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Original Article

Association between SOD2 rs6917589, rs2842980, rs5746136 and rs4880 polymorphisms and primary open angle glaucoma in a northern Chinese population

Yanlai Zhang[1,2], Chengzhi Zhang[1], Siyuan Guan[1], Lin Zhou[1], Qi Li[1,2]

[1]The First Affiliated Hospital of Chongqing Medical University, Chongqing, China; [2]Chongqing Key Laboratory of Ophthalmology, Chongqing Eye Institute, Chongqing, China

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Abstract: We performed an analysis of the association between SOD2 polymorphisms and development of POAG in a Northern Chinese population. A total of 261 patients and 312 healthy controls were enrolled in our study. Genotyping of rs6917589, rs2842980, rs5746136 and rs4880 was done in a 384-well plate format on the sequenom MassARRAY platform (Sequenom, San Diego, USA). We found that individuals carrying the TT genotype of rs2842980 manifested an increased risk of POAG in comparison with those carrying the AA genotype (OR=3.30, 95% CI=1.83-5.98). In recessive model, we observed that the TT genotype of rs2842980 showed an increased risk of POAG compared with the AA+AT genotype (OR=3.17, 95% CI=1.83-5.49) in recessive model. Moreover, subjects with the AA genotype of rs5746136 presented a high risk of POAG than the GG genotype or G allele carriers in co-dominant (OR=1.95, 95% CI=1.09-3.49) and recessive models (OR=1.80, 95% CI=1.03-3.13). The haplotype analysis revealed that rs6917589 and rs4880 showed linkage disequilibrium (D'=0.59, r^2=0.019). The ATAT haplotype showed a decreased risk of a reduction risk in DTC risk (OR=0.55, 95% CI=0.35-0.85, P=0.01), while the GAGT haplotype indicated an elevated risk in POAG risk (OR=1.52; 95% CI=1.06-2.20; P=0.01). In conclusion, our study firstly suggests that SOD2 polymorphism plays an important role in the development of POAG in Northern Chinese population.

Keywords: SOD2, polymorphism, primary open angle glaucoma, Northern China

Introduction

Glaucoma is the main cause of irreversible blindness. It is estimated that about 90 million people suffer from glaucoma. Primary glaucoma affects about 60.5 million people in 2010, and it is forecasted that 79.6 million people would suffer from glaucoma and 11.2 million would be blindness due to this disease [1]. Primary open angle glaucoma (POAG) is the main type of glaucoma in all population worldwide, and patients with POAG usually show no obvious ocular abnormality. The real mechanisms leading to the development of POAG are not clearly understood. Some environmental factors are involved in the pathogenesis of POAG, including a higher age, males, refractive error and a first relative history of glaucoma [2-4]. However, not all individuals with hazardous factors of primary open angle glaucoma would develop this disease, which means some genetic and biological factors may contribute to its progression.

Oxidative stress reflects the imbalance of reactive oxygen species in human body, and it is involved in the development of POAG. As a second messenger, reactive oxygen species play an important role in regulating protein and participating in retinal ganglion cell death signaling pathway. The low activity of anti-oxidative enzymes and low level of molecular weight antioxidants reflect the oxidative stress [5-7]. Imbalance of oxidants and antioxidants in the aqueous humor causes an increase in the production of reactive oxygen species which may induce the trabecular meshwork damage, finally leading to the pathogenesis of POAG [8, 9]. Many antioxidant enzymes are found in aqueous humor, such as orgotein superoxide dis-

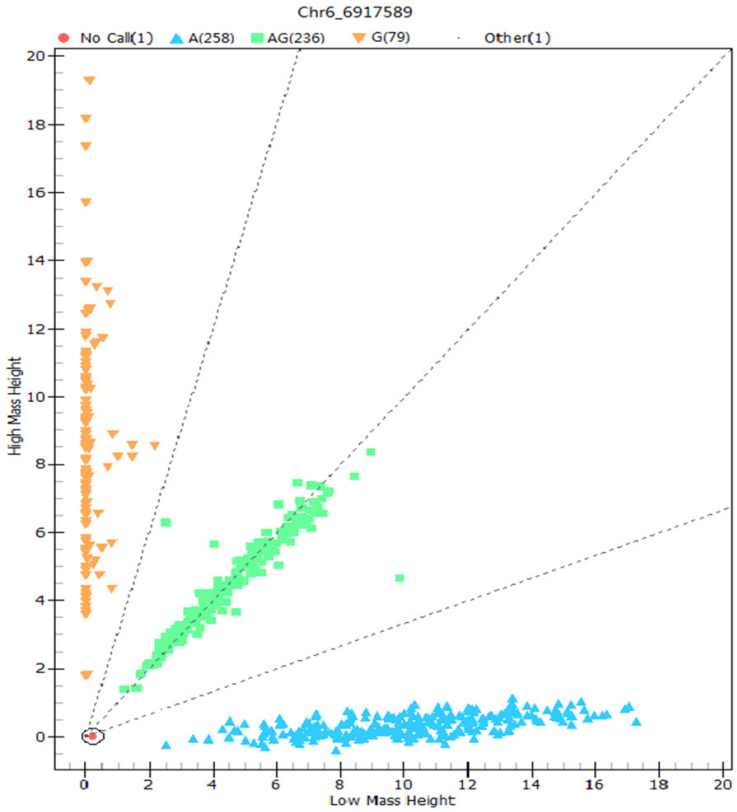

Figure 1. Scatter plot of SOD2 SNP rs6917589.

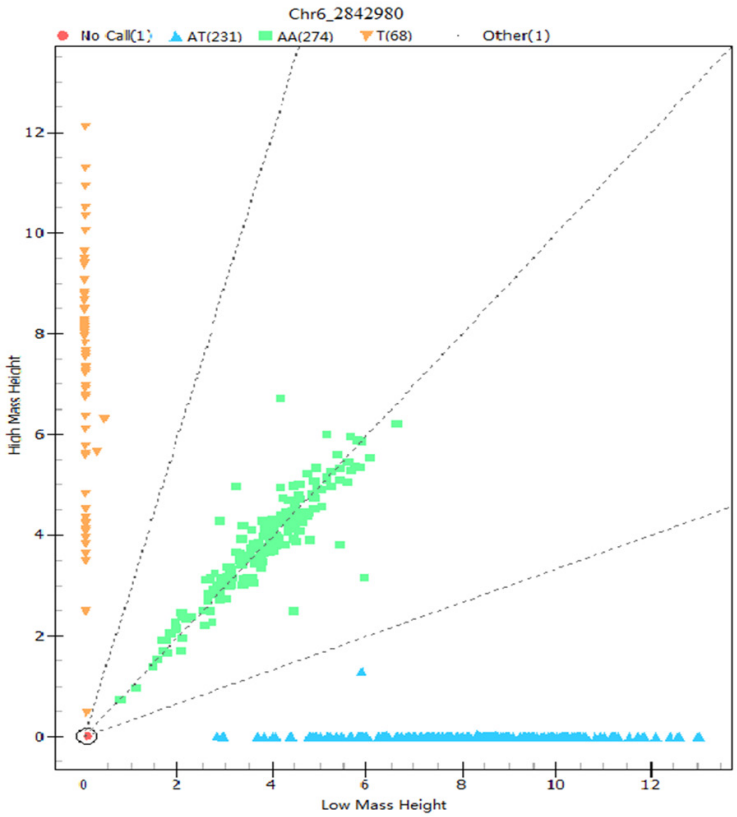

Figure 2. Scatter plot of SOD2 SNP rs2842980.

mutase (SOD), catalase (CAT) and glutathione peroxidase (GPX) [6, 10]. The expression of SOD2 is altered in aqueous humor of patients [10], and the activity of SOD is also low in serum of patients with POAG [11]. SOD2 encodes Mn-dependent superoxide dismutase and a kind of mitochondrial antioxdative enzymes, which is responsible of eliminating reactive oxygen species of mitochondrion and preventing oxidative stress response. Only two studies reported the role of SOD2 polymorphisms in the risk of POAG [12, 13]. Therefore, we firstly performed an analysis of the association between SOD2 polymorphisms and development of POAG in a Northern Chinese population.

Materials and methods

Subjects

A hospital-based case-control study was performed in this study. A total of 261 patients and 312 healthy controls were enrolled in our study. The diagnosis of POAG was according to the following criteria: (1) Intraocular pressure >22 mmHg or more in each eye without therapy; (2) Wide anterior chamber angle (Shaffer III or IV); (3) Glaucomatous optic nerve damage (cup-to-disc ratio >0.5, focal loss of the nerve fiber layer and firm nerve hemorrhage); (4) Visual field loss in line with optic nerve damage. The exclusion criteria for patients with POAG were congenital glaucoma, any secondary glaucoma such as exfoliation syndrome or a history of ocular trauma. These 261 patients were collected from The First Affiliated Hospital of Chongqing Medical University between January 2013 and December 2015.

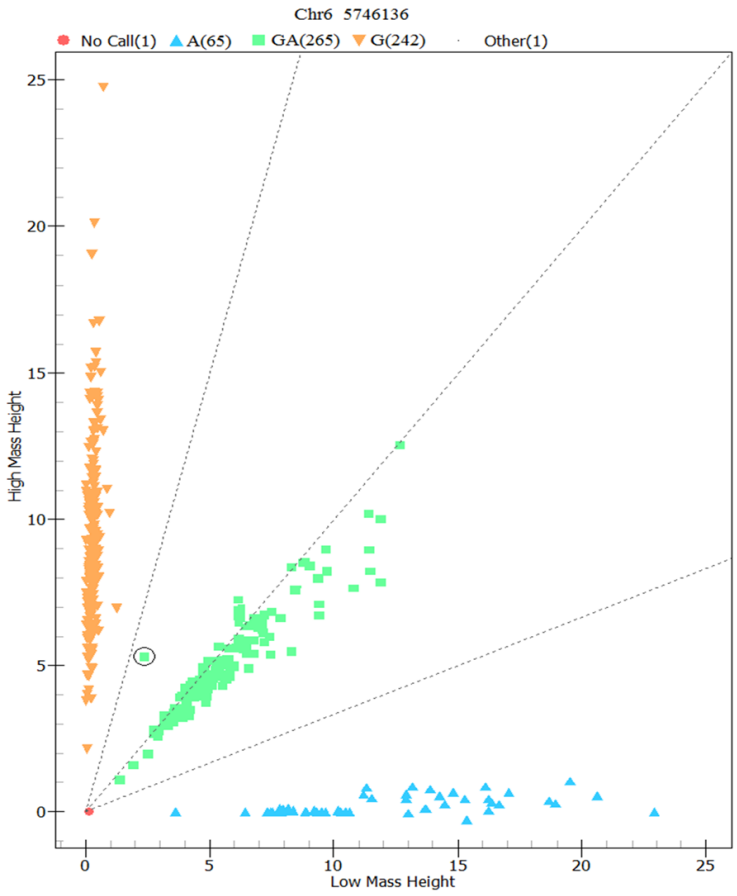

Figure 3. Scatter plot of SOD2 SNP rs5746136.

During the period mentioned above, the healthy controls were enrolled from individuals who received healthy examination in the Inner Mongolia Autonomous Region People's Hospital. All the controls were pronounced of congenital glaucoma and any secondary glaucoma. The average age of patients with POAG was 55.21±8.14 years (ranged 31 to 82 years). The average age of controls was 53.70±9.30 years (ranged 33-79 years). The mean intraocular pressures of patients with POAG and controls were 27.40±2.73 mmHg and 16.72±3.14 mmHg, respectively. The mean cup-to-disc ratio of patients with POAG and controls were 0.79±0.12 and 0.33±0.079, respectively.

The demographic and clinical characteristics of patients with POAG and controls were collected from the medical records. Performance of this study was authorized by the Hospital's Ethics Committee. Written informed consents were obtained from all participants.

DNA extraction and genotyping

Each patient was asked to provide a 3-mL peripheral venous blood sample before receiving any anti-cancer treatment. These samples were kept in tubes containing 0.5 M ethylenediaminetetraacetic acid. Genomic DNA was isolated from whole blood using a TIANamp Blood DNA Kit (Tiangen, Beijing, China) according to the manufacturer's instructions, and the DNA samples were stored in -20°C until using. We selected four potentially function SNPs in the SOD2, and the SNPs were selected based on population and MAF >5% using dbSNP (http://www.ncbi.nlm.nih.gov/projects/SNP). Four SNPs of SOD2 were selected for analysis, including rs6917589, rs2842980, rs5746136 and rs4880.

SNP genotyping was done in a 384-well plate format on the sequenom MassARRAY platform (Sequenom, San Diego, USA). Primers for polymerase chain reaction amplification and single base extension assays were designed by Sequenom Assay Design 3.1 software. The PCR reaction for genotyping SOD2 rs6917589, rs2842980, rs5746136 and rs4880 was performed in 5 μL, following by the SAP and iPLEX reaction. The samples are then desalted, dispensed to a SpectroCHIP and analyzed with MALDI-TOF MS (**Figures 1-4**).

Statistical analysis

Demographic and clinical data are reported as means ± standard deviations, or frequencies and percentages. Comparison of these data between groups was performed using the students test or chi-square test. Deviation from the Hardy-Weinberg equilibrium of SOD2 rs6917589, rs2842980 and rs5746136 in controls was analyzed by Pearson Chi-square (χ^2) test. Relationship between SOD2 rs6917589, rs2842980 and rs5746136 and risk of POAG was analyzed by multivariate logistic regression

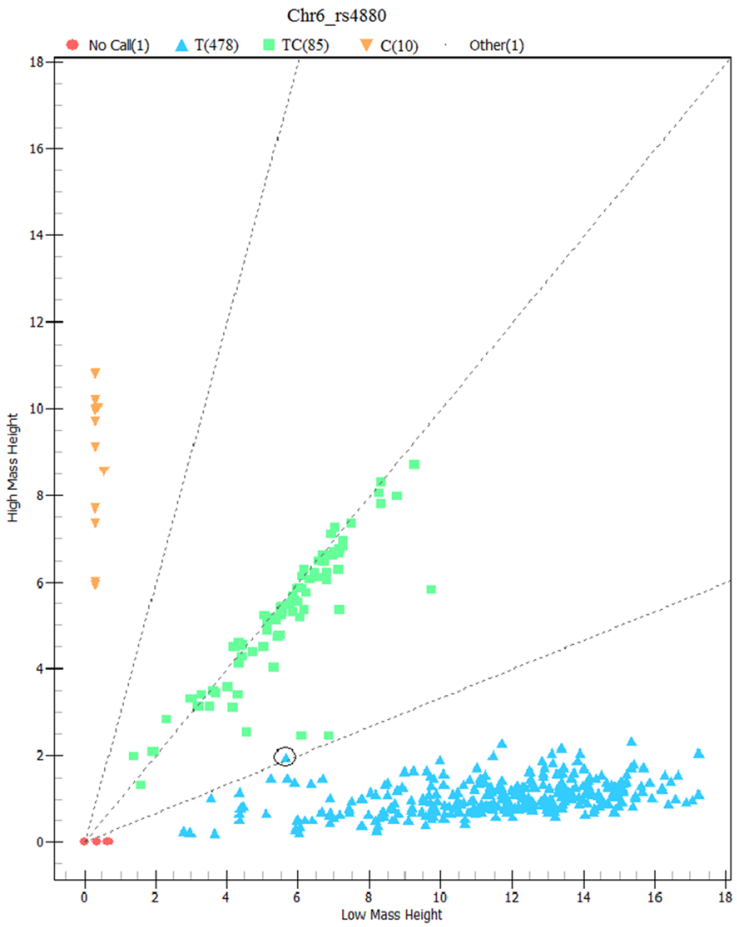

Figure 4. Scatter plot of SOD2 SNP rs4880.

analysis, with results being reported as odds ratios (ORs) and 95% confidence intervals (CIs). The linkage disequilibrium and haplotype analysis were analyzed by SHEsis software (http://analysis.bio-x.cn/myAnalysis.php) [14]. The statistical analysis was performed by IBM SPSS Statistics for Windows, Version 21.0. (Armonk, NY: IBM Corp). *P*-value <0.05 was considered to represent statistically significant differences.

Results

Comparison with those of the controls, patients with POAG had higher values of age (t=2.14, P=0.03), intraocular pressures (t=47.80, P< 0.001) and cup-to-disc ratio (t=54.64, P< 0.001), and had a family history of glaucoma (χ^2=17.95, P<0.001) (**Table 1**).

The chi-square test indicated significant differences between the patients with POAG and controls in terms of the genotype frequencies of SOD2 rs2842980 (χ^2=15.20, P<0.001) and rs5746136 (χ^2= 8.54, P=0.01), while no significant difference was seem in regard to rs6917589 (χ^2=1.24, P=0.54) and rs4880 (χ^2=0.98, P=0.61) (**Table 2**). The genotype distributions of SOD2 rs6917589, rs2842980 and rs5746136 were in line with the Hardy-Weinberg equilibrium in the controls (*P* value for HWE >0.05), whereas the rs4880 was not (*P* value for HWE=0.007).

The multivariate logistic regression analysis indicated that individuals carrying the TT genotype of rs2842980 manifested an increased risk of POAG in comparison with those carrying the AA genotype (OR=3.30, 95% CI=1.83-5.98) (**Table 3**). In recessive model, we observed that the TT genotype of rs2842980 showed an increased risk of POAG compared with the AA+AT genotype (OR=3.17, 95% CI=1.83-5.49) in recessive model. Moreover, subjects with the AA genotype of rs5746136 conferred a high risk of POAG than the GG genotype or G allele carriers in codominant (OR=1.95, 95% CI=1.09-3.49) and recessive models (OR=1.80, 95% CI=1.03-3.13). However, no significant association was found between SOD2 rs6917589 and rs4880 polymorphisms and risk of POAG.

The haplotype analysis revealed that rs6917589 and rs4880 showed linkage disequilibrium (D'=0.59, r^2=0.019) (**Figure 5**). Nine common haplotypes (frequency >0.03 in either the patients with POAG and controls has been selected) accounted for the main of the haplotypes in patients with POAG and controls (**Table 4**). The ATAT haplotype showed a reduction risk in DTC risk (OR=0.55, 95% CI= 0.35-0.85, P=0.01), while the GAGT haplotype was associated with an elevated risk in POAG risk (OR=1.52; 95% CI=1.06-2.20; P=0.01). However, the other seven haplotypes were not related to the risk of POAG.

Table 1. Demographic and clinical characteristics of patients with POAG and controls

Variables	Patients N=261	%	Controls N=312	%	t or χ^2 test	*P* value
Sex						
Female	140	53.64	150	48.08		
Male	121	46.36	162	51.92	1.76	0.19
Age	61.53±10.20		59.64±10.74		2.14	0.03
Family history of glaucoma						
No	238	91.19	308	98.72		
Yes	23	8.81	4	1.28	17.95	<0.001
Smoking						
No	166	63.60	219	70.19		
Yes	95	36.40	93	29.81	2.80	0.09
Intraocular pressures	27.34±2.40		16.58±2.95		47.31	<0.001
Cup-to-disc ratio	0.79±0.12		0.33±0.08		54.64	<0.001

Table 2. Distributions of SOD2 rs6917589, rs2842980, rs5746136 and rs4880 between the two study groups

Genes	Patients N=261	%	Controls N=312	%	χ^2	*P* value	χ^2 for HWE	*P* value
rs6917589								
AA	122	46.74	136	43.59				
AG	101	38.70	135	43.27				
GG	38	14.56	41	13.14	1.24	0.54	0.67	0.41
rs2842980								
AA	116	42.34	158	57.66				
AT	99	42.86	132	57.14				
TT	46	67.65	22	32.35	15.20	<0.001	0.62	0.43
rs5746136								
GG	108	41.38	134	42.95				
GA	112	42.91	153	49.04				
AA	41	15.71	25	8.01	8.54	0.01	1.72	0.19
rs4880								
TT	218	83.52	260	83.33				
TC	37	14.18	48	15.38				
CC	6	2.30	4	1.28	0.98	0.61	7.25	0.007

Discussion

Single nucleotide polymorphisms (SNPs) are DNA sequence polymorphisms caused by a single nucleotide variation. The mutations include the transformation of a single base by transversion, insertion, or deletion, and SNPs are thought to affect susceptibility to human diseases [15]. Genetic polymorphism affects the expression and activity of SOD2, and thereby influences the risk to diseases. In the present study, we analyzed the correlation of SOD2 rs6917589, rs2842980, rs5746136 and rs4880 with the susceptibility to POAG, and we showed that the TT genotype of rs2842980 and AA genotype of rs5746136 were associated with an increased risk of POAG in co-dominant and recessive genotype models. The ATAT and GAGT haplotypes had association with the risk of this disease.

SOD2 is located on the chromosome 6q25 and includes five exons, encoding mitochondrial protein to form homologous tetramer. SOD2 has a function of eliminating active oxygen species (ROS) in mitochondria, thereby preventing

Table 3. Association between SOD2 rs6917589, rs2842980, rs5746136 and rs4880 and risk of POAG

Genes	Patients N=261	%	Controls N=312	%	OR (95% CI)[1]	*P* value
rs6917589						
Co-dominant						
AA	122	46.74	136	43.59	1.0 (Ref.)	
AG	101	38.7	135	43.27	0.97 (0.57-1.66)	0.91
GG	38	14.56	41	13.14	1.17 (0.68-2.01)	0.56
Dominant						
AA	122	46.74	136	43.59	1.0 (Ref.)	
AG+GG	139	53.26	176	56.41	0.89 (0.63-1.25)	0.49
Recessive						
AA+AG	223	85.44	271	86.86	1.0 (Ref.)	
GG	38	14.56	41	13.14	1.10 (0.67-1.81)	0.71
rs2842980						
Co-dominant						
AA	116	44.44	158	50.64	1.0 (Ref.)	
AT	99	37.93	132	42.31	1.02 (0.71-1.48)	0.91
TT	46	17.62	22	7.05	3.30 (1.83-5.98)	<0.001
Dominant						
AA	116	44.44	158	50.64	1.0 (Ref.)	
AT+TT	145	55.56	154	49.36	1.32 (0.94-1.86)	0.11
Recessive						
AA+AT	215	82.38	290	92.95	1.0 (Ref.)	
TT	46	17.62	22	7.05	3.17 (1.83-5.49)	<0.001
rs5746136						
Co-dominant						
GG	108	41.38	134	42.95	1.0 (Ref.)	
GA	112	42.91	153	49.04	1.60 (0.89-2.88)	0.12
AA	41	15.71	25	8.01	1.95 (1.09-3.49)	0.03
Dominant						
GG	108	41.38	134	42.95	1.0 (Ref.)	
GA+AA	153	58.62	178	57.05	0.99 (0.70-1.39)	0.94
Recessive						
GG+GA	220	84.29	287	91.99	1.0 (Ref.)	
AA	41	15.71	25	8.01	1.80 (1.03-3.13)	0.04
rs4880						
Co-dominant						
TT	218	83.52	260	83.33	1.0 (Ref.)	
TC	37	14.18	48	15.38	2.18 (0.59-8.01)	0.24
CC	6	2.3	4	1.28	2.65 (0.67-10.37)	0.16
Dominant						
TT	218	83.52	260	83.33	1.0 (Ref.)	
TC+CC	43	16.48	52	16.66	0.91 (0.57-1.44)	0.68
Recessive						
TT+TC	255	97.7	308	98.71	1.0 (Ref.)	
CC	6	2.3	4	1.28	2.26 (0.62-8.25)	0.22

1. Adjusted for sex, age, family history of glaucoma and smoking habit.

oxidative stress response. Because the eyes are sensitive to oxidative stress, the more light, ultraviolet radiation and environmental pollution could reduce production of ROS and failure

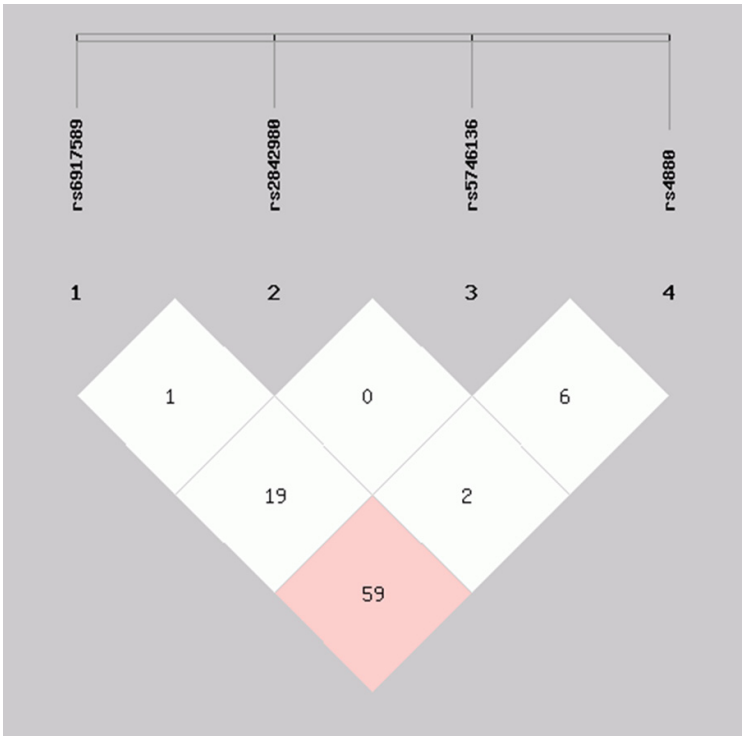

Figure 5. The linkage disequilibrium of SOD2 rs6917589, rs2842980, rs5746136 and rs4880.

of oxidation mechanism [16, 17]. Oxidative stress response causes the damages of eye tissue lipid, protein and nucleic acid, and plays an important role in the risk of glaucoma [5]. The expression of SOD2 gene in aqueous humour significantly increased in patients with POAG [18, 19]. Majsterek et al. reported an significantly decreased of SOD expression in glaucoma patients with comparison to controls [20]. Ghanem et al. reported that an increased level of aqueous humor SOD may contribute to the development of POAG [21].

Currently, only a few studies reported the correlation between SOD2 polymorphisms and POAG risk [12, 13, 22]. Zhou et al. performed a study in Western China, and reported the relationship between rs6917589 rs2842980, rs5746136 and rs4880 polymorphisms and POAG risk [12]. Zhou et al. revealed that SNPs rs6917589 and rs5746136 conferred an increased risk of POAG [12], which was partly in line with our results. Celojevic et al. conducted a study with 239 patients and 185 controls in Sweden, indicating that rs2842980 in SOD2 was significantly correlated with the pathogenesis of POAG in a multivariate analysis [13]. Our results also reported similar results that the SOD2 SNP rs2842980 was correlated with an elevated risk.

Moreover, we firstly reported significant linkage disequilibrium between rs6917589 and rs4880, and we observed that the haplotype ATAT conferred a reduced risk of POAG, whereas the haplotype GAGT showed an increased risk. A previous study indicated a significant relationship between haplotype ATGT and POAG risk [12]. The differences of these results may be attributed to different populations and sample sizes. Therefore, further studies with more subjects are greatly needed to confirm our results.

There were two limitations to our study. First, the POAG patients and controls were enrolled from one hospital only, which may bring selection bias in our study. Only 261 patients and 312 healthy controls were collected into our study, and the results may be undervalued due to the limited number of subjects.

In conclusion, our study firstly suggests that SOD2 rs2842980 and rs5746136 polymorphisms play an important role in the development of POAG in Northern Chinese population, and we offers insight into the influence of SOD2 gene in the susceptibility of POAG.

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Disclosure of conflict of interest

None.

Address correspondence to: Qi Li, The First Affiliated Hospital of Chongqing Medical University, Chongqing, China; Chongqing Key Laboratory of Ophthalmology, Chongqing Eye Institute, Chongqing, China. Tel: 86-23-89012155; Fax: 86-23-89012536; Email: yz4005@126.com

Table 4. Haplotype analysis of the association between SOD2 rs6917589-rs2842980-rs5746136-rs4880 and POAG risk

Haplotype	Patients	%	Controls	%	OR (95% CI)	*P* value
AAAT	87	13.90	78	15.00	0.92 (0.66-1.29)	0.64
AAGC	22	3.51	22	4.23	0.84 (0.46-1.54)	0.57
AAGT	165	26.36	117	22.50	1.26 (0.95-1.65)	0.10
ATAT	36	5.75	52	10.00	0.55 (0.35-0.85)	0.01
ATGT	79	12.62	56	10.77	1.21 (0.841-1.75)	0.30
GAAT	40	6.39	36	6.92	0.93 (0.58-1.48)	0.75
GAGT	116	18.53	69	13.27	1.52 (1.06-2.20)	0.01
GTAT	20	3.19	11	2.12	1.62 (0.76-3.45)	0.21
GTGT	25	3.99	54	10.38	0.35 (0.22-0.58)	<0.001

Overall P<0.001.

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