·Basic Research·

Correlation between polymorphisms in the *MFN1* gene and myopia in Chinese population

Yun-Chun Zou[1], *Jia-Hong Lei*[2], *Ying Wang*[1], *Shuang Xu*[1]

[1]Department of Ophthalmology and Optometry, North Sichuan Medical College, Nanchong 637000, Sichuan Province, China

[2]Department of Laboratory Medicine, North Sichuan Medical College, Nanchong 637000, Sichuan Province, China

Correspondence to: Yun-Chun Zou. Department of Ophthalmology and Optometry, North Sichuan Medical College, Nanchong 637000, Sichuan Province, China. zychun03@163.com

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Abstract

• **AIM: To explore whether genetic variations in the *MFN1* gene are associated with low to moderate myopia in Chinese population.**

• **METHODS: The case-control association analysis was used. The study included 100 independent myopia patients (-0.75 D ≤ spherical refraction ≤-8.00 D) and 100 sex-matched healthy controls (with binocular spherical equivalent ranges between -0.50 D and +0.50 D). Four single nucleotide polymorphism (SNP) tags (rs3976523, rs13098637, rs6762399 and rs7618348) were selected for genotyping by direct sequencing. The frequencies of genotypes and their alleles were calculated based on the number of SNP genotypes in each sample. The Chi-square test was used to examine the difference in the frequency between the myopia cases and controls.**

• **RESULTS: Genotype distributions in the four SNPs were all in accordance with the Hardy-Weinberg equilibrium; analysis showed that rs13098637 was significantly associated with low to moderate myopia (P=0.003 and empirical P=0.010). There were no statistically significant differences observed for the genotype or allele frequencies of the other three SNPs between the myopia cases and controls in the Chinese population in this study.**

• **CONCLUSION: The current study has revealed that the C allele of rs13098637 in *MFN1* had a significant association with low to moderate myopia.**

• **KEYWORDS:** myopia; *MFN1* gene; single nucleotide polymorphism; association analysis

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INTRODUCTION

Myopia is the most common eye disease of visual impairment in the world, involving 80% of Asian youth [1,2]. The incidence of myopia is very high in the developing countries, especially in Asian countries where the incidence has been increased every year. The incidence of myopia is as high as 40%-70% in the Asian population [3,4], and even higher in China[5-7].

Myopia is a developmental disease. The change in the expression of genes that control eye development or growth will lead to the occurrence and development of myopia. In recent years, some progress has been made toward the molecular mechanisms of the disease, a number of candidate genes have been identified, and many genes and loci related to the disease have been found through gene linkage and correlation studies[8,9]. However, most of these genes have not been independently validated and no confirmed causal gene has been found for the disease.

The *MFN1* gene codes for the mitochondrial fusion protein 1 (MFN1), which is located on the outer membrane of cell. *MFN1* is a protein-coding gene of 45.5 kb with 18 exons. The MFN1 protein plays a pivotal role in mediating mitochondrial fusion and morphology in mammalian cells[10]. Mitochondrial fusion is a complex process, where MFN1 is the main molecule that regulates mitochondrial fusion and plays an important role in maintaining the homeostasis of mitochondrial morphology. It facilitates the binding of mitochondria in the early stage of the fusion. *MFN1* plays an important role in the movement of mitochondria and acts together with intra-membrane protein optic atrophy 1 (OPA1). OPA1 is localized in the intermembrane space of mitochondria and endometrium, and is widely distributed in retinal ganglion cells as a dynein-related protein. OPA1 is essential for synaptic structure of retinal ganglion cells [11]. Studies have shown that the deletion of *OPA1* in the optic nerve atrophy model rats can lead to the structure change of dendrites in retinal neural cells [12]. Therefore, MFN1 assists OPA1 in regulating mitochondrial function [13]. Together, MFN1 and OPA1 may protect the cell against spontaneous apoptosis [14], and have impact on the adjustment of retinal

mitochondria. The eye is a high energy consuming organ and changes in mitochondrial function may affect the development of myopia, suggesting that pathogenesis of myopia may be associated with the mitochondria.

Not much has been done about the role of *MFN1* gene on myopia. In this study, 4 loci within the *MFN1* gene were analyzed for correlation with low and moderate myopia.

SUBJECTS AND METHODS

Subjects This study was approved by the Human Subjects Ethics Subcommittee of the North Sichuan Medical College and adhered to the tenets of the Declaration of Helsinki. Every study subject gave written informed consent. All participants were recruited by Visual Center of North Sichuan Medical College, and most of them are students or teachers and all of them are Han people with no genetic relationship among them.

Inclusion criteria: cases had spherical equivalent (SE) between -0.75 D and -8.00 D, and controls had SE within ±0.50 D. Exclusion criteria: people with eye disease such as turbid cornea and crystalline lens, with genetic system diseases related to myopia, such as the Stickler syndrome and Marfan syndrome. Patients with obvious ocular trauma or a long-term high fever childhood were also excluded.

All subjects were asked in detail about the history of myopia, and underwent a complete eye examination (visual acuity, refraction and slit lamp examination and so on). The diopter was precisely determined using a phoropter. The axial length of eye, corneal curvature, and anterior chamber depth were measured with IOL Master. Lens thickness and vitreous chamber depth were measured by an ophthalmological A-mode ultrasound.

Six milliliter of venous peripheral blood was extracted from each participant in tube with EDTA anticoagulant. The blood was used for genomic DNA extraction using the modified salting-out method.

Single Nucleotide Polymorphism Selection and Genotyping We selected four tag single nucleotide polymorphisms (SNPs). Table 1 showed the position of these four tag SNPs and the minor allele frequencies in cases and controls. All tag SNPs were genotyped by direct sequencing. The primers used in the polymerase chain reaction (PCR) were shown in Table 2.

Statistical Analysis Clinical measurement data were expressed as mean ±SD; significance in difference between groups was determined by t-test. The partial correlation analyses of myopia diopter and refractive factors, such as the axis length (AL), lens thickness (LT), anterior chamber depth (ACD) and the average corneal power (CP), were calculated using the SPSS software (version 17.0 for Windows, SPSS Inc., Chicago, IL, USA).

Table 1 SNP tags information from the Hapmap project

SNP	Position	Allele	Minor allele	Frequency of minor allele
rs6762399	180545401	C	T	0.386
rs13098637	180575508	T	C	0.125
rs3976523	180581873	A	C	0.3
rs7618348	180627433	C	T	0.289

Table 2 PCR primers of all the tag SNP

SNP (rs number)	Primer sequence
rs6762399	F 5' GATTAGCCAGGATGGTCTCG 3'
	R 5' GGAGGAACAAACGATGGAAT 3'
rs13098637	F 5' GAAGGACTAGAATTGGATTAAAGG 3'
	R 5' TGTAACTTTAACCCAAACAGAAAT 3'
rs3976523	F 5' TTGTTAAAGAGGCAAGTAGGTGG 3'
	R 5' CGTATTCAAACTAAGCAGCACAG 3'
rs7618348	F 5' TCCACTTCTACTGTCTGGGTCC 3'
	R 5' AGAGAAGTCTTTGAACAACTGGAAC 3'

F: Forward primer; R: Reverse primer.

Genotypes were tested for Hardy-Weinberg equilibrium (HWE) in case and control subjects with a threshold P value of 0.01 for being significantly deviated from HWE. The inequilibrium may be attributed to birth, population stratification, or natural selection, and may also indicate a correlation with disease. In the control group, if the distribution of tag SNP genotype does not fit the HWE, the tag SNP will be excluded for further correlation analysis.

Genotypes were obtained by direct DNA sequencing and differences in the observed genotype were examined by the Chi-square test. The Single-marker association analysis was performed with Plink (v1.07) and Fisher's exact test under three genetic models (allelic, dominant and recessive), and multiple comparisons were corrected by permuting the case-control status of the subjects 10 000 times [15]. P<0.05 and corrected P<0.05 were considered statistically significant.

RESULTS

Average Refractive Parameters Bilateral myopia was present in 100 subjects (42 men, 58 women) with age ranged from 18 to 37y and there were 100 control subjects (45 men, 55 women) with age from 20 to 38y. For subjects with myopia, the spherical refractive errors of the right and left eyes were -3.76±1.79 D and -3.57±1.12 D, respectively. The average refractive parameters for control and case groups are shown in Table 3. Results of partial correlation analysis are shown in Table 4.

Distribution of Single Nucleotide Polymorphism Genotypes and Frequency of Allele The PCR products from the four loci were visualized on 1% agarose gel after electrophoresis at 150 V and 100 mA for 20min (Figure 1).

Single Nucleotide Polymorphism Genotyping Table 5 shows the distribution of genotypes in cases and controls. All genotypes of examined SNPs were tested for HWE, and no significant deviations were found (P>0.05). The allele frequency of the four tag SNPs are shown in Table 6. This

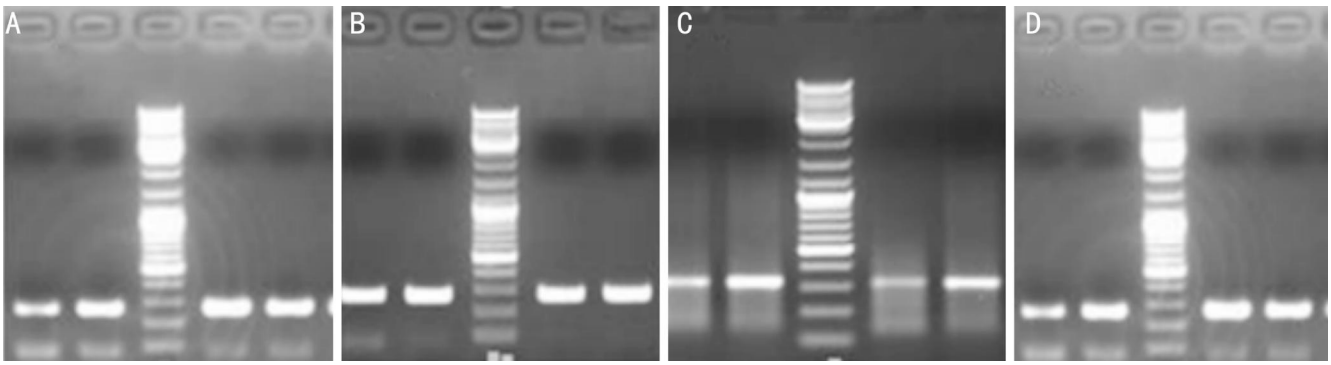

Figure 1 The PCR results of these four SNP tags A, B, C, D represent the PCR products elctrophorogram of rs3976523, rs13098637, rs6762399, and rs7618348 and the PCR products length of this four tag SNPs is 262 bp, 274 bp, 325 bp and 263 bp respectively.

Table 3 Measurements of biological parameters from 400 eyes

Eye parameter (mm)	HCCR	VCCR	AL	LT	ACD	VCD
Total	7.84±0.25	7.66±0.26	24.00±1.11	3.67±0.27	3.22±0.29	16.71±1.03
Controls	7.91±0.02	7.74±0.02	23.26±0.06	3.77±0.02	3.08±0.02	16.07±0.06
Myopia cases	7.77±0.01	7.58±0.02	24.74±0.06	3.57±0.02	3.35±0.02	17.35±0.06
T value	6.11	6.67	-17.55	8.19	-10.31	-15.75
P	＜0.01	＜0.01	＜0.01	＜0.01	＜0.01	＜0.01

HCCR: Horizontal corneal curvature radius; VCCR: Vertical corneal curvature radius; AL: Axis length; LT: Lens thickness; ACD: Anterior chamber depth; VCD: Vitreous chamber depth.

Table 4 Results of partial correction analysis on eye-related biological parameters

Eye parameter	D and HCCR	D and VCCR	D and AL	D and VCD	AL and VCD	AL and CCR (H/V)
r	0.81	0.84	-0.90	-0.89	0.95	0.78/0.82
P	＜0.01	＜0.01	＜0.01	＜0.01	＜0.01	＜0.01

D: Diopter; HCCR: Horizontal corneal curvature radius; VCCR: Vertical corneal curvature radius; AL: Axial length; VCD: Vitreous chamber depth; H: Horizontal; V: Vertical.

Table 5 Summary of genotyping results for the four SNP tags

SNP (rs number)	Genotype	Obs (exp) genotype counts controls/cases	χ^2	*P*
	CC	31 (30.8) / 35 (36)		
rs6762399	CT	49 (49.4) / 50 (48)	0.967	0.617
	TT	20 (19.8) / 15 (16)		
	TT	79 (77.44) / 57 (58.52)		
rs13098637	TC	18 (21.12) / 39 (35.96)	11.64	0.003
	CC	3 (1.44) / 4 (5.52)		
	AA	46 (49) / 40 (44.22)		
rs3976523	AC	48 (42) / 53 (44.56)	0.743	0.690
	CC	6 (9) / 7 (11.22)		
	CC	51 (49) / 47 (47.61)		
rs7618348	CT	38 (42) / 44 (42.78)	0.802	0.67
	TT	11 (9) / 9 (9.61)		

Obs (exp) genotype counts: Observed (expected) genotype counts.

analysis revealed that the rs13098637 polymorphism was strongly associated with myopia. The *P* value of a Chi-square test for the trend was 0.003, and was 0.012 after the permutation test. The frequency of the allele C of rs13098637 was significantly higher in the myopia than in the control group with an OR of 2.25, indicating that the allele may be a dangerous allele.

DISCUSSION

According to the causes, primary myopia might be divided into high myopia and simple myopia [16]. For high myopia of single gene inheritance, diopter is more than -6.00 D, and mostly over -8.00 D. Simple myopia of multiple gene inheritance is determined both genetically and environmentally with a genetic index of about 60%-75%. The genetic factors are polygenic. The diopter in myopia of multifactorial inheritance was believed to be below -6.00 D. However, in recent years, there is a sharp rise in the incidence of myopia and high myopia and the incidence of

Table 6 Summary of number of alleles in the four tag SNPs

SNP	Allele	Controls	Cases	χ^2	*P*	OR	Pc1	Pc2
rs6762399	C	111	120	0.83	0.362	0.83	1.0	0.844
	T	89	80					
rs13098637	T	76	53	9.06	0.003	2.25	0.01	0.012
	C	24	47					
rs3976523	A	140	133	0.57	0.452	1.18	1.0	0.898
	C	60	67					
rs7618348	C	150	138	0.047	0.828	1.05	1.0	0.999
	T	50	62					

Pc1: *P* value corrected with the Bonferroni method; Pc2: *P* value after the permutation test (with 10 000 replacements).

myopia above -6.00 D is as high as up to 5%-10%. The myopia is mainly due to excessive speculation. It was speculated that in high myopia cases whose spherical refractive errors ranged from -6.00 D to -8.00 D, some of them might be those of multifactorial inheritance with higher refractive diopter. Therefore, in this study, patients with myopia less than -8.00 D were selected for investigation. Eye refractive status is determined by many factors, among them, the axial length is the most important factor [17]. In this study, adult older than 18y were selected to rule out the interference from the axial variation due to eye development. Our results showed that the longer the eye axis, the higher the degree of myopia, and that there was a close relationship between the ocular axial length and diopter (r=0.90, P<0.01). This was in agreement with most of the previous results that myopia was mainly resulted from the increased axial length of eyeball [18].

Myopia is complex in nature with multifactorial etiology. Twin and family studies have provided convincing evidence for significant genetic influence on myopia with environmental factors having very modest effect. The mechanisms underlying the pathogenesis of myopia have been the focus of research in recent years [19-21]. Among the numerous methods used, identification of genes susceptible to myopia is an important way and approach to reveal the mechanisms and to prevent and treat the disease. In a 2008 report[21], five SNP loci around the *MFN1* gene was found in a British cohort study that are strongly associated with myopia (including high, medium and low myopia). Especially the SNP locus of rs6794192 and rs7618348 showed lower *P* values. However, in our previous study, after genotyping rs3976523 which is linked with rs6794192 (r^2=0.893) in the high myopia population, no correlation was found between rs3976523 and high myopia. For rs7618348, we found the *P* values in the allele difference between controls and myopia patients was slightly less than 0.05 before correction but more than 0.05 after the correction. Our earlier study also showed that there are other two loci (rs6762399 and rs13098637) in the *MFN1* gene may be associated with high myopia (Sichuan university library databases). Because in the British study, most of the population is low to moderate myopia (the mean SE of cases was -3.92 D) [22], and in our previous studies, the results were from the patients with a threshold SE of -8.00 D and the mean SE of our previous case subjects was about -10 D. This might explain the difference. So this study was undertaken to continue investigate the correlation of the four loci with myopia (mainly low and moderate myopia) using the case-control analysis in the Chinese population.

In this study, we did not find any relationship between myopia and rs7618348 SNP flanking the *MFN1* gene on 3q26.33, nor myopia and rs6762399 and rs3976523 within in the *MFN1* gene. On contrast, we found that rs13098637 locus within the *MFN1* gene was related to the disease, suggesting that the locus might be a candidate gene region for myopia in the Han population. The single SNP (rs13098637) that was associated with myopia in this study is located in an intron at the centre of the *MFN1* gene. This suggests that *MFN1* is very likely the myopia susceptibility gene at 3q26.

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