GENETIC TESTING AND MOLECULAR BIOMARKERS
Volume 18, Number 6, 2014
© Mary Ann Liebert, Inc.
Pp. 446–452
DOI: 10.1089/gtmb.2013.0479

Evaluation of *MYOC*, *ACAN*, *HGF*, and *MET* as Candidate Genes for High Myopia in a Han Chinese Population

Xian Yang,[1,*] Xiaoqi Liu,[2–4,*] Jie Peng,[5,*] Hong Zheng,[2,*] Fang Lu,[2] Bo Gong,[2] Guiqiu Zhao,[1] Yan Meng,[1] Hongzai Guan,[1] Meizhen Ning,[1] Zhenglin Yang,[2,4] and Yi Shi[2–4]

Aim: To investigate the association between high myopia (HM) and single nucleotide polymorphisms (SNPs) in the myocilin (*MYOC*), hepatocyte growth factor (*HGF*), hepatocyte growth factor receptor (*MET*), and aggrecan (*ACAN*) genes in a Han Chinese population. *Methods:* Sixteen SNPs were genotyped by the SNaPshot method in a subject group composed of 1052 HM patients and 1070 controls. Statistical analysis was performed to determine the association between the SNPs and the susceptibility of HM. *Results:* Two SNPs (rs3784757 and rs1516794) in *ACAN* were significantly associated with HM ($p = 0.0334$ and 0.0236, odds ratio [OR] = 0.83 and 0.79, respectively). The risk haplotype CA and the protective haplotype TT, generated by rs3784757 and rs1516794, showed significant association with HM ($p = 0.0327$ and 0.0304, OR = 1.21 and 0.80, respectively). Two SNPs (rs38857 and rs10215153) in *MET* and one SNP (rs3784757) in *ACAN* showed significant association with HM ($p = 0.0064$, 0.0113, and 0.0373; OR = 4.14, 5.74 and 0.52; respectively) in the recessive model. None of the other SNPs showed significant association with HM. *Conclusions:* Our results suggested that genetic variants in *ACAN* and *MET* are associated with HM. Functional roles of *ACAN* and *MET* in the development of HM need to be further investigated.

Introduction

High myopia (≤ -6.0 diopters) is a disorder that can cause poor eyesight by refractive error, bringing about a loss of visual acuity due to severe complications of glaucoma, retinal or choroid detachment (Celorio and Pruett, 1991), and macular degeneration (Burton, 1989). It has the highest prevalence in East Asian populations. The prevalence of high myopia (HM) in a rural Chinese adult population and Singapore adult Chinese population was 1.8% and 9.1% (Wong *et al.*, 2000; Liang *et al.*, 2009). However, the treatments for HM are limited and the pathogenesis of HM remains unclear.

It is believed that myopia is a complex disease caused by the interaction of multiple genetic and environmental factors. However, HM is highly heritable, where genetic predisposition seems to be a dominant factor (Dirani *et al.*, 2006). Identification of genes responsible for myopia, especially HM, is very important but very difficult, although several loci for HM have been mapped (Yang *et al.*, 2009; Ma *et al.*, 2010; Shi *et al.*, 2011a, 2011b; Wojciechowski, 2011). However, no convincing causal genes have yet been identified at these loci. Recently, the candidate gene association studies of HM or refractive error have led to the identification of several susceptible genes including the myocilin (*MYOC*) gene, the hepatocyte growth factor (*HGF*) gene, the hepatocyte growth factor receptor (*MET*) gene, and the aggrecan (*ACAN*) gene (Han *et al.*, 2006; Tang *et al.*, 2007; Vatavuk *et al.*, 2009; Yanovitch *et al.*, 2009; Yip *et al.*, 2011). To further investigate whether these genes are associated with HM in Han Chinese, 16 single nucleotide polymorphisms (SNPs), which were previously reported to be associated with myopia or HM of the *MYOC*, *HGF*, *MET*, and *ACAN* genes, have been chosen to be tested in this study in a Han Chinese population composed of 1052 HM cases and 1070 normal controls.

[1]Department of Ophthalmology, Qingdao University School of Medicine, Affiliated Hospital of Qingdao University School of Medicine, Qingdao, China.
[2]The Sichuan Provincial Key Laboratory for Human Disease Gene Study, Clinical Laboratory Department, Sichuan Academy of Medical Sciences & Sichuan Provincial People's Hospital, Chengdu, China.
[3]Key Laboratory for NeuroInformation of Ministry of Education, School of Life Science and Technology, University of Electronic Science and Technology of China, Chengdu, China.
[4]School of Life Science and Engineering, Southwest Jiaotong University, Chengdu, China.
[5]Department of Ophthalmology, Sichuan Academy of Medical Sciences & Sichuan Provincial People's Hospital, Chengdu, China.
*These authors contributed equally to this work.

Materials and Methods

Ethics statement

All procedures used in this study conformed to the tenets of the Declaration of Helsinki. The Institutional Review Board and the Ethics Committee of the Affiliated Hospital of Qingdao University School of Medicine of China and Sichuan Academy of Medical Sciences & Sichuan Provincial People's Hospital approved the protocols used. Informed consent was obtained from all participants.

Subjects

All of the participants in this study were drawn from the Chinese Han population. All individuals (HM patients and normal controls) were recruited at the clinic and ward of the ophthalmic department, the Affiliated Hospital of Qingdao University School of Medicine of China. All subjects underwent standard ophthalmologic examinations, including uncorrected visual acuity testing, best-corrected visual acuity testing, dilated pupillary indirect ophthalmoscopic examination of the fundus, slit-lamp biomicroscopic examination of the anterior chamber, intraocular pressure examination by Goldman applanation tonometer, keratometry by keratometer, optometry, and measurement of axial length by ultrasound (CINE SCAN, French). The diagnosis for HM in this study required the spherical equivalent to be less than or equal to -6.0 diopter sphere (DS) in at least one eye and the axial length of the eye globe to be longer than or equal to 26.0 mm. Individuals were excluded from the study if they had undergone ocular procedures that might alter refraction, or if they had other symptoms besides HM (e.g., besides eye problems, individuals with Stickler syndrome suffer from distinctive facial abnormalities, hearing loss, and joint phenotypes). For the controls, the criteria were a spherical equivalent from -1.0 to $+1.0$ DS and no evidence of disease in either eye. All of the controls were unrelated individuals to the HM cases and to themselves. A total of 1052 unrelated patients with HM and 1070 normal controls were analyzed in this study (Table 1).

SNP selection and genotyping

We selected 16 SNPs that were previously reported to be associated with myopia or HM at the loci of the *MYOC*, *HGF*, *MET*, and *ACAN* genes (Han *et al.*, 2006; Tang *et al.*, 2007; Vatavuk *et al.*, 2009; Yanovitch *et al.*, 2009; Yip *et al.*, 2011). Venous blood from each subject was drawn and collected in an EDTA tube. Genomic DNA was extracted from the blood by serial phenol/chloroform extraction and ethanol precipitation. SNP genotyping was performed with the dye terminator-based SNaPshot method (Applied Biosystems, Foster City, CA). SNP analysis was performed on the ABI 3130 Genetic Analyzer (Applied Biosystems). The SNP reported in this article has a genotyping success rate of 97% accuracy as judged by random re-genotyping of 5% of the samples in the cohort.

Haplotype analysis

The linkage disequilibrium (LD) block structure was examined using the program Haploview (Vision 4.2). The D' values and r-values for all pairs of SNPs were calculated, and the haplotype blocks were estimated using the program Haploview (Vision 4.2). The haplotype frequencies between cases and controls were compared using χ^2 analysis.

Statistical analysis

The Hardy–Weinberg equilibrium (HWE) for the SNPs was calculated by the χ^2 test. All analyses were adjusted for age and sex on total cohorts. Allele and genotype frequencies between cases and controls were compared with the χ^2 analysis. Statistical significance was defined as $p < 0.05$. All statistical analyses were performed using the software SPSS version 10.0. Correction for multiple testing was analyzed by the Bonfferoni method. Genetic power was calculated by using the software ''Power and Sample Size Program'' (PS version 3.0.43) (Dupont and Plummer, 1998).

Results

SNP analysis

The 16 selected SNPs were successfully genotyped and were within HWE in both case and control groups ($p > 0.001$, Table 2). The SNP frequencies of the controls in this study were similar to those of Han Chinese Beijing available in HapMap database, which implied reliable genotyping data in the study.

We found that rs3784757 and rs1516794 in the *ACAN* locus were significantly associated with HM in this study (allelic $p = 0.0334$ and 0.0236, respectively; odds ratio [OR] [95% confidence interval (CI)] = 0.83 [0.70, 0.99] and 0.79 [0.64, 0.97], respectively; Table 2). None of the other 14 SNPs showed significant difference between the case and control groups (allelic $p > 0.05$, Table 2). We also tested the association between these SNPs and HM by using dominant and recessive models. We found that two SNPs (rs38857 and rs10215153) in the *MET* gene and one SNP (rs3784757) in the *ACAN* gene were significantly associated with HM of recessive model ($p = 0.0064$, 0.0113, and 0.0373, respectively; OR = 4.14 [95% CI: 1.38, 12.43], 5.74 [95% CI: 1.27, 25.95], and 0.52 [95% CI: 0.28, 0.97], respectively; Table 3), while the other SNPs showed no association between the

Table 1. Characteristics of High Myopia Cases and Controls in the Study

Group	Total number	Male (%)	Female (%)	Average age (years)[a]	Age range (years)	Refractive errors (diopter)		Axial length (mm)	
						OD	OS	OD	OS
Cases	1052	598 (56.8)	467 (43.2)	47.65 ± 16.48	11–78	−9.14 ± 2.77	−9.45 ± 2.93	27.82 ± 1.71	28.03 ± 1.94
Controls	1070	587 (54.9)	602 (45.1)	61.12 ± 12.03	40–75	−0.45 ± 0.41	−0.48 ± 0.39	23.02 ± 0.53	23.11 ± 0.47

[a]The values are given in ± standard deviation.
OD, right eye; OS, left eye.

Table 2. Association Between High Myopia and 16 Single Nucleotide Polymorphisms of the *MYOC*, *HGF*, *MET*, and *ACAN* Genes in the Han Chinese Population

Gene	*SNP*	*Chr*	*BP*	*Minor allele*	*Number (case/control)*	*MAF (case/control)*	p_*HWE* *(case/control)*	p_*allelic*[a]	*Odds ratio (95% CI)*
MYOC	rs235858	1	169863125	C	1052/1070	0.494/0.503	0.322/0.087	0.5588	0.96 (0.86, 1.09)
	rs2421853	1	169866576	A	1046/1064	0.210/0.194	0.843/0.033	0.1887	1.11 (0.95, 1.29)
	rs7545646	1	169873362	C	1019/1039	0.012/0.013	0.704/0.671	0.7242	0.91 (0.52, 1.57)
	rs16864720	1	169878890	A	1048/1064	0.012/0.013	0.684/0.675	0.9341	0.98 (0.57, 1.68)
	rs7523603	1	169880993	C	1046/1062	0.259/0.278	0.878/0.363	0.1601	0.91 (0.79, 1.04)
	rs235920	1	169888598	C	1050/1064	0.436/0.428	0.403/0.001	0.6173	1.03 (0.91, 1.17)
	rs2075537	1	169890456	T	1045/1068	0.437/0.459	0.266/0.694	0.1603	0.92 (0.81, 1.04)
HGF	rs2286194	7	81193385	A	1050/1064	0.184/0.193	0.006/0.490	0.4859	0.95 (0.81, 1.10)
	rs3735520	7	81238875	T	1052/1066	0.438/0.443	0.103/0.709	0.7648	0.98 (0.87, 1.11)
MET	rs9641562	7	116110027	C	1050/1068	0.090/0.104	0.004/0.034	0.1128	085 (0.69, 1.04)
	rs38857	7	116152649	T	1052/1066	0.110/0.096	0.295/0.041	0.1305	1.17 (0.96, 1.42)
	rs10215153	7	116186367	A	1052/1070	0.105/0.091	0.842/0.012	0.1147	1.18 (0.96, 1.44)
	rs2073560	7	116210397	A	1049/1068	0.288/0.304	0.790/0.434	0.2703	0.93 (0.81, 1.06)
	rs1621	7	116224842	G	1050/1068	0.132/0.122	0.707/0.579	0.3197	1.10 (0.91, 1.31)
ACAN	rs3784757	15	87204408	T	1052/1068	0.131/0.154	0.401/0.390	0.0334	0.83 (0.70, 0.99)
	rs1516794	15	87205907	T	1052/1068	0.084/0.104	0.584/0.418	0.0236	0.79 (0.64, 0.97)

[a]*p*-values were adjusted for age and sex.

BP, base position; Chr, chrosome; MAF, frequency of minor allele; *p*_HWE, the *p*_value of Hardy–Weinberg equilibrium; CI, confidence interval; SNP, single nucleotide polymorphism.

cases and controls in both dominant and recessive models ($p > 0.05$, Table 3).

We performed a power analysis to rule out false negatives. We have calculated the genetic power of all the tested SNPs by using the software ''Power and Sample Size Program'' (PS version 3.0.43) and the power values ranged from 0.951 to 0.986.

Haplotype analysis

We then performed haplotype analysis by using Haploview 4.2 software to examine the LD block structure of the 16 SNPs in the *MYOC*, *HGF*, *MET*, and *ACAN* genes. As shown in Figure 1A, two LD blocks were located in the *MYOC* gene including three and two SNPs, respectively. rs2285194 and rs3735520 of the *HGF* gene were not in the same LD block with the D′ of 0.82 (Fig. 1B). rs2073560 and rs1621 were in the same LD block of the *MET* gene with the D′ of 0.97 (Fig. 1C). rs3784757 and rs1516794 in the *ACAN* gene were in the same LD block with the D′ of 0.99 (Fig. 1D).

The risk haplotype CA and the protection haplotype TT generated by rs3784757 and rs1516794 were significantly associated with HM ($p = 0.0327$ and 0.0324, respectively; Table 4) with OR of 1.21 (95% CI: 1.02, 1.43) and 0.80 (95% CI: 0.65, 0.98), respectively. However, the other haplotypes showed no significant association between the case and control groups ($p > 0.05$, Table 5 and 6).

Discussion

Recently, *MYOC*, *HGF*, *MET*, and *ACAN* SNPs were reported to lead susceptibility for HM (Han *et al.*, 2006; Tang *et al.*, 2007; Vatavuk *et al.*, 2009; Yanovitch *et al.*, 2009; Yip *et al.*, 2011). However, controversy exists in the previous studies regarding the association between them and HM (Khor *et al.*, 2009; Schache *et al.*, 2009; Zayats *et al.*, 2009). In this study, we found that SNPs in the *MET* and *ACAN* genes but not in the *MYOC* and *HGF* genes were associated with HM in a Han Chinese population.

The *myocilin* gene (*MYOC*; OMIM 601652), located on chromosome 1q24-q25, is expressed in many human eye tissues, including the trabecular meshwork cells (TMC), sclera, ciliary body, iris, choroids, and retina (Adam *et al.*, 1997), and the secreted protein is present in the aqueous

Table 3. Genotype Analysis of rs38857, rs10215153, and rs3784757 in High Myopia Cases and Controls

Gene	*SNP*	*Chr*	*BP*	*Genotype*	*Case* n *(%)*	*Control* n *(%)*	p	*OR (95% CI)*
MET	rs38857	7	116152649	TT	16 (1.5)	4 (0.4)	0.0064	4.14 (1.38, 12.43)
				CT	199 (18.9)	196 (18.4)		
				CC	837 (79.6)	866 (81.2)		
MET	rs10215153	7	116186367	AA	11 (1.0)	2 (0.2)	0.0113	5.74 (1.27, 25.95)
				AG	199 (18.9)	190 (17.8)		
				GG	842 (80.0)	878 (82.1)		
ACAN	rs3784757	15	87204408	TT	15 (1.4)	29 (2.7)	0.0373	0.52 (0.28, 0.97)
				CT	246 (23.4)	271 (25.4)		
				CC	791 (75.2)	768 (71.9)		

p, the *p*-value of recessive model; OR, the odds ratio of homozygote.

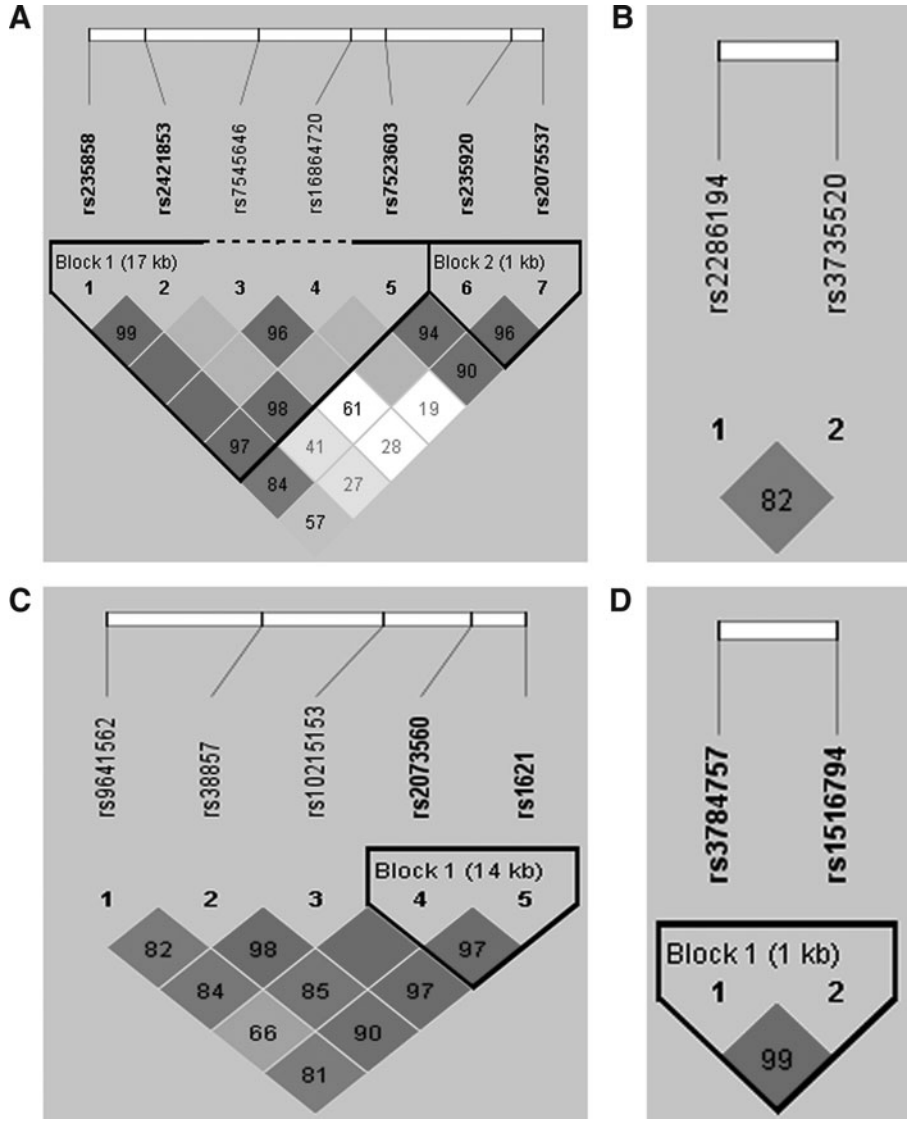

FIG. 1. The linkage disequilibrium (LD) pattern of the 16 SNPs in *MYOC*, *HGF*, *MET*, and *ACAN* gene **(A–D**, respectively). The LD block structure was examined using the program Haploview (Vision 4.2) based on the genotype data. The D' values and r^2 values for all pairs of SNPs were calculated, and the haplotype blocks were generated by the program Haploview.

humor (Tamm, 2002). MYOC is well known for its role in glaucoma (Polansky, 2003; Gong *et al.*, 2004). From clinical statistics, myopia patients are susceptible to open-angle glaucoma (Perkins and Phelps, 1982; Mastropasqua *et al.*, 1992; Grødum *et al.*, 2001), and there is evidence of higher intraocular pressure in myopic eyes compared with emmetropic eyes (Wu *et al.*, 2000). Additionally, some factors, such as bFGF, that stimulate myocilin expression in TMC have also been implicated in the regulation of postnatal eye growth and myopia (Rohrer *et al.*, 1997; Lin *et al.*, 2006; Andrew *et al.*, 2008). Association between *MYOC* and HM was first reported in a population-based case–control study of Chinese ethnicity in Singapore (Wu *et al.*, 1999). However, the results of the following studies between *MYOC* and HM were inconsistent and were even conflicting in the same ethical origin populations (Leung *et al.*, 2000; Zayats *et al.*, 2009). In the present study, we examined the association between seven SNPs in the *MYOC* gene and their haplotypes and HM in a Han Chinese population. We found no evidence to support a significant association between *MYOC* polymorphisms and HM (Table 2). More work should be carried out to verify the influence of MYOC on HM susceptibility in Han Chinese.

Table 4. *ACAN* Haplotype Association with High Myopia in the Han Chinese Population

	Haplotype		*Frequency*			
ACAN	*rs3784757*	*rs1516794*	*Case*	*Control*	p	*OR (95% CI)*
1	C	A	0.869	0.846	0.0327	1.21 (1.02, 1.43)
2	T	T	0.084	0.103	0.0304	0.80 (0.65, 0.98)
3	T	A	0.048	0.051	0.5881	0.93 (0.70, 1.22)

Table 5. *MYOC* Haplotype Association with High Myopia in the Han Chinese Population

	Haplotype			*Frequency*			
Block 1	*rs235858*	*rs2421853*	*rs7523603*	*Case*	*Control*	p	*OR (95% CI)*
1	T	G	T	0.293	0.303	0.475	0.95 (0.84, 1.09)
2	C	G	C	0.254	0.276	0.1055	0.89 (0.78, 1.02)
3	C	G	T	0.24	0.226	0.2848	1.08 (0.94, 1.25)
4	T	A	T	0.207	0.192	0.2292	1.10 (0.94, 1.28)
Block 2	*rs235920*	*rs2075537*		*Case*	*Control*	p	*OR (95% CI)*
1	T	T		0.433	0.452	0.2064	0.93 (0.82, 1.04)
2	C	G		0.432	0.421	0.4758	1.05 (0.93, 1.18)
3	T	G		0.131	0.120	0.2958	1.10 (0.92, 1.32)

The hepatocyte growth factor (HGF) and its receptor (MET), broadly expressed in retina, pigment epithelium, and choroid, play an important role in matrix metalloproteinases and tissue inhibitors of metalloproteinase pathways, and may play an active role in scleral remodeling, axial elongation, and myopia development as well (Hamasuna *et al.*, 1999; Gong *et al.*, 2003; Han *et al.*, 2006). It was initially shown as a candidate for myopia in an analysis of mice eye weight (Zhou and Williams, 1999) and was confirmed to have significant effects on eye size, lens weight, and retinal area. *HGF* had previously been reported to be associated with HM in a Han Chinese population by family-based analysis (128 nuclear families with 133 severely myopic offspring) (Han *et al.*, 2006). A strong association has been found between the mild to moderate myopia group and the *HGF* SNP rs3735520 and the *HGF* haplotypes rs2286194-rs3735520-rs17501108 and rs12536657-rs2286194, and a moderate association of the extreme HM with rs2286194 in a Caucasian family dataset (649 individuals) (Yanovitch *et al.*, 2009). However, no evidence of association between the *MET* gene and myopia was found in the same study (Yanovitch *et al.*, 2009). Analysis of a Singaporean children cohort (1126 individuals) suggested that carriage of rs2073560 (*MET*+ 110703) A allele was associated with increased susceptibility to myopia and was also associated with a faster rate of change in refractive error (Khor *et al.*, 2009). A current study (Schache *et al.*, 2009) found that there is likely no genetic association of the *MET* gene with myopia, axial length, anterior chamber depth, and corneal curvature in a Caucasian population. In our study, no genetic association between the *HGF* gene and HM has been found (Table 2). Two SNPs (rs38857 and rs10215153) in the *MET* gene showed significant association with HM in the recessive model (Table 3). Because of the relatively larger sample used in this study, we suggested it is not *HGF* but *MET* that is more likely to be susceptible for HM in Han Chinese. However, given our findings and the previous studies together, it indicated that HGF and MET might play different role in different populations and in different type of myopia. We need to examine the association of HGF and MET between moderate, high, and extreme HM (≤ -10.0 diopters) respectively in the future.

Aggrecan (*ACAN*) is a proteoglycan gene in eye sclera. It was first reported in form-deprivation myopia animal models. In chicks, it accumulated in increased amounts in the presence of increased turnover rate in the cartilaginous layer of the posterior sclera, in parallel with the scleral growth of the myopic eye (Rada *et al.*, 1994; Rada *et al.*, 1998). However, in mammals such as tree shrew and monkey, the changes were opposite (Marzani and Wallman, 1997; McBrien *et al.*, 2000). In the last year, a Hong Kong group reported an evaluation of proteoglycan gene polymorphisms as risk factor in the genetic susceptibility to HM (Yip *et al.*, 2011). They found that rs3784757 and rs1516794 in the *ACAN* gene showed a weak association in group 1 subjects (300 patients with HM and 300 emmetropic controls) but was not replicated in group 2 subjects (356 HM patients and 354 controls). To our knowledge, the allele frequency of an SNP may be changed in different cohorts, even those from the same ethnic origin; thus, the results of association studies need to be further replicated in different cohorts or increase the sample size. In this study, our results showed borderline association between these two SNPs and HM (Table 2). However, after multiple testing by using the Bonferroni correction, rs3784757 and rs1516794 showed no significant differences between HM and control groups ($p = 0.534$ and 0.378, respectively). Nevertheless, haplotype frequencies of these SNPs in the *ACAN* gene were statistically associated with HM (Table 4). Therefore, to avoid filtering real myopia genes, the role of ACAN in the pathogenesis of myopia

Table 6. *MET* Haplotype Association with High Myopia in the Han Chinese Population

	Haplotype		*Frequency*			
MET	*rs2073560*	*rs1621*	*Case*	*Control*	P	*OR (95% CI)*
1	G	A	0.581	0.574	0.6833	1.03 (0.91, 1.16)
2	A	A	0.287	0.303	0.2427	0.92 (0.81, 1.05)
3	G	G	0.131	0.122	0.3668	1.09 (0.91, 1.30)

requires more refinement in both animal models and human genetic epidemiologic studies.

Taking our data and previous studies together, the association between genetic variants and the *MYOC*, *ACAN*, HGF, and *MET* genes were inconsistent in different populations. This may be explained by the genetic heterogeneity of HM among different ethnicities. It is also suggested that HM genetics are still not clearly defined and that a multifactorial model as yet to be determined will likely be the etiology.

Acknowledgments

We thank all the subjects for their participation. This work was supported by the following grants: the Outstanding Young Scientist Research Award Foundation of Shandong Province (BS2010SF009, to X.Y.); the National Basic Research Program of China (973 Program, 2011CB504604 to Z.Y.); the Natural Science Foundation of China (81170882 to Y.S.; 81271047 to X.L.; 81300768 to J.P.); the Department of Science and Technology of Sichuan Province, China (2012JQ0023 to Y.S.).

Declaration of Funding Sources

The funding organization had no role in the design or conduct of this research.

Author Disclosure Statement

All authors have declared that no competing interests exist.

References

Adam MF, Belmouden A, Binisti P, *et al.* (1997) Recurrent mutations in a single exon encoding the evolutionarily conserved olfactomedin-homology domain of TIGR in familial open-angle glaucoma. Hum Mol Genet 6:2091–2097.

Andrew T, Maniatis N, Carbonaro F, *et al.* (2008) Identification and replication of three novel myopia common susceptibility gene loci on chromosome 3q26 using linkage and linkage disequilibrium mapping. PLoS Genet 4:e1000220.

Burton TC (1989) The influence of refractive error and lattice degeneration on the incidence of RD. Trans Am Ophthalmol Soc 87:143–155.

Celorio JM, Pruett RC (1991) Prevalence of lattice degeneration and its relation to axial length in severe myopia. Am J Ophthalmol 111:20–23.

Dirani M, Chamberlain M, Shekar SN, *et al.* (2006) Heritability of refractive error and ocular biometrics: the Genes in Myopia (GEM) twin study. Invest Ophthalmol Vis Sci 47:4756–4761.

Dupont WD, Plummer WD (1998) Power and sample size calculations for studies involving linear regression. Control Clin Trials 19:589–601.

Gong G, Kosoko-Lasaki O, Haynatzki GR, *et al.* (2004) Genetic dissection of myocilin glaucoma. Hum Mol Genet 13 Spec No 1:R91–R102.

Gong R, Rifai A, Tolbert EM, *et al.* (2003) Hepatocyte growth factor modulates matrix metalloproteinases and plasminogen activator/plasmin proteolytic pathways in progressive renal interstitial fibrosis. J Am Soc Nephrol 14:3047–3060.

Grødum K, Heijl A, Bengtsson B (2001) Refractive error and glaucoma. Acta Ophthalmol Scand 79:560–566.

Hamasuna R, Kataoka H, Moriyama T, *et al.* (1999) Regulation of matrix metalloproteinase-2 (MMP-2) by hepatocyte growth factor/scatter factor (HGF/SF) in human glioma cells: HGF/SF enhances MMP-2 expression and activation accompanying up-regulation of membrane type-1 MMP. Int J Cancer 82:274–281.

Han W, Yap MK, Wang J, *et al.* (2006) Family-based association analysis of hepatocyte growth factor (HGF) gene polymorphisms in high myopia. Invest Ophthalmol Vis Sci 47:2291–2299.

Khor CC, Grignani R, Ng DP, *et al.* (2009) cMET and refractive error progression in children. Ophthalmology 116:1469–1474, 1474.e1.

Leung YF, Baum L, Lam DS, *et al.* (2000) TIGR/MYOC proximal promoter GT-repeat polymorphism is not associated with myopia. Hum Genet 107:404–405.

Liang YB, Wong TY, Sun LP, *et al.* (2009) Refractive errors in a rural Chinese adult population the Handan eye study. Ophthalmology 116:2119–2127.

Lin HJ, Wan L, Tsai Y, *et al.* (2006) The TGFbeta1 gene codon 10 polymorphism contributes to the genetic predisposition to high myopia. Mol Vis 12:698–703.

Ma JH, Shen SH, Zhang GW, *et al.* (2010) Identification of a locus for autosomal dominant high myopia on chromosome 5p13.3-p15.1 in a Chinese family. Mol Vis 16:2043–2054.

Marzani D, Wallman J (1997) Growth of the two layers of the chick sclera is modulated reciprocally by visual conditions. Invest Ophthalmol Vis Sci 38:1726–1739.

Mastropasqua L, Lobefalo L, Mancini A, *et al.* (1992) Prevalence of myopia in open angle glaucoma. Eur J Ophthalmol 2:33–35.

McBrien NA, Lawlor P, Gentle A (2000) Scleral remodeling during the development of and recovery from axial myopia in the tree shrew. Invest Ophthalmol Vis Sci 41:3713–3719.

Perkins ES, Phelps CD (1982) Open angle glaucoma, ocular hypertension, low-tension glaucoma, and refraction. Arch Ophthalmol 100:1464–1467.

Polansky JR (2003) Current perspectives on the TIGR/MYOC gene (Myocilin) and glaucoma. Ophthalmol Clin North Am 16:515–527.

Rada JA, Achen VR, Rada KG (1998) Proteoglycan turnover in the sclera of normal and experimentally myopic chick eyes. Invest Ophthalmol Vis Sci 39:1990–2002.

Rada JA, Matthews AL, Brenza H (1994) Regional proteoglycan synthesis in the sclera of experimentally myopic chicks. Exp Eye Res 59:747–760.

Rohrer B, Tao J, Stell WK (1997) Basic fibroblast growth factor, its high- and low-affinity receptors, and their relationship to form-deprivation myopia in the chick. Neuroscience 79:775–787.

Schache M, Chen CY, Dirani M, *et al.* (2009) The hepatocyte growth factor receptor (MET) gene is not associated with refractive error and ocular biometrics in a Caucasian population. Mol Vis 15:2599–2605.

Shi Y, Li Y, Zhang D, *et al.* (2011a) Exome sequencing identifies ZNF644 mutations in high myopia. PLoS Genet 7:e1002084.

Shi Y, Qu J, Zhang D, *et al.* (2011b) Genetic variants at 13q12.12 are associated with high myopia in the Han Chinese population. Am J Hum Genet 88:805–813.

Tamm ER (2002) Myocilin and glaucoma: facts and ideas. Prog Retin Eye Res 21:395–428.

Tang WC, Yip SP, Lo KK, *et al.* (2007) Linkage and association of myocilin (MYOC) polymorphisms with high myopia in a Chinese population. Mol Vis 13:534–544.

Vatavuk Z, Skunca Herman J, Bencić G, *et al.* (2009) Common variant in myocilin gene is associated with high myopia in

isolated population of Korcula Island, Croatia. Croat Med J 50:17–22.
Wojciechowski R (2011) Nature and nurture: the complex genetics of myopia and refractive error. Clin Genet 79:301–320.
Wong TY, Foster PJ, Hee J, *et al.* (2000) Prevalence and risk factors for refractive errors in adult Chinese in Singapore. Invest Ophthalmol Vis Sci 41:2486–2494.
Wu H, Yu XH, Yap EP (1999) Allelic association between trabecular meshwork-induced glucocorticoid response (TIGR) gene and severe myopia. Invest Ophthalmol Vis Sci 40: S600.
Wu SY, Nemesure B, Leske MC (2000) Glaucoma and myopia. Ophthalmology 107:1026–1027.
Yang Z, Xiao X, Li S, *et al.* (2009) Clinical and linkage study on a consanguineous Chinese family with autosomal recessive high myopia. Mol Vis 15:312–318.
Yanovitch T, Li YJ, Metlapally R, *et al.* (2009) Hepatocyte growth factor and myopia: Genetic association analyses in a caucasian population. Mol Vis 15:1028–1035.
Yip SP, Leung KH, Ng PW, *et al.* (2011) Evaluation of proteoglycan gene polymorphisms as risk factors in the genetic susceptibility to high myopia. Invest Ophthalmol Vis Sci 52:6396–6403.
Zayats T, Yanovitch T, Creer RC, *et al.* (2009) Myocilin polymorphisms and high myopia in subjects of European origin. Mol Vis 15:213–222.
Zhou G, Williams RW (1999) Eye1 and Eye2: gene loci that modulate eye size, lens weight, and retinal area in the mouse. Invest Ophthalmol Vis Sci 40:817–825.

Address correspondence to:
Yi Shi, PhD
The Sichuan Provincial Key Laboratory for Human Disease Gene Study
Sichuan Academy of Medical Sciences & Sichuan Provincial People's Hospital
32 the First Ring Road West 2
Chengdu
Sichuan 610072
China

E-mail: shiyi197911@gmail.com

Xian Yang, MD, PhD
Department of Ophthalmology
Affiliated Hospital of Qingdao University School of Medicine
16th Jiangsu Road
Qingdao
Shandong 266003
China

E-mail: yangxian_zhao@126.com