Lack of association between \textit{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 $\alpha_{2B}$-adrenergic receptor gene ($\textit{ADRA2B}$). 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 $\textit{ADRA2B}$ 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 $\alpha_{2B}$-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 $\alpha_{2B}$-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 $\beta$-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, $\textit{ADRA2B}$ 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 $\beta$2-adrenoceptor...
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’-ACGTGTAGAGGAAGAGG-3’ and reverse primer 5’-CGTTCGGCAATGTCTGGGATC-3’. PCR was performed in a total volume of 37.5 μL, including 3.75 μL 10×PCR buffer, 1.5 mmol/L MgCl₂, 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