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LABORATORY STUDY

Association of *ZNF644*, *GRM6*, and *CTNND2* genes with high myopia in the Han Chinese population: Jiangsu Eye Study

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Abstract

Aims **High myopia is a common visual disorder in the world. The *ZNF644*, *GRM6*, and *CTNND2* genes are expressed in the retina. This study aims to investigate the associations of these genes with high myopia in Han Chinese population.**

Methods **The case–control association included high myopia cases ($n = 430$) and controls ($n = 430$) recruited from a population-based study, 'Jiangsu Eye Study'. Fourteen single-nucleotide polymorphisms (SNPs) in three genes were genotyped by the TaqMan method using the real-time PCR system.**

Results **Three SNPs *GRM6*-rs11746675, *GRM6*-rs2067011, and *GRM6*-rs2645339 were associated with high myopia (odds ratio (OR) = 0.74, $P = 0.003$; OR = 0.78, $P = 0.018$; and OR = 0.78, $P = 0.023$; respectively). The significances of rs2067011 and rs2645339 disappeared after multiple testing corrections. Rs11746675 remained significant after correction for multiple testing. The genetic model analysis found that *GRM6*-rs11746675 and *GRM6*-rs2067011 were suggestively associated with high myopia in the recessive model (OR = 0.54, $P = 0.004$; OR = 0.52, $P = 0.003$; respectively). Haplotype GAT for *GRM6* markers rs2067011-rs2645339-rs762724 showed significance ($P = 0.0239$), but such association did not remain significant after multiple testing corrections.**

Conclusions **Our data suggested that genetic variants in *GRM6* are associated with high myopia. The mechanism of *GRM6* in the development of high myopia need to be further investigated.**

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Introduction

Myopia is a common visual disorder in the worldwide with significant concerns in public health, especially in East Asian populations. High myopia (−6.00 diopters (D) or less) is one of the leading cause of vision loss or even irreversible blindness with pathologic complications such as myopic retinopathy, maculopathy, choroidal neovascularization, retinal detachment, posterior staphyloma, cataract, and primary open-angle glaucoma.[1]

The prevalence of myopia varies across ethnic groups and has rapidly increased in the past few decades. Among adult Chinese in the United States, the prevalence of myopia (spherical equivalent (SE) ≤ −1.0D) and high myopia (SE ≤ −5.0D) was 37.2 and 11.8%, respectively.[2] In Singapore adults, the prevalence of myopia (SE ≤ −0.5D) and high myopia (SE ≤ −5.0D) was 38.9 and 8.4%, respectively.[3] Chinese adults had higher prevalence of myopia and high myopia compared with non-Chinese adults in above surveys. A study reported that the prevalence of myopia (SE < −0.5D) and high myopia (SE < −6.0D) was 95.5 and 19.5%, respectively, in college students of Shanghai.[4] In the long term, such problems will impose a heavy burden on the health-care system and the economy of the society concerned.

Myopia is a multifactorial disease.[1] Familial aggregation studies have estimated sibling recurrence risks of common forms of refractive errors to range from 3 to 5 for myopia.[5] Recent twin study of myopia estimated heritabilty was 78% for SE in Koreans.[6] So far, almost 22 loci (MYP1–MYP3, MYP5–MYP23) have been identified for myopia susceptibility through family-based linkage analyses and genome-wide association studies. Unfortunately, no candidate gene has been consistently replicated.

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Although the pathophysiology of high myopia is far from clearly understood, a common pathologic structural abnormality is the excessive lengthening of the posterior segment of the ocular globe. A visually triggered signaling cascade from the retina ultimately guides the scleral remodeling that leads to eye growth.[7] The zinc-finger protein 644 gene (*ZNF644*) is a zinc-finger protein that functions as a transcriptional factor and is expressed in the retina.[8] The glutamate receptor metabotropic 6 gene (*GRM6*) is important in the function of the ON-bipolar cells, which is a major controller of dopamine release.[9] The catenin (cadherin-associated protein), delta 2 (*CTNND2*) encodes an adhesive junction-associated protein of the armadillo/beta-catenin superfamily and is implicated in brain and eye development and cancer formation.[10] Here we selected 14 common single-nucleotide polymorphisms (SNPs) of *ZNF644*, *GRM6*, and *CTNND2* to evaluate whether these gene variations are associated with high myopia in Han Chinese populations.

Materials and methods

The project was approved by the ethics committee of Affiliated Hospital of Nantong University. A written informed consent was obtained from each participant. The research followed the tenets of the Declaration of Helsinki.

This study was a part of the Jiangsu Eye Study, a population-based epidemiologic study focusing on common eye diseases and health-related parameters.[11] One rural district/county within southern and northern Jiangsu was selected as the sampling area, which are Binhu District and Funing County, respectively. The surveys were carried out by randomly selecting individuals within each district/county which was similar with the method we described previously.[12] Sample size was based on estimating an anticipated 2.6% prevalence for SE ≤ -6.0D within an error bound (precision) of 25% with 95% confidence interval (CI).[13] Assuming an examination response rate of 85%, and a design effect of 1.5 to account for inefficiencies associated with the cluster sampling design, a sample of 4062 persons was required for each district/county. The participants were unrelated and self-identified Han Chinese. All individuals were randomly selected from each district/county. Geographically defined cluster sampling included 6722 individuals in Binhu District from January to December 2010. Actually, 6106 subjects were examined with the response rate of 90.8%. The same sampling was used in 6145 randomly selected individuals in Funing County from September 2010 to May 2011 and actually 5947 subjects were examined with the response rate of 96.8%.

All study participants underwent a detailed ocular examination. The participants underwent a non-cycloplegic refraction test with auto refractometer (AR-610; NIDEK, Aichi, Japan), subjective refraction and retinoscope. The inclusion criteria for high myopia included: (i) myopia in both eyes and a SE ≤ -6.00D in at least one eye,[14] (ii) without nuclear cataract, (iii) without corneal lesions, (iv) without any previous history of ocular procedures. Finally, we examined 430 high myopia patients. The criteria of control individuals in this study had uncorrected visual acuity of 20/20 or better by Early Treatment Diabetic Retinopathy Study (ETDRS) chart and without other known eye or systemic diseases. In all, 430 individuals were included as normal controls. The ocular examinations were performed by professionals according to the uniform standard. Peripheral venous blood was collected in an ethylene diamine tetraacetic acid anti-coagulation tube. Genomic DNA was isolated from leukocytes by the phenol–chloroform method. Details of ocular examination, blood collection, and DNA extraction have been reported previously.[11]

Selection and genotyping of SNPs

Fourteen SNPs were selected from three candidate genes (*ZNF644*, *GRM6*, *CTNND2*). We selected haplotype-tagging SNP by searching Han Chinese data from the International HapMap Project (http://hapmap.ncbi.nlm.nih.gov/) using the Tagger program. All SNPs were selected with a minor-allele frequency $>5\%$ in the HapMap CHB population. Details of tested SNPs and TaqMan assays are listed in Table 1. Genotyping of all SNPs were conducted with a commercial gene-expression assay (TaqMan Assay; Applied Biosystems, Foster City, CA, USA, with the 7500 Real-Time PCR System; Applied Biosystems), as described in our previous publication.[11]

Statistical analysis

Statistical analyses were performed with a commercial statistical software program (Stata 8.0; Stata Corp, College

Table 1 Taqman SNP genotyping assays information

Gene	*SNP ID*	*Chromosome*	*Minor/major*	*Maf*	*SNP type*
ZNF644	rs358693	Chr1	A/C	0.385	Intron
ZNF644	rs358695	Chr1	A/G	0.263	Intron
ZMF644	rs358698	Chr1	T/C	0.384	Intron
ZNF644	rs358690	Chr1	A/G	0.302	Intron
ZNF644	rs12724992	Chr1	G/A	0.143	Intron
GRM6	rs11746675	Chr5	T/C	0.426	Silent mutation
GRM6	rs17078853	Chr5	G/T	0.057	UTR 3'
GRM6	rs762724	Chr5	C/T	0.463	Intron
GRM6	rs2067011	Chr5	A/G	0.472	Missense mutation
GRM6	rs2645339	Chr5	G/A	0.476	Nonsense mutation
GRM6	rs2071247	Chr5	T/C	0.253	Silent mutation
CTNND2	rs1479617	Chr5	A/G	0.407	Intron
CTNND2	rs6885224	Chr5	C/T	0.348	Intron
CTNND2	rs12716080	Chr5	G/T	0.468	Intron

Station, TX, USA). χ^2 was used to test the allelic and genotypic associations between cases and controls and to estimate odds ratio (OR) and 95% CI. Hardy–Weinberg equilibrium (HWE) of each SNP in control subjects were also tested using a χ^2 test. Correction for multiple comparisons was performed by permutation. Multiple testing was corrected by generating empirical *P*-values based on 50 000 permutations across all SNPs and haplotype blocks for a given sample set as appropriate. The haplotype blocks were estimated by using software Haploview Version 4.2 (Mark Daly's Laboratory, Cambridge, MA, USA). $P<0.05$ was considered as statistically significant.

Results

The demographic information for the study participants was generalized in Table 2. All of the tested SNPs are in HWE in the control population, except *GRM6*-rs17078853 that was excluded from further analysis. The call rates of all SNPs genotyping were >98%.

In allele frequency analysis (Table 3), three SNPs of *GRM6* were associated with high myopia ($P<0.05$): rs11746675 (OR = 0.74, $P=0.003$, 95% CI: 0.60–0.91), rs2067011 (OR = 0.78, $P=0.018$, 95% CI: 0.63–0.96), rs2645339 (OR = 0.78, $P=0.023$, 95% CI: 0.63–0.96). The frequency of the minor T allele of rs11746675 was 35.9% in the high myopia group and 42.9% in the control group, indicating a protective role. In addition, this SNP of *GRM6* remained significant ($Pc=0.009$), whereas the significance of other two SNPs disappeared after correction for permutation test.

In genotype-frequency analysis (Table 4), *GRM6*-rs11746675, *GRM6*-rs762724, *GRM6*-rs2067011, and *GRM6*-rs2645339 had significant differences between cases and controls in recessive model ($P=0.004$, $P=0.03$, $P=0.003$, $P=0.005$, respectively). The frequency of the TT genotype of rs11746675 was 10.9% in the high myopia group and 18.1% in the control group and conferred an OR of 0.54 in the recessive model, indicating a protective role in developing high myopia.

In haplotype analysis (Table 5), four SNPs of *ZNF644*, three SNPs of *GRM6* genotyped in this study were in the same LD block. One haplotype block of *GRM6* showed statistical significance between cases and controls: GAT (SNP rs2067011-rs2645339-rs762724, $P=0.024$). However, their significance disappeared after correction for multiple testing ($Pc=0.08$).

Discussion

This study presented the evidence of involvement of *GRM6* in the susceptibility of high myopia. Our subjects

Table 2 Demographic information of study participants

Group	*n*	*Age (mean ± SD; years)*	*Gender*		*Spherical equivalent*	
			Female (%)	*Male (%)*	*OD*	*OS*
Control	430	59.37 ± 4.06	265 (61.63)	165 (38.37)	NA	NA
High myopia	430	65.72 ± 7.49	277 (64.42)	153 (35.58)	− 10.73 ± 3.32	− 10.52 ±3.24

Table 3 Distribution of minor allele of tested 14 SNPs and their association with high myopia

Gene name	*SNPs minor/major*	*Controls minor/major (%)*	*Cases minor/major (%)*	*OR (95% CI)*	P-*value*
ZNF644	rs358693 A/C	320/540 (37.2)	290/570 (33.7)	0.94 (0.76–1.17)	0.13
	rs358695 A/G	372/488 (43.3)	401/459 (46.6)	1.12 (0.91–1.37)	0.16
	rs358698 T/C	324/536 (37.7)	291/569 (33.8)	0.92 (0.74–1.14)	0.10
	rs358690 A/G	134/726 (15.6)	114/746 (13.3)	0.84 (0.63–1.13)	0.17
	rs12724992 G/A	47/813 (5.5)	57/803 (6.6)	1.36 (0.88–2.10)	0.31
GRM6	rs11746675 T/C	369/491 (42.9)	309/551 (35.9)	0.74 (0.60–0.91)	0.003/0.005(Pa)
	rs17078853 G/T	110/750 (12.8)	117/743 (13.6)	—	—
	rs762724 C/T	351/509 (40.8)	311/549 (36.2)	0.82 (0.66–1.01)	0.048
	rs2067011 A/G	357/503 (41.5)	309/551 (35.9)	0.78 (0.63–0.96)	0.018/0.021(Pa)
	rs2645339 G/A	359/501 (41.7)	313/547 (36.4)	0.78 (0.63–0.96)	0.023/0.021(Pa)
	rs2071247 T/C	325/535 (37.8)	334/526 (38.8)	1.04 (0.84–1.29)	0.66
CTNND2	rs1479617 A/G	224/636 (26.0)	213/647 (24.8)	0.95 (0.75–1.2)	0.54
	rs6885224 C/T	180/680 (20.9)	192/668 (22.3)	1.04 (0.81–1.34)	0.48
	rs12716080 G/T	183/677 (21.3)	205/655 (23.8)	1.11 (0.87–1.43)	0.20

Abbreviation: Pa, after age correction. All HWE $P>0.05$ except GRM6-rs17078853 $P<0.05$.

Table 4 Genotype frequency of SNPs in control and high myopia subjects

SNP		*Genotype distribution n (%)*		*Dominant model*		*Recessive model*	
		Control	*High myopia*	*OR (95% CI)*	*Pa*	*OR (95% CI)*	*Pa*
GRM6-rs11746675	CC	139 (32.3)	168 (39.1)	0.74 (0.55–1.00)	0.05	0.54 (0.35–0.83)	0.004
	TC	213 (49.5)	215 (50.0)				
	TT	78 (18.1)	47 (10.9)				
GRM6-rs762724	TT	152 (35.3)	166 (38.6)	0.85 (0.63–1.15)	0.29	0.62 (0.40–0.95)	0.03
	TC	205 (47.7)	217 (50.5)				
	CC	73 (17.0)	47 (10.9)				
GRM6-rs2067011	GG	149 (34.7)	166 (38.6)	0.83 (0.61–1.12)	0.23	0.52 (0.34–0.81)	0.003
	GA	205 (47.7)	219 (50.9)				
	AA	76 (17.7)	45 (10.5)				
GRM6-rs2645339	AA	147 (34.2)	165 (38.4)	0.82 (0.61–1.11)	0.20	0.54 (0.35–0.84)	0.005
	GA	207 (48.1)	217 (50.5)				
	GG	76 (17.7)	48 (11.2)				

Abbreviation: Pa, after age correction.

Table 5 Haplotype analysis of *ZNF644* and *GRM6* in control and high myopia subjects

Gene name	*Haplotype*	*Haplotype frequency*		*OR (95% CI)*	P-*value*
		Controls	*Cases*		
ZNF644 (rs358695-rs358693-rs358698-rs358690)	ACCG	0.43	0.46	1.15 (0.95–1.39)	0.16
	GATG	0.22	0.21	0.90 (0.71–1.12)	0.36
	GCCG	0.19	0.20	1.04 (0.81–1.32)	0.77
	GATA	0.15	0.13	0.86 (0.66–1.13)	0.29
GRM6 (rs2067011-rs2645339-rs762724)	GAT	0.58	0.63	1.25 (1.03–1.52)	0.024/0.08(Pc)
	AGC	0.40	0.36	0.83 (0.68–1.00)	0.05

Abbreviation: Pc, after permutation correction.

were from the Jiangsu Eye Study, a population-based epidemiologic study. The design of a population-based study can minimize the sample-selection bias often present in a hospital-based case–control study.

Previous studies indicate that genes responsive to visual signals are involved in the biological pathways of ocular growth.[7] The abnormality of sclera in highly myopic eyes of human and animal models is a result of changes in scleral extracellular matrix metabolism.[15] Therefore, we hypothesize that genes directly responsive to visual signals such as *ZNF644*, *GRM6* and *CTNND2* might functionally contribute to the development of high myopia.[8–10]

GRM6 mapped to 5q35, contained 10 exons and encoded an 877 amino acid protein, mGluR6. As a member of Group III mGluRs (mGluR4, 6, 7, and 8), it contains a signal peptide, a large bi-lobed extracellular NH2-terminal domain containing the glutamate-binding site, seven G protein-coupled receptor transmembrane domains and an intracellular COOH-terminal domain. Mutations in *GRM6* have been identified in patients with congenital stationary night blindness (CSNB) in previous studies.[9] High myopia is usually observed in CSNB1B patients with *GRM6* mutations,[16] and myopia is not always associated with CSNB, except in cases resulting from mutations in *NYX* and *GRM6*.[17,18] Xu *et al*[16] identified three novel variations with potential functional consequences in the *GRM6* of patients with high myopia, suggesting a potential role in the development of myopia in rare cases. Our findings support *GRM6* as a susceptibility gene for high myopia.

An essential step in intricate visual processing is the segregation of visual signals into ON and OFF pathways by retinal bipolar cells.[19] A mouse model lacking the *GRM6* gene showed a loss of ON response, but an unchanged OFF response to light, demonstrating its essential role in ON synaptic transmission.[20] Disruption of ON and OFF pathway transmission perhaps alter eye growth. *GRM6* is important in the function of the ON-bipolar cells, which is a major controller of dopamine release. This may be important because the evidence on experimental myopia suggests that dopamine release may be an important factor in the control of eye growth. However, the specific mechanism is not clear and need to be investigated in the future.

ZNF644 belongs to the Krüppel C2H2-type zinc-finger protein family, which contains 7 C2H2-type zinc fingers. Shi *et al*[8] first used exome sequencing to identify mutations in zinc-finger protein 644 in a large pedigree with autosomal dominant high myopia, and then replicated in a Chinese cohort. *ZNF644* is predicted to be a transcription factor and is expressed in the retina. It may regulate genes involved in eye development, a mutant ZNF644 protein may impact the normal eye development and therefore underlie the axial elongation of the eye globe in high myopia patients.[8,21] Tran-Viet *et al*[21] identified two novel variants in *ZNF644* in US cohort, in addition to a known variant that demonstrated association. Mutations of *ZNF644* identified in Mendelian inheritance families and sporadic cases, explain only a small proportion of the subjects with high myopia. Therefore, we evaluated in a larger cohort of patients with well-characterized high myopia and normal controls to determine whether these variants are associated with the clinical outcome or not. However, we did not find the association of the selected SNPs of *ZNF644* with high myopia in this study.

CTNND2 gene on chromosome 5p15 previously found to be linked to high myopia in a family segregation study of three Hong Kong Chinese pedigrees (LOD = 4.68).[22] This gene encodes an adhesive junction-associated protein of the armadillo/beta-catenin superfamily and is implicated in brain and eye development and cancer formation.[10] Two SNPs (rs12716080 and rs6885224) of *CTNND2* in the meta-analysis of Singaporean Chinese data sets were identified strong association with high myopia.[23] However, Yu *et al*[24] failed to replicate the association in a Chinese Population. Instead, they suggested that mutation in the rs1479617 region of *CTNND2* may be important for pathological myopia development.[24] In our study, we failed in the repetition of these three SNPs (rs12716080, rs6885224, and rs11479617) in the role of myopia.

The possible explanations to the inconsistency of the association studies could be the differences in the sample sizes of subjects, the recruitment criteria, and/or ethnic groups recruited in the studies. Our study has certain limitations. With older age, several age-related confounding factors may come to play. But we make strict standard to select the group. It also can avoid the influence of some environmental factors, for example, outdoor activities, near work, education. Because most of the subjects are farmers.

In conclusion, we found that *GRM6* genetic variants are associated with the risk of high myopia in Chinese population. To further confirm the role of *GRM6* in the pathogenesis of high myopia, the current results from this population-based study will serve as the baseline for prospective observation of the role of genetic factors in the development of high myopia.

Summary

What was known before

- High myopia is a common visual disorder in the world. The *ZNF644*, *GRM6*, and *CTNND2* genes are expressed in the retina.

What this study adds

- We have investigated the associations of *ZNF644*, *GRM6*, and *CTNND2* genes with high myopia and concluded that *GRM6* gene might be involved in high myopia pathogenesis in the Han Chinese population.

Conflict of interest

The authors declare no conflict of interest.

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