# High myopia is not associated with single nucleotide polymorphisms in the *COL2A1* gene in the Chinese population

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Abstract. Association studies have revealed that the rs1635529 polymorphism in the *COL2A1* (collagen, type II,  $\alpha$  1) gene may be a potential candidate for myopia development and may be associated with myopia in Caucasians. The aim of this study was to investigate the association of high myopia with 5 polymorphisms including rs1635529 in COL2A1 in a Chinese population. Genomic DNA samples were selected from our Genomic DNA Repository for Myopia. A total of 581 high myopia individuals (refraction  $\leq$ -6.00D, spherical equivalent) and 384 normal controls (refraction between -0.50D and +2.00D, spherical equivalent) from a Han Chinese cohort participated in the association study. Allelic discrimination assay was performed on five single nucleotide polymorphisms (SNPs) (rs1635529, rs60542319, rs1635530, rs1635531 and rs954326) in COL2A1 by direct sequence analysis of polymerase chain reaction products. The genotype, allele and haplotype frequencies of the SNPs between cases and controls were compared by Chi-square test for association analysis. Based on the results, SNPs at rs1635530 and rs1635531 were found to be monomorphic in all subjects. There was no statistically significant difference in genotype, allele and haplotype frequencies for the other three SNPs (rs1635529, rs60542319 and rs954326) between the high myopia group and the control group. We do not find evidence to support an association of SNP rs1635529 in COL2A1 with high myopia in the Chinese population studied, nor of the other two SNPs (rs60542319 and rs954326).

#### Introduction

The global prevalence of refractive errors has been estimated from 800 million to 2.3 billion (1). Myopia is an increasingly crucial global public health issue (2-4). The prevalence of myopia has been reported as high as 70-90% in certain Asian countries, 30-40% in Europe and the US, and 10-20% in Africa (3,5-10). Its extreme form, high myopia, is the fourth most common cause of irreversible blindness in the world (11,12).

Myopia is a common, complex and multifactorial disorder. More and more research into genetic factors of myopia give rise to its importance, with improved understanding of its biological mechanism (2,13-18). The association study of candidate genes and loci has been evaluated and the available evidence suggests the involvement of certain genes and loci in myopia in different ethnic populations (19-26).

The *COL2A1* gene (OMIM120140), mapping to 12q13.11, encodes the  $\alpha$ -1 chain of type II collagen, a fibrillar collagen widely expressed in cartilage and the vitreous humor of the eye. *COL2A1* mutations are reported to cause Stickler syndrome type I (OMIM 108300) (27), with severe myopia as a consistent phenotype. Recently, a significant association was identified between SNP rs1635529 located in the *COL2A1* locus and myopia in Caucasian family-based association studies in two separate reports (23,28).

We used a case-control association study approach to conduct candidate genetic analysis to investigate the association between high myopia and SNP markers in intron 1 of the *COL2A1* gene (rs1635529 and a number of adjacent SNPs). It is the first report of a *COL2A1* SNP site association study in a large Han Chinese cohort

#### Materials and methods

Subjects. In total, 965 unrelated individuals participated in this study, including 581 high myopia cases (sphere at each meridian  $\leq$ -6.00D), and 384 university students without myopia (spherical equivalent between -0.50 and +2.00D) were used as normal controls.

Refractive errors were measured by an autorefractor (Topcon KR-8000, Paramus, NJ, USA) after mydriasis with compound tropicamide (Mydrin<sup>®</sup>-P; Santen Pharmaceutical Co. Ltd., Osaka, Japan). All cases and controls received an ocular biometry examination using IOL master V5 (Carl Zeiss Meditec AG, Jena, Germany). Written informed consent, conforming to the tenets of the Declaration of Helsinki, was obtained from each participant prior to the study. This study

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Table I. Primers for amplifying the fragment encompassing the five single nucleotide polymorphisms of interest.





Figure 1. Schematic representation of five SNPs (rs1635529, rs60542319, rs1635530, rs1635531 and rs954326) showing their location on the *COL2A1* gene (not to scale). In the present study, all five SNPs were selected for COL2A1. Vertical arrows show the SNPs with their rs numbers. Black boxes indicate the position of exons (based on NM\_001844.4, Genebank). <sup>†</sup>Single nucleotide polymorphism associated with myopia as reported in a Caucasian population..



Figure 2. Sequence analysis of three SNPs: (A) rs1635529 (A/C), (B) rs60542319 (-/C) and (C) rs954326 (A/C) for genotype. The different sequence resulting from the polymorphism at this point is shown by an arrow.

was approved by the Institutional Review Board of Zhongshan Ophthalmic Center.

The inclusion criteria for individuals in each group are as follows. (A) Control group: i) best unaided visual acuity of 1.0 or better; ii) bilateral refraction between -0.50D and +2.00D (spherical equivalent, SE); iii) no other known eye or related systemic diseases; and iv) no family history of high myopia or hyperopia. (B) High myopia group: i) best corrected visual acuity of 0.8 or better; ii) spherical refraction at each meridian at least  $\leq$ -6.00D; and iii) no other known eye or related systemic diseases.

*SNP genotyping*. Genomic DNA was prepared from venous blood. Genotyping was carried out by sequencing the fragments with SNPs. Polymerase chain reaction was used to amplify a 561-bp DNA fragment encompassing five SNPs of interest in intron 1 of *COL2A1* (NCBI human genome build 36.3, NC\_000012.11 for genomic DNA and NM\_001844.4 for mRNA),including rs1635529,rs60542319,rs1635530,rs1635531 and rs954326 [http://www.ncbi.nlm.nih.gov/snp/, National

Center for Biotechnology Information (NCBI), Bethesda, MD, USA], using a pair of primers (Table I). Sequences of the amplicons were determined by Sanger dideoxy sequencing with ABI BigDye Terminator cycle sequencing kit version 3.1 (Applied Biosystems, Foster City, CA, USA) on an ABI 3100 Genetic Analyzer (Applied Biosystems). Sequencing results from patients as well as *COL2A1* consensus sequences from the NCBI Human Genome Database (NC\_000005.8) were imported into the SeqManII program of the Lasergene package (DNAStar Inc., Madison, WI, USA) and then aligned to identify the genotyping at each site with a polymorphism.

*Statistical analysis*. Initially, Hardy-Weinberg equilibrium was tested by the Chi-square test (p-values <0.05 were considered significant). Haplotype estimation and inference was determined using PHASE (29,30) (PHASE software version 2; Dr Matthew Stephens, University of Chicago, Chicago, fIL, USA). Distribution of genotype, allele frequency and haplotype in the high myopia group were compared with that in the control group by using the Chi-square test. P<0.05 was used as

(2/1)	U		Gen	lotype			All	lele	
	- 1	/1	1/2	2/2	<sup>a</sup> P-value	MAF [1]	<sup>a</sup> P-value	OR	95% CI
rs1635529 A/C HM 581 MNC 384	84	84 57	280 185	217 142	0.984	0.386 0.389	0.867	0.984	(0.816-1.187
rs60542319 -/C HM 581 MNC 384	81 84	84 57	280 185	217 142	0.984	0.386 0.389	0.867	0.984	(0.816-1.187
rs1635530 C/G HM 581 MNC 384	81 84 3 3 3	581 884	0 0	0 0	1	0.000	1	0	0
rs1635531 C/G HM 581 MNC 384	81 84 82 82	581 884	0 0	0 0	1	0.000 0.000	1	0	0
rs954326 A/C HM 581 MNC 384	81 84	15 8	145 86	421 290	0.558	0.151 0.133	0.275	1.158	(0.890-1.506

## Results

Five SNPs: rs1635529 (A/C), rs60542319 (-/C), rs1635530 (C/G), rs1635531 (C/G) and rs954326 (A/C) (Fig. 1) were selected for the association study, for their localization within *COL2A1* intron 1 using the HapMap database (http://www.ncbi.nlm.nih.gov/SNP/ provided in the public domain by the NCBI, Bethesda, MD, USA).

The genotype for each SNP was identified by direct sequencing of all subjects. Genotype and allele frequencies in the myopia and control groups are shown in Table II. Polymorphisms for rs1635530 and rs1635531 were not detected, as the C allele was present in all 965 subjects recruited. The other three SNPs (rs1635529 A/C, rs60542319 -/C and rs945326 A/C) revealed three types of genotypes, respectively (Fig. 2). There was no significant deviation from HWE for these three SNP distributions in the case and controls (data not shown).

In all high myopia subjects (n=581), allele diversity of rs1635529 (allele A: 448/1162=0.39 and allele C=0.61) and rs954326 (allele A: 175/11162=0.15 and allele C=0.85) were confirmed to HapMap data (http://www.ncbi.nlm.nih.gov/SNP/). In this study, we provide allele diversity data for rs60542319 (allele -: 448/1162=0.39 and allele C=0.61) in a Han Chinese cohort, while only data for Western and Northern Europeans are listed.

For total myopia cases (n=581), compared with the control group (n=384), there was no significant distribution difference for genotype, allele (Table II) and haplotype (rs1635529, rs60542319 and rs954326) frequencies (Table III).

## Discussion

In the present study, SNP rs1635529, from previous reports (23,28), and 4 more adjacent SNPs in *COL2A1* were selected to evaluate the possible relevance to high myopia. In the current study, a Chinese high myopia population composed of 581 subjects (spherical equivalent  $\leq$ -6.00D) and 384 matched controls (-0.50D  $\leq$  spherical equivalent  $\leq$  +2.00D) participated in this association study. Our results found that no association was present between high myopia and *COL2A1* SNP rs1635529, rs60542319 and rs954326. The genotype, allele and haplotype frequencies were not significantly different (P>0.05). It is the first report in a Chinese population to identify an association of rs1635529 with high myopia and obtain a negative result.

To date, there are two positive association results of rs1635529 with myopia, both in a Caucasian cohort. One study showed highly significant over-transmission to a cohort of 342 myopes ( $\leq 0.75D$ ) with mixed, but Caucasian-dominant, ethnicity (23). Another study identified a significant association between rs1635529 with high-grade myopia ( $\leq$ -5.00D) in two independent Caucasian family datasets, Duke (146 cases) and Cardiff (130 cases) (28). However, our results here presented negative results in the Chinese myopia group. Combined with

d)

Group	n	Haplotype <sup>a</sup>				
		A-C	A-A	CCC	CCA	<sup>b</sup> P-value
HM	581	449	0	538	175	0.367
MNC	384	298	1	369	100	

Table III. Haplotype (rs1635529, rs60542319 and rs954326) frequency of COL2A1 between high myopia cases and controls.

HM, high myopia; MNC, myopia normal controls. <sup>a</sup>Haplotype estimation and inference were determined using PHASE. <sup>b</sup>P-value was calculated by the Chi-square test between the high myopia and normal control.

no association of *COL2A1* with myopia in a Hong Kong Chinese population (130 nuclear families,  $\leq$ -0.50D) after four *COL2A1* SNPs (in exons 1, 7, 34 and 53) were genotyped (31), *COL2A1* does not associate with myopia in the Chinese cohort. This may be due to the ethnic difference and more confirmation will be required in different cohorts. In addition, no linkage study has reported evidence of a strong linkage between *COL2A1* and high or common myopia to date, (32), other than one study with a suggestive linkage result to the *COL2A1* locus on 12q, as mentioned in the same Duke and Cardiff cohort (28). It is possible that it may be due to a shared linkage disequilibrium pattern with a causative variant in a nearby gene (2).

Numerous studies have strongly implicated the role of certain genetic factors in myopia development. Association studies are widely applied to explore possible factors that can play a role in myopia formation, since its mechanism of pathogenesis remains to be clarified. *COL2A1* is responsible for certain cases of Stickler Syndrome. To date, a number of studies have attempted to identify an association between non-syndromic high myopia with common SNPs in genes responsible for syndromic high myopia but, unfortunately, the results are inconclusive and generally controversial (33).

In conclusion, our study does not support an association of high myopia with rs1635529 and other SNPs in *COL2A1*, and further studies are required to identify candidate genes responsible for myopia.

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