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Association between lumican gene −1554 T/C polymorphism and high myopia in Asian population: a meta-analysis

Xuan Liao[1,2], Xu-Bo Yang[1], Meng Liao[1], Chang-Jun Lan[3], Long-Qian Liu[1]

[1]Department of Ophthalmology, West China School of Medicine, Sichuan University, Chengdu 610041, Sichuan Province, China

[2]Department of Ophthalmology and Optometry, North Sichuan Medical College, Nanchong 637007, Sichuan Province, China

[3]Department of Ophthalmology, Affiliated Hospital of North Sichuan Medical College, Nanchong 637007, Sichuan Province, China

Correspondence to: Long-Qian Liu. Department of Ophthalmology, West China School of Medicine, Sichuan University, 37 Guoxue Xiang, Chengdu 610041, Sichuan Province, China. b.q15651@hotmail.com

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Abstract

• AIM: To investigate the association between lumican gene −1554 T/C polymorphism and high myopia susceptibility.

• METHODS: We searched the published literature in the Medline, Embase, and CBM databases from inception to July 2013. A meta-analysis was performed by the programs RevMan 5.1 and Stata 12.0, and the odds ratio (OR) with 95% confidence interval (CI) was calculated in fixed or random effect model based on heterogeneity test among studies.

• RESLUTS: Seven case-control studies with a total of 1 233 cases and 936 controls were included. A statistical significant association with high myopia was observed in the recessive model (TT *vs* CT+CC: OR=1.92; 95% CI= 1.14-3.23) and codominant model (TT *vs* CT: OR=1.81, 95%CI=1.19-2.75).

• CONCLUSION: The present meta-analysis suggested that lumican −1554 T/C polymorphism might be moderately associated with high myopia susceptibility. This conclusion warrants confirmation by further studies.

• KEYWORDS: lumican; high myopia; gene polymorphism; meta-analysis

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INTRODUCTION

Myopia is a prevalent ocular disorder affecting about 500 million people worldwide [1]. Its extreme form, high myopia, also termed pathologic myopia in certain cases, is defined as a refractive error of at least -6.00 diopter (D). High myopia predisposes individuals to ocular abnormalities such as cataract, glaucoma, chorioretinal degeneration, or retinal detachment, which can lead to irreversible vision loss or even blindness [2]. Epidemiological investigations have shown that the prevalence of high myopia ranges from 2.8% -4.6% in Caucasian populations to more than 8% in Asian populations [3,4]. High myopia is one of the major causes of visual impairment worldwide, and therefore is one of the most significant public health problems, imposing increasing economic and social burden[5-7].

Myopia is currently thought as a complex disease that is modulated by a combination of genetic and environmental factors, as well as their interactions. The view is supported by compelling evidence from epidemiological, experimental and clinical studies [8]. While environmental and behaviorial factors play an important role in development, the majority of the variance of myopia is deemed to attribute to hereditary factors. Family, twin and population studies provide considerable evidence to demonstrate a significant genetic contribution to myopia[9-11]. Over ten affirmative gene loci are known for high myopia, including Xq28 (MYP1), 18p11.31 (MYP2), 12q21-q23 (MYP3), 7q36 (MYP4), 17q21-q22 (MYP5), 2q37.1, 4q22-q27, Xq23-q25, 15q12-13, and 5p15.33-p15.2, and a number of candidate genes associated with high myopia have been reported to date [8].

The morphological characteristics underlying high myopia, including scleral thinning and localized ectasia and axial elongation, are associated with extracellular matrix alterations during the scleral remodeling process, which involve increased collagen degradation and decreased synthesis of collagen and proteoglycans [12,13]. The sclera is a fibrous connective tissue constituting the main structural framework of the eyeball, whose major extracellular matrix

consists of structural macromolecules and small leucine-rich repeat proteoglycans (SLRPs). Proteoglycans not only contribute to providing shape and biomechanical strength to the eyeballs, but also exhibit direct and indirect cell signaling properties [14]. Lumican, a member of the SLRP family, appears to play an essential role in the control of scleral collagen fibril assembly and interaction [15]. Alteration of lumican gene expression is likely to affect scleral collagen fibrillogenesis, subsequent axial elongation, and the development of high myopia. Additional support for this idea has been obtained from experiments on Lum null mice and Lum/Fmod double null mice, which lack funcational lumican genes, and display significant functional defects in scleral collagen fibrillogenesis and certain features of high myopia such as scleral thinning, axial elongation, and retinal detachment[16,17]. Analogously, knockdown of lumican gene in zebrafish results in disruption of the scleral collagen fibril arrangement and scleral thinning [18]. The effect of lumican gene on significance in scleral collagen fibrillogenesis suggests lumican could be used to indicate susceptibility to high myopia. The human lumican gene is located on chromosome 12q22; it spans about 7.5kb of genomic DNA, and is composed of 3 exons separated by introns of 2.2kb[19]. It falls within the chromosome 12q21-23 MYP3 interval, on which the potential genetic locus for autosomal dominant high myopia has been identified[20,21].

A number of single nucleotide polymorphisms (SNPs) in lumican gene have been reported, and its -1554 T/C polymorphism has been found to be correlated with high myopia susceptibility [22]. However, research results on the association between lumican gene -1554 T/C polymorphism and high myopia risk are inconclusive. Therefore, we conducted a meta-analysis to examine whether there is such an association.

MATERIALS AND METHODS

Literature Search Strategy We searched Medline, Embase, and Chinese Biomedical Database (CBM) from inception to July 2013 to retrieve papers on lumican gene -1554 T/C polymorphism and the risk for high myopia in any language. The keywords and MeSH terms used were: ["lumican" (MeSH) OR "LUM" OR "proteoglycan genes"] AND ["Polymorphism, Single Nucleotide" (MeSH) OR "Polymorphism, Genetic" (MeSH) OR "Polymorphism" OR "Polymorphisms" OR "SNP" OR "SNPs"] AND ["myopia" (MeSH) OR "high myopia" OR "pathological myopia"]. In addition, manual searches of the reference lists of relevant original studies and review articles were performed to identify other potentially eligible studies. We contacted authors of original studies for additional information when necessary.

Inclusion Criteria Eligible studies had to meet all the following criteria: 1) evaluated lumican gene -1554 T/C polymorphism and high myopia susceptibility; 2) case-control design that compared high myopia cases and healthy controls; 3) sufficient published data available to estimate an odds ratio (OR) with 95% confidence interval (CI); and 4) original research articles, not reviews or comments.

Data Extraction The following data were extracted by two investigators independently: first author's name; publication year; country where conducted; study design; ethnicity; mean age, and gender of the study subjects; gene and polymorphism analyzed; genotyping method; numbers of cases and controls; and numbers of cases and controls with different genotypes. Disagreements were resolved by discussion until a consensus was achieved. Otherwise, a third investigator was consulted to resolve the dispute.

Statistical Analysis ORs with 95% CIs were computed to assess the strength of the association between lumican gene -1554 T/C polymorphism and high myopia risk. The significance of the pooled ORs was determined by the Z-test, with $P<0.05$ considered statistically significant. The pooled ORs were calculated for allelic effect (C allele *vs* T allele), dominant model (CT+TT *vs* CC), recessive model (TT *vs* CT+CC), and codominant model (TT *vs* CC; TT *vs* CT). We calculated the Q statistic to estimate the heterogeneity and the I^2 statistic to quantify the extent of heterogeneity[23,24]. A $P \leqslant 0.1$ was considered statistically significant for the Q-statistic test. I^2 represents the percentage of total variation across studies that are attributable to heterogeneity rather than chance. An I^2-value of 0% indicates no observed heterogeneity, and larger values show increasing heterogeneity. If heterogeneity existed among the studies, a random-effects model (the DerSimonian and Laird method) was used to compute the summary risk estimate [25]. If there was no heterogeneity, a fixed-effects model (the Mantel-Haenszel method) was used [26]. Where possible, we assessed whether genotype frequencies of controls in individual studies were consistent with the expected distribution, that is, in Hardy-Weinberg equilibrium (HWE), by using the χ^2 test, in which $P<0.05$ was considered significant. To test the robustness of the association, we performed a one-way sensitivity analysis by sequential omission of individual studies or non-HWE studies. The possibility of publication bias was assessed by visual inspection of a funnel plot in which the standard error of log (OR) of each study was plotted against its corresponding log (OR). An asymmetric plot indicates possible publication bias. Funnel plot asymmetry was evaluated with the Begg's rank correlation test and the Egger's linear regression test (significance set as $P<0.05$) [27-29]. All statistical analyses were performed by RevMan 5.1 (Revman; The Cochrane Collaboration, Oxford, UK) and Stata 12.0 (StataCorp, The College Station, Texas, USA).

RESULTS

Study Characteristics Our literature search retrieved 17 potentially relevant studies (Figure 1). Of these, seven studies were excluded because they were reviews, letters, animal studies, or not related to high myopia and lumican gene -1554 T/C polymorphism. After full-text review, two papers were excluded, one because it was a duplicate publication and the other because it used a family-based design. Another paper was excluded due to failure to extract sufficient data. Finally, seven case-control studies, which comprised 1 233 cases and 936 controls, were included in this meta-analysis [30-36]. The publication years of the included studies ranged from 2006 to 2013, and all were written in English. Genotyping methods included polymerase chain reaction (PCR), polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP), DNA sequencing and mass spectrometer (MS). All genomic DNA was extracted from venous blood (Table 1). The Hardy-Weinberg equilibrium test was performed on all included studies, and the result showed that the lumican gene -1554 T/C genotype frequencies in controls deviated from the HWE in only one of the studies[34] (Table 2).

Main Results Results of pooled analysis on the associations between lumican -1554 T/C polymorphism and high myopia susceptibility are shown in Table 3. On the whole, a statistical significant increase in high myopia susceptibility was associated with the TT variant genotype in the recessive model (OR=1.92; 95%CI=1.14-3.23; random effects model), and the heterogeneity among the individual studies was significant (P<0.01, I^2=80%). A significant trend was observed in the codominant model (TT *vs* CT: OR=1.81, 95%CI=1.19-2.75; random effects model) with the significant heterogeneity (P=0.01, I^2=64%) as well. Unfortunately, we did not conduct a subgroup analysis to explore the source of heterogeneity due to the limited studies and incomplete information available (Table 3).

Sensitivity Analysis We performed a sensitivity analysis to assess the influence of each individual study on the pooled OR by sequential omission of individual studies in every comparison. None of the individual studies significantly affected the pooled ORs. Furthermore, exclusion of the one non-HWE study had little effect on the pooled OR, changing it from 1.81 (95%CI: 1.19, 2.75) to 1.48 (95%CI: 1.15, 2.87), which indicated that the study was not a potential source of heterogeneity[34].

Publication Bias Visual inspection of the funnel plots revealed no obvious asymmetry (Figure 2). In addition, Egger's linear regression test on the natural logarithm scale of the OR found no evidence of publication bias for the allelic contrast model (P=0.44), dominant model (P=0.31), recessive model(P=0.30), or codominant model (P=0.74 for TT *vs* CC, P=0.77 for TT *vs* CT) (Table 4).

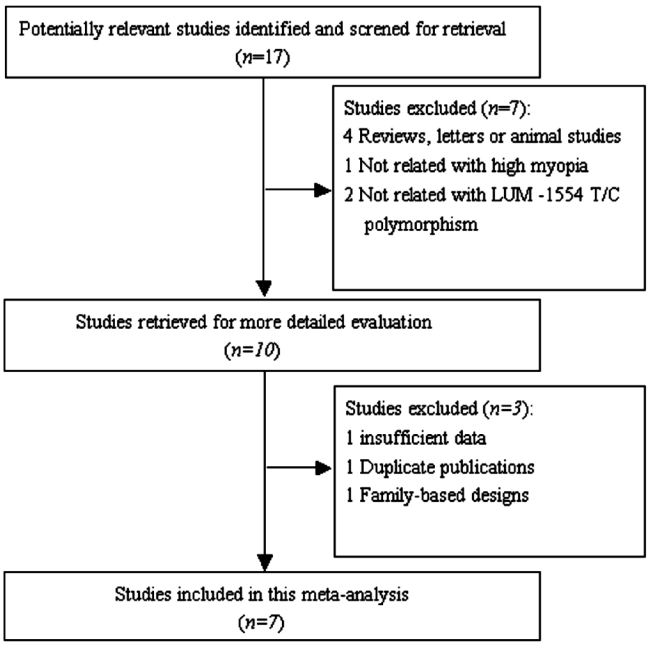

Figure 1 Flow diagram of article selection process.

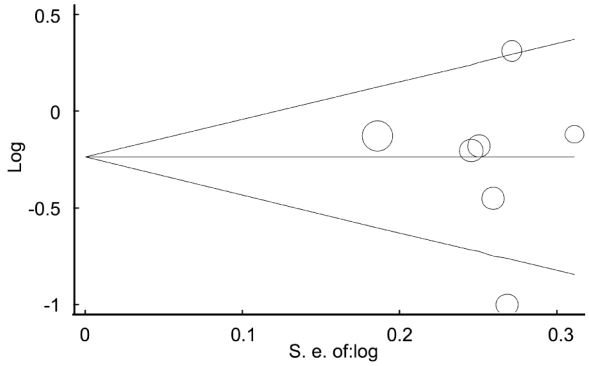

Figure 2 Funnel plot of publication bias for the association between Lumican −1554 T/C polymorphism and high myopia (TT/CT) Each point represents a separate study for the indicated association. Vertical coordinates mean natural logarithm of OR. Horizontal ordinate mean standard error of Log OR.

DISCUSSION

Steady advances have been made in the field of human myopia genetics, but there is still much remain to be worked out. So far, the importance of the genetic variation in high myopia has been well established in twin, family and population studies, and more than 10 loci have been identified. Nonetheless, the genes responsible have not yet been discovered. Lumican is known to play important roles in maintaining tissue homeostasis and regulating cellular functions including cell proliferation, migration, adhesion, and differentiation, as well as regulating collagen fibrillogenesis[37]. Empirical studies have shown that lumican may modulate scleral collagen fibril assembly and interaction, based on the defects observed in scleral collagen fibril diameter and organization in lumican-deficient mice and zebrafish [17-19]. The specific function of lumican in the formation of scleral collagen fibrils makes it a strong candidate for an indicator of high myopia susceptibility.

Table 1 Basic characteristics of included studies

Authors, Ref	Country	Genotyping method	Age (mean±SD, a)		Gender (M/F)		Refractive degree (diopter)		Axial length (mm)	
			Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls
Wang I *et al*[30]	China, T	PCR-sequencing	34.4±15.2	41.9±8.5	50/70	77/60	≤-10.00	-1.50~+0.50	29.47±1.84	24.08±0.65
Zhang F *et al*[31]	China	PCR-sequencing	37.0±12.7	36.0±13.1	44/50	45/45	-9.50±3.00	0.00±0.25	28.18±1.02	24.23±0.27
Wang P *et al*[32]	China	PCR-RFLP	21.8±16.2	27.3±7.3	NA	NA	[1]≤-6.00	[1]-0.50~+1.00	NA	NA
Lin H *et al*[33]	China, T	PCR-RFLP	18.0±3.4	18.0±2.9	112/70	45/33	[1]-8.83±2.50	[1]0.02±0.32	26.80±1.80	23.56±0.78
Lin H *et al*[34]	China, T	PCR-RFLP	16~25	16~25	129/72	55/31	[1]≤-6.00	-0.50+0.508	NA	NA
Dai L *et al*[35]	China	PCR-MS	NA	NA	NA	NA	≤-12.75	-0.50	27.16±2.00	23.08±2.28
Park S *et al*[36]	Korean	PCR-RFLP	32.5	40.5	61/67	114/121	[1]11.26±3.88	[1]-0.27±1.95	27.89±1.96	22.16±2.07

Ref: References; T: Taiwan; PCR: Polymerase chain reaction; RFLP: Restriction fragment length polymorphism; MS: Mass spectrometry; NA: Not applicable; [1]SE: Spherical equivalent.

Table 2 Genotype distribution and frequency of included studies

Author, Ref	Cases					Controls					HWE test (controls)	
	Total	CC	CT	TT	MAF	Total	CC	CT	TT	MAF	χ^2	*P*
Wang I *et al*[30]	120	61	32	27	0.358	137	55	68	14	0.350	1.119	0.290
Zhang F *et al*[31]	94	6	31	57	0.229	90	45	32	13	0.322	3.113	0.078
Wang P *et al*[32]	288	21	112	155	0.267	208	27	87	94	0.339	0.923	0.337
Lin H *et al*[33]	182	74	95	13	0.332	79	41	35	3	0.259	1.845	0.174
Lin H *et al*[34]	201	104	83	14	0.274	86	37	45	4	0.308	4.440	0.035
Dai L *et al*[35]	220	21	74	125	0.264	101	8	38	55	0.267	0.158	0.691
Park S *et al*[36]	128	8	34	86	0.195	235	11	72	152	0.200	0.426	0.514

Ref: references; MAF: Minimum allele frequency; HWE: Hardy-Weinberg equilibrium.

Table 3 Main results of pooled ORs in the meta-analysis

Inherited model	Fixed effects model			Random effects model			Heterogeneity test	
	OR (95%CI)	*P*	Weight (%)	OR (95%CI)	*P*	Weight (%)	I^2	*P*
T/C	0.73 (0.64, 0.84)	<0.01	51.3	0.70 (0.45, 1.10)	0.12	23.6	90	<0.01
CT+TT/CC	1.27 (1.02, 1.59)	0.03	14.5	1.38 (0.71, 2.67)	0.34	20.2	86	<0.01
TT/CT+CC	1.65 (1.34, 2.03)	<0.01	15.1	1.92 (1.14, 3.23)	0.01	19.7	80	<0.01
TT/CC	2.22 (1.63, 3.02)	<0.01	5.8	2.16 (0.93, 5.03)	0.07	17.2	83	<0.01
TT/CT	1.57 (1.26, 1.96)	<0.01	13.3	1.81 (1.19, 2.75)	0.006	19.3	64	0.01

OR: Odds ratio; 95%CI: 95% confidence interval.

Table 4 Evaluation of publication bias by Egger's linear regression test

Inherited model	Coefficient	SE	*t*	*P*>|t|	95%CI
T/C	-6.60	7.77	-0.85	0.44	-26.58, 13.38
CT+TT/CC	-2.40	2.15	-1.12	0.31	-7.92, 3.12
TT/CT+CC	4.61	3.97	1.16	0.30	-5.60, 14.81
TT/CC	-1.48	4.20	-0.35	0.74	-12.29, 9.32
TT/CT	-1.21	3.97	-0.3	0.77	-11.42, 9.00

SE: Standard error; 95%CI: 95% confidence interval.

A growing number of studies suggest that genetic variation plays a significant role in determining individual susceptibility to complex multifactorial disease traits. Genetic polymorphisms that alter the level of gene expression are expected to have an essential influence on disease risk. Previous studies have reported several lumican gene polymorphisms; some of them are not significantly associated with high myopia, while others are. A possibly protective variation c.893-105G>A in the lumican gene was observed for high myopia in a Caucasian population [38]. Yip *et al* [39] indicated that six proteoglycan genes including lumican rs3759222, rs10859110, rs2300588 were not associated with high myopia in a Chinese population. Wang *et al* [30] explored the association between lumican polymorphism and high myopia in Taiwanese individuals, and found a significant difference in SNP rs3759223 between cases and controls, suggesting an association between lumican gene and high myopia susceptibility. The association was clarified by the result of a population-based haplotyping, in which rs3759223 demonstrated a statistical significance and composed a haplotype block within lumican in relation to high myopia susceptibility [22]. Similarly, Zhang *et al*[31] found that in a Northern Chinese population, including 12 pedigree cases and 82 sporadic cases with pathological myopia, the variation ratio of SNP (rs3759223) of the

lumican gene was significantly higher in a cases group than in a control group. However, three studies, two in the Chinese population and one in the Korean population, showed no association between SNP (rs3759223) of the lumican gene and high myopia[32,35,36].

The -1554 T/C gene polymorphism is located in -4.006bp in the 5'regulatory region (5'RR) of the lumican gene (4.406bp upstream from exon 1). It is a putative regulatory element of the lumican gene that affects promoter activities and scleral collagen fibrillogenesis during the development of myopia. However, the connection between lumican gene -1554 T/C polymorphism and high myopia remains controversial as the above. Therefore, we performed this meta-analysis to estimate the association. This meta-analysis involving 1 233 cases and 936 controls from seven studies demonstrated an association between lumican gene -1554 T/C polymorphism and increased high myopia susceptibility in Asian populations. The present study restricted to the Asian population may reduce the chance of false positives from population stratification and minimize the impact of environmental confounders. Yet even so, we found significant heterogeneity among the results of individual studies. The sensitivity analysis indicated that the results of the meta-analysis were stable, and we found no evidence of publication bias among the included studies.

One limitation of our meta-analysis is that some relevant studies could not be included due to publication limitation, resulting in a relatively small sample size that limited the statistical power of this analysis. In addition, all the data in this meta-analysis were from the Asian populations, and might not be applicable to other ethnicities. It is also possible that unaccounted-for factors influenced our results. For instance, the differences among included studies in the sources (population-based or family-based) and severity of disease (refractive error -6.00D or -10.00D) may have caused bias from non-differential misclassification. Finally, our analysis was based on unadjusted data, which may have allowed the influence of confronting factors, such as age, gender, environment, and lifestyle.

In summary, the present meta-analysis suggests that lumican gene -1554 T/C polymorphism is associated with high myopia susceptibility. However, due to the above-mentioned limitations, our results must be considered preliminary and interpreted with caution. Further studies are merited to assess the association in greater detail including any combination of SNP-SNP and SNP-covariate interactions. Gene-gene and gene-environment interactions should also be considered for a comprehensive understanding of the association between the lumican gene -1554 T/C polymorphism and high myopia susceptibility.

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