SHORT COMMUNICATION

Systematic assessment of the tagging polymorphisms of the *COL1A1* gene for high myopia

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Abstract Reduced scleral collagen accumulation has been found in the development of myopia. Single nucleotide polymorphisms (SNPs) at the type I collagen α-1 gene (*COL1A1*) may cause different susceptibilities to myopia. We conducted a case–control study to systematically examine *COL1A1* as a candidate gene for high myopia. A case was defined as spherical refraction ≤–6 D and control ≥–1.5 D. The study comprised 471 cases and 623 controls, and ten tagging SNPs were genotyped. None of the SNPs reached the significant level of 0.05. Subset analysis on cases with a strong family history did not demonstrate significant results. We could not find an interaction between gene and near work. Exploratory analyses by changing the cutoff values to re-define cases and controls did not improve the results. Haplotype analysis did not yield significant association with myopia. This study failed to demonstrate *COL1A1* as a significant risk factor for high myopia.

Keywords SNP · COL1A1 · Haplotype · Myopia · Case–control study

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Introduction

Myopia is a common eye condition worldwide. Taiwan is among the highest risk areas in the world. The prevalence of high myopia is 18% among young Taiwanese men and 24% among young Taiwanese women (Lin et al. 2001). Using family data, we reported that a family history was a significant risk factor for high myopia (Liang et al. 2004). However, genetic studies on non-syndromic myopia are relatively sparse. More efforts are needed to discover genetic loci influencing susceptibility to common myopia and to fill the gap in our knowledge of the underlying genetic mechanism.

The development of high myopia is associated with marked thinning of the sclera. Collagen accounts for 90% of scleral dry weight (Norton and Rada 1995), and the majority is type I collagen (Keeley et al. 1984). It was found in animal studies that type I collagen mRNA expression significantly decreased in the sclera of the myopic eyes relative to the contralateral control

eyes (Gentle et al. 2003). The study also found that posterior scleral collagen degradation was significantly increased in eyes developing myopia. Genetic variants at the type I collagen α-1 gene (*COL1A1*) may influence gene expression, which causes different susceptibilities to myopia. Paluru et al. (2003) reported a linkage of high myopia to 17q21-22, where *COL1A1* is located. Accordingly, *COL1A1* can be a candidate gene for myopia. Although Paluru et al. (2003) did not find causal mutations in the coding region of *COL1A1*, this does not completely exclude a possibility of a polymorphism in the non-coding region conferring a risk for myopia. We conducted a case–control study to test for the *COL1A1* gene as a susceptibility locus to high myopia.

Methods

Subjects

The participants were recruited from young male military conscripts between 2003 and 2006. All subjects were of Han Chinese descent and between the ages of 18–25 years. Individuals with spherical refraction ≤–6.0 D in one eye and ≤–4.0 D in the other eye were classified as cases. A subject was recruited as a control if his worse eye had spherical refraction ≥–1.5 D. The refractive error was measured with an autorefractometer (Topcon KR-8100) for all eyes. Each participant was given a questionnaire in which details of family history and environmental factors were asked. The details of the questionnaire can be found in our previous publication (Liang et al. 2004). All participants gave their informed consent. The study was approved by the Institutional Review Board at the Kaohsiung Medical University, Taiwan.

SNP selection and genotyping

Tagging single nucleotide polymorphisms (tSNPs) at *COL1A1* were selected from the phase II data of the HapMap Project (Altshuler et al. 2005) using the Tagger Pairwise method (Barrett et al. 2005). Seven tSNPs were selected according to the following criteria: $r^2 \geq 0.8$ and minor allele frequency (MAF) ≥ 10% in the Han Chinese population (http://www.hapmap.org). To increase the coverage of this candidate gene, we also manually selected three additional common SNPs (rs2075559, rs2249492 and rs1061237) with a MAF > 40%. Genotyping was carried out by using the TaqMan technology. To reduce the genotyping cost,

we did not type every subject for all SNPs. However, the number of genotyping for every SNP was more than 960 to assure enough statistical power.

Statistics

Hardy–Weinberg equilibrium (HWE) was tested among cases and controls separately. We examined the effect of the common allele in three genetic models (dominant, additive and recessive). We also used the Hap-Clustering program (Tzeng et al. 2006) to perform haplotype analysis for each haplotype block. Education level is a surrogate of near work, and it has been repeatedly shown as a risk factor for myopia (Mutti et al. 2002; Liang et al. 2004). We further tested whether education can be an effect modifier to interact with the genotypes. The education level was dichotomized into high (i.e., college or graduate) education level and low education level, to be consistent with our previous study (Liang et al. 2004). To enrich the genetic component, we also performed a subset analysis only on cases with highly myopic parents.

We used 450 cases and 550 controls to estimate the power to detect two different odds ratios given the risk allele frequency of 10% in the general population. The results showed that our data provided a power of 93% and 79% for an odds ratio (OR) of 1.9 and 1.7, respectively.

Results

A total of 471 cases and 623 controls was included. SNP rs2141279 was not in HWE ($P < 1.0 \times 10^{-9}$) and was removed for further statistical analysis. None of the SNPs reached a nominal significant level of 0.05 (Table 1). Subset analysis on cases with highly myopic parents did not yield any new significant result except for rs2277633, with a *P* value of 0.04 under the recessive model (supplementary Table 1). Similarly, subjects stratified by educational level did not demonstrate any significant result or a significant gene × education interaction (supplementary Table 2). We further performed exploratory analyses by increasing the cutoff values to re-define cases (≤–7 D, –8 D, –9 D or –10 D) and decreasing the cutoff values to re-define controls (≥–1.0 D or ≥–0.5 D). However, the re-defined cases and controls did not improve the results. The ten SNPs form three small haplotype blocks, but haplotype analyses did not yield significant results for any block (overall *P* > 0.9 for each block).

Table 1 Ten SNPs and their relationships with high myopia under three genetic models (dominant, additive and recessive)

SNP	Myopia	Genotype (n)			P		
					Dominant	Additive	Recessive
rs2075555	Case	GG (183)	GT (161)	TT (36)	0.94	0.88	0.63
	Control	GG (296)	GT (269)	TT (53)			
rs2141279	Case	CC (190)	CT (179)	TT (1)	NA[a]	NA[a]	NA[a]
	Control	CC (298)	CT (300)	TT (7)			
rs2586494	Case	AA (96)	AC (194)	CC (90)	0.61	0.64	0.20
	Control	AA (162)	AC (278)	CC (166)			
rs2696247	Case	AA (172)	AG (160)	GG (43)	0.78	0.81	0.93
	Control	AA (266)	AG (257)	GG (69)			
rs2075559	Case	CC (142)	CG (211)	GG (118)	0.31	0.89	0.18
	Control	CC (167)	CG (312)	GG (132)			
rs2586488	Case	GG (118)	AG (173)	AA (88)	0.33	0.76	0.59
	Control	GG (174)	AG (308)	AA (134)			
rs2277633	Case	GG (272)	AG (87)	AA (13)	0.98	0.77	0.32
	Control	GG (455)	AG (153)	AA (15)			
rs2277632	Case	AA (165)	AG (167)	GG (43)	0.37	0.42	0.75
	Control	AA (254)	AG (289)	GG (75)			
rs2249492	Case	TT (111)	CT (180)	CC (87)	0.48	0.98	0.47
	Control	TT (167)	CT (316)	CC (129)			
rs1061237	Case	AA (111)	AG (174)	GG (87)	0.68	0.73	0.30
	Control	AA (178)	AG (316)	GG (128)			

[a] No statistical results because not in HWE

Discussion

We systematically investigated ten tSNPs at *COL1A1* using a large Taiwanese population. We analyzed the genetic effects of each SNP on high myopia, but none of them was significantly associated with the phenotype. Various analyses were also performed, including: subset analysis on subjects with a strong family history; subset analysis according to education level; and analyses based on different cutoff points to re-define cases and controls. Although we found one *P* value of 0.04 for the cases with a strong family history, this *P* value should not be considered significant after adjustment for multiple testing. No other approaches yielded any significant finding. Given the large sample size in the present study, and the use of tSNPs, it is unlikely that the existence of a common genetic variant at *COL1A1* would influence susceptibility to high myopia.

Our study design has strengths and limitations. We recruited only young men to avoid the heterogeneity due to gender in the phenotype, because women have been shown to have a higher risk for high myopia (Liang et al. 2004). Therefore, our study was not confounded by the gender issue. The study population was relatively homogenous in terms of ethnicity, geographic location and age. As a result, several undetectable confounding factors could be minimized. It needs to be noted that our results cannot exclude the existence of a rare causal genetic polymorphism at *COLIA1*. Using both empirical and simulated data,

Zeggini et al. (2005) showed that common tSNPs have limited capacity to tag variants with minor allele frequencies of <5%. Although using haplotypes can increase the power to detect a rare causal allele, the power is still not satisfied (de Bakker et al. 2005).

We did not examine the Sp1 polymorphism that is the most commonly studied marker at *COLIA1*, because it is not polymorphic in East Asians (Lei et al. 2003).

In conclusion, our systematic evaluation indicated that the *COL1A1* gene is unlikely to play an important role in high myopia.

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