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Association of CALCA and RAMP1 gene polymorphisms with migraine in a Chinese population

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Abstract

Background & Objective: The calcitonin gene-related peptide (CGRP) has a central role in the pathogenesis of migraine, but variations in CGRP-related genes, including the calcitonin gene-related polypeptide-alpha (*CALCA*) gene and the receptor activity modifying 1 (*RAMP1*) gene, have not been found to link with migraine in Australian population. The goals of this study were to determine whether variants in the two genes are related to migraine in Chinese population. *Methods:* Using a case-control approach, rs3781719 and rs145837941 in the *CALCA* gene and rs3754701 and rs7590387 at the *RAMP1* locus was analyzed in a cohort of 504 migraine cases and 529 ethnically matched controls. Genotyping was performed using Sequenom MALDI-TOF mass spectrometry iPLEX platform. *Results:* The *CALCA* gene rs145837941 variant was not found in migraine or control group. No significant difference in genotypic and allelic distribution was observed in the other three polymorphisms between migraine cases and controls. All the three SNPs were also not selected as significant factors that independently contributed to susceptibility to migraine in multivariate analysis. In the subgroup analysis, the *CALCA* rs3781719 seemed to be a significant risk for migraine with aura, but was not statistically significant after FDR correction. Moreover, there was no synergistic relationship between the three SNPs in the multifactor dimensionality reduction analysis for explore locus–locus interactions.
Conclusion: Our data suggested that variants in *CALCA* gene and *RAMP1* gene were not associated with migraine in the Han-Chinese population.

Keywords: Migraine, *CALCA, RAMP1*, CGRP, polymorphism, China

INTRODUCTION

Migraine is a common and chronic neurovascular disease, affecting approximately 9.3% of the population in Mainland China.[1] The main clinical features include throbbing and severe unilateral head pain, usually accompanied by nausea, vomiting, neurological disturbance, photophobia and phonophobia. Based on the presence of an aura preceding headache in the early stages of the attack, migraine is divided into two main types: migraine with aura or without aura.[2] Although the exact pathogenesis of migraine remains to be determined, headache is most likely caused by activation of the trigeminovascular system and associated release of calcitonin gene-related peptide (CGRP).[3]

CGRP is a potent vasodilator neuropeptide with long-lasting effects. Over the past years; clinical studies have shown that CGRP plays an important role in the migraine process. Several studies have demonstrated serum CGRP levels elevated in the external jugular vein during a spontaneous migraine attack, with reduction following headache cessation.[4] Furthermore, intravenous injection of CGRP triggers migraine-type headache for migraineurs, and non-migraineurs only described headache that did not meet criteria for experimentally induced migraine.[5] Moreover, several selective CGRP receptor antagonists have been developed for treatment of migraine and some of them have demonstrated clinical efficacy.[6]

CGRP is a 37 amino acid neuropeptide and exists in two forms, α-CGRP and β-CGRP. Human α-CGRP is the main form expressed in trigeminal ganglia neurons and encoded by the calcitonin gene-related polypeptide-alpha (*CALCA*) gene.[7] The receptor activity modifying protein 1 (*RAMP1*) gene encoding for one subunit of the CGRP receptor is required for trafficking to the cell surface and for CGRP binding.[8]

In view of the relations between CGRP and migraine, we hypothesized that single nucleotide polymorphisms (SNP) of CGRP-related genes may be associated with migraine susceptibility.

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Against all expectations, such research in Australian population was negative.[9,10] So in the present study, we investigated rs3781719 and rs145837941 in the *CALCA* gene, and rs3754701 and rs7590387 at the *RAMP1* locus in a Chinese population of migraineurs and matched controls. Our association study of these four variants was conducted in a large sample involving 1033 individuals from a single institution in southern Fujian province of China.

METHODS

A total of 504 migraineurs were investigated in this study. They were recruited from the specialized headache clinic at Department of Neurology of the First Affiliated Hospital of Xiamen University between April 2010 and August 2015. Diagnosis of migraine was made by the chief physician of neurology, and all the patients fulfilled the international classification of headache disorders (ICHD-III beta) based on the results of neurological examination, CT or MRI.[2] A total of 529 non-headache healthy volunteers were included in the control group. They were recruited from the nurses in our hospital or attendees at the physical examination department who can provide a history of headache. The two groups were matched for age and sex and recruited from the same geographic areas. Subjects with tumor, or a history of depression and other co-morbid psychiatric disorders were excluded. Written informed consent was obtained from all participants, and the study was approved by the hospital ethics committee.

Genetic analysis

Genomic DNA was extracted from peripheral blood lymphocytes using the TIANamp Blood DNA Kit (Tiangen Biotech, Beijing, China) in strict accordance with laboratory procedures and stored at −80 °C for genotyping. All subjects were genotyped for SNPs using Sequenom iPLEX Assay technology as reported.[11]

Statistical analysis

Hardy-Weinberg equilibrium was verified for observed genotype frequencies of each SNP using the public statistics web-tool http://ihg.gsf.de/cgi-bin/hw/hwa1.pl. Age difference between two groups was assessed by the Student's t-test. Chi-square analysis was performed to compare allele and genotype frequencies in the patient and control groups. Statistical analysis was performed using the Statistical Package for the Social Sciences version 22.0 (SPSS, version 22.0) for Windows. Potential locus-locus interactions in three SNPs were performed using the multifactor dimensionality reduction (MDR) software (version 3.0.2).

To correct the error of multiple comparisons, the Benjamini–Hochberg false discovery rate (FDR) test were used and yielded P_{fdr}, and a P_{fdr} value less than 0.05 was considered significant. Moreover, in the subgroup analysis, the Bonferroni correction was also used, a P_{fdr} value less than 0.05/2 was considered significant.

RESULTS

Characteristics of patients

The study population consisted of 504 migraine patients including 77 migraine with aura and 427 migraine without aura. The mean age of the cases, including 71 males and 433 females, was 35.2±10.2 years. And the mean age of the 529 controls including 57 males and 472 females, was 34.6±8.9 years. The migraine patients and healthy controls were not statistically different in terms of age and sex ($P = 0.339$ and $P = 0.106$, respectively).

Gene polymorphisms

The polymorphism of *CALCA* rs145837941 was not found in migraine or control group, all the allele was the ancestral A allele. So this locus was excluded from the following analysis.

The genotypes of the remaining three SNPs in patients and controls followed the Hardy–Weinberg equilibrium. However, no significant difference in genotypic and allelic distribution was observed in the three polymorphisms between migraine cases and controls (Table 1). In the results of subgroup analysis, between migraine without aura patients and controls, no differences in the genotypic and allelic distributions of the three SNPs were observed. Between migraine with aura patients and controls, the allelic distribution of *CALCA* rs3781719 was different, the frequency of the A allele was higher in migraine with aura than in controls, but this result was no statistically significant after Bonferroni correction ($P \leqslant 0.05/2$). In addition, no significant difference in allelic distribution was observed in the polymorphisms of rs3754701 and rs7590387 in *RAMP1* between migraine with aura patients and controls.

To further explore locus–locus interactions, all the three SNPs were included in the MDR analysis.

Table 1: Genotype and allele frequencies of gene polymorphism in Chinese migraine case-control population

SNP	Group	Genotypes			P [a]	P_{fdr}	Alleles		P [a]	P_{fdr}
rs3781719(CALCA)		AA (%)	AG (%)	GG (%)			A	G		
	Control	365(69.0)	145(27.4)	19(3.6)			875(82.7)	183(17.3)		
	Migraine	334(66.3)	157(31.2)	13(2.6)	0.305	0.498	825(81.8)	183(18.2)	0.610	0.610
	MO	271(63.5)	145(34.0)	11(2.6)	0.074	0.222	687(80.4)	167(19.6)	0.204	0.306
	MA	64(83.1)	12(15.6)	1(1.3)	0.037	0.111	140(90.9)	14(9.1)	0.010	0.030[b]
rs3754701(RAMP1)		AA	AT	TT			A	T		
	Control	115(21.7)	251(47.4)	163(30.8)			481(45.5)	577(54.5)		
	Migraine	94(18.7)	249(49.4)	161(31.9)	0.466	0.498	437(43.4)	571(56.6)	0.335	0.503
	MO	79(18.5)	211(49.4)	137(32.1)	0.465	0.465	369(43.2)	485(56.8)	0.324	0.324
	MA	15(19.5)	38(49.4)	24(31.2)	0.899	0.899	68(44.2)	86(55.8)	0.761	0.785
rs7590387(RAMP1)		GG	GC	CC			G	C		
	Control	295(55.8)	210(39.7)	24(4.5)			800(75.6)	258(24.4)		
	Migraine	268(53.2)	206(40.9)	30(6.0)	0.498	0.498	742(73.6)	266(26.4)	0.296	0.503
	MO	223(52.2)	178(41.7)	26(6.1)	0.394	0.465	624(73.1)	230(26.9)	0.204	0.306
	MA	45(58.4)	28(36.4)	4(5.2)	0.844	0.899	118(76.6)	36(23.4)	0.785	0.785

MA = migraine with aura; MO = migraine without aura; SNP = single nucleotide polymorphism.
a: P values were calculated byχ^2 analysis; $P_{fdr:}$ After false discovery rate correction, significance is taken at $P_{fdr} \leqslant 0.05$ for migraine, and $P_{fdr} \leqslant 0.05/2$ for subgroup analysis.
b: The CALCA rs3781719 seems to be a significant risk for MA, but lost statistically significant after FDR correction.

As shown in Table 2, the best interaction model was the three-factor model with a testing accuracy of 49.09% and a perfect CVC of 10, but it was not statistically significant (P = 0.854). Moreover, the interaction dendrogram showed that there was no synergistic relationship between these three factors in the best model.

DISCUSSION

The well-documented relationship between CGRP and migraine makes polymorphisms in the *CALCA* and *RAMP1* genes a good choice to study the susceptibility to migraine. However, this study indicated that the rs3781719 and rs145837941 in the *CALCA* gene, and rs3754701 and rs7590387 at the *RAMP1* locus were not associated with the whole migraine in the Han-Chinese population. In the subgroup analysis, the *CALCA* rs3781719 seems to be a risk for migraine with aura but was not statistically significant after strict statistical corrections. This result was similar to the Sutherland's report in Australians and Baburhan's research in Turkey[9,12], which may highlight the fact that *CALCA* gene and *RAMP1* gene were not associated with migraine without regard to race.

The human *CALCA* gene is located on chromosome 11p15.2-p15.1. And rs3781719 polymorphism in the promoter of the gene has been linked to essential hypertension and alcohol-drinking Psoriasis vulgaris in Chinese populations.[13,14] The rs145837941 site is located in the coding sequence of the *CALCA* gene

Table 2: MDR models of multi-genotypes of *CALCA* and *RAMP1* for migraine susceptibility

Model	Testing accuracy	CVC	P	OR (95% CI)
rs3781719(*CALCA*)	0.4811	6/10	0.698	0.8563(0.3915~1.8726)
rs3781719(*CALCA*), rs7590387(*RAMP1*)	0.4758	7/10	0.616	0.8172(0.3715~1.7926)
rs3781719(*CALCA*), rs3754701(*RAMP1*), rs7590387(*RAMP1*)[a]	0.4909	10/10	0.854	0.93(0.4298~2.0121)

MDR, multifactor dimensionality reduction; CVC, cross-validation consistency; a, The model with the maximum testing accuracy and maximum CVC was considered the best model.

and is likely to disrupt structure of the *CALCA* propeptide. Our research suggests it is highly conserved in Chinese population. Alpha-CGRP is the predominant isoform produced by sensory neurons, especially in trigeminal neurons, and is presumed to be the primary contributor to pain mechanisms.[15] In the past decade, the role of CGRP in migraine has been firmly established by clinical and animal studies. The mechanism of CGRP that could potentially contribute to migraine includes roles in light aversion, neurogenic inflammation, peripheral and central sensitization of nociceptive pathways, cortical spreading depression and so on.[16] Our result revealed that rs3781719 in *CALCA* gene seems to be a significant risk for migraine with aura, but taking the small size of migraine with aura samples into account, it is necessary to use a larger population study to increase the power of research.

RAMP1 appears to be the functional rate-limiting subunit of CGRP receptor and is essential for the binding of CGRP to the receptor.[17] In addition, several small molecule antagonists of *RAMP1*/calcitonin receptor-like receptor complexes are in development for the treatment of migraine.[18] SNP rs3754701 is in the *RAMP1* gene promoter and rs7590387 locates 1.4 kb downstream of the RAMP1 gene. A recent research suggested that *RAMP1* rs7590387 may have a role in the transformation of episodic migraine into medication overuse headache.[19] So the role of non genetic hereditary mechanisms in migraine needs to be considered. Such exploratory research has been conducted by Yu *et al*. in Chinese migraineurs. Their study provided the first evidence that DNA methylation at RAMP1 promoter might play a role in migraine.[20]

In conclusion, our present study results suggested that variants in *CALCA* gene and *RAMP1* gene were not associated with migraine in the Han-Chinese population. However, given the important role that CGRP potentially plays in migraine pathogenesis, further comprehensive studies into the association of genetic variations of *CALCA* and *RAMP1* genes with migraine from a more genetic point of view are needed.

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DISCLOSURE

Conflicts of interest: None

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