Neurosci Bull August 1, 2010, 26(4): 322-326. http://www.neurosci.cn
DOI: 10.1007/s12264-010-0301-0

·Original Article·

Lack of association between *ADRA2B* -4825 gene insertion/deletion polymorphism and migraine in Chinese Han population

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Abstract: Objective The present study aimed to estimate the association between susceptibility to migraine and the 12-nucleotide insertion/deletion (indel) polymorphism in promoter region of α_{2B}-adrenergic receptor gene (*ADRA2B*). **Methods** A case-control study was carried out in Chinese Han population, including 368 cases of migraine and 517 controls. Genomic DNA was extracted from blood samples, and DNA fragments containing the site of polymorphism were amplified by PCR. Data were adjusted for sex, age, migraine history and family history, and analyzed using a logistic regression model. **Results** There was no association between indel polymorphism and migraine, at either the allele or the genotype level. **Conclusion** These findings do not support a functional significance of *ADRA2B* indel polymorphism at position -4825 relative to the start codon in the far upstream region of the promoter in the present migraine subjects.

Keywords: migraine; promoter of α_{2B}-adrenergic receptor gene; insertion/deletion polymorphism; genetic association

1 Introduction

Migraine is a highly prevalent neurovascular disorder with a complex inheritance pattern, and it affects a significant proportion of adult population worldwide[1]. Clinically, it is divided into 2 main subtypes based on the absence or presence of an aura: migraine without aura (MO) and migraine with aura (MA)[2]. Since migraine has a strong genetic component, identification of genetic factors will be important for understanding the pathophysiological mechanism of this disease. Studies on the association of some candidate genes with migraine have been conducted. These candidate genes are mainly involved in serotonin and dopamine pathways, and also in other pathways with an already suspected function in migraine pathophysiology[3]. The dysfunction of the sympathetic nervous system, in which α_{2B}-adrenergic receptor (ADRA2B) plays an important physiologic role, is a striking feature of migraine patients[4-6]. The receptor ADRA2B is critical for regulating neurotransmitter release from sympathetic nerves and from adrenergic neurons in the central nervous system. In addition, antagonists of β-adrenoceptors have shown effectiveness in preventive treatment of migraine[7]. Due to its wide expression on vascular smooth muscle cells, cerebral and peripheral vasculature, endothelium and prejunctional nerve terminals, *ADRA2B* gene has become one of the important candidates involved in the pathophysiology of migraine. However, a recent study has revealed that there is no association between 3 functional single nucleotide polymorphisms (SNPs) of β2-adrenoceptor

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Article ID: 1673-7067(2010)04-0322-05
Received date: 2010-03-02; Accepted date: 2010-06-23

gene (*ADRB2*) and migraine[8]. Despite this finding, the dysfunction of the sympathetic nervous system in migraine may also be related to other sympathetic co-transmitters or their receptors, such as α-adrenergic receptor. More recently, a population study has identified a novel 12-nucleotide (GGGACGGCCCTG) insertion/deletion (indel) polymorphism at position -4825 relative to the start codon in the far upstream region of the *ADRA2B* promoter (-4825 indel). Besides, this indel polymorphism is shown to be common and in complete linkage with the deletion polymorphism at position +901 and a G/C substitution at position -98[9]. The present study was aimed to investigate whether the 12-bp indel polymorphism is associated with migraine in Chinese Han population.

2 Materials and methods

2.1 Subjects A total of 368 unrelated migraine patients (Chinese Han ethnic), from the headache clinic of the Department of Neurology at the First Affiliated Hospital of Soochow University between 2008 and 2009, were employed in the present study. Migraine (MO or MA) was diagnosed by at least 2 experienced clinical neurologists, based strictly on the 2nd Edition of International Classification of Headache Disorders criteria[2]. In addition, patients with hypertension were excluded. Besides, a total of 517 unrelated healthy blood donors (Chinese Han ethnic) without any kind of headache, from a community nutritional survey conducted in the same region during 2008-2009, were recruited as the control. Through finishing a self-reported questionnaire, any subject with migraine or having a family history of migraine was excluded from the control group. To minimize the potential bias from population stratification, cases and controls were matched for sex and age. All participants had given their informed consent to participate in the study, and the design of the study was approved by the Ethical Committee of Soochow University.

2.2 Analysis of *ADRA2B* -4825 indel polymorphism Genomic DNA was extracted from blood samples by using the Chelex method[10]. DNA fragments containing the polymorphism site were amplified with forward primer 5'-ACGTGTAGAGGAAGAGGAAGG-3' and reverse primer 5'-CGTTCGGCAATGTCTGGAATAC-3'. PCR was performed in a total volume of 37.5 μL, including 3.75 μL 10×PCR buffer, 1.5 mmol/L $MgCl_2$, 0.25 mmol/L dNTPs, 0.5 mmol/L of each primer, 100 ng genomic DNA, and 1.5 U of Taq DNA polymerase. PCR was performed at 94 °C for 5 min, followed by 35 cycles of 94 °C for 30 s, 60 °C for 30 s, and 72 °C for 30 s, with final elongation at 72 °C for 5 min. The PCR products were analyzed by 7% non-denaturing polyacrylamide gel electrophoresis (PAGE) and visualized by silver staining[11]. The genotypes were determined by the number and the sizes of bands on the gel. The 12-bp deletion allele yielded a 200-bp band and the insertion allele yielded a 212-bp band. During genotyping, the experimenter had no knowledge of whether the sample was from a migraine patient or a control subject. Ten percent of the samples were randomly selected and tested in duplicate by different persons, and the reproducibility was 100%.

2.3 Statistics The association between polymorphism and migraine was evaluated by unconditional logistic regression. Results were adjusted according to sex and age. Additional stratification analyses were performed based on sex, age of disease onset (less than and equal to or greater than 26 years) and family history of migraine. These statistical analyses were conducted using Statistic Analysis System software (version 8.0, SAS Institute). $P < 0.05$ was considered as statistically significant. Power analysis was conducted by running the Power for Association With Error (PAWE) program[12], and a power of at least 0.95 was estimated with the whole sample for allelic and genotypic association (an error set at 0.01) to obtain an odds ratio (OR) of 2.0 or higher. The same statistical power calculations were performed for subgroups, and a power of at least 0.75 was obtained for all the subgroups (except the MA and MO subgroups, due to the small number of samples).

3 Results

The characteristics of migraine cases and controls were summarized in Table 1. There was no statistically significant difference between cases and controls in the frequency distribution of sex or age. Genotype distribution had no deviation from Hardy-Weinberg equilibrium in either migraine or control group ($P > 0.05$). Allele and genotype frequencies of

Table 1. A summary of the characteristics of migraine cases and controls

Characteristics	Migraine		Control		*P*-value
	n=368	Frequencies (%)	*n*=517	Frequencies (%)	
Age (mean±SD)	34.2±11.9		35.4±10.2		0.87 [a]
Sex					
Male	108	29.4	150	29.0	0.91 [b]
Female	260	70.6	367	71.0	
Age of migraine onset	26.9±10.7				
Family history of migraine					
Positive	133	36.1			
Negative	235	63.9			
Classification of migraine					
MA	14	3.8			
MO	354	96.2			
Duration of migraine (years)	7.3±7.4				

[a] Two-sided two-sample *t*-test between cases and controls. [b] χ^2 test for differences between cases and controls.

Table 2. Genotype distribution and allele frequencies of *ADRA2B* -4825 indel polymorphism among migraine cases and controls, and the risk of migraine

Genotype/Allele	MO and MA	*n* (%)	MO	*n* (%)	Control	*n* (%)	OR[a] (95% CI)[a]		*P* value	
							MO and MA	MO	MO and MA	MO
12N ins/ins	149	40.4	140	39.6	216	41.8	1.00 (Reference)	1.00 (Reference)		
12N ins/del	168	45.7	164	46.3	239	46.2	0.98(0.74-1.31)	0.95(0.70-1.30)	0.90	0.72
12N del/del	51	13.9	50	14.1	62	12.0	0.85(0.54-1.33)	0.82(0.53-1.26)	0.42	0.34
P_{trend}									0.49	0.37
12N ins	466	63.3	444	62.7	671	64.9	1.00 (Reference)	1.00 (Reference)		
12N del	270	36.6	264	37.3	363	35.1	0.93(0.76-1.14)	0.93(0.76-1.14)	0.49	0.35

[a] Adjusted for sex and age.

the indel polymorphism were shown in Table 2. Results showed that after adjustment for sex and age, the 12-bp indel polymorphism was not associated with migraine, at either the genotype or the allele level (OR=0.85, 95% CI between 0.54-1.33, *P*=0.49; OR=0.93, 95% CI between 0.76-1.14, *P*=0.49, respectively). Stratification analyses based on sex, age of disease onset and family history of migraine were shown in Table 3. There were no statistically significant differences between cases and controls after stratification.

4 Discussion

The lack of easily measured biological markers for migraine presents a great challenge for the identification of migraine genes. A bidirectional relation exists between migraine and several neurological diseases, such as epilepsy, stroke and depression[13]. Since migraine can be part of the clinical spectra of neurological diseases, revealing the genetic factors may help understand the molecular mechanisms underlying migraine, thus providing unique opportunities to unravel the common pathological molecular mechanisms[14].

Table 3. Stratification analyses based on sex, age of disease onset and family history of migraine

Groups	Genotypes			Alleles	
	Ins/Ins	Ins/Del	Del/Del	Ins	Del
Sex					
Male (case)	41	55	12	137	79
Male (control)	52	78	20	182	118
P value		0.54[a]		0.52	
Female(case)	108	113	39	329	191
Female(control)	141	163	63	445	289
P value		0.37[a]		0.34	
Age of disease onset					
<26 (median)	67	91	21	225	133
⩾ 26(median)	82	77	30	241	137
P value		0.79[b]		0.80	
Family history of migraine					
Positive	49	68	16	166	100
Negative	100	100	35	300	170
P value		0.68[c]		0.70	
Classification of migraine					
MA	9	4	1	22	6
MO	140	164	50	444	264
P value		0.10[c]		0.09	

[a]Adjusted for age. [b]Adjusted for sex. [c]Adjusted for sex and age.

In human, α_{2B}-adrenoceptor is encoded by *ADRA2B* gene which is located at chr2 11q11.1. We chose this gene on the basis of its biological relevance. Several reports have revealed associations of various cardiovascular and metabolic phenotypes with *ADRA2B* +901 indel polymorphism[15-20]. Furthermore, the *ADRA2B* deletion variant is associated with increased responsivity and connectivity of brain regions implicated in emotional memory, which have a bi-directional relation with migraine[21].

As far as we know, the present work is the first study to evaluate the association between *ADRA2B* polymorphism and migraine. The present case-control association study indicates that *ADRA2B* -4825 indel polymorphism is not associated with migraine, which is consistent with a previous report[7]. Since the -4825 indel polymorphism is in complete linkage with the indel polymorphism at position +901 and a G/C substitution at position -98[9], combined with previously reported data for female migraine subjects[8], our findings do not support a major role of the polymorphisms within adrenergic receptor systems in migraine pathology.

However, several points should be taken into consideration when explaining the results. Firstly, migraine is a complex disease with a polygenic origin, a wide phenotypic distribution, and various potential comorbidities. The association between a genetic variant and susceptibility to migraine could influence separately some but not all of its components. Thus, ADRA2B may act only in a subgroup of patients suffering from certain comorbid conditions. Secondly, there is no replication of our study through investigating in another accompanying population. Thirdly, here the sample size is relatively small, which may also influence the detection of the association between migraine and *ADRA2B* indel polymorphism. Thus, further studies involving a large population are needed.

Acknowledgements: This work was supported by the National Natural Science Foundation of China (No. 30800621) and China Postdoctoral Science Foundation (No. 20080431121, 200902530).

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中国汉族人群 *ADRA2B* 基因 -4825 插入 / 缺失多态与偏头痛易感性无关

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摘要：目的 研究α2B肾上腺素受体(α_{2B}-adrenergic receptor, ADRA2B)基因启动子区的12碱基插入/缺失多态与偏头痛易感性之间的关系。**方法** 在中国汉族人群中，对368例偏头痛患者和517例健康对照者进行病例对照研究。从受试者外周血中提取基因组DNA，采用PCR法对该多态进行分型。得到的数据采用逻辑回归方法分析，并经过性别、年龄、偏头痛病史及家族史校正。**结果** 在等位基因和基因型水平，该多态均未显示出与偏头痛易感性的相关性。**结论** 从本样本基础上得到的结果并不表明*ADRA2B*基因启动子区的12碱基插入/缺失多态在偏头痛的发生中起作用。

关键词： 偏头痛；α2B 肾上腺素受体基因启动子；插入 / 缺失多态；基因关联