

Genetic Association Study Revealed Three Loci Were Associated Risk of Myopia Among Minors

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Background: Myopia has raised a predominant public concern among minors. A recent genome-wide association study (GWAS) identified six novel loci in Asian adults. Whether these genetic loci works for myopia in minors remains unknown and worthy of exploration.

Methods: In order to validate the findings, here we performed a case-control study (600 myopia minors, 110 high myopia (HM) minors, and 800 non-myopia minors as controls) utilizing the TaqMan single nucleotide polymorphism (SNP) genotyping assays. Adjusted odds ratios (ORs) and 95% confidence intervals (CIs) was adopted.

Results: The median ages in controls, myopia, and HM were 15.1, 15.0, and 15.1, respectively, while the means \pm standard deviations for them were 0.32 ± 0.41 , -3.2 ± 1.6 , and -9.8 ± 2.2 , respectively. We found rs2246661 (allelic OR: 1.29; 95% CI: 1.09–1.52; $P = 0.003$), rs74633073 (allelic OR: 1.41; 95% CI: 1.12–1.78; $P = 0.004$), and rs76903431 (allelic OR: 1.42; 95% CI: 1.11–1.81; $P = 0.005$) were significantly associated with increased risk of myopia. Rs2246661 was also significantly associated with increased risk of HM in minors (OR: 1.37; 95% CI: 1.02–1.84; $P = 0.035$).

Conclusion: We identified three loci contributed to myopia in minors and these findings gave new insight into the genetic susceptibility mechanisms of myopia at the molecular level.

Keywords: myopia, genome-wide association study; GWAS, SNP, minors

Introduction

Myopia, the most common refractive error, results in a significant threat on global public health worldwide.¹ As the myopic population increases globally, the severity of its impact is predicted.² Children with early onset are particularly susceptible to myopia-related complications, like high myopia (HM) and myopic macular degeneration.³ According to a recent school-based epidemiology study of myopia in China with 14,551 participants (ages ranging from 5 to 16 years), the overall prevalence of myopia is 78.2%.⁴ Myopia is a complex disease which is contributed by various environmental and genetic factors. Environmental factors includes low outdoor time and near work, dim light exposure, the use of LED lamps for home-work, low sleeping hours, and short reading distance.^{3,5} Meanwhile, there is growing evidence that susceptibility genes play a crucial role in the risk of myopia and single nucleotide polymorphisms (SNPs) may contribute to the risk of myopia.^{6–10}

Recently, a genome-wide association study (GWAS) identified six novel loci (rs2246661, rs74633073, rs76903431, rs698047, rs17029206, and rs72748160) in Asian adults, and revealed the important role of genes in the nervous system in the pathogenesis of myopia.¹¹ The findings highlighted a nervous system role in

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pathogenesis of myopia. Minors are better suited to studying the genetic factors of myopia. Whether these genetic loci works for myopia in minors remains unknown and worthy to be explored. Thus, here we aimed to evaluate the potential role of these GWAS identified loci in occurrence of myopia in this case-control study including 600 myopia minors, 110 HM minors and 800 non-myopia minors.

Patients and Methods

Study Population

A total of 600 consecutive myopia minors, 110 HM minors, and 800 non-myopia minors, which were frequency-matched by age and gender, were recruited in this case-control study. All subjects were Chinese Han population. Myopia was defined as mean spherical equivalent (MSE) of both eyes ≤ -0.5 diopters (D), while HM was defined as MSE less than or equal to -6.0 D.¹² Patients with a predisposition to myopic eye disease, other known ocular or systemic diseases were excluded. Controls were selected from subjects coming for routine vision screening. The criteria for the control group were as follows: minors with MSE between -0.5 D and $+1.0$ D, best unaided visual acuity ≥ 0.8 , and no other known ocular or systemic diseases.¹³ Patients are tested for refractive errors using an automated refractometer (Topcon RM-8000B, Topcon

Co., Tokyo, Japan). The refraction was taken under cycloplegia. Information of all participants, including age, gender, body mass index (BMI, calculated using weight/height²), self-reported outdoors time, self-reported time using electronic equipment, and parental myopia, was collected through questionnaire responses, and all subjects donated 5 mL peripheral venous blood. The study protocol was approved by the ethics committee of Nanjing Tongren Hospital. All subjects gave their written informed consent, and the study complied with the Declaration of Helsinki.

DNA Extraction and Genotyping

Genomic DNA was extracted by blood DNA extraction kit (Promega, Madison, Wisconsin, USA) and stored in TE buffer. Genotyping was performed by TaqMan assay in 384-well ABI 7900HT Real-Time PCR system (Applied Biosystems [ABI], Foster City, CA). The qPCR reactions proceeded in a final volume of 10 μ L mix including 5 μ L TaqMan Genotyping Master Mix (Thermo Fisher Scientific), 0.5 μ L pre-designed TaqMan probe (Thermo Fisher Scientific), 20 ng genomic DNA and ultrapure water. Each plate included blank samples as negative controls to verify genotyping quality. Genotype data were analyzed using their System SDS Allelic Discrimination Software version 2.3 (Applied Biosystems). For quality control, about 5% of the samples were genotyped

Table 1 Characteristics of Participating Minors

	Non-Myopia Minors (N=800)	Myopia Minors		High Myopia Minors	
		N=600	P value	N=110	P value
Age (years)	15.1 (14.1, 15.9)	15.0 (14.0, 15.9)	0.787	15.1 (13.9, 16.0)	0.919
Gender					
Boys	436 (54.4%)	338 (56.3%)	0.495	62 (56.4%)	0.713
Girls	364 (45.6%)	262 (43.7%)		48 (43.6%)	
BMI (kg/m ²)	18.3 (4.8)	18.9 (4.1)	0.014	18.8 (4.3)	0.300
Self-reported outdoors time (h/day)	1.3 (0.7)	1.1 (0.6)	<0.001	1.0 (0.5)	<0.001
Self-reported time using electronic equipment (h/day)	1.1 (0.6)	1.2 (0.6)	0.002	1.8 (1.1)	<0.001
Parental Myopia					
Yes	153 (19.1%)	122 (20.3%)	0.573	32 (29.7%)	0.015
No	647 (80.9%)	478 (79.7%)		78 (70.3%)	
Mean spherical equivalent (diopters)	0.32 (0.41)	-3.2 (1.6)	<0.001	-9.8 (2.2)	<0.001

Notes: Values are means (SD), median (quartiles), or absolute numbers (percentages). P value in bold means statistically significant.

repeatedly with Sanger sequencing and the results of both methods were in good agreement.

Statistical Analysis

SPSS 22.0 (SPSS, Chicago, IL) was used for statistical analysis, and a two-sided P-value of less than 0.05 was used as statistical significance. Chi-square goodness-of-fit test was adopted to derive the Hardy-Weinberg equilibrium (HWE). In the case-control study, Student *t*-test and/or Chi-square test were used to demonstrate how demographic and clinical characteristics and frequency of genotypes differ between case and control groups. Using unconditional logistic regression model, adjusted odds ratios (ORs) and 95% confidence intervals (CIs) were adopted (only significant variables in Table 1 were included for adjustment) to evaluate the effects of SNPs and to quantify the association between the SNPs and myopia in minors.

Results

Characteristics of Study Population

Table 1 presented the 600 myopia minors, 110 HM minors, and 800 non-myopia minors in this case-control study. The groups were comparable in age, and gender ($P>0.05$). While the highly myopic spent more time using electronic devices ($P<0.001$), less time outdoors ($P<0.001$) and had more myopic parents than non-myopic ones ($P=0.015$). The median ages in controls, myopia, and HM were 15.1, 15.0, and 15.1, respectively, while the means \pm standard deviation for them were 0.32 ± 0.41 , -3.2 ± 1.6 , and -9.8 ± 2.2 , respectively.

Genetic Association Study of Myopia

All six SNPs analyzed were in HWE in non-myopia controls, indicating that the sampled subjects were representative of the population and did not show any bias in genotype frequency ($p>0.05$). Subsequently, we evaluated the associations between the selected SNPs and the risk of myopia adjusting for BMI, self-reported outdoors time, and self-reported time using electronic equipment. Table 2 showed the results of genotypic frequency analysis for selected loci. SNP rs2246661 (allelic OR: 1.29; 95% CI: 1.09–1.52; $P=0.003$), rs74633073 (allelic OR: 1.41; 95% CI: 1.12–1.78; $P=0.004$), and rs76903431 (allelic OR: 1.42; 95% CI: 1.11–1.81; $P=0.005$) significantly contributed to elevated susceptibility of myopia. Under additive genetic model, all of three SNPs showed statistically significant associations. For rs2246661, the CT genotype was

Table 2 Associations Between Candidate Loci and Myopia in Minors

Variants	Myopia (n=600)	Controls (n=800)	OR (95% CI)*	P
rs2246661				
TT	281	442	1.00 (reference)	
CT	257	296	1.42 (1.12–1.81)	0.004
CC	62	62	1.64 (1.1–2.43)	0.014
C vs T			1.29 (1.09–1.52)	0.003
rs74633073				
CC	449	645	1.00 (reference)	
CT	137	147	1.39 (1.05–1.85)	0.022
TT	14	8	2.61 (1.12–6.08)	0.026
T vs C			1.41 (1.12–1.78)	0.004
rs76903431				
TT	457	660	1.00 (reference)	
AT	131	133	1.48 (1.11–1.97)	0.007
AA	12	7	2.57 (1.04–6.37)	0.041
A vs T			1.42 (1.11–1.81)	0.005
rs698047				
CC	163	233	1.00 (reference)	
CG	298	397	1.12 (0.77–1.62)	0.563
GG	139	170	1.22 (0.85–1.74)	0.286
G vs C			1.06 (0.91–1.24)	0.452
rs17029206				
TT	288	349	1.00 (reference)	
CT	241	346	0.88 (0.74–1.04)	0.124
CC	71	105	0.85 (0.66–1.1)	0.227
C vs T			0.91 (0.78–1.06)	0.234
rs72748160				
GG	484	674	1.00 (reference)	
GT	110	121	1.32 (0.96–1.8)	0.087
TT	6	5	1.74 (0.52–5.83)	0.371
T vs G			1.22 (0.94–1.59)	0.144

Notes: *Adjusted for BMI, self-reported outdoors time, and self-reported time using electronic equipment. P value in bold means statistically significant.

associated with a 1.42-fold increased risk (95% CI = 1.12–1.81; $P=0.004$), while the TT genotype conferred 1.64-fold increased risk of myopia (95% CI = 1.1–2.43; $P=0.014$), compared with the TT genotype. For rs74633073, the CT genotype was associated with a 1.39-fold increased risk (95% CI = 1.05–1.85; $P=0.022$), while the TT genotype conferred 2.61-fold increased risk of myopia (95% CI = 1.12–6.08; $P=0.026$). For rs76903431, genotype GG was associated with a 1.48-

Table 3 Associations Between Candidate Loci and High Myopia in Minors

Variants	High Myopia (n=110)	Controls (n=800)	OR (95% CI)*	P
rs2246661				
TT	47	442	1.00 (reference)	0.044
CT	50	296	1.55 (1.01–2.37)	
CC	13	62	1.88 (1.03–3.43)	
C vs T			1.37 (1.02–1.84)	
rs74633073				
CC	82	645	1.00 (reference)	0.214
CT	25	147	1.39 (0.83–2.34)	
TT	3	8	3.07 (0.86–10.9)	
T vs C			1.35 (0.86–2.14)	
rs76903431				
TT	84	660	1.00 (reference)	0.142
AT	24	133	1.47 (0.88–2.48)	
AA	2	7	2.33 (0.5–10.96)	
A vs T			1.31 (0.82–2.09)	
rs698047				
CC	28	233	1.00 (reference)	0.446
CG	57	397	1.24 (0.71–2.17)	
GG	25	170	1.27 (0.66–2.45)	
G vs C			1.07 (0.79–1.46)	
rs17029206				
TT	54	349	1.00 (reference)	0.342
CT	44	346	0.85 (0.62–1.18)	
CC	12	105	0.77 (0.44–1.33)	
C vs T			0.99 (0.72–1.37)	
rs72748160				
GG	88	674	1.00 (reference)	0.353
GT	20	121	1.32 (0.74–2.35)	
TT	2	5	3.19 (0.67–15.05)	
T vs G			1.23 (0.74–2.05)	

Notes: *Adjusted for self-reported outdoors time, self-reported time using electronic equipment, and parental myopia. P value in bold means statistically significant.

fold increased risk (95% CI = 1.11–1.97; $P = 0.007$), while the GG genotype conferred 2.57-fold increased risk of myopia (95% CI = 1.04–6.37; $P = 0.041$), compared with the CC genotype.

Genetic Association Study of HM

We further evaluated the associations of these six candidate SNPs with HM adjusting for self-reported outdoors

time, self-reported time using electronic equipment, and parental myopia. We only found rs2246661 (OR: 1.37; 95% CI: 1.02–1.84; $P = 0.035$), significantly contributed to elevated susceptibility of HM (Table 3). Under additive genetic model, the CT genotype was associated with a 1.55-fold increased risk (95% CI = 1.01–2.37; $P = 0.044$), while the TT genotype conferred 1.88-fold increased risk of myopia (95% CI = 1.03–3.43; $P = 0.040$), compared with the TT genotype.

Discussion

The current study investigated the potential function of six GWAS identified loci in occurrence of minors' myopia in a case-control study in Chinese population. We found three loci, including rs2246661, rs74633073, and rs76903431, significantly contributed to elevated risk of myopia. Besides, we also found rs2246661 significantly contributed to HM in minors. Our results confirm the GWAS findings in Asian adults and further provide a causal explanation for the occurrence of myopia at the molecular level.

The prevalence of myopia grew rapidly in minors.^{3,5,14} Finding the causes of the disease and taking effective preventive measures are vital to controlling the damage caused by myopia in young people. To date, a series of GWASs have been conducted to characterize the molecular mechanism responsible for myopia worldwide.^{11, 15–20} However, not all could be replicated. For example, Wang et al²¹ replicated findings of two Japanese GWAS in a Chinese population, and got null results. This was because myopia in adults was a genetically heterogeneous disease, which was influenced by inborn genetic factors and acquired environmental factors. On the contrary, minors are better suited to exploring the genetic factors of myopia. Thus, we attempted to classify the occurrence of myopia in minors was affected by GWAS loci identified in adults in this case-control study.

In the current study, rs2246661, rs74633073, and rs76903431 were identified to be associated risk of myopia in minors. Through searching Pubmed, we did not find any other genetic associations. According to RegulomeDB 2.0, rs2246661 and rs74633073 were located at the transcription factor (TF) binding site, which could affect the combination of TFs and their targets.²² HaploReg v4.1 revealed rs2246661 could cause Ets Motifs change, and rs74633073 could cause AP-2, RFX5 Motifs change, while rs76903431 could cause CDP, Pbx-1, RXRA Motifs change.²³ This evidence supported the important role of these genetic loci.

Our study had several limitations. First, the selection bias of a case-control study design cannot be avoided. Second, early-onset myopia, which refers to myopia occurring before the age of 11 years, was not evaluated in current study, due to the limitations of sample size.²⁴ Third, based on existing sample size, the associations might not have the strength to achieve real results, especially for HM. Fourth, the biological function of these SNPs and its detailed effect on occurrence of myopia need to be deep investigated by further biological studies. There are also several strengths in our research, including the detailed inspection and accurate diagnosis of cases, structured questionnaire by well-trained interviewers, and strict quality control of genotyping.

Conclusions

Conclusively, this study provides the evidence of the promotional role of rs2246661, rs74633073, and rs76903431 loci on the susceptibility of myopia. Replicated researches in independent ethnic samples and functional investigation are needed to confirm our findings.

Disclosure

The authors declare that they have no conflict of interest.

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