• Basic Research •

The association of lumican polymorphisms and high myopia in a Southern Chinese population

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Received: 2016-11-17 Accepted: 2017-02-06

Abstract

● AIM: To investigate the correlation between lumican (LUM) gene and high myopia in a Southern Chinese population.

● METHODS: The study comprised of 95 high myopia patients with a spherical equivalent ≤−6.5 diopters (D). The control group recruited 95 individuals with a spherical equivalent ranging from −0.5 D to +0.5 D. Direct sequencing was used to detect the single nucleotide polymorphisms (SNPs) of LUM gene in coding region. Genotype distributions were tested for Hardy-Weinberg disequilibrium. Genotypic and allelic frequencies were analyzed through Chi-square test or Fisher's exact test.

● RESULTS: We identified 3 SNPs of the LUM gene: LUM c.32 (rs577456426), LUM c.507 (rs17853500) and LUM c.849 (rs181915277). Among the three SNPs, the genotype and allele frequencies of rs17853500 showed a significant difference between patients and control subjects (P<0.05). However, there were no significant differences in rs181915277 and rs577456426 between the two groups (P>0.05).

● CONCLUSION: LUM c.507 polymorphism may be a risk factor for the pathogenesis of high myopia in the Southern Chinese population.

● KEYWORDS: lumican; sclera; high myopia; single nucleotide polymorphisms

DOI:10.18240/ijo.2017.10.06

Citation: Wang GF, Ji QS, Qi B, Yu GC, Liu L, Zhong JX. The association of lumican polymorphisms and high myopia in a Southern Chinese population. *Int J Ophthalmol* 2017;10(10):1516-1520

INTRODUCTION

Myopia is a common eye disease and a significant public health issue. Simple myopia can be corrected with spectacles or contact lenses, whereas high (pathological) myopia is one of the main causes leading to low vision or blindness. Pathological myopia is defined as a refractive error of at least −6.00 diopters (D) with pathological changes including temporal crescent, pigment epithelial thinning, leopard retina, Fuch's macula, and retina-choroidal atrophy. Epidemiological evidence suggests increasing prevalence of myopia, especially in Asian populations[1-2], causing profound economic cost to the society. Therefore, it is important to identify the etiology of high myopia.

Active remodeling mechanism of the sclera is an important process during the pathogenesis of myopia. Scleral remodeling involves reduced production of collagen and proteoglycans and increased collagen degradation. The sclera contains a collagen-rich extracellular matrix that undergoes significant biochemical and biomechanical remodeling during the development of myopia[3-5]. Alteration in the expression levels of extracellular matrix components may affect scleral morphology, as in myopia. Lumican (LUM), a keratan sulfate proteoglycan belonging to the small leucine-rich proteoglycan (SLRP) family, is one of the major extracellular matrix components of the sclera. LUM-deficient mice displayed corneal opacity, skin fragility, abnormally large collagen fibril diameters, and disorganized interfibrillar spacing[6]. This role implicated LUM could be responsible for the control of collagen fibrillogenesis. And Chakravarti *et al*'s[7] study further showed that mice deficient in LUM and fibromodulin manifested certain features of high myopia. LUM knockdown zebrafish caused scleral thinning and increased size of scleral coats[8]. LUM mRNA level was significantly down-regulated during the development of lens-induced myopia[9]. It's suggested that altered expression of LUM may contribute to myopia. Increasing studies have showed that single nucleotide polymorphisms (SNPs) in the LUM gene were associated with high myopia[10-13]. Majava *et al*[14] found that a novel SNP c.893-105G>A may contribute to pathogenesis of high myopia. However, some other studies suggested that polymorphisms in LUM gene were not associated with high myopia[15-18]. Case-control studies about the relationship between LUM polymorphisms and high myopia were summarized in Table 1. Therefore, we want to

Table 1 Summary of case-control studies about the relationship between LUM polymorphisms and high myopia

First author (year)	Region	Subjects (*n*)	Refractive status (D)	Mean age (a)	Conclusions
Lin HJ[11] (2010)	Taiwan, China	Myopia: 201 Control: 86	≤-6.0 ±0.5	16 to 25 16 to 25	LUM rs17853500, rs3832846, rs17018757, and rs3759223, and the novel SNP c.1567 C>T contributes to the development of high myopia
Zhang F[10] (2009)	China	Myopia: 94 Control: 90	≤-6.0 ±0.5	37 36	LUM rs3759223 and rs17853500 were associated significantly with high myopia
Chen ZT[12] (2009)	Taiwan, China	Myopia:120 Control: 137	≤-10.0 -1.5 to 0.5	NA	rs3759223 and rs3741834 were associated significantly with high myopia
Majava M[14] (2007)	Finland	Myopia: 125 Control: 308	≤-6.0 ±2.0	NA	LUM c.893-105 G>A may contribute to pathogenesis of high myopia
Okui S[13] (2016)	Japan	Myopia: 1585 Control: 1011	≤-9.0 ±1.0	12 to 78 20 to 78	LUM rs3759223 contributes to the risk of very high myopia
Wang P[15] (2009)	China	Myopia: 288 Control: 208	≤-6.0 -0.5 to +1.0	21 27	LUM rs3759223 was not associated with high myopia
Paluru PC[17] (2004)	USA	Myopia: 10 Control: 5	-32 to -7.5 -3.2 to +1.2	NA	No polymorphism in LUM was associated with high myopia
Yip SP[16] (2011)	Hong Kong, China	Myopia: 656 Control: 654	≤-8.0 ±1.0	26 26	LUM was not associated with high myopia
Park SH[18] (2013)	Korea	Myopia: 128 Control: 235	≤-9.25 -1.5 to 0	32.5 40.5	rs3759222 and rs3759223 were not associated with high myopia

investigate whether LUM gene polymorphisms are correlated with high myopia in the Southern Chinese population. LUM gene was extracted from the blood of 95 patients with high myopia and 95 individuals in the control group, and sequenced the polymorphisms of LUM coding region by direct sequencing.

SUBJECTS AND METHODS

Patients and Study Design From January to December 2012, we recruited 500 volunteers and examined their refractive status in detail at the First Affiliated Hospital of Jinan University. All of the participants were unrelated and of Chinese Han Nationality. The eligible volunteers were divided into the pathological myopia group and control group with 95 members in each group. LUM gene was extracted from the blood of two groups, and sequenced the polymorphisms of LUM coding region by direct sequencing. This research followed the Declaration of Helsinki and conformed to the principles of medical ethics. For each subject, the study protocol and procedure were fully explained, and written consent was obtained. The experiment was authorized by the First Affiliated Hospital of Jinan University Ethics Committee.

Subject Enrollment The two group were given detailed ophthalmic examinations, including uncorrected visual acuity and best corrected visual acuity, subjective refraction, ocular movements, slit-lamp examination (Topcon, Japan), non-contact intraocular pressure (IOP; Topcon, Japan), optical coherence tomography (Zeiss Humphrey, Germany) and fundus color photography (Topcon, Japan). Anterior chamber depth (ACD) and axial length (AXL) is measured by IOL master (Zeiss Humphrey, Germany). In accordance with pathological myopia diagnostic criteria, clinical presentation of the disease included: 1) binocular myopic spherical equivalent (SE) >6.0 D; 2) AXL >26 mm; 3) with one of the following ocular fundus changes: leopard retina, comus, lacquer crack, macular hemorrhage, Fuchs spot, posterior staphyloma, peripheral chorioretinal lesions and retinal detachment. Inclusion criteria for the control group included: 1) binocular SE between -0.50 D to +0.50 D; 2) AXL <24 mm; 3) no family history of pathological myopia. Participants with eye disease, history of intraocular surgery, cataract, glaucoma, retinal disorders, or laser treatment were excluded.

Lumican Gene Sequencing The genomic DNA was extracted from 5 to 10 mL of venous blood from all participants. DNA was purified from lymphocyte pellets according to standard procedures using a Puregene DNA isolation kit (Tiangen, Beijing, China). The primer sequences used in the polymerase chain reaction (PCR) are as follows: LUM-1F: GCACGTGTACGCAATCTAACC; LUM-1R: GTTGTGCAGCCCAGGTATTT; LUM-2F: ACTGTGCCATTTTGGTAGCC; LUM-2R: GGCCTGCCTTTCATCTTTTC. The PCR was performed in a 30 μL reaction volume containing 3 μL 10× rTaq buffer (Tiangen, Beijing, China), 200 μmol/L of dNTP, 0.2 μmol/L of each primer, 1.0 unit of rTaq DNA polymerase (Tiangen,

Beijing, China), and 80 ng of genomic DNA. The conditions, after initial denaturation at 95℃ for 5min, were 30 cycles of 30s at 95℃, 30s at 55℃, and 45s at 72℃, followed by final extension at 72℃ for 5min. The PCR products remained at 12℃ and were purified using a Multi Screen-PCR plate (Millipore, Boston, USA). The purified PCR products were bidirectionally sequenced using the ABI3730XLDNA sequencer (Applied Biosystems, Foster, USA).

Statistical Analysis Characteristics between the two groups were tested using independent-samples *t*-test or Levene's test for equality of variances. Genetic analysis and Hardy-Weinberg equilibrium were analyzed through Chi-square test or Fisher's exact test and *P*-value were calculated by SPSS 16. A *P*-value <0.05 was considered significant.

RESULTS

Patient Characteristics The study group was comprised of 95 patients with high myopia and the control group consisted of 95 individuals with normal vision. The patients enrolled in the study group were with age 18-30y, male:female ratio was 1.1:1.0, mean ACD 3.46 mm, mean cornea diopter (CD) 43.4 D, and mean IOP 16 mm Hg. The volunteers in the control group were with age 18-30y, male: female ratio was 0.7:1.0, mean ACD 2.99 mm, mean CD 43.2 D, and mean IOP 15.7 mm Hg (Table 2). There was no significant difference between the control and study groups in age, gender, CD, ACD, and IOP.

Hardy-Weinberg Equilibrium Analysis SNPs in the coding region of LUM gene were determined for investigating whether LUM gene polymorphisms correlate with high myopia. Direct-sequencing analysis found three SNPs of the LUM gene in coding region, which were LUM c.32 (rs577456426), LUM c.507 (rs17853500) and LUM c.849 (rs181915277). The genotype distributions were obtained by direct counting and allele frequencies were calculated subsequently. The genotype distributions of the three SNPs between the patients and control subjects were consistent with Hardy-Weinberg equilibrium (Table 3).

Lumican Gene Sequencing Analysis This study detected three polymorphisms of the LUM gene, which are LUM c.32 (rs577456426), LUM c.507 (rs17853500) and LUM c.849 (rs181915277). The genotype distributions and allelic frequencies of the three SNPs between the high myopia and control groups were shown in Tables 3 and 4. For the polymorphism rs17853500, the genotype frequencies of T/T:T/C:C/C were 26.3%:58.9%:14.7% respectively, in the high myopia group and 50.5%:40.0%:9.5% respectively, in the control group. The allelic frequencies of T:C was 55.8%:44.2% in the high myopia group and 70.5%:29.5% in the control group. There was a significant relationship between rs17853500 of LUM gene and high myopia ($P<0.05$). However, there were no significant differences in genotype distributions and allelic frequencies of rs577456426 and rs181915277 between the two groups ($P>0.05$) (Tables 4, 5).

Table 2 Characteristics of the high myopia patients and control subjects mean±SD

Characteristics	Case *n*=95	Control *n*=95	*t*	*P*
Age (a)	23.5±3.8	23±3.9	0.85	0.40
SE (D)	-9.15±3	0.02±0.3	-20.6	0.00
AXL (mm)	28.3±1.7	23.4±0.8	7.52	0.00
CD (D)	43.4±1.4	43.2±1.4	0.73	0.47
ACD (mm)	3.33±0.4	3.11±0.3	2.90	0.01
IOP (mm Hg)	16±2.6	15.7±2.1	0.09	0.93

SE: Spherical equivalent; AXL: Axial length; CD: Cornea diopter; ACD: Anterior chamber depth; IOP: Intraocular pressure; SD: Standard deviation.

DISCUSSION

As a member of the SLRP family, LUM is widely expressed in fibrous connective tissue, participating in the regulation of stromal collagen fibrils assembly, corneal transparency, scleral morphology, skin elasticity and so on[19-21]. LUM is located at 12q21-q23 (MYP3), a genetic locus often associated with high myopia[22]. Gene knockout studies[6-8] suggested that LUM was a potential pathological myopia gene. The study explored the association of LUM polymorphisms and high myopia in the Southern Chinese population.

This genetic association study revealed that there was a significant difference in the polymorphism rs17853500 of LUM between high myopia patients and normal controls, whereas no significant difference was detected in the other two polymorphisms (rs577456426 and rs181915277). These results indicated that LUM rs17853500 at the coding region of might be associated with increased susceptibility to high myopia. Our results are consistent with Zhang *et al*'s[10] and Lin *et al*'s[11] studies. Zhang *et al*[10] revealed that SNPs rs17853500 and rs3759223 in LUM were associated significantly with pathological myopia in Northern Han Chinese, and they also found that there was no difference in the rate of mutation between pedigree and sporadic group. Lin *et al*'s[11] study revealed that haplotype distributions of LUM polymorphisms (rs17853500, rs3832846, rs17018757 and rs3759223) contributed to the pathogenesis of high myopia in Taiwan-born Han Chinese. They sequenced all three exons, intron-exon boundaries, and promoter regions of LUM, while we detected the SNPs of LUM gene in coding region. However, Paluru *et al*[17] and Park *et al*[18] did not support an association of LUM polymorphisms with high myopia in the USA and Korea respectively. The contradict result from these studies might be explained by racial and region differences. Moreover, these studies used different genotyping methods. They analyzed the SNPs genotyping by restriction fragment length polymorphism (RFLP), mass spectrometry or DNA pooling. We detected the

Table 3 Hardy-Weinberg equilibrium analysis of the three SNPs in the high myopia patients and control subjects

SNPs	Case				Control			
	Genotype	Observed value	Expected value	*P*	Genotype	Observed value	Expected value	*P*
rs17853500	T/T	25	29.58		T/T	48	47.25	0.96
	T/C	56	46.86	0.27	T/C	39	38.57	
	C/C	14	21.28		C/C	9	7.29	
rs577456426	C/C	54	56.33		C/C	51	53.44	0.51
	C/T	39	33.65	0.44	C/T	41	35.63	
	T/T	2	5.02		T/T	3	5.94	
rs181915277	C/C	34	40.13		C/C	38	42.65	0.21
	C/T	56	43.23	0.79	C/T	52	42.00	
	T/T	5	11.64		T/T	5	10.35	

Table 4 Genotype distributions of the three SNPs in the high myopia patients and control subjects *n* (%)

SNPs	Genotype	Case (*n*=95)	Control (*n*=95)	*P*
rs17853500	T/T	25 (26.3)	48 (50.5)	0.03
	T/C	56 (58.9)	38 (40.0)	
	C/C	14 (14.7)	9 (9.5)	
rs577456426	C/C	54 (56.8)	51 (53.7)	0.85
	C/T	39 (41.1)	41 (43.2)	
	T/T	2 (2.1)	3 (3.2)	
rs181915277	C/C	34 (35.8)	38 (40)	0.83
	C/T	56 (58.9)	52 (54.7)	
	T/T	5 (5.3)	5 (5.3)	

Table 5 Allelic frequencies of the three SNPs in the high myopia patients and control subjects' eyes *n* (%)

SNPs	Allelic frequencies	Case *n*=190	Control *n*=190	*P*
rs17853500	T	106 (55.8)	134 (70.5)	0.03
	C	84 (44.2)	56 (29.5)	
rs577456426	C	147 (77.4)	143 (75.3)	0.63
	T	43 (22.6)	47 (24.7)	
rs181915277	C	124 (65.3)	128 (67.4)	0.66
	T	66 (34.7)	62 (32.6)	

SNPs of LUM encoding region by direct sequencing, which was known as the gold standard for detecting polymorphisms. Nevertheless, direct sequencing requires the PCR amplification of each site, and is not suitable for the screening of the whole genome.

This study investigated the relationship between LUM polymorphisms and high myopia by a case-control study, which is an effective, economic and simple method to study the etiology and risk factors of the disease. It can be applied to the evaluation of disease risk factors, monitoring of drug efficacy[23], evaluation of vaccine immunological effect[24], and outbreak investigation[25], *etc*. However, the results of case-control studies were influenced by the researcher's design flaws, such as the source and selection of cases and controls, the size of the sample, the measurement and regulation of the relevant factors, and so on. Wang *et al*[15] did not support an association of LUM rs3759223 with high myopia in China. In their study, they just detected one SNP rs3759223 of LUM by RFLP analysis, whereas we analyzed the SNPs of LUM gene in coding region by direct sequencing. The control individuals from their study had bilateral refraction ranging from -0.5 to 1.0 D, whereas in our study, the control subjects had refractive errors between -0.50 and +0.5 D. In addition, the participants in their study were larger than that in our group. These different selection criteria might contribute to the discrepancy between the findings in the two studies. This present study has some limitations, including regional limitations of population selection and small sample size. Further studies can be carried out in a multicenter study with a number of hospitals across the country, so as to increase the sample size and reduce the selection bias.

In conclusion, our findings demonstrated that rs17853500 polymorphism was correlated with high myopia. LUM might play an important role in regulating scleral extracellular matrix interactions, thus affecting the development of myopia. Further research is required to confirm whether LUM is the virulence gene of high myopia, and the role of LUM gene mutation in the pathogenesis of high myopia.

ACKNOWLEDGEMENTS

Foundations: Supported by Natural Science Foundation of Guangdong Province, China (No.2015A030310158; No.2014A030313359); the Science and Technology Planning Project of Guangdong Province, China (No.2015B020226003); the Scientific and Cultivation Foundation of the First Affiliated Hospital of Jinan University (No.2015201).

Conflicts of Interest: Wang GF, None; **Ji QS,** None; **Qi B,** None; **Yu GC,** None; **Liu L,** None; **Zhong JX,** None.

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