size in each subgroup. Therefore, the statistical power to detect significance in this cohort might have been limited. Furthermore, the recruitment of all subjects was performed in Beijing Tongren hospital, which might have introduced potential selection and referral biases.

In conclusion, we replicated the associations of two SNPs, rs10483727 and rs33912345, in the *SIX1/SIX6* locus with HTG, NTG, and overall POAG in a Chinese cohort. Both SNPs showed stronger association with NTG than with HTG in terms of *P* values and *OR*s, which suggests their role in increasing subject susceptibility to glaucomatous neurodegeneration. Further work should be performed to investigate the role of the *SIX6* gene in the pathogenesis of POAG.

MATERIALS AND METHODS

Sample collection

Chinese patients with POAG and control subjects were recruited from the eye clinics of Beijing Tongren hospital. This study was approved by the Beijing Tongren hospital ethics board and conducted in accordance with the tenets of the Declaration of Helsinki. Written informed consent was obtained from every participant.

POAG was defined by the presence of open angles on gonioscopy and typical glaucomatous optic neuropathy with compatible vision field impairment (Weinreb and Khaw, 2004). All patients underwent comprehensive ophthalmological examinations including visual acuity, intraocular pressure measurement, slit lamp examination, gonioscopy, stereo photography of the optic nerve head (Kowa, Japan), and visual field perimetry (central 24-2, full threshold program, Humphrey Field Analyzer; Zeiss Meditec AG, Germany). All patients with NTG underwent 24 h IOP measurement without any anti-glaucomatous medications or had ceased all medications at least 4 weeks prior to the IOP measurement. For the diagnosis of NTG, the peak intraocular pressure during the 24 h measurement was required to be lower than 21 mmHg. The 24-hour IOP was measured at 2, 6, 8, and 10 am and 2, 6, and 10 pm in a sitting position using a non-contact tonometer. The normal controls, all of whom were 50 years or older, were all subjected to both slip-lamp biomicroscopy and fundoscopy, and were con-

firmed to represent subjects with an IOP below 21 mmHg without glaucomatous neuropathy (no disc cupping and vertical cup-to-disc ratio less than 0.4) and without family history of glaucoma.

Genotyping

Genomic DNA was extracted from 4 mL peripheral blood using the salting-out procedure described by Miller et al. (Miller et al., 1988). For genotyping, the DNA from each individual was prepared at a concentration of 50 ng μL^{-1}. Genotyping of the SNPs rs10483727, rs33912345, and rs146737847 was performed with the MassARRAY system (Sequenom Inc, San Diego, USA) via matrix assisted laser desorptionionization time-of-flight mass spectrometry (MALDI-TOF MS) on the 866 patients with POAG and 266 normal controls according to the manufacturer's instructions. Variant calling was performed by Sequenom MassARRAY Typer 4.0 software. The call rates of all three SNPs were greater than 95%.

Statistical analysis

Statistical analysis was performed using SPSS software Version 19.0 (USA). Comparison of continuous variables between patients and controls was performed using unpaired *t*-tests. The three SNPs were tested for HWE using the χ^2 test. Comparison of genotype and allele frequencies was performed using χ^2 tests or Fisher's exact test. Allelic association was evaluated by logistic regression to calculate the *ORs* and 95% *CIs*. Bonferroni correction was adopted in multiple testing. A *P*-value of less than 0.025 (=0.05/2) was considered as statistically significant in the association analysis. The patients were stratified into a late-onset subgroup, defined by a diagnosis age over 40 years old, and an early-onset subgroup, defined by a diagnosis age between 20–40 years. Association was determined for each disease-subgroup by comparing the patients in that subgroup with the whole control group (Chen et al., 2012). The Breslow-Day test was used to assess whether the *ORs* had statistical differences between the early-onset and late-onset HTG and NTG subgroups.

Compliance and ethics *The author(s) declare that they have no conflict of interest.*

Acknowledgements *This work was supported by National Natural Science Foundation of China (81271005, 81300767), and the Beijing Scholar Program.*

Author contributions *Ningli Wang and Liyun Jia conceived and designed the study. Jinghong Sang, Bowen Zhao, Huaizhou Wang, and Nihong Zhang collected samples and clinical profiles. Jinghong Sang performed the data analysis and drafted the manuscript. All authors revised the manuscript. All authors had full access to all data in the study.*

Anderson, D.R., Drance, S.M., and Schulzer, M. (2001). Natural history of normal-tension glaucoma. Ophthalmology 108, 247–253.

Burdon, K.P., Mitchell, P., Lee, A., Healey, P.R., White, A.J., Rochtchina, E., Thomas, P.B., Wang, J.J., and Craig, J.E. (2015). Association of open-angle glaucoma loci with incident glaucoma in the Blue Mountains Eye Study. Am J Ophthalmol 159, 31–36.e1.

Carnes, M.U., Liu, Y.P., Allingham, R.R., Whigham, B.T., Havens, S., Garrett, M.E., Qiao, C., Katsanis, N., Wiggs, J.L., Pasquale, L.R., Ashley-Koch, A., Oh, E.C., and Hauser, M.A. (2014). Discovery and functional annotation of SIX6 variants in primary open-angle glaucoma. PLoS Genet 10, e1004372.

Chen, L.J., Tam, P.O., Leung, D.Y., Fan, A.H., Zhang, M., Tham, C.C., Chiang, S.W., Fan, B.J., Wang, N., and Pang, C.P. (2012). SNP rs1533428 at 2p16.3 as a marker for late-onset primary open-angle glaucoma. Mol Vis 18, 1629–1639.

Chen, Y., Hughes, G., Chen, X., Qian, S., Cao, W., Wang, L., Wang, M., and Sun, X. (2015). Genetic variants associated with different risks for high tension glaucoma and normal tension glaucoma in a Chinese population. Invest Ophthalmol Vis Sci 56, 2595–2600.

Cheng, C.Y., Allingham, R.R., Aung, T., Tham, Y.C., Hauser, M.A., Vithana, E.N., Khor, C.C., and Wong, T.Y. (2015). Association of common SIX6 polymorphisms with peripapillary retinal nerve fiber layer thickness: the Singapore Chinese Eye Study. Invest Ophthalmol Vis Sci 56, 478–483.

Fan, B.J., Wang, D.Y., Pasquale, L.R., Haines, J.L., and Wiggs, J.L. (2011). Genetic variants associated with optic nerve vertical cup-to-disc ratio are riskfactors for primary open angle glaucoma in a US Caucasian population. Invest Ophthalmol Vis Sci 52, 1788–1792.

Gallardo, M.E., Lopez-Rios, J., Fernaud-Espinosa, I., Granadino, B., Sanz, R., Ramos, C., Ayuso, C., Seller, M.J., Brunner, H.G., Bovolenta, P., and Rodriguez, D.C.S. (1999). Genomic cloning and characterization of the human homeobox gene SIX6 reveals a cluster of SIX genes in chromosome 14 and associates SIX6 hemizygosity with bilateral anophthalmia and pituitary anomalies. Genomics 61, 82–91.

Iglesias, A.I., Springelkamp, H., van der Linde, H., Severijnen, L.A., Amin, N., Oostra, B., Kockx, C.E., van den Hout, M.C., van Ijcken, W.F., Hofman, A., Uitterlinden, A.G., Verdijk, R.M., Klaver, C.C., Willemsen, R., and van Duijn, C.M. (2014). Exome sequencing and functional analyses suggest that SIX6 is a gene involved in an altered proliferation-differentiation balance early in life and optic nerve degeneration at old age. Hum Mol Genet 23, 1320–1332.

Iwase, A., Suzuki, Y., Araie, M., Yamamoto, T., Abe, H., Shirato, S., Kuwayama, Y., Mishima, H.K., Shimizu, H., Tomita, G., Inoue, Y., and Kitazawa, Y. (2004). The prevalence of primary open-angle glaucoma in Japanese: the Tajimi Study. Ophthalmology 111, 1641–1648.

Kawakami, K., Sato, S., Ozaki, H., and Ikeda, K. (2000). Six family genes–structure and function as transcription factors and their roles in development. Bioessays 22, 616–626.

Kuo, J.Z., Zangwill, L.M., Medeiros, F.A., Liebmann, J.M., Girkin, C.A., Hammel, N., Rotter, J.I., and Weinreb, R.N. (2015). Quantitative trait locus analysis of *SIX1-SIX6* with retinal nerve fiber layer thickness in individuals of European descent. Am J Ophthalmol 160, 123-130.e1.

Li, X., Perissi, V., Liu, F., Rose, D.W., and Rosenfeld, M.G. (2002). Tissue-specific regulation of retinal and pituitary precursor cell proliferation. Science 297, 1180–1183.

Liang, Y.B., Friedman, D.S., Zhou, Q., Yang, X., Sun, L.P., Guo, L.X., Tao, Q.S., Chang, D.S., and Wang, N.L. (2011). Prevalence of primary open angle glaucoma in a rural adult Chinese population: the Handan eye study. Invest Ophthalmol Vis Sci 52, 8250–8257.

Liu, Y., Hauser, M.A., Akafo, S.K., Qin, X., Miura, S., Gibson, J.R., Wheeler, J., Gaasterland, D.E., Challa, P., Herndon, L.W., Ritch, R., Moroi, S.E., Pasquale, L.R., Girkin, C.A., Budenz, D.L., Wiggs, J.L., Richards, J.E., Ashley-Koch, A.E., and Allingham, R.R. (2013). Investigation of known genetic risk factors for primary open angle glaucoma in two populations of African ancestry. Invest Ophthalmol Vis Sci 54, 6248–6254.

Mabuchi, F., Sakurada, Y., Kashiwagi, K., Yamagata, Z., Iijima, H., and Tsukahara, S. (2015). Involvement of genetic variants associated with

primary open-angle glaucoma in pathogenic mechanisms and family history of glaucoma. Am J Ophthalmol 159, 437–444.e2.

Macgregor, S., Hewitt, A.W., Hysi, P.G., Ruddle, J.B., Medland, S.E., Henders, A.K., Gordon, S.D., Andrew, T., McEvoy, B., Sanfilippo, P.G., Carbonaro, F., Tah, V., Li, Y.J., Bennett, S.L., Craig, J.E., Montgomery, G.W., Tran-Viet, K.N., Brown, N.L., Spector, T.D., Martin, N.G., Young, T.L., Hammond, C.J., and Mackey, D.A. (2010). Genome-wide association identifies ATOH7 as a major gene determining human optic disc size. Hum Mol Genet 19, 2716–2724.

Miller, S.A., Dykes, D.D., and Polesky, H.F. (1988). A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 16, 1215.

Osman, W., Low, S.K., Takahashi, A., Kubo, M., and Nakamura, Y. (2012). A genome-wide association study in the Japanese population confirms 9p21 and 14q23 as susceptibility loci for primary open angle glaucoma. Hum Mol Genet 21, 2836–2842.

Philomenadin, F.S., Asokan, R.,N,V., George, R., Lingam, V., and Sarangapani, S. (2015). Genetic association of SNPs near ATOH7, CARD10, CDKN2B, CDC7 and SIX1/SIX6 with the endophenotypes of primary open angle glaucoma in Indian population. PLoS One 10, e0119703.

Quigley, H.A., and Broman, A.T. (2006). The number of people with glaucoma worldwide in 2010 and 2020. Br J Ophthalmol 90, 262–267.

Ramdas, W.D., van Koolwijk, L.M., Ikram, M.K., Jansonius, N.M., de Jong, P.T., Bergen, A.A., Isaacs, A., Amin, N., Aulchenko, Y.S., Wolfs, R.C., Hofman, A., Rivadeneira, F., Oostra, B.A., Uitterlinden, A.G., Hysi, P., Hammond, C.J., Lemij, H.G., Vingerling, J.R., Klaver, C.C., and van Duijn, C.M. (2010). A genome-wide association study of optic disc parameters. PLoS Genet 6, e1000978.

Ramdas, W.D., van Koolwijk, L.M., Lemij, H.G., Pasutto, F., Cree, A.J., Thorleifsson, G., Janssen, S.F., Jacoline, T.B., Amin, N., Rivadeneira, F., Wolfs, R.C., Walters, G.B., Jonasson, F., Weisschuh, N., Mardin, C.Y., Gibson, J., Zegers, R.H., Hofman, A., de Jong, P.T., Uitterlinden, A.G., Oostra, B.A., Thorsteinsdottir, U., Gramer, E., Welgen-Lussen, U.C., Kirwan, J.F., Bergen, A.A., Reis, A., Stefansson, K., Lotery, A.J., Vingerling, J.R., Jansonius, N.M., Klaver, C.C., and van Duijn, C.M. (2011). Common genetic variants associated with open-angle glaucoma. Hum Mol Genet 20, 2464–2471.

Weinreb, R.N., and Khaw, P.T. (2004). Primary open-angle glaucoma. Lancet 363, 1711–1720.

Wiggs, J.L., Yaspan, B.L., Hauser, M.A., Kang, J.H., Allingham, R.R., Olson, L.M., Abdrabou, W., Fan, B.J., Wang, D.Y., Brodeur, W., Budenz, D.L., Caprioli, J., Crenshaw, A., Crooks, K., Delbono, E., Doheny, K.F., Friedman, D.S., Gaasterland, D., Gaasterland, T., Laurie, C., Lee, R.K., Lichter, P.R., Loomis, S., Liu, Y., Medeiros, F.A., McCarty, C., Mirel, D., Moroi, S.E., Musch, D.C., Realini, A., Rozsa, F.W., Schuman, J.S., Scott, K., Singh, K., Stein, J.D., Trager, E.H., Vanveldhuisen, P., Vollrath, D., Wollstein, G., Yoneyama, S., Zhang, K., Weinreb, R.N., Ernst, J., Kellis, M., Masuda, T., Zack, D., Richards, J.E., Pericak-Vance, M., Pasquale, L.R., and Haines, J.L. (2012). Common variants at 9p21 and 8q22 are associated with increased susceptibility to optic nerve degeneration in glaucoma. PLoS Genet 8, e1002654.

Williams, S.E., Carmichael, T.R., Allingham, R.R., Hauser, M., and Ramsay, M. (2015). The genetics of POAG in black South Africans: a candidate gene association study. Sci Rep 5, 8378.