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Research report

Association of PDE4D and IL-1 gene polymorphism with ischemic stroke in a Han Chinese population

Nan Li[1], Zhiyi He*, Jialiang Xu[2], Fang Liu[3], Shumin Deng[4], Hui Zhang[5]

Department of Neurology, The First Affiliated Hospital of China Medical University, Shenyang 110001, China

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ABSTRACT

Background: The single-nucleotide polymorphisms (SNPs) of the phosphodiesterase 4D (PDE4D) and interleukin-1 (IL-1) genes are associated with increased risk for the development of ischemic stroke (IS) in whites. However, little is known about whether this association could also occur in Han Chinese.
Method: A total of 371 patients with IS and unrelated healthy controls were recruited and the SNPs of the PDE4D (83T/C), (87T/C), IL-1 (−889C/T) and IL-1 (−511C/T) were characterized, respectively, by polymerase chain reactions-restriction fragment length polymorphism (PCR-RFLP). The genotype and allele frequencies of these SNPs in this population were statistically analyzed.
Results: The genotype and allele frequencies of the PDE4D (87T/C) and IL-1 (−511C/T) were similar between IS patients and controls. In contrast, the frequencies of CC genotype and C allele of the PDE4D (83T/C) and the T allele frequency of IL-1 (−889C/T) in IS patients were significantly higher than that in healthy controls ($p = 0.001$, $p = 0.003$ and $p = 0.02$, respectively), independent of the conventional risk factors. The values of odds ratio (OR) reached at OR = 1.603; 95%CI = 1.032–2.489; $p = 0.036$ for the CC genotype of the PDE4D (83T/C) and OR = 1.913; 95%CI = 1.621–2.375; $p = 0.034$ for the TT genotype of the IL-1 (−889C/T), respectively.
Conclusions: the SNPs of the PDE4D (83T/C) and IL-1 (−889C/T) were associated with increased risk for the development of IS in Northern Han Chinese.

1. Introduction

Stroke is a major cause of morbidity and mortality in the world. Stroke caused death of near 5.7 million people in 2005 and more than 50% of these deaths occurred in Asian countries, such as China, India, Korea, and Japan (WHO report: http://www.who.int/Cardiovascular_diseases/). Stroke is the second leading cause of death in China and the incidence rate of stroke is about 170.3/100,000[1]. Ischemic stroke (IS) accounts for more than 70% of the stroke cases in China. IS is commonly caused by extracranial embolism, intracranial thrombosis, or decreased cerebral blood flow. The development of IS has been attributed to the interaction of multiple factors, including genetic variants, chronic diseases, risk behaviors and inflammation [2]. While hypertension, diabetes, hyperlipidemia and heavy smoking are risk factors for the development of IS, inflammation-related atherosclerosis is one of the major causes of stroke [3,4]. Furthermore, epidemiological studies in families and in twins revealed that genetic factors are crucial for the predisposition to IS [5–8]. Therefore, determination of the association of inflammation-related gene variants with the development of IS in a given population will be of great significance in the predisposition to IS.

Phosphodiesterase 4D (PDE4D) and IL-1 are pro-inflammatory factors, involved in the pathogenesis of atherosclerosis [9–11]. PDE4D can result in hydrolysis of cAMP and is expressed in multiple types of cells, including immunocompetent lymphocytes and antigen presenting cells as well as vascular smooth cells [12]. PDE4D can also promote the proliferation and migration of vascular smooth muscle cells, contributing to the development of inflammation and atherogenesis [13]. IL-1 can induce the expression of adherence molecules on endothelial cells, attracting circulating leukocytes, and promote the synthesis of IL-6, fibrinogen, C-reactive protein and other pro-inflammatory components, participating into the pathogenesis of atherosclerosis [11,14]. Indeed, high levels of IL-1 mRNA transcripts have been detected in the

* Corresponding author. Tel.: +86 02483282515.
E-mail addresses: lnan.2010@163.com (N. Li), zhiyih.ln@163.com (Z. He), jialiangxucmu@163.com (J. Xu), Liufang219@163.com (F. Liu), shumdeng@163.com (S. Deng), Zhanghuicmu2h@126.com (H. Zhang).
[1] Tel.: +86 13147831958/13897876326; fax: +86 04167101508.
[2] Tel.: +86 13840044655.
[3] Tel.: +8613591664420.
[4] Tel.: +8613516036195.
[5] Tel.: +86 13066652736.

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Table 1
The selected characteristics of subjects.

	Case (n = 371)	Control (n = 371)	P value
Age in years ± SD	63.88 ± 7.36	62.87 ± 7.57	0.064
Male gender, n (%)	230 (62)	247 (66.6)	0.220
Hypertension, n (%)	129 (34.8)	79 (21.3)	<0.001
Diabetes, n (%)	92 (24.8)	52 (14)	<0.001
Hypercholesterolemia, n (%)	4 (3.8)	8 (2.2)	0.279
Smoking, n (%)	153 (41.2)	92 (24.8)	<0.001

n: Number; SD: standard deviation; continuous and categorical variables were tested by t-test and χ^2-analysis, respectively.

plaques of human patients with atherosclerosis [11]. Both IL-1α and IL-1β have similar functions.

The association of specific PDE4D single-nucleotide polymorphisms (SNPs) and haplotypes with stroke is initially identified in an Icelandic population [12] and further reported in whites [15–17]. In addition, significantly different levels of PDE4D mRNA transcripts are detected in B lymphoblastoid cells between stroke patients and control subjects [12]. The SNPs of IL-1α (−889C/T), IL-1β (−511C/T), and PDE4D (83T/C) and (87T/C) have been found significant association with increased risk of IS in whites and Korean [12,18]. However, studies from other populations failed to find the association [19]. Hence, whether these SNPs are associated with IS remains controversial. Recently, the PDE4D (83T/C) has found association with IS and haemorrhagic stroke while the SNP of IL-1β (−511C/T) seems not to be associated with increased risk for IS in a Southern Chinese population [20,21]. Given that there are about 56 ethnic populations in China how these SNPs are associated with IS in Northern Han Chinese remains to be determined.

In this study, we explored the potential association of the SNPs of IL-1α (−889C/T) (rs1800587), IL-1β (−511C/T) (rs16944), and PDE4D (83T/C) (rs966221) and (87T/C) (rs2910829) with risk for the development of IS in a Northern Chinese Han population. We found that the SNPs of IL-1α (−889C/T) and PDE4D (83T/C), but not IL-1β (−511C/T) and PDE4D (87T/C), were significantly associated with increased risk for the development of IS in Northern Han Chinese. We discussed the implication of our findings in the predisposition to IS.

2. Subjects and methods

2.1. Subjects

A total of 371 patients with IS and 371 unrelated healthy control subjects were Han Chinese and recruited from outpatient and inpatient services at the First Affiliated Hospital of China Medical University and Jin Zhou Central Hospital from March 2008 to January 2009. All patients with IS were diagnosed at the onset with a sudden loss of global or focal cerebral function and corresponding infarction on brain imaging of computed tomography (CT) or magnetic resonance imaging (MRI). Patients with transient ischemic attack were excluded from the study. The unrelated control subjects were from the same geographic areas and their clinical histories were obtained through an interview by physicians. Individuals who had any history or occurrence of cerebrovascular or cardiovascular diseases were excluded. Subjects who had history of or current hypertension, diabetes mellitus, smoking habit, and hypercholesterolemia were recorded. Arterial hypertension was defined if systolic and diastolic blood pressure were ≥140 and ≥90 mmHg, respectively, for at least two different occasions. Diabetes mellitus was diagnosed, according to the standard of American Diabetes Association (http://www.diabetes.org/home.jsp) and hypercholesterolemia was determined if total fasting plasma cholesterol ≥6.5 mmol/L. Written informed consents were obtained from individual participants and experimental protocols were approved by the Ethics Committee of both hospitals. The selected characteristics of those subjects were summarized in Table 1.

2.2. Biochemical parameters and genotype determination

Venous blood (10 mL) was collected from individuals after fasting overnight and subjected to Ficoll gradient centrifuge for the isolation of peripheral blood mononuclear cells (PBMC). Genomic DNA was extracted from individuals' PBMC using the Wizard genomic DNA purification kit (Promega, Madison), according to the manufacturer's instructions. The fragments of the IL-1α, IL-1β and PDE4D genes were amplified by polymerase chain reactions (PCR) using the isolated genomic DNA as the template and specific primers. The sequences of primers, the PCR conditions and the profiles of DNA fragments were presented in Table 2 [9,17]. The amplificants were further digested with the specific endorestriction enzyme (Table 2) and characterized by 3% agarose gel electrophoresis. The resulting restriction fragment length polymorphism (RFLP) was used to determine the SNPs of these genes. Two PBMC samples with known each genotype, determined by our preliminary studies, were used as the positive controls to ensure the specificity and sensitivity of genotyping. Some DNA fragments from each genotype of samples were further cloned and their authenticities were determined by DNA sequencing. Allele and genotype frequencies were determined by gene counting, and the deviation from Hardy–Weinberg (HW) equilibrium was tested [13].

The levels of blood plasma glucose and total cholesterol of individual subjects were measured by routine glucose oxidase procedure and biochemical methods [1,13], respectively.

2.3. Statistical analysis

Categorical data are reported as the frequency in the population and continuous data are expressed as mean ± SD of each group. Continuous and categorical variables of biological and clinical measures between cases and controls were analyzed student's t and χ^2 tests, respectively. For each gene variant, odds ratio (OR) was calculated, along with the 95% confidence interval (CI) to measure the genetic association with IS. Association of each studied polymorphism with IS was analyzed by stratifying individual confounding factor and then assessed by logistic regression analysis, adjusting for confounding variables, including diabetes mellitus, smoking habit, hypertension and hypercholesterolemia with 0 for negative while 1 for positive. In addition, the association was also tested by a two-point haplotypic analysis. All the statistical tests were performed using the SPSS software for Windows (Ver. 13.0). Haplotype analysis of the 2 PDE4D SNPs was performed using the SHESIS program (http://analysis.Bio-x.cn/my analysis.php). A 2-tailed P value <0.05 was considered statistically significant.

Table 2
The primer sequences, PCR conditions, and genotyping profiles.

SNP	Primer sequences (fragment size)	Restriction enzyme	Annealing temperature (°C)/extension (s)/cycles	Profiles of DNA fragments
PDE4D (83C/T)	F: 5′TTGTTTCTAGTTAGCCTTG3′ R: 5′ATTTGGCCTTGCAATATAC3′ (359 bp)	Tail	50/60/40	T/T:359 C/C:264, 95 C/T:359, 264, 95
PDE4D (87C/T)	F: 5′AAGATGAGGAAGAATAATGG3′ R: 5′ATGAAGACACCTGAAAGATC3′ (486 bp)	SSP1	52/60/40	T/T: 486 C/C:264, 222 C/T:486, 264, 222
IL-1α (−889C/T)	F: 5′GGGGGCTTCACTATGTTGCCCACA CTGGACTAA3′ R: 5′GAAGGCATGGATTTTTACATATGA CCTTCCATG3′ (309 bp)	Nco I	57/60/40	T/T:309 C/C:266, 43 C/T:309, 266, 43
IL-1β (−511C/T)	F: 5′TGGCATTGATCTGGTTCATC3′ R: 5′GTTTAGGAATCTTCCCACTT3′ (304 bp)	Nco I	56/60/35	T/T:304 C/C:190, 114 C/T:304, 190, 114