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Haplotype Analysis of Association of the *MYOC* Gene with Primary Angle-Closure Glaucoma in a Han Chinese Population

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Purpose: The aim of this study is to examine whether or not myocilin (*MYOC*) genetic variations are associated with susceptibility to primary angle-closure glaucoma (PACG) in the Han Chinese population. *Methods:* Four single-nucleotide polymorphisms (SNPs)—rs235913, rs183532, rs12076134, and rs235875—in the *MYOC* gene were genotyped in 212 adult patients with PACG and 255 age-, sex-, and ethnic-matched healthy controls by using a polymerase chain reaction–restriction fragment length polymorphism assay. Data were analyzed by chi-square analysis. *Results:* The four SNPs in the *MYOC* gene were in the Hardy–Weinberg equilibrium in all the subjects. The frequencies of A allele rs183532 were significantly different between the PACG patients and the controls (0.238 vs. 0.169, $p=0.008$; OR = 1.541; 95% CI: 1.117–2.127). The frequencies of the AA genotype and A allele of rs235913 were increased in PACG patients compared with controls, but the difference was not significant ($p=0.037$, $p=0.017$, respectively). A comparison of the distributions of the genotypes and alleles of rs12076134 and rs235875 showed no statistically significant differences between the PACG patients and the controls ($p>0.05$). Haplotype analysis indicated that the frequency of the AATG and AATA haplotypes was significantly higher for PACG patients than for control subjects (both $p<0.001$). However, the frequency of CGGA and CGTG haplotypes was lower for PACG patients than for control subjects ($p<0.001$). *Conclusions:* Our study suggests that rs183532 is associated with an increased risk of PACG in the Chinese Han population.

Introduction

Glaucoma is one of the largest causes of blindness worldwide. Globally, there are more than 70 million patients with glaucoma. It is estimated that there will be 79.6 million subjects suffering with glaucoma and about 11.2 million patients with bilateral blindness by 2020 (Quigley and Broman, 2006). In China, it is reported that primary angle-closure glaucoma (PACG) has relatively high visual morbidity rates and accounts for half of all blind glaucoma patients (Casson, 2008). According to the epidemiological survey, the majority of patients with PACG live in Asian regions, especially in China (Congdon *et al.*, 1992; Foster *et al.*, 1996). The characteristics of PACG consist of increased thickness of the lens (Foster *et al.*, 1996), a shallow anterior chamber (Lin *et al.*, 1997), and a short axial length (Abu-Amero *et al.*, 2007), often accompanied by hypermetropic refraction error (Congdon *et al.*, 1996; Dai *et al.*, 2008). To date, the mechanism of PACG remains largely unknown. It is generally accepted that PACG is a multifactorial disorder resulting from the interaction between a genetic predisposition and environmental elements (Amerasinghe *et al.*, 2011). It is demonstrated that the risk of developing PACG is much higher in families, in which first-degree relatives have the disease (Wang *et al.*, 2002). Recently, genome-wide association studies have identified three susceptibility loci (rs110224102 in PLEKHA7, rs3753841 in COL11A1, and rs1015213 located between PCMTD1 and ST18 on chromosome 8q) for PACG (Vithana *et al.*, 2012). Association studies also suggest the involvement of several single-nucleotide polymorphisms (SNPs) of the matrix metalloproteinase-9 (MMP-9) gene (Cong *et al.*, 2009; Awadalla *et al.*, 2011), optineurin (Rezaie *et al.*, 2002), ABCC5 (Karla *et al.*, 2009), and tumor necrosis factor-a (Wang *et al.*, 2012). However, these genes only partly explain the genetic predisposition to PACG, and more research is needed to determine the causative genes of this disease.

MYOC is the first gene identified to be responsible for POAG (Sheffield *et al.*, 1993; Stone *et al.*, 1997). Mutations in *MYOC* account for over 8% of JOAG and 3–4% of adult-onset

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Table 1. Clinical Characteristics of Study Participants

Clinical features	*PACG patients*	*Controls*
Age (mean ± SD)	55.2 ± 10.3	56.5 ± 10.6
Number	212	255
Sex (*n*, % female)	115 (54.2)	137 (53.7)
IOP in mmHg (mean ± SD)	25.2 ± 3.4	12.2 ± 4.3
C/D (mean ± SD)	0.71 ± 0.10	0.20 ± 0.10

IOP, intraocular pressure; PACG, primary angle-closure glaucoma; SD, standard deviation.

POAG (Adam *et al.*, 1997; Mansergh *et al.*, 1998). *MYOC* consists of three exons and encodes 504 amino acid residues. Myocilin is an acidic protein that contains an NH2-terminal myosin-like domain and a COOH-terminal olfactomedin-like domain (Waryah *et al.*, 2013). Almost 80 mutations have been found in *MYOC* and about 90% of the mutations are located in the olfactomedin-like domain encoded by exon 3 (Fingert *et al.*, 1999; Kanagavalli *et al.*, 2003; Cheng *et al.*, 2012; Waryah *et al.*, 2013; Zhou *et al.*, 2013). Although several studies indicated that the *MYOC* mutation was associated with POAG (Cai *et al.*, 2012; Liu *et al.*, 2012; Mendoza-Reinoso *et al.*, 2012), the relationship between *MYOC* genetic polymorphism and PACG remains unclear. Although in the Quebec population, mutation in *MYOC* has been reported in isolated patients with PACG (Faucher *et al.*, 2002), the association of *MYOC* mutation with PACG in the Chinese population is inconsistent. Dai *et al.* (2008) found that *MYOC* gene mutation was associated with PACG in a Chinese population. However, the study of Aung *et al.* (2005) did not support a role for *MYOC* mutations in the pathogenesis of PACG in the Chinese. Therefore, we performed this case–control study to clarify the relationship between *MYOC* and PACG in a Chinese population.

Subjects and Methods

Subjects

A total of 212 patients with PACG and 255 age-, sex-, and ethnic-matched healthy controls were recruited from General Hospital of Chinese People's Liberation Army, and No. 181 Hospital of Guilin from 2007 to 2013. Both the patients and healthy controls underwent a full ophthalmic examination, including a visual acuity test, a slit-lamp examination of the anterior chamber, a gonioscopy, the measurement of central corneal thickness and intraocular pressure (IOP), a fundus examination with special attention to optic disc parameters, and a visual field test. A total of 212 patients were selected for the PACG group according to the following diagnostic criteria: the presence of glaucomatous optic neuropathy with a cup:disc ratio ≥ 0.7, peripheral visual loss, an IOP of more than 21 mmHg, and the presence of at least 180 degrees of closed angle in which the trabecular meshwork (TM) was not visible on a gonioscopy. Patients with secondary angle-closure glaucoma, which was caused by uveitis, trauma, or lens subluxation, were excluded. The healthy individuals came from the same geographical regions as the PACG patients,

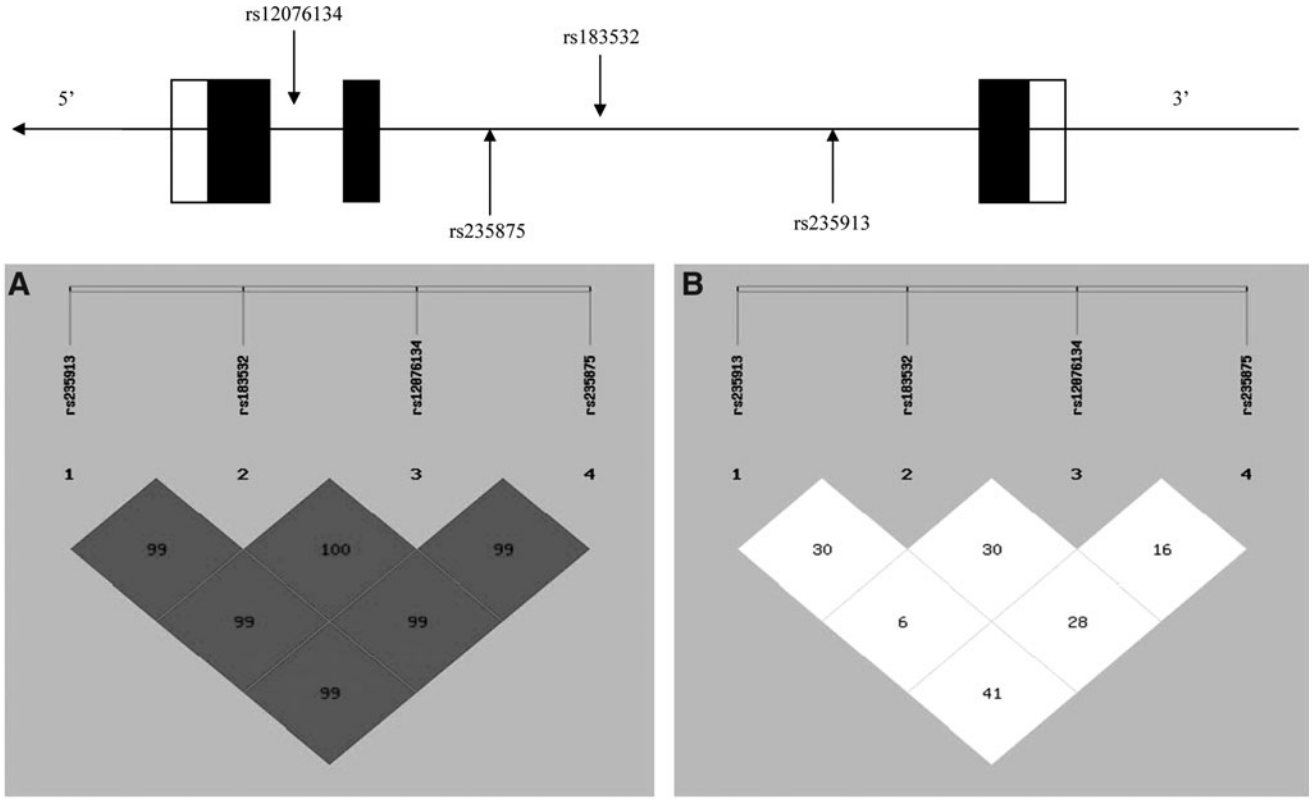

FIG. 1. *Top:* The structure of the human *MYOC* gene. This gene consists of three exons separated by two introns. *Boxes* indicate exons, and *lines* indicate introns and intergenic regions. *Filled boxes* indicate coding regions. *Arrows* mark the locations of polymorphisms. *Below:* The patterns of linkage disequilibrium in the *MYOC* gene, with their $|D'|$ **(A)** and r^2 values **(B)**.

and the controls were age-, sex-, and ethnic-matched with the PACG patients. The clinical characteristics of these subjects are shown in Table 1. The study was approved by the ethics committee of the General Hospital of Chinese People's Liberation Army and met the tenets of the Declaration of Helsinki. Informed consent was obtained from all of the subjects.

SNP selection and genotyping

There are 541 SNPs for the human *MYOC* gene listed in the National Center for Biotechnology Information SNP database (www.ncbi.nlm.nih.gov/SNP). We also screened the data for the TagSNPs on the International HapMap Project website (www.hapmap.org/). According to the Xie's protocol (Xie *et al.*, 2010, 2011), we utilized the Haploview 4.2 software and the HapMap phase II database and obtained four tagging SNPs (rs235913, rs183532, rs12076134, and rs235875) for Chinese Hans using minor allele frequency ≥ 0.10 and linkage disequilibrium (LD) patterns with $r^2 \geq 0.8$ as a cutoff.

Blood samples were collected from all participants, and genomic DNA was extracted from the peripheral blood leukocytes by phenol and chloroform extraction. Genotyping was confirmed by TaqMan methods according to the manufacturer's instructions. To ensure the results were verified, we used sequenced genomic DNAs as positive controls in our assays.

Quality control

To ensure the results were verified, of the genotyped samples, 10% were duplicated and there was at least one positive and one negative control per 96-well DNA plate in our assays. The accuracy of the genotyping was determined by the genotype concordance between duplicate samples. We obtained a 100% concordance between the genotyped duplicate samples for each of the SNPs. The genotyping success rate for each SNP was 100%.

Statistical analysis

The differences in age and gender between the cases and the controls were assessed by the t-test and χ^2 test, respectively. The Hardy–Weinberg equilibrium was tested using the χ^2 test. The significance of the differences in the allele and genotype distribution between the patients and the controls was evaluated with the use of the χ^2 test performed by using SPSS (version 17.0; SPSS, Inc., Chicago, IL). When the number of genotypes or alleles was fewer than 5 counts, the Fisher's exact test was used. Based on the genotype data of the genetic variations, we performed the LD analysis and haplotype-based case–control analysis, using the expectation maximization algorithm and the software SNPAlyze version 3.2 (Dynacom Co, Ltd.,Yokohama, Japan). The pairwise LD analysis was performed using four SNP pairs. We used $|D'|$ values of >0.5 to assign SNP locations to one haplotype block. SNPs with an r^2 value of <0.5 were selected as tagged. In the haplotype-based case–control analysis, haplotypes with a frequency of <0.03 were excluded. The frequency distribution of the haplotypes was calculated by performing a permutation test using the SHEsis software. In addition, logistic regression analysis was performed to assess the contribution of the major risk factors. A Bonferroni cor-

Table 2. Distribution of Genotypes and Alleles

SNP	*Group*	n	*Genotypes*			p	*Alleles*		p	*OR [95% CI]*
rs235913			A/A	A/C	C/C		A	C		
	PACG	212	46 (21.7%)	123 (58.0%)	43 (20.3%)	0.037	215 (0.507)	209 (0.493)	0.017	1.366 [1.055–1.776]
	Control	255	39 (15.3%)	141 (55.3%)	75 (29.4%)		219 (0.429)	291 (0.571)		
rs183532			A/A	A/G	G/G		A	G		
	PACG	212	13 (0.061)	75 (0.354)	124 (0.585)	0.024	101 (0.238)	323 (0.762)	**0.008**	1.541 [1.117–2.127]
	Control	255	6 (0.024)	74 (0.290)	175 (0.686)		86 (0.169)	424 (0.831)		
rs12076134			G/G	G/T	T/T		G	T		
	PACG	212	10 (0.047)	74 (0.349)	128 (0.604)	0.800	94 (0.222)	330 (0.778)	0.527	0.905 [0.666–1.230]
	Control	255	15 (0.059)	92 (0.361)	148 (0.580)		122 (0.239)	388 (0.761)		
rs235875			A/A	A/G	G/G		A	G		
	PACG	212	33 (0.156)	97 (0.458)	82 (0.387)	0.989	163 (0.384)	261 (0.616)	0.905	0.984 [0.755–1.282]
	Control	255	40 (0.157)	118 (0.463)	97 (0.380)		198 (0.388)	312 (0.612)		

Bold value indicates the frequency of the AATG and AATA haplotypes was significantly higher for PACG patients than for control subjects (both $p<0.001$).
SNP, single-nucleotide polymorphism.

Table 3. The Frequency of Haplotypes in Case and Control

Haplotypes	*Case (freq)*	*Control (freq)*	χ^2	*Pearson's* p	*OR [95% CI]*
AAGA	62.15 (0.147)	85.94 (0.169)	0.476	0.490	0.882 [0.618–1.259]
CGGA	6.14 (0.014)	36.00 (0.071)	16.070	**<0.001**	0.200 [0.084–0.476]
AGTA	55.28 (0.130)	75.00 (0.147)	0.276	0.599	0.905 [0.622–1.316]
AATG	52.59 (0.124)	22.05 (0.043)	22.076	**<0.001**	3.259 [1.946–5.457]
AATA	14.95 (0.035)	7.00 (0.014)	15.187	**<0.001**	2.350 [1.595–7.145]
CGTG	171.21 (0.404)	289.94 (0.569)	20.616	**<0.001**	0.545 [0.419–0.709]

Bold values indicate the frequency of the AATG and AATA haplotypes was significantly higher for PACG patients than for control subjects (both $p < 0.001$).

rected *p*-value of less than 0.0125 was considered statistically significant.

Results

Study population characteristics

The patients in the PACG cohort numbered 212 and included 97 male and 115 female subjects. The average age of the PACG patients was 55.2 ± 10.3 years. The healthy controls cohort included 255 subjects (90 males, 135 females) with a mean age of 56.5 ± 10.6 years. The demographic characteristics between the PACG cases and the healthy controls were similar. There was no significant difference between the cases and the controls with respect to age and gender. The clinical features of the patients with PACG and the healthy controls are summarized in Table 1.

Association between MYOC *polymorphisms and glaucoma*

The four SNPs in the *MYOC* gene were in the Hardy–Weinberg equilibrium in all the subjects. The frequencies of A allele rs183532 were significantly different between the PACG patients and the controls (0.238 vs. 0.169, $p = 0.008$; OR = 1.541; 95% CI: 1.117–2.127, Table 2). The frequencies of the AA genotype and A allele of rs235913 were increased in PACG patients compared with controls, but the difference was not significant ($p = 0.037$, $p = 0.017$, respectively, Table 2). A comparison of the distributions of the genotypes and alleles of rs12076134 and rs235875 showed no statistically significant differences between the PACG patients and the controls ($p > 0.05$, Table 2).

Figure 1 shows patterns of LD in the *MYOC* gene, with their $|D'|$ and r^2 values. All four SNPs are located in one haplotype block because all $|D'|$ are beyond 0.5. All four SNPs were available for the performance of a haplotype-based case–control study because all of the r^2 values were below 0.5.

In the haplotype-based case–control analysis, haplotypes were established through the use of four SNPs (Table 2). The frequency of the AATG and AATA haplotypes was significantly higher for PACG patients than for control subjects (both $p < 0.001$, Table 3). However, the frequency of the CGGA and CGTG haplotypes was lower for PACG patients than for control subjects ($p < 0.001$, Table 3).

Discussion

In this study, we found that the genetic polymorphisms and haplotypes of *MYOC* were associated with the risk of PACG in the Chinese Han population. At present, it is generally accepted that PACG is a complex multifactorial and polygenic disorder, in which multiple environmental and genetic factors are simultaneously involved. The foundation for human studies examining putative causative genes that may be involved in PACG is based on a candidate gene approach. This involves selecting a functionally relevant gene to study and subsequently investigating its association with the PACG phenotype.

MYOC encodes the protein myocilin, which is believed to have a role in the cytoskeletal function. *MYOC* is expressed in many ocular tissues, including the TM, and was revealed to be the TM glucocorticoid-inducible response protein (TIGR). The TM is a specialized eye tissue essential in regulating IOP, and mutations in *MYOC* have been identified as the cause of hereditary juvenile-onset open-angle glaucoma. The previous study indicated that *MYOC* mutation is associated with the risk for POAG (Kanagavalli *et al.*, 2003; Cheng *et al.*, 2012; Liu *et al.*, 2012). However, the relationship between *MYOC* polymorphism and PACG remains unclear. *MYOC* is preferentially expressed in the anterior segment of the eye, where high amounts of myocilin mRNA have been detected in the TM, sclera, ciliary body, and iris. The finding that mutant *MYOC* proteins form aggregates that are not secreted suggests that mutant *MYOC* accumulates in TM cells and disturbs normal cellular function, resulting in impaired outflow of aqueous humor, elevated IOP, and glaucoma (Adam *et al.*, 1997). However, as *MYOC* is expressed in the retina, it is also possible that *MYOC* causes glaucoma at the retinal ganglion cell level (Ortego *et al.*, 1997). However, the role of the myocilin gene in PACG remains to be established. Worldwide, the most common form of PACG is the chronic asymptomatic type, in which affected individuals have painless progressive visual loss associated with increased IOP and optic disc cupping. The clinical phenotype has some similarities to POAG, the main differences being the configuration of the angle and a stronger association between IOP and severity of optic neuropathy (Tamm *et al.*, 1999). Previous studies have reported the presence of *MYOC* mutations in individuals with PACG (Faucher *et al.*, 2002; Aung *et al.*, 2005; Dai *et al.*, 2008). These reports provided preliminary evidence that PACG subjects may carry *MYOC* mutations, but were limited by a small sample size.

In the present study, we find that rs183532 of the *MYOC* gene was significantly associated with the risk of glaucoma. The AA or AG genotype of rs183532 significantly differed between PACG patients and control subjects, indicating that the risk of PACG is increased in subjects with the A allele of rs183532. In addition, we successfully established

haplotypes for the *MYOC* gene from the different combinations of the four SNPs. The AATG and AATA haplotypes were significantly higher for PACG patients than for control subjects. However, the frequency of CGGA and CGTG haplotypes was lower for PACG patients than for control subjects.

In conclusion, the present results indicate that PACG is associated with the polymorphism rs183532 of the *MYOC* gene in the Chinese population.

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Author Disclosure Statement

No competing financial interests exist.

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