ORIGINAL RESEARCH

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Association between *PRDM16*, *MEF2D*, *TRPM8*, *LRP1* gene polymorphisms and migraine susceptibility in the She ethnic population in China

Abstract

Background: The prevalence of migraines in the She population, a minority in China, is significantly higher than that in Han Chinese and other Asian populations. Two single nucleotide polymorphisms (SNPs) have been found to be associated with migraine susceptibility in the She population.

Purpose: This study investigated four SNPs, identified in genome-wide association studies, within migraine-susceptible loci in Han Chinese for their association with migraine susceptibility in the She population.

Methods: Two-hundred unrelated migraine patients and 200 healthy controls were recruited. The SNPs examined included rs2651899 (*PRDM16*), rs2274316 (*MEF2D*), rs7577262 (*TRPM8*) and rs11172113 (*LRP1*). Genotyping of the SNPs was performed by allele-specific polymerase chain reaction and direct sequencing.

Results: No significant differences between the participants with migraines and controls (participants without migraines) were demonstrated in genotypes, alleles and allele carriage frequencies for the four SNPs. A subgroup analysis found that migraine with aura had a lower frequency of C allele positivity in rs2651899 than in healthy controls (59.6% vs. 74.5%, respectively; $P < 0.034$). Univariate analyses indicated that no genotype of the four SNPs had a significant association with migraines. Males had a lower risk of migraines, and advanced age was a significant risk factor for migraines in females.

Conclusion: The SNPs in four migraine susceptible loci in Han Chinese were not risk factors for migraines in a relatively small sample of the She population.

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Migraine is a common chronic neurologic condition and neurovascular disease characterized by a unilateral headache lasting 4–72 hours that is accompanied by nausea, emesis, phonophobia, photophobia, visual disturbances and disability [1-3]. The lifetime incidence of migraine is 6.5% to 7.4% for males, and 18.2% to 21% for females, resulting in a male-to-female ratio of about 1:3 [4,5]. The prevalence of migraine varies worldwide: China, 4.32%; Japan, 8.4%; Korea, 6.0%; France, 7.9%; United States, 13.2%; Sweden, 13.2%; and Spain, 8.4% [6,7].

Migraine is a multifactorial disorder resulting from complex interactions between multiple predisposing genes, environmental factors and other factors, including hormonal, vascular, neuronal and pain pathways [8-12]. Migraine is characterized by accompanying symptoms such as auras, ataxia and periodic muscle paralysis; however, genetic studies have suggested that migraines with auras and migraine without auras are different clinical manifestations of the same genetic disease [8,12,13]. Moreover, studies have shown that migraines result from interactions between genetic factors and environmental factors and genetic predisposition and family susceptibility have been demonstrated [14]. With advanced detection techniques and methods, and the emergence of genome-wide association (GWA) analysis, some susceptibility genes have been confirmed to be related to the pathogenesis of migraines [15-17].

A genome-wide meta-analysis, including 23,285 migraine patients and 95,425 case-controls, identified 142 single-nucleotide polymorphisms (SNPs) at 12 loci that were associated with migraine susceptibility, including five newly identified loci [18]. Another genome-wide meta-analysis that included 59,674 migraine patients and 316,078 controls from 22 genome-wide association studies (GWAS) identified 44 independent single-nucleotide polymorphisms (SNPs) that were significantly associated with migraine risk. Those SNPs were mapped to 38 distinct genomic loci, including 28 loci not previously reported [8].

The She ethnic population in Fujian Province, China, has its own lifestyle, cultural traditions, and genetic background that differ from those of Han Chinese and other Asian populations [6,7]. In our previous epidemiological study on migraines in the She population, the prevalence of migraines was as high as 10.53%, which is significantly higher than that in China overall (4.32%) [6,19]. Based on the five newly identified polymorphic loci from the above-mentioned meta-analysis [17], we investigated the genotype distribution and allele frequency of those five polymorphic

loci in the She population and Han Chinese to explore their correlation with migraines [20,21]. Our results showed that the SNPs rs4379368 (in *c7orf10*) and rs13208321 (in *FHL5*) were potential genetic markers for migraines in the She population [20]. We subsequently found that rs4379368 and rs10504861 (near *MMP16*) were markers for susceptibility to migraines with aura, and that rs12134493 (near *TSPAN2*) is a marker for risk of migraines without aura in the Han population in Fujian Province, China [21], suggesting that the migraine susceptible loci are different between the Han and She populations. Because evidence suggests that SNPs in those four migraine susceptible loci might not be risk factors for migraines in the She population, we wanted to explore whether the four SNPs reported to be associated with migraines in Han Chinese (i.e., rs2651899 in *PRDM16* [22-27], rs2274316 in *MEF2D* [23,28-30], rs7577262 in *TRPM8* [18,28] and rs11172113 in *LRP1* [27-31]) were similarly associated with migraine in the She population, which is already shown to have a high prevalence of migraine. The purpose of the present study was to investigate the four SNPs identified within migraine susceptible loci in Han Chinese for their association with migraine susceptibility in the She population.

Materials and Methods

Participants

Based on our previous epidemiological study on migraines in the She ethnic population [6], 200 unrelated She migraine patients and 200 healthy controls without migraine were recruited from Nigde Municipal Hospital, Fujian Province, China (Figure 1). A multi-stage, age- and gender-stratified sampling method was used. Inclusion criteria were both parents of She ethnicity, age >15 years, permanent resident of Fujian province and able to describe their own symptoms clearly. Participants diagnosed with other diseases or headache-causing disorders were excluded. In the final stage of sampling, participants were stratified by gender and age distribution based on the 2005 Chinese census data of the She population. Migraine was diagnosed according to the International Diagnostic criteria for migraines developed by the Headache Classification Committee of International Headache Society 2004 [3]. The study protocol was approved by the Ethics Committee of our hospital, and signed informed consent was obtained from each subject before the study.

FIGURE 1 Study sample selection

Allele-specific polymerase chain reaction

Genomic DNA was extracted using a blood DNA extraction kit (Omega Bio-Tek Inc., Norcross, GA, USA) according to the manufacturer's instructions.

Target segments comprising the four SNPs were amplified using a polymerase chain reaction (PCR) amplification kit (Sangon Biotech, Shanghai, China) and primers were synthesized by Sangon Biotech Co., Ltd. (Shanghai, China; Table 1). The PCR mixture was composed of ddwater (8.5 μ L), 2 $\times$ PCR Master (12.5 μ L), DNA (3 μ L) and 50 μ M primers (1 μ L). Conditions used for the PCR were as follows: pre-denaturation at 94°C for 3 min for a total of 35 cycles of denaturation at 94°C for 30 s; annealing at 58°C for 30 s with extension at 72°C for 45 s; and a final extension at 72°C for 5 min. The products were subject to sequencing at Boshang Biotech Co., Ltd. (Shanghai, China). The alleles of rs2651899 included TT, CT, and CC; the alleles of rs2274316 included AA, AC, and CC; the alleles of rs7577262 included GG, AG, and AA; and the alleles of rs11172113 included TT, CT, and CC. Primers used in this study are listed in Table 1.

Statistical analysis

Age is presented as mean $\pm$ standard deviation (SD) and was compared using the independent t-test. Categorical variables are shown as frequency and percentages, and were tested using chi-square tests. Frequencies involving genotype, allele and allele carrier (the number of individuals having an allele that is either homozygous or heterozygous) were calculated by counting directly. A univariate logistic regression analysis was implemented to examine the associations between risk

factors and outcomes. Subgroup analyses were carried out according to migraine type and gender. All statistical analyses, including Hardy-Weinberg equilibrium, were performed using IBM SPSS statistical software (version 22 for Windows; IBM Corp., Armonk, NY, USA). All statistical analyses were two-sided and evaluated at a significance level of 0.05; a P-value of < 0.05 was considered to be strong evidence of a significant difference between groups.

Results

This study included 400 participants, and 50% of the study participants were patients with migraines. The mean age of the two groups of participants was similar, but the migraine group had a higher percentage of females than the control group (67.5% vs. 47.0%, respectively; $P < 0.001$) (Table 2).

The genetic study included TT, CT, and CC genotypes of rs2651899 (PRDM16 gene), AA, AC, and CC genotypes of rs2274316 (MEF2D), GG, AG, and AA genotypes of rs7577262 (TRPM8) and TT, CT and CC genotypes of rs11172113 (LRP1).

Additional subgroup analyses were performed by migraine type and gender, given that two prevalent subtypes of migraine are noted, migraine with aura and migraine without aura [8], and because females appear to be more susceptible to migraines than males [7]. Neither migraine type nor gender affected the results regarding allele frequencies for the four SNPs between the two groups. The C allele positivity for the SNP rs2651899 was significantly lower for migraine patients with aura versus controls (59.6% vs. 74.5%, respectively; $P = 0.034$); the case and control groups had similar allele carriage frequencies for the other SNPs regardless of migraine type or gender (Tables 3 and 4).

Results of univariate logistic regression analysis are summarized in Table 5. Overall, no genotype in four SNPs showed a significant association with migraines. Gender was the only significant contributor for migraines, and males had a lower risk of migraines (odds ratio (OR) = 0.43, 95% confidence interval (CI): 0.28-0.64, $P < 0.001$). In a subgroup analysis, similar results were found for migraine with aura and migraine without aura. No risk factors were identified for migraines in males, while advanced age was associated with an increased risk of migraines in females (OR = 1.04, 95% CI: 1.02-1.06, $P = 0.001$).

TABLE 1. Primers used for PCR of four polymorphic loci

Polymorphisms	Primers	Sequence
rs2651899	Forward	5'-AACACGCACCCTCCAACTG-3'
	Reverse	5'-TTGTCCCTAACCTGAGGCT-3'
rs2274316	Forward	5'-CCTCGCACACGTACATACAGA-3'
	Reverse	5'-GCCACCCTACTCTTATGCAGA-3'
rs7577262	Forward	5'-GTCITTCTCAATCCAAGTTTCGG-3'
	Reverse	5'-CCTTGACTCAAGCTTGTCAATG-3'
rs11172113	Forward	5'-ACTCAGAATGGAAGCGGAGA-3'
	Reverse	5'-CAGCAGAAGGAGAGATAGGCT-3'

TABLE 2. Participants' demographic characteristics

	Control (n = 200)	Migraine without aura (n = 148)	Migraine with aura (n = 52)	P
Age (years)	44.8 ± 14	47.8 ± 12.4	45.1 ± 11.9	0.099
Gender				< 0.001
Male	106 (53)	50 (33.8)	15 (28.8)	
Female	94 (47)	98 (66.2)	37 (71.2)	

Values expressed as number (%) and mean ± standard deviation.

Bold values indicate significance between the two groups, $P < 0.05$

Discussion

This study investigated the associations of well-known migraine susceptibility SNPs (rs2651899, rs2274316, rs7577262 and rs11172113) in the She ethnic population of Fujian Province in China. Our previous study investigated the genotype distribution and allele frequency of five polymorphic loci in the She and Han Chinese populations to explore their correlation with migraines [20,21]. Those five loci were the newly identified polymorphic loci from the above-mentioned meta-analysis of 29 GWAS [18]. We found that rs4379368 (in *c7orf10*) and rs13208321 (in *FHL5*) were potential genetic markers for migraines in the She population [20]. In the present study, four SNPs were chosen based on their association with migraines identified in previous studies [22-31] as well as in a meta-analysis of 29 GWAS [20]. The rs2651899 SNP is localized to PRDM16, which is associated

with myelodysplastic syndromes, acute myeloid leukemia and metabolic syndrome [24]. The rs2651899 SNP of PRDM16 has been found to be closely associated with migraines in the Han Chinese population [23,23,27]. The rs2274316 SNP is localized to MEF2D, which is a member of the muscle cell enhancement factor 2 (MEF2) transcription factor family, and plays a crucial role in the activation of muscle-specific gene transcription in skeletal, cardiac, and smooth muscle cells [32]. The rs2274316 SNP of MEF2D has been found to be closely associated with migraines in the Han Chinese population [23]. The rs7577262 SNP is localized to TRPM8, which controls cold and pain sensations and is mainly expressed in sensory neurons and spinal dorsal root ganglion neurons [25]. The rs11172113 SNP is localized to LRP1. LRP1 is responsible for neuronal signal transmission, and is mainly expressed in the brain and blood vessels. Interaction

TABLE 3. Allele frequency and allele carriage frequency in all participants by migraine type

Single nucleotide polymorphism	Control (n = 200)	Migraine without aura (n = 148)	P^a	Migraine with aura (n = 52)	P^a
rs2651899					
Allele frequencies			0.452		0.056
C	49.0%	45.9%		38.5%	
T	51.0%	54.1%		61.5%	
Allele carriage frequencies					
C allele positive	74.5%	70.3%	0.382	59.6%	0.034
T allele positive	76.5%	78.4%	0.679	82.7%	0.339
rs2274316					
Allele frequencies			0.266		0.882
A	74.8%	78.4%		74.0%	
C	25.3%	21.6%		26.0%	
Allele carriage frequencies					
A allele positive	92.5%	96.6%	0.102	96.2%	0.349
C allele positive	43.0%	39.9%	0.558	48.1%	0.537
rs7577262					
Allele frequencies			0.542		0.610
A	48.0%	50.3%		45.2%	
G	52.0%	49.7%		54.8%	
Allele carriage frequencies					
A allele positive	70.5%	74.3%	0.432	71.2%	0.927
G allele positive	74.5%	73.6%	0.858	80.8%	0.347
rs11172113					
Allele frequencies			0.260		0.781
C	30.3%	26.4%		28.8%	
T	69.8%	73.6%		71.2%	
Allele carriage frequencies					
C allele positive	53.5%	48.6%	0.371	50.0%	0.652
T allele positive	93.0%	95.9%	0.243	92.3%	0.771

^a Comparison between respective group and control group

TABLE 4. Allele frequency and allele carriage frequency in all participants by gender

Single nucleotide polymorphism	Male			Female		
	Control (n = 106)	Migraine (n = 65)	P	Control (n = 94)	Migraine (n = 135)	P
rs2651899						
Allele frequencies			0.103			0.337
C	46.7%	37.7%		51.6%	47.0%	
T	53.3%	62.3%		48.4%	53.0%	
Allele carriage frequencies						
C allele positive	72.6%	61.5%	0.129	76.6%	70.4%	0.297
T allele positive	79.2%	86.2%	0.255	73.4%	76.3%	0.619
rs2274316						
Allele frequencies			0.552			0.688
A	74.1%	76.9%		75.5%	77.4%	
C	25.9%	23.1%		24.5%	22.6%	
Allele carriage frequencies						
A allele positive	91.5%	95.4%	0.336	93.6%	97.0%	0.213
C allele positive	43.4%	41.5%	0.812	42.6%	42.2%	0.960
rs7577262						
Allele frequencies			0.352			0.510
A	46.7%	41.5%		49.5%	52.6%	
G	53.3%	58.5%		50.5%	47.4%	
Allele carriage frequencies						
A allele positive	67.9%	63.1%	0.516	73.4%	78.5%	0.370
G allele positive	74.5%	80.0%	0.615	74.5%	73.3%	0.848
rs11172113						
Allele frequencies			0.464			0.056
C	27.8%	31.5%		33.0%	24.8%	
T	72.2%	68.5%		67.0%	75.2%	
Allele carriage frequencies						
C allele positive	50.0%	58.5%	0.282	57.4%	44.4%	0.053
T allele positive	94.3%	95.4%	0.766	91.5%	94.8%	0.317

TABLE 5. Risk factors for a migraine in all participants and in patients classified by migraine type or gender^a

	Overall univariate		Type of migraine		Gender	
			Without aura	With aura	Male	Female
	OR (95% CI)	P	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
Age, years	1.01 (0.99, 1.03)	0.077	1.02 (1, 1.03)	1.00 (0.98, 1.03)	1 (0.98, 0.98)	1.04 (1.02, 1.06) ^b
Gender						
Female	Reference		Reference		-	-
Male	0.43 (0.28, 0.64)	< 0.001*	0.45 (0.29, 0.70)*	0.36 (0.19, 0.70)*	-	-
rs2651899						
TT	Reference		Reference		Reference	
CC	1.38 (0.87, 2.19)	0.168	1.22 (0.74, 2.02)	1.91 (0.96, 3.79)	1.53 (0.77, 3.06)	1.36 (0.71, 2.58)
CT	0.95 (0.57, 1.57)	0.831	0.96 (0.56, 1.66)	0.89 (0.38, 2.08)	0.73 (0.3, 1.77)	0.95 (0.5, 1.82)
rs2274316						
AA	Reference		Reference		Reference	
CC	0.94 (0.62, 1.42)	0.762	1.03 (0.65, 1.61)	0.73 (0.39, 1.37)	0.98 (0.51, 1.88)	0.93 (0.53, 1.61)
AC	0.43 (0.17, 1.12)	0.083	0.44 (0.15, 1.28)	0.41 (0.09, 1.94)	0.51 (0.13, 2.09)	0.43 (0.11, 1.63)
rs7577262						
GG	Reference		Reference		Reference	
AA	0.82 (0.52, 1.32)	0.421	0.82 (0.49, 1.36)	0.85 (0.42, 1.73)	1.13 (0.56, 2.29)	0.75 (0.39, 1.43)
AG	1.13 (0.7, 1.84)	0.613	0.97 (0.58, 1.63)	0.65 (0.29, 1.46)	0.77 (0.34, 1.74)	0.96 (0.51, 1.83)
rs11172113						
TT	Reference		Reference		Reference	
CC	1.16 (0.77, 1.74)	0.475	1.15 (0.74, 1.78)	1.18 (0.63, 2.23)	0.68 (0.36, 1.29)	1.63 (0.94, 2.82)
CT	0.75 (0.32, 1.79)	0.523	1.66 (0.6, 4.53)	1.21 (0.36, 4.03)	0.67 (0.16, 2.87)	0.76 (0.26, 2.26)

Abbreviations: CI: confidence interval; OR: odds ratio

^a A univariate logistic regression analysis was performed. Multivariable analysis was not undertaken as only one significant result was revealed in the univariate analysis.

Dash denotes data unavailable.

^b Indicates significant findings, $P < 0.05$

exists between LRP1 and glutamate receptors, both of which localize in neurons [25]. A two-stage case-controlled GWAS of migraines without aura in the Han Chinese population of

Taiwan found positive association signals between rs10166942 in TRPM8 and rs11172113 in LRP1 and migraines, which was similar to a previous finding in a

GWAS of Caucasians [31]; however, other study of the Han Chinese population failed to find a significant association between alleles, genotypes of the two SNPs (rs10166942 and rs111721132) and migraines [27].

In the present study, no significant differences between the case and control groups were demonstrated in the genotypes, alleles and allele carriage frequencies for the four well-documented SNPs in the She population. Our results showed only a marginally lower frequency of C allele positivity for rs2651899 in migraine patients with aura versus controls in the She population. Univariate analyses indicated that no genotype of the four SNPs had a significant association with migraines, suggesting that the four SNPs in migraine susceptible loci may not be risk factors for migraines in the She population. These findings imply that differences in migraine susceptible loci exist between the Han and She populations, as well as between the She population and other ethnic groups in different countries (Table 6).

TABLE 6. An allele frequency comparison of the 4 SNPs used in this study between Han Chinese, She populations and general population

SNP	Minor allele	Population	OR (95% CI)	Reference
rs2651899	C	General	1.09 (1.07–1.12)	18
		Han Chinese	1.324 (1.004-1.746)	24
		Han Chinese	1.382 (1.100-1.736)	27
		She	Not significant	This study
rs2274316	C	General	1.07 (1.04–1.09)	18
		Han Chinese	2.783 (1.662-4.659)	23
		She	Not significant	This study
rs7577262	A	General	0.87 (0.84–0.90)	18
		She	Not significant	This study
rs1172113	C	General	0.90 (0.88–0.92)	18
		Han Chinese	1.23 (1.04-1.45)	31
		She	Not significant	This study

Previous studies have demonstrated that migraine is more prevalent in women. In the She population, 6.18% of

males and 13.82% of females experience migraines [6]. The average prevalence of migraines in the United States of America was 20.2% in females and 9.4% in males between 2005 and 2012 [9]. The present study confirmed that gender was the only significant contributor for migraines, and males had a lower risk of migraines; no risk factors for migraines were found in males, while advanced age was associated with a modest but significant increased risk of migraines in females. In a previous study, C allele positivity for MEF2D rs2274316 was found to be a significant risk factors for females [23]; however, in our study no genotype of the four SNPs showed a significant association with migraines, regardless of gender. This is consistent with a previous study that showed no significant interactions between gender and genotype of the SNPs within the MEF2D locus for migraines without aura, and all SNPs had relatively similar ORs for both genders [30].

This study had some limitations. According to the calculation method of sample size and statistical power for genetic association studies, as previously described [33], our sample size was relatively small, especially compared with GWA studies, which require large samples for statistical power. Genetic association studies attempting to detect causal genes require a sample size with sufficient statistical power; because our study lacked sufficient statistical power, interpretation of our results is limited. We did not perform additional control measures (e.g., Bonferroni, Sidak or FDR) to correct for the Family-Wise Error Rate (FWER), which may result in a Type I error. Population distribution was also uneven in the present study, with more females than males in the migraine group.

In conclusion, SNPs in four migraine susceptible loci in Han Chinese (rs2651899 in PRDM16, rs2274316 in MEF2, rs7577262 in TRPM8, rs11172113 in LRP1) were not associated with migraine in a relatively small sample of the She population and thus may not be risk factors for migraines in the She population, regardless of the subtype of migraine and gender. Additional multicenter studies with a larger sample size are necessary to confirm these findings in the She population and in other ethnic and racial groups.

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Conflict of interest: The authors declare that they have no conflicts of interest.

Authors' Contribution

Jing Yang: literature research, experimental studies, data acquisition, data analysis and statistical analysis, manuscript preparation.

Xiaoyang Wu: clinical studies

Qifang Lin: literature research, clinical studies.

Yuli Zeng: clinical studies.

Qiaoqing Xia: clinical studies.

Luoyuan Cao: experimental studies.

Baoying Huang: experimental studies.

Genbin Huang: guarantor of integrity of the entire study, study design.

Xianguo Fu: guarantor of integrity of the entire study, study design.

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