Genetic Variants Associated With Different Risks for High Tension Glaucoma and Normal Tension Glaucoma in a Chinese Population

Yuhong Chen,[1] Guy Hughes,[2] Xueli Chen,[1] Shaohong Qian,[1] Wenjun Cao,[3] Li Wang,[1] Min Wang,[1] and Xinghuai Sun[1,4]

[1]Department of Ophthalmology and Vision Science, Shanghai Medical College, Eye and Ear Nose Throat Hospital, Fudan University, Shanghai, China
[2]University of California Irvine School of Medicine, Irvine, California, United States
[3]Department of Clinical Laboratory, Eye and Ear Nose Throat Hospital, Fudan University, Shanghai, China
[4]State Key Laboratory of Medical Neurobiology, Institutes of Brain Science, Fudan University, Shanghai, China

Correspondence: Xinghuai Sun, Department of Ophthalmology and Vision Science, Shanghai Medical College, Eye and Ear Nose Throat Hospital, Fudan University, 83 Fenyang Road, Shanghai, China, 200031;
xhsun@shmu.edu.cn.

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Purpose. We investigated the association of genetic factors with primary open angle glaucoma (POAG), including high tension glaucoma (HTG) and normal tension glaucoma (NTG), in a Han Chinese population.

Methods. We recruited 1157 POAG cases, including 860 HTG and 297 NTG, and 934 normal controls. A total of 13 previously reported single nucleotide polymorphisms (SNPs) located at four gene regions (*TMCO1, CDKN2B-AS1, ATOH7*, and *SIX1/SIX6*) was genotyped. Distributions of allele frequencies were compared between cases and controls as well as in the HTG and NTG subgroups. The IOP, vertical cup-to-disc ratio (VCDR), central corneal thickness (CCT), axial length (AL), and age at diagnosis also were investigated as quantitative phenotypes with genotypes of these SNPs.

Results. The SNPs rs4656461 and rs7555523 at *TMCO1*, rs523096 and rs2157719 at *CDKN2B-AS1*, as well as rs33912345 and rs10483727 at *SIX1/SIX6* showed statistically significant association with POAG. The SNPs at *ATOH7* did not show statistically significant association with POAG in our dataset. In the subgroup analysis of HTG and NTG, multiple variants at *CDKN2B-AS1* and *SIX1/SIX6* showed stronger association with NTG than HTG. The SNPs rs523096 and rs2157719 at *CDKN2B-AS1* as well as rs33912345 and rs10483727 at *SIX1/SIX6* were found to be associated with IOP where the minor alleles were associated with an increase in IOP. In contrast, SNPs at *TMCO1* showed significant association with HTG only.

Conclusions. Genetic variants in *CDKN2B-AS1*, *SIX1/SIX6*, and *TMCO1* were associated with POAG in a Han Chinese population. Genes *CDKN2B-AS1* and *SIX1/SIX6* seem to harbor a tendency toward POAG with lower IOP, while carriers of risk alleles at *TMCO1* seem to be predisposed to developing POAG with higher IOP.

Keywords: genetics, glaucoma, POAG, HTG, NTG

Primary open angle glaucoma (POAG) is the most common type of glaucoma and China will have roughly 20% of the patients worldwide by 2020.[1] The condition of POAG is characterized by progressive retinal ganglion cell death with corresponding visual field loss. Elevated IOP is a major risk factor for POAG, but not a necessary criterion for diagnosis, and thus, POAG can be clinically classified into two subgroups: high tension glaucoma (HTG), in which elevated IOP is a major feature, and normal tension glaucoma (NTG), in which IOP does not rise outside of the normal range.

Genetics have been shown to have an important role in the pathogenesis of POAG, a genetically heterogeneous disorder. Except for a small proportion of glaucoma patients showing classical Mendelian inheritance patterns, most cases are sporadic and present in the common complex form. Increasingly, genome-wide association studies (GWAS) have been applied to investigate the molecular basis of this polygenic disease. These studies have successfully identified genetic associations that are significant at the genome-wide level, elucidating multiple genes involved in the common complex forms of POAG, including *CAV1/CAV2* (caveolin 1/caveolin 2),[2] *ATOH7* (atonal homolog 7),[3] *TMCO1* (transmembrane and coiled-coil domains 1),[4,5] *CDKN2B-AS1* (cyclin-dependent kinase inhibitor 2B antisense RNA 1),[4,5] and *SIX1/SIX6* (sin oculis homeobox 1/sin oculis homeobox 6).[5] A GWAS in Australians of European descent identified susceptibility loci at *TMCO1* and *CDKN2B-AS1* that contributed to severe forms of glaucoma.[4] Wiggs et al.[5] also found associations between the *CDKN2B-AS1* region, the *SIX1/SIX6* region, and POAG, specifically with respect to NTG. Ramdas et al.[3] used meta-analysis data from six separate studies to find significant evidence that common variants of *CDKN2B* (rs1063192), *ATOH7* (rs190004), and *SIX1* (rs10483727) are associated with POAG. In addition, a GWAS performed in an Icelandic population identified significant associations between POAG and single nucleotide polymorphisms (SNPs) close to the caveolin 1 (*CAV1*) and caveolin 2 (*CAV2*) genes on chromosome

7q31.[2] All of these selected genes primarily point to pathways involved in optic nerve development and retinal ganglion cell (RGC) apoptosis. For instance, *ATOH7* is known to have a key role in RCG formation,[6] *SIX1/SIX6* are transcription factors involved in eye development,[7,8] and *CDKN2B-AS1* codes for an antisense RNA that regulates the expression of *CDKN2B*, which in turn regulates cell cycle maintenance and apoptosis of RGCs.[5,9] The *TMCO1* gene encodes a transmembrane protein with a coiled-coil domain that may localize to the Golgi apparatus and endoplasmic reticulum or to the mitochondria in different cell types, also with a proposed role in apoptosis.[4,10] The *CAV1* and *CAV2* are members of the caveolin gene family which regulate adult neural stem cell proliferation, and *CAV1* has been shown to be involved in both nitric oxide and TGF-β signaling, which have been implicated as culprits in the pathogenesis of POAG.[2]

Multiple studies have confirmed or replicated these genetic associations in populations from Europe,[11–14] the United States,[13,15–17] Japan,[18–21] and Africa.[22,23] However, these loci have not been validated in a large sample size Han Chinese population. Genetic associations generally are more biologically meaningful if they are replicated across different ethnic groups and this is essential for establishing the credibility of a genotype-phenotype association. In this study, we aimed to replicate previously reported SNPs in POAG in a large sample size Han Chinese population and investigate their association predilection toward two major subphenotypes of POAG.

Materials and Methods

Patients

The study was approved by the ethical committee of Eye and ENT hospital, Fudan University, and all procedures were conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from each individual.

The samples used in the study were collected in the Eye and ENT hospital. Each participant underwent a complete eye examination including best corrected visual acuity, slit-lamp examination of the anterior chamber, measurement of IOP, and fundus examination. Central corneal thickness (CCT), axial length (AL), gonioscopy, and visual field (VF) were examined if POAG was suspected.

Subjects with POAG were unrelated and met the following inclusion criteria[22]: glaucomatous optic neuropathy in at least one eye and VF loss consistent with optic nerve damage in at least one eye. Glaucomatous optic neuropathy was defined as a vertical cup-to-disc ratio (VCDR) greater than 0.7 or focal loss of the nerve fiber layer resulting in a notch in the neuroretinal rim, associated with a glaucomatous VF defect and open angles detected during a gonioscopic examination. The exclusion criteria for POAG subjects included the diagnosis or history of any secondary glaucoma, history of ocular trauma, or significant use of systemic or ocular glucocorticoids. Patients from pedigrees with glaucoma inherited as a Mendelian trait also were excluded from this study. The POAG cases were further stratified into HTG or NTG based on maximum recorded IOP >21 mm Hg or ≤ 21 mm Hg, respectively. The examined control subjects were unrelated and met the following criteria: no known first degree relative with glaucoma, IOP less than 21 mm Hg in both eyes without treatment, and no evidence of glaucomatous optic neuropathy in either eye.

Methods

Genomic DNA was extracted from leukocytes of the peripheral blood for each participant. It was purified by the Qiagen QIAmp Blood Kit (Qiagen, Hilden, Germany). A total of 13 previously reported SNPs was chosen for validation, including rs4656461 and rs7555523 at *TMCO1*; rs1063192, rs523096, rs7049105, rs2157719, rs4977756, and rs10116277 at *CDKN2B-AS1*; rs7916697, rs1900004, and rs3858145 at *ATOH7*; as well as rs33912345 and rs10483727 at *SIX1/SIX6*. The SNP rs4236601 in the *CAV1/CAV2* region has shown a monomorphic minor allele frequency in Asian populations including those of Chinese[2] and Japanese ancestry.[20] Additionally, rs4236601 has failed validation in some replication studies.[23,24] Thus, it was not included in this study as it is less likely to affect POAG risk to a great extent in the Han Chinese population.

The SNP genotyping was performed using iPLEX Gold chemistry on the MassARRAY system (Sequenom, Inc., San Diego, CA, USA) by means of matrix assisted laser desorption ionization time-of-flight mass spectrometry method (MALDI-TOF) according to the manufacturer's instructions. Genotype calling was performed in real time with MassARRAY RT software version 3.0.0.4 and analyzed using the MassARRAY Typer software version 3.4 (Sequenom, Inc.). Each SNP with call rate greater than 95% was analyzed in the next step.

Genotype and allele frequencies were calculated for each SNP. All genotyping results were screened for deviations from Hardy–Weinberg equilibrium (HWE; $P > 0.01$). Association analyses were conducted using PLINK (1.07). Logistic regression was used to calculate odds ratios (OR) with 95% confidence intervals (CI) and adjusted for age and sex. Each SNP was assessed for association with each quantitative trait, including age at diagnosis, CCT, IOP, AL, and VCDR, using linear regression under an additive genetic model. Continuous variables were expressed as mean ± SD and compared across groups using a Student's *t*-test. Correction for multiple comparisons was done using a Bonferroni correction, generating a required *P* value of 0.004 to account for testing 13 SNPs. The analysis of CCT and AL was performed using the averaged data from both eyes. The analysis of IOP and VCDR was performed using the greater values between eyes. The IOP with correction for CCT was calculated using the Kohlhaas method,[25] as $\Delta\text{IOP} = (-0.0423 \times \text{CCT}) + 23.28$.

Results

We enrolled 1157 POAG cases and 934 normal controls were enrolled in this study. As expected, when compared to the normal population, POAG cases had elevated IOP and larger VCDR. The average age of POAG cases was 48.81 years and that of controls was 53.37 years. Of the cases 32.24% were female, while 59.74% of the controls were female. Age and sex were corrected for in all of the subsequent analyses. The case group contained 860 HTG and 297 NTG. Approximately 74.33% of the cohort had a highest recorded IOP more than 21 mm Hg and the mean (±SD) highest recorded IOP was 30.10 ± 11.37 mm Hg for all POAG patients, 33.75 ± 10.60 mm Hg for HTG patients, and 18.47 ± 2.22 mm Hg for NTG patients. Demographic and other phenotypic information is provided in Tables 1 and 2.

All SNPs passed quality control and genotyping efficiency criteria (>95% with all the samples) and were in HWE in cases and controls ($P > 0.01$). Allele frequencies and *P* values for the 13 SNPs for POAG patients and control subjects are shown in Table 3. In the full case control dataset, we observed significant association of POAG with rs4656461 ($P = 0.002$, OR = 3.18 [1.52–6.65]) and rs7555523 ($P = 0.003$, OR = 3.31 [1.52–7.20]) at the *TMCO1* region, rs1063192 ($P = 0.047$, OR = 0.85 [0.72–1.00]), rs523096 (5.833×10^{-5}, OR = 0.65 [0.53–0.80]), rs7049105 (0.006, OR = 0.82 [0.71–0.94]), and rs2157719 ($P =$

Table 1. Demographic and Clinical Features for POAG Cases and Controls

	POAG	Controls
Number	1157	934
Female %*	32.24%	59.74%
Age, y*	48.81 ± 16.28	53.37 ± 14.83
Maximum IOP, mm Hg*	30.10 ± 11.37	14.85 ± 2.84
VCDR*	0.85 ± 0.12	0.34 ± 0.11

* Indicates statistically significant difference ($P < 0.05$) between cases and controls.

3.528×10^{-5}, OR = 0.64 [0.52–0.79]) at the *CDKN2B-AS1* region, and rs33912345 ($P = 5.503 \times 10^{-4}$, OR = 0.75 [0.64–0.88]) and rs10483727 ($P = 0.003$, OR = 0.78 [0.67–0.92]) at the *SIX1/SIX6* region. Among them, rs4656461 and rs7555523 at *TMCO1*, rs2157719 and rs523096 at *CDKN2B-AS1*, and rs33912345 and rs10483727 at *SIX1/SIX6* remained significant after Bonferroni correction for multiple testing. The minor alleles for SNPs at *CDKN2B-AS1* and *SIX1/SIX6* showed protective effects with lower frequencies in cases than controls. Low minor allele frequencies of the 2 SNPs (rs4656461 and rs7555523) at *TMCO1* were detected, with both at a frequency less than 2% in cases versus 0.5% in controls (Table 3).

Next, we stratified the cases by IOP into HTG and NTG. In the HTG subgroup, we identified significant association with SNPs rs4656461 ($P = 8.465 \times 10^{-4}$, OR = 3.70 [1.72–7.97]) and rs7555523 ($P = 0.001$, OR = 3.85 [1.72–8.62]) at *TMCO1*, rs523096 ($P = 0.002$, OR = 0.70 [0.56–0.87]), rs7049105 ($P = 0.029$, OR = 0.84 (0.72–0.98]), and rs2157719 ($P = 0.002$, OR = 0.70 [0.56–0.88]) at *CDKN2B-AS1*, as well as rs33912345 ($P = 0.015$, OR = 0.80 (0.68–0.96]) and rs10483727 ($P = 0.046$, OR = 0.84 (0.71–1.00]) at *SIX1/SIX6*. Only rs4656461 and rs7555523 at *TMCO1* as well as rs523096 and rs2157719 at *CDKN2B-AS1* survived correction for multiple testing (Table 4). In the NTG subgroup, rs523096 ($P = 1.765 \times 10^{-4}$, OR = 0.52 (0.36–0.73]) and rs2157719 ($P = 3.445 \times 10^{-5}$, OR = 0.47 [0.33–0.67]) at *CDKN2B-AS1* as well as rs33912345 ($P = 4.288 \times 10^{-4}$, OR = 0.64 (0.50–0.82]) and rs10483727 ($P = 8.518 \times 10^{-4}$, OR = 0.66 (0.51–0.84]) at *SIX1/SIX6* showed stronger association with lower *P* values than those found in the comparison of HTG and normal controls. All four SNPs survived correction for multiple testing (Table 4). The SNPs at *TMCO1* did not exhibit statistical significance in the comparison of NTG versus controls.

In the case cohort, association analysis was conducted for all 13 SNPs with the 5 quantitative traits: age at diagnosis, IOP, CCT, AL and VCDR. Only IOP showed statistically significant association with multiple SNPs in *CDKN2B-AS1* (rs523096 [$P = 0.001$], rs2157719 [$P = 0.002$]) and *SIX1/SIX6* (rs33912345 [$P = 0.002$], rs10483727 [$P = 0.002$]) after adjustment for age and sex (Table 5) and remained significant after Bonferroni correction. At all associated SNPs the glaucoma risk alleles were associated with a lower IOP. No significant associations were observed for age at diagnosis, CCT, VCDR, and AL.

To exclude the potential confounding effect of CCT on IOP measurement, the IOP also was analyzed after correction for CCT, using the same method as Kohlhaas.[25] The mean uncorrected IOP (30.10 ± 11.36 mm Hg) and corrected IOP (30.48 ± 11.16 mm Hg) were found to be very close. According to the definition used in our study, after IOP correction, the number of HTG patients and NTG patients would be reclassified to 919 and 238, respectively, from the initial 860 and 297. However, the association results showed almost no change before and after correction when comparing HTG or NTG versus controls. The *P* values of SNPs rs33912345 and rs10483727 at *SIX1/SIX6* after correction for CCT were one order of magnitude lower than those without correction in the quantitative analysis of IOP (Supplementary Tables S1, S2). A high similarity between *P* values also was acquired when doing linear regression analysis of maximum IOP after adjusting for CCT (Supplementary Table S3).

Table 2. Clinical Characteristics of HTG and NTG

	HTG, $n = 860$	NTG, $n = 297$
Female %*	29.77%	39.53%
Age at diagnosis	42.14 ± 16.49	49.92 ± 14.41
Maximum recorded IOP*	33.75 ± 10.60	18.47 ± 2.22
CCT *	545.04 ± 35.52	528.37 ± 34.99
AL	24.93 ± 1.70	24.87 ± 1.62
Max VCDR	0.85 ± 0.12	0.83 ± 0.11

* Indicates statistically significant difference ($P < 0.05$) between HTG and NTG.

Discussion

In this study, we replicated the association of POAG with 4 identified loci including *TMCO1*, *CDKN2B-AS1*, *ATOH7*, and *SIX1/SIX6* in a Chinese Han population. The SNPs rs4656461 and rs7555523 at *TMCO1*, rs523096 and rs2157719 at *CDKN2B-AS1*, as well as rs33912345 and rs10483727 at *SIX1/SIX6* were validated, exhibiting association with POAG in our cohorts. The minor alleles for SNPs at *CDKN2B-AS1* and *SIX1/SIX6* demonstrated protective effects for POAG. After stratification by IOP, the association of rs523096 and rs2157719 at *CDKN2B-AS1* remained significant in the HTG and NTG subgroups; however, they showed stronger association in the NTG subgroup. The two SNPs at *SIX1/SIX6* showed significant association in the HTG subgroup, but only marginal association in the HTG subgroup. In contrast, the SNPs at *TMCO1* showed statistically significant association only in the HTG subgroup and not in the NTG subgroup.

The most significant region to be identified so far by independent research groups as having an association with POAG in different ethnic populations is the *CDKN2B-AS1* region on chromosome 9p21.[3–5,13,19–21,23,26,27] This association was confirmed by our study in a Han Chinese population as well. Several studies so far have reported the association of *CDKN2B-AS1* as restricted to the NTG subgroup and not present in the HTG subpopulation when stratified by IOP.[5,18,19,21] In our results, we see significant association with *CDKN2B-AS1* SNPs in the full POAG cohort and in both the NTG and HTG subgroups. However, our results showed stronger association with the NTG subgroup than the HTG subgroup, which is consistent with previous studies by Burdon et al.[26] Although a number of studies report rs1063192 as the most significant SNP in the *CDKN2B-AS1* region, in our cohorts, rs523096 yielded the strongest association signals among all SNPs at *CDKN2B-AS1*, a finding consistent with a Japanese NTG GWAS.[19,21]

The *SIX1/SIX6* region was first reported to be associated with an increased VCDR,[28] implying an increased risk for POAG. Then, the SNP rs10483727 was observed to actually infer risk for POAG.[3,5,13,15,20] Later, exome sequencing of four independent population-based cohorts in the Netherlands revealed another variant, rs33912345 (His141Asn) in *SIX6*, associated with POAG.[11] In our study, we also have identified a strong association of these two SNPs (rs10483727, rs33912345) at *SIX1/SIX6* with POAG in our full dataset and in the NTG subgroup; there is only mild association in our HTG subgroup. Our results demonstrated that *SIX1/SIX6* may

Table 3. Allele Frequencies for 13 SNPs and Association With POAG Adjusted for Age and Sex

Gene	CHR	SNP	BP	MA	MAF_Case	MAF_Control	OR	*P*
TMCO1	1	rs4656461	165687205	G	0.018	0.005	3.18 (1.52–6.65)	0.002
TMCO1	1	rs7555523	165718979	C	0.016	0.005	3.31 (1.52–7.20)	0.003
CDKN2B-AS1	9	rs1063192	22003367	C	0.184	0.204	0.85 (0.72–1.00)	0.047
CDKN2B-AS1	9	rs523096	22019129	C	0.095	0.135	0.65 (0.53–0.80)	5.833×10^{-5}
CDKN2B-AS1	9	rs7049105	22028801	A	0.319	0.361	0.82 (0.71–0.94)	0.006
CDKN2B-AS1	9	rs2157719	22033366	G	0.092	0.133	0.64 (0.52–0.79)	3.528×10^{-5}
CDKN2B-AS1	9	rs4977756	22068652	G	0.214	0.227	0.89 (0.76–1.04)	0.128
CDKN2B-AS1	9	rs10116277	22081397	G	0.302	0.311	0.95 (0.82–1.09)	0.454
ATOH7	10	rs7916697	69991853	A	0.388	0.391	0.97 (0.85–1.11)	0.661
ATOH7	10	rs1900004	70000881	A	0.389	0.391	0.97 (0.84–1.11)	0.651
ATOH7	10	rs3858145	70011838	G	0.385	0.393	0.95 (0.83–1.09)	0.445
SIX1/SIX6	14	rs33912345	60976537	A	0.201	0.251	0.75 (0.64–0.88)	5.503×10^{-4}
SIX1/SIX6	14	rs10483727	61072875	C	0.209	0.254	0.78 (0.67–0.92)	0.003

The BP position of each SNP was in reference to NCBI build 37.5. Chr, Chromosome; BP, base pair position; MA, minor allele; MAF, minor allele frequency; MAF_case, minor allele frequencies in cases; MAF_control, minor allele frequencies in controls.

influence the phenotypic features in patients with NTG more than HTG.

The IOP is a well-established risk factor for glaucoma and an important inheritable endophenotype in glaucoma.[29,30] Multiple SNPs at *CDKN2B-AS1* (rs523096, rs2157719) and *SIX1/SIX6* (rs33912345, rs10483727) also exhibited association with IOP. Consistent with previous studies,[26] the minor alleles, which also were the protective alleles, were associated with higher IOP. Thus, the SNPs at *CDKN2B-AS1* and *SIX1/SIX6* were associated with IOP as a quantitative trait and as a dichotomous variable. The enrichment of the risk alleles in patients with glaucoma, but with IOP in the normal range suggests that in patients with these particular risk alleles, glaucoma is more likely to develop in the absence of elevated IOP. This is consistent with our findings of a stronger association between these two genes and NTG than with HTG.

Additionally, it has been demonstrated that *CDKN2B-AS1* and *SIX1/SIX6* SNPs contribute to variation in VCDR in normal individuals[11,18,28] and in POAG patients.[15,17,26] However, VCDR showed no association with SNPs at any of the four loci in our dataset. It is possible that because VCDR is a diagnostic feature of POAG and was used as an inclusion criterion for the definition of glaucoma, the sample was skewed toward the top end of the normal distribution for this phenotype, and this would decrease the effective power. When the controls with recorded measurements of VCDR were added to the analysis to test this potential bias, the SNPs at both *CDKN2B-AS1* (rs523096, rs2157719) and *SIX1/SIX6* (rs33912345, rs10483727) did reach statistical significance (Supplementary Table S4).

The CCT is well known to confound the measurement of IOP and significantly influence IOP readings obtained by applanation tonometry.[25] Although the influence of CCT on an individual's IOP is controversial, designating patients as elevated or normal IOP after CCT correction might provide a more accurate dichotomy between the arbitrary distinction of NTG and HTG. In this study, we used the method of Kohlhaas et al.[25] to correct IOP due to the relatively conservative adjustment to the IOP and the similarity to several other metrics assessed by Brandt et al.[31] Consistent with results from Burdon et al.,[26] the correction of IOP had little to no effect on the association statistics, before and after adjustment for other covariates (Supplementary Tables S1, S2). Only during the quantitative trait analysis did the Kohlhaas-corrected IOP produce *P* values one order of magnitude more significant than the uncorrected IOP analysis for SNPs at *SIX1/SIX6* (Supplementary Table S3). The SNPs at the *CDKN2B-AS1* and *SIX1/SIX6* loci were associated both with IOP as a quantitative trait and as a dichotomous variable, with and without correction for CCT. Although CCT is among the strongest independent predictors for the development of POAG,[31] none of the genotyped SNPs in our study yielded statistically significant association with CCT.

Table 4. Association Results in Comparison of HTG and NTG Patients Versus Controls Adjusted for Age and Sex

				HTG			NTG		
Gene	SNP	MA	MAF Control	MAF_Case	OR (95%CI)	*P*	MAF_Case	OR (95%CI)	*P*
TMCO1	rs4656461	G	0.005	0.020	3.70 (1.72–7.97)	8.465×10^{-4}	0.014	2.55 (0.96–6.80)	0.062
TMCO1	rs7555523	C	0.005	0.018	3.85 (1.72–8.62)	0.001	0.012	2.61 (0.92–7.35)	0.071
CDKN2B-AS1	rs1063192	C	0.204	0.186	0.88 (0.73–1.05)	0.146	0.177	0.79 (0.61–1.01)	0.063
CDKN2B-AS1	rs523096	C	0.135	0.101	0.70 (0.56–0.87)	0.002	0.078	0.52 (0.36–0.73)	1.765×10^{-4}
CDKN2B-AS1	rs7049105	A	0.361	0.320	0.84 (0.72–0.98)	0.029	0.314	0.79 (0.64–0.98)	0.033
CDKN2B-AS1	rs2157719	G	0.133	0.099	0.70 (0.56–0.88)	0.002	0.071	0.47 (0.33–0.67)	3.445×10^{-5}
CDKN2B-AS1	rs4977756	G	0.227	0.213	0.90 (0.76–1.07)	0.235	0.218	0.88 (0.69–1.11)	0.280
CDKN2B-AS1	rs10116277	G	0.311	0.306	0.98 (0.84–1.15)	0.818	0.289	0.87 (0.70–1.08)	0.200
ATOH7	rs7916697	A	0.391	0.398	1.02 (0.88–1.18)	0.797	0.356	0.83 (0.68–1.02)	0.075
ATOH7	rs1900004	A	0.391	0.399	1.02 (0.88–1.19)	0.784	0.356	0.83 (0.68–1.02)	0.073
ATOH7	rs3858145	G	0.393	0.397	1.00 (0.86–1.16)	0.998	0.350	0.79 (0.65–0.98)	0.029
SIX1/SIX6	rs33912345	A	0.251	0.210	0.80 (0.68–0.96)	0.015	0.176	0.64 (0.50–0.82)	4.288×10^{-4}
SIX1/SIX6	rs10483727	C	0.254	0.219	0.84 (0.71–1.00)	0.046	0.181	0.66 (0.51–0.84)	8.518×10^{-4}

Table 5. Linear Regression Analysis of Maximum IOP

Gene	SNP	MA	IOP	
			β	P
TMCO1	rs4656461	G	−0.153	0.927
TMCO1	rs7555523	C	0.622	0.723
CDKN2B-AS1	rs1063192	C	1.387	0.016
CDKN2B-AS1	rs523096	C	2.400	0.001
CDKN2B-AS1	rs7049105	A	0.538	0.265
CDKN2B-AS1	rs2157719	G	2.367	0.002
CDKN2B-AS1	rs4977756	G	1.118	0.041
CDKN2B-AS1	rs10116277	G	0.965	0.056
ATOH7	rs7916697	A	0.391	0.407
ATOH7	rs1900004	A	0.333	0.481
ATOH7	rs3858145	G	0.318	0.501
SIX1/SIX6	rs33912345	A	1.748	0.002
SIX1/SIX6	rs10483727	C	1.742	0.002

The SNP rs4656461 was the initial SNP at *TMCO1* reported to be associated with POAG.[4,14] Later, another SNP at *TMCO1*, rs7555523, also was found to be related to increased IOP in a Caucasian population.[12] In our study, although *TMCO1* loci showed no association with IOP or the subgroup of NTG patients, statistically significant association was observed clearly in the HTG subgroup and also in the full dataset of POAG. This leads us to believe that *TMCO1* may contribute to POAG through the pathway of IOP elevation. However, similar to *CAV1/CAV2*, due to the very low minor allele frequencies of SNPs in *TMCO1*, this gene may have limited effect on POAG risk in a population of Chinese Han when compared to Caucasians.

The *ATOH7* (rs190004) gene has been reported to be strongly associated with optic disc area and VCDR in a GWAS involving Australian and U.K. cohorts.[32] It then was replicated in a GWAS in The Netherlands and a GWAS in Asia.[28,33] Additionally, it was reported to be suggestively associated with POAG in Caucasians[3]; however, in this study, it was neither recognized to be associated with VCDR nor detected in be associated with POAG, only observed as marginally associated with NTG. Similar results also have been seen in previous reports,[18,34] and may result from the different methods applied for the VCDR measurement. In our study, the VCDR was estimated by using a 90-diopter lens, instead of being measured on the stereoscopic fundus photo. The inconsistent results also may be due to the genetic heterogeneity between ethnic groups. Interestingly, in the analysis of NTG cases versus controls, the 3 SNPs at the *ATOH7* region yielded marginal *P* values, ranging from 0.02 to 0.07, compared to no difference seen in the analysis of HTG cases versus controls. This result suggests that *ATOH7* still could be mildly related to POAG as a non-IOP related genetic factor.

A variety of genetic factors contribute to optic neuropathy in POAG and can be classified into two main types: high-IOP–related and non-IOP–related (RGC vulnerability–related) genetic factors. It is presumed that non–IOP-related genetic factors would predominate in patients with NTG, whereas high-IOP–related genetic factors would predominate in patients with HTG.[18] Our results indicated that *CDKN2B-AS1* and *SIX1/SIX6* may contribute to glaucomatous optic neuropathy as non–IOP-related genetic factors because these loci showed much stronger association with NTG than HTG. In contrast, *TMCO1*, together with recently published gene *ABCA1*,[35–37] may contribute to glaucoma as high-IOP–related genetic factors. Furthermore, it is possible that *CDKN2B-AS1* SNPs are involved in IOP- and non-IOP–related pathological pathways, because it showed association with NTG and HTG phenotypes. This also may indicate that non-IOP–related factors (RGC vulnerability factors) have a potential role in the pathogenesis of HTG as well.[38]

Our findings suggested similar genetic associations to those initially found in Caucasian populations; *TMCO1*, *CDKN2B-AS1*, and *SIX1/SIX6* showed association with POAG in a Han Chinese population, while *CDKN2B-AS1* and *SIX1/SIX6* loci harbor a tendency toward association with NTG compared to HTG. In contrast, *TMCO1* loci harbor a tendency toward association with HTG compared to NTG. Clarification of the different genetic factors and pathophysiological pathways causing each subtype of glaucoma may contribute to understanding the pathogenesis of the disease.

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