• Investigation •

Association between interleukin-10 genetic polymorphisms and risk of primary open angle glaucoma in a Chinese Han population: a case-control study

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Abstract

• AIM: To investigate the association between interleukin-10 (IL-10) genetic polymorphisms and risk of POAG through a case-control study in a Han population of China.

• METHODS: A total of 210 patients with POAG and 420 normal subjects were recruited during the period from Dec. 2013 to Dec. 2016. The IL-10 -1082A>G (rs1800870), -819T>C (rs1800871) and -592C>A (rs1800872) polymorphisms were determined using iPlex GOLD SNP genotyping analysis (the SequenomMassARRAY® System, Sequenom, San Diego, USA). The association between IL-10 -1082A>G (rs1800870), -819T>C (rs1800871), and -592C>A (rs1800872) polymorphisms and risk of POAG was assessed by singlelogistic regression analysis.

• RESULTS: We observed that those carrying the CC genotype of rs1800871 was associated with an increased risk of POAG when compared with those harboring the TT genotype (OR=1.84, 95%CI=1.01-3.38). Those with AA genotype of rs1800872 had a 10.62 fold risk of POAG in comparison to the CC genotype (OR=10.62, 95%CI, 3.41-33.09). A completely linkage disequilibrium was found between IL-10 rs1800871-rs1800872 (D'=1.00, r^2=0.16). The A-C-A (OR=2.60, 95%CI, 1.48-4.58) and G-T-A (OR=2.34, 95%CI, 1.42-3.86) haplotypes were associated with an increased risk of POAG, while the A-T-C haplotype showed a decreased risk of POAG (OR=0.63, 95%CI, 0.49-0.81).

• CONCLUSION: Our data suggest that IL-10 rs1800871 and rs1800872 can be predictive factors for the pathogenesis of POAG in the Chinese population.

• KEYWORDS: primary open angle glaucoma; IL-10; polymorphism; haplotype
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INTRODUCTION

Glaucoma is the leading cause of irreversible blindness[1], and this disease has became one of the important public health issues worldwide[2]. It is estimated that about 60.5 million people with primary glaucoma by 2010, increasing to 79.6 million by 2020 with bilateral blindness in 5.9 million primary open angle glaucoma (POAG)[3]. Almost half of the world's glaucoma population occur in Asian counties. A recent study suggests that POAG prevalence is about 0.7% in mainland China[4]. The etiology of developing POAG is still uncertain, but it is well known that many environmental and lifestyle factors contribute to the development of this disease, such as intraocular pressure, age, alcohol drinking, cigarette smoking, high body mass index, systemic hypertension[5]. However, individuals with similar risk factors of POAG would not develop this disease, suggesting that genetic factors play an important role in the pathogenesis of POAG. Currently, many studies have indicated many genetic factors contribute to the development of POAG, such as MTHFR C677T, vitamin D, GSTM1, OPA1, CYP46A1, PPARγ2, Toll-Like Receptor 2 and SOD2[6-11]. Use of genetic biomarkers may play an important role in identifying individuals at high-risk, and contribute to the prevention of POAG.

Studies have linked inflammation and immune reaction play critical roles in the processes of POAG[12-13]. Interleukin-10 (IL-10), also known as human cytokine synthesis inhibitory factor (CSIF), is a multifunctional cytokine which is mainly come from human T helper type1 (Th1) cells and Th2 cells, macrophages/monocytes and dendritic cells[14]. IL-10 exhibits complex immunosuppressive and immunostimulatory properties. For instance, IL-10 promotes B cell-mediated

functions, enhancing proliferation, differentiation, and antibody production, although it can inhibit the functions of T cells and antigen presenting cells (APCs), host type 1 immune responses were inhibited by reducing the production of IL-2, IFN-gamma, and other cytokines, IL-10 itself inhibits the production of IFN-gamma from Th1 cells, and the lack of IFN-gamma increases APC inactivation[15]. Further research revealing more about important roles of IL-10 in the development of eye related diseases[16-18]. Single nucleotide polymorphisms (SNPs), are one of the most common type of genetic variation among people, and making up more than 90% of these variations[19]. IL-10 is encoded by the IL-10 gene which was located on human chromosome 1q31-32, it spans 4.8 kb and contains 5 exons and 4 introns that encode 178 amino acids[20]. Ouma *et al*[21] reported three most common SNPs[-1082A>G (rs1800870), -819T>C (rs1800871) and -592C>A (rs1800872)] in IL-10 promoter region that significantly affect the gene transcription and expression. Currently, no study reported the association between IL-10 genetic polymorphisms and risk of POAG. Therefore, we aimed to perform a case-control study in a Han population of China, and investigation the association between IL-10 -1082A>G (rs1800870), -819T>C (rs1800871), and -592C>A (rs1800872) and risk of POAG.

SUBJECTS AND METHODS

Ethical Approval A case-control study design was used. In this study, 210 patients with POAG without any blood relationship were recruited from the Renmin Hospital of Wuhan University between December 2013 and December 2016. Informed written consent was obtained from all participants for use of their blood sample for genotyping for this study. The study protocol was approved by the Institutional Review Board of Renmin Hospital of Wuhan University, China (No.201601005).

Subjects All the patients were diagnosed by the following criteria: 1) abnormal appearance of the disc or retinal nerve fiber layer; 2) visual field loss according to optic nerve damage; 3) glaucomatous optic nerve damage and cup-to-disc ratio (CDR) above 0.5; 4) intraocular pressure above 21 mm Hg in any one eye, and visual acuity less than 0.05. The exclusion criteria for POAG patients were those with secondary glaucoma, primary angle-closure glaucoma (PACG), congenital glaucoma, and a history of steroid use as well as ocular trauma.

Simultaneously, a total of 420 controls were recruited from the individuals attending the routine health examination and with no previous history of glaucoma. The patients and controls were genetically unrelated Chinese Han population.

DNA Extraction Totally 5 mL of peripheral venous blood were obtained from each respondent. The white cell was handled with Qiagen Blood DNA extraction kit to extract genomic DNA for genotyping analysis according to the manufacturer's protocol, and stored at -80℃ until use. Take the mixed anticoagulation whole blood 1 mL, add the buffer, and mix well. 70℃ water bath pot incubated for 10min and added 1 mL ethanol to fully oscillate. Transfer the liquid to the adsorption column, centrifuge 3000 rpm for about 3min, discard the supernatant, and add 200 μL AE eluent, kept for 5min, and 5000 rpm centrifuge for 2min. Genomic DNA was isolated from peripheral blood samples with QiagenBlood DNA extraction kit.

IL-10 Genotyping The IL-10 -1082A>G (rs1800870), -819T>C (rs1800871), and -592C>A (rs1800872) polymorphisms were determined using iPlex GOLD SNP genotyping analysis using the SequenomMassARRAY® System (Sequenom, San Diego, USA). A multiplexed SNP reaction containing 36 SNPs was run against 94 HapMap DNAs in duplicate. Briefly, polymerase chain reactions (PCR) were carried out in a 96-Well GeneAmp PCR System 9700 (Applied Biosystems) with mixes consisting of 5 μL mixture reaction, containing 1.8 μL $dddH_2O$, 0.5 μL 10× buffer, 0.4 μL Mg^{2+}, 0.1 μL dNTP, 0.2 μL Hotstar, 1 μL forward primer/reverse primer and 1 μL DNA sample (10 ng/μL). Thermal cycle conditions were performed as follows: denaturation at 95℃ for 2min; followed by 45 cycles of 95℃ for 30s, 55℃ for 30s, and 72℃ for 60s; a final annealing and extension at 72℃ for 5min. The PCR products were desalted and crystallized, and analyzed by SpectroCHIP and MALDI-TOF MS reaction. The fluorescence data of IL-10 -1082A>G (rs1800870), -819T>C (rs1800871) and -592C>A (rs1800872) were analyzed by Typer 4.0 software. The primer sequences were as follows: 5' arm forward primers: IL-10 F1 (5'-CAGCTCCTTTGAGAATGGCA-3') and IL-10F2 (5'-CCTGACAGTTTCAAAACGTGG-3'). Reverse primers: IL-10 R1 (5'-AGGAACTGCTTCCTTCACGA-3') and IL-10 R2 (5'-CCTAGGAATGCTCGTCAAGA-3').

Statistical Analysis Demographic and clinical characteristics, and genotype and allele frequencies of the IL-10 -1082A>G (rs1800870), -819T>C (rs1800871) and -592C>A (rs1800872) between the two groups were compared by Chi-square (χ^2) test or student *t* test. The Hardy-Weinberg equilibrium was assessed for IL-10 -1082A>G (rs1800870), -819T>C (rs1800871) and -592C>A (rs1800872) in both the patient and control groups by Chi-square (χ^2) test with one degree of freedom. The frequencies of IL-10 -1082A>G (rs1800870), -819T>C (rs1800871) and -592C>A (rs1800872) haplotypes as well as linkage disequilibrium in controls and patients were estimated using SHEsis software[22]. Haplotypes with frequencies of <5% were excluded.

The association between IL-10 -1082A>G (rs1800870), -819T>C (rs1800871) and -592C>A (rs1800872) polymorphisms and risk of POAG was assessed by multiple logistic regression analysis. The results were expressed by odds ratios (OR) and 95% confidence intervals (CI). All the data were analyzed with the software IBM SPSS Statistics for Windows, Version 19.0. (IBM Corp., Armonk, NY, USA). Two tailed $P<0.05$ was considered as statistical significant difference.

RESULTS

The demographic and clinical variables of 210 patients with POAG and 420 healthy controls are shown in Table 1. No significant difference was found between patients with POAG and controls in terms of age ($\chi^2=0.76$, $P=0.86$), sex ($\chi^2=0.003$, $P=0.96$), BMI ($\chi^2=0.02$, $P=0.89$) and smoking status ($\chi^2=0.03$, $P=0.86$). However, we found that patients with POAG were more likely to have a history of POAG ($\chi^2=11.84$, $P=0.001$), diabetes ($\chi^2=9.84$, $P=0.002$) and hypertension ($\chi^2=10.86$, $P=0.001$), a habit of drinking ($\chi^2=6.30$, $P=0.01$), and higher levels of intraocular pressure ($t=44.68$, $P<0.001$) and CDR ($t=49.19$, $P<0.001$).

We observed that the TT, TC, and CC genotypes of rs1800871 showed significantly differences between patients with POAG and controls ($\chi^2=6.19$, $P=0.04$), and the CC, CA and AA genotypes of rs1800872 also revealed a significantly differences ($\chi^2=28.81$, $P<0.001$; Table 2). Moreover, we found that the rs1800870, rs1800871 and rs1800872 were in line with Hardy-Weinberg equilibrium in both patients and controls.

Using conditional logistic regression analysis, we observed that those carrying the CC genotype of rs1800871 were associated with an increased risk of POAG when compared with those harboring the TT genotype (OR: 1.84, 95%CI: 1.01-3.38). Those with AA genotype of rs1800872 had a 10.62 fold risk of POAG in comparison to the CC genotype (OR: 10.62, 95%CI: 3.41-33.09; Table 3).

In addition, we found that individuals with a history of POAG (OR: 9.65, 95%CI: 1.99-46.79), diabetes (OR: 1.98, 95%CI: 1.15-3.43) and hypertension (OR: 1.93, 95%: 1.29-2.89), and a habit of drinking status (OR: 1.46, 95%CI: 1.01-2.09) had an increased risk of POAG, when compared with the reference group.

A completely linkage disequilibrium was found between IL-10 rs1800871 and rs1800872 (D': 1.00, r^2: 0.16; Figures 1 and 2). The A-C-A (OR: 2.60, 95%CI: 1.48-4.58) and G-T-A (OR: 2.34, 95%CI: 1.42-3.86) haplotypes were associated with an increased risk of POAG (Table 4), while the A-T-C haplotype showed a decreased risk of POAG (OR: 0.63, 95%CI: 0.49-0.81).

DISCUSSION

A misbalance in the physiological equilibrium may shift from regulatory immunity into a neuroinflammatory degenerative process, what may lead to a predisposition to glaucoma. As

Table 1 Demographic and clinical characteristics of patients with POAG and healthy controls *n* (%)

Variables	Patients (n=210)	Controls (n=420)	χ^2 or t	P
Age, y			0.76	0.86
<45	33 (15.71)	64 (15.24)		
45-60	57 (27.14)	128 (30.48)		
60-75	85 (40.48)	162 (38.57)		
>75	35 (16.67)	66 (15.71)		
Sex			0.003	0.96
Female	94 (44.76)	187 (44.52)		
Male	116 (55.24)	233 (55.48)		
BMI, kg/m^2			0.02	0.89
<24	163 (77.62)	328 (78.10)		
≥24	47 (22.38)	92 (21.90)		
History of POAG			11.84	0.001
No	201 (95.71)	418 (99.52)		
Yes	9 (4.29)	2 (0.48)		
History of diabetes			9.84	0.002
No	175 (83.33)	385 (91.67)		
Yes	35 (16.67)	35 (8.33)		
History of hypertension			10.86	0.001
No	146 (69.52)	341 (81.19)		
Yes	64 (30.48)	79 (18.81)		
Smoking			0.03	0.86
Never	131 (62.38)	265 (63.10)		
Ever	79 (37.62)	155 (36.90)		
Drinking			6.30	0.01
Never	118 (56.19)	279 (66.43)		
Ever	92 (43.81)	141 (33.57)		
Intraocular pressure, mm Hg	26.51±2.27	15.93±2.93	44.68	<0.001
Cup-to-disk ratio	0.75±0.11	0.34±0.09	49.19	<0.001

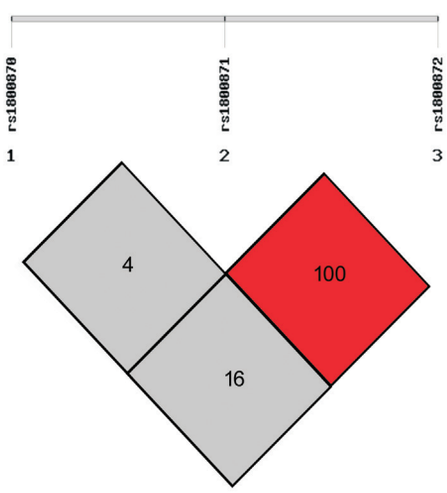

Figure 1 D' of linkage disequilibrium test for IL-10 rs1800870, rs1800871, and rs1800872.

POAG has been characterized as a neurodegenerative disorder which similar to other neurodegenerative diseases, increasing research concerning the role of the immune system in POAG has been performed in the recent years that demonstrates the

Table 2 Genotype distributions of IL-10 -1082A>G (rs1800870), -819T>C (rs1800871) and -592C>A (rs1800872) between patients and controls *n* (%)

SNPs	Patients (*n*=210)	Controls (*n*=420)	χ^2	*P* value	Patients		Controls	
					χ^2 for HWE	*P* for HWE	χ^2 for HWE	*P* for HWE
rs1800870			3.55	0.17	0.21	0.56	0.34	0.56
AA	82 (39.05)	197 (46.90)						
AG	105 (50.00)	185 (44.05)						
GG	23 (10.95)	38 (9.05)						
rs1800871			6.19	0.04	0.05	0.82	0.42	0.39
TT	85 (40.48)	178 (42.38)						
TC	96 (45.71)	210 (50.00)						
CC	29 (13.81)	32 (7.62)						
rs1800872			28.81	<0.001	3.42	0.08	0.012	0.91
CC	142 (67.62)	340 (80.95)						
CA	50 (23.81)	76 (18.10)						
AA	18 (8.57)	4 (0.95)						

Table 3 Logistic regression analysis of association between IL-10 polymorphisms and risk of POAG patients

Variables	*β*	S.E.	Wals	OR	95%CI	*P*
rs1800870						
AA					1.0 (Ref.)	-
AG	0.28	0.19	2.04	1.32	0.90-1.92	0.153
GG	0.12	0.33	0.13	1.12	0.59-2.13	0.719
rs1800871						
TT					1.0 (Ref.)	-
TC	0.20	0.20	1.05	1.22	0.83-1.80	0.306
CC	0.61	0.31	3.91	1.84	1.01-3.38	0.048
rs1800872						
CC					1.0 (Ref.)	-
CA	0.35	0.22	2.48	1.41	0.92-2.17	0.115
AA	2.36	0.58	16.62	10.62	3.41-33.09	<0.001
History of POAG						
No					1.0 (Ref.)	-
Yes	2.27	0.81	7.92	9.65	1.99-46.79	0.005
History of diabetes						
No					1.0 (Ref.)	-
Yes	0.68	0.28	6.03	1.98	1.15-3.43	0.014
History of hypertension						
No					1.0 (Ref.)	-
Yes	0.66	0.21	10.32	1.93	1.29-2.89	0.001
Drinking habit						
Never					1.0 (Ref.)	-
Ever	0.38	0.19	4.12	1.46	1.01-2.09	0.042

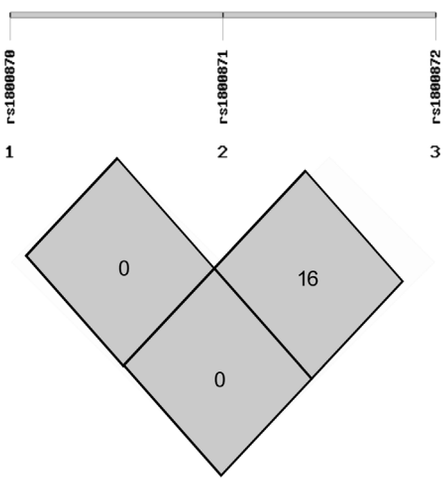

Figure 2 r^2 of linkage disequilibrium test for IL-10 rs1800870, rs1800871, and rs1800872.

Table 4 Haplotype analysis of IL-10 rs1800870, rs1800871, and rs1800872 with POAG risk *n* (%)

Haplotype	Patients	Controls	OR (95%CI)	*P*
A-C-A	28 (6.67)	23 (2.74)	2.60 (1.48-4.58)	<0.001
A-C-C	82 (19.52)	154 (18.33)	1.12 (0.83-1.51)	0.47
A-T-C	141 (33.57)	377 (44.88)	0.63 (0.49-0.81)	<0.001
G-C-C	38 (9.05)	93 (11.07)	0.83 (0.56-1.24)	0.36
G-T-A	34 (8.10)	31 (3.69)	2.34 (1.42-3.86)	<0.001
G-T-C	73 (17.38)	132 (15.71)	1.16 (0.84-1.59)	0.36

immune system definitely plays a role in the pathogenesis of POAG[23-25].

Among the many immune cytokines, IL-10 exhibits a double-faced role during the development of cancers and inflammation related diseases, inducing both immunosuppressive and anti-angiogenic effect. Previous studies revealed that cytokine gene polymorphisms contributed to the development of ocular involvement and many eye related diseases[26-29]. It is reported that early acute inflammatory condition occurs in eye with current acute primary angle-closure, and anti-inflammatory treatment could be a useful method for acute primary angle-closure[30]. POAG is correlated with an aqueous inflammatory response in the aqueous humor, and the inflammatory response

is significantly elevated in eyes[31-32]. Therefore, expression of IL-10 may be associated with the pathogenesis of POAG. Another hypothesis has gained strength in recent years, variants SNPs in the population may contribute significantly to genetic risk for common diseases including age-related disorders. It is well-known for a long time that many primary eye diseases, including POAG, have genetic components. Polymorphisms of the related genes of POAG, have been shown to have some role in the development of glaucoma[33-36]. In our study, we found that the IL-10 rs1800871 and rs1800872 were significant associated with an increased risk of POAG, and a completely linkage disequilibrium was found between IL-10 rs1800871 and rs1800872.

SNPs which play an important role in the regulation the expression of protein, can contribute to the differences between individuals in the susceptibility to a disease and its severity. The human IL-10 gene is located on chromosome 1q31-1q32 and composed of five exons and four introns[20]. In the IL-10 gene promoter region, the alleles of -1082G, -819C, and -592C for three common SNPs have been associated with increased production of IL-10, and thus influence the expression and function of protein[20,37-39]. Currently, many genome-wide association studies have revealed that various genetic loci contributes to the onset and progression of breast cancer, especially for inflammation related genes[40-44]. Currently, many studies reported that interleukin genetic polymorphisms contribute to the development of many eye related diseases, such as IL-6, IL-1β and IL-1α, but the results are inconsistent[45-50]. Markiewicz *et al*[51] performed a case-control study in 511 unrelated Caucasian subjects, and found that the -1607 1G/2G MMP1, -1562 C/T MMP9, -511 C/T IL-1β gene polymorphisms were risk factors for the development of POAG. Lin *et al*[52] reported that E2 allele of IL-1 exon5 could be considered as a marker to predict the risk of POAG in Chinese population. However, some studies reported no association between polymorphisms of interleukin factors and risk of POAG[45,47,53]. Currently, no study reported the association between IL-10 genetic polymorphisms and risk of POAG. Our study firstly reported that CC genotype of rs1800871 and AA genotype of rs1800872 were significant associated with an increased risk of POAG, and A-C-A, G-T-A and A-T-C haplotypes of IL-10 rs1800870, rs1800871, and rs1800872 were associated with risk of POAG. Further studies are greatly needed to confirm our findings.

There are three limitations should be mentioned in the present study. First, since patients and controls were only enrolled from one place of China, these participants may not well represent individuals in other places, and the selection bias may be inevitable. Second, since the incidence of POAG was low, the sample size of patients was relatively small, which may result in a low statistical power to identify differences between groups. Third, this is a case-control study, which could not explain the causal relationship between risk factors and diseases.

In conclusion, our data suggest that IL-10 rs1800871 and rs1800872 could be considered as a predictive factor for the pathogenesis of POAG in the Chinese population.

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