

Original Article

Association of CYP2J2 gene polymorphisms with ischemic stroke

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Abstract: Objective: The present study aims to investigate the relationship between CYP2J2 gene polymorphisms and ischemic stroke (IS) in a Chinese Han population. Methods: We performed a case-control study including 300 stroke patients and 300 healthy control subjects to compare the distribution of genetic polymorphism G-50T in CYP2J2 gene. Results: We found GT genotype of G-50T in CYP2J2 gene was associated with the risk for IS (13.7% vs. 7.7%, $P = 0.037$). After adjustment of confounders, the difference remains significant (OR = 1.890, 95% CI: 1.042-3.011). Conclusion: CYP2J2 gene polymorphism might increase the risk of stroke in Chinese population.

Keywords: Ischemic stroke, CYP2J2 gene, single nucleotide polymorphism

Introduction

Stroke is a major cause of morbidity and mortality worldwide [1]. It is general accepted that stroke was a complex polygenic disease resulting from the interaction between genetic variation and environmental factors [2]. Previous studies indicated that several susceptibility gene of the stroke have been identified such as interleukin (IL) 10 [3, 4], ALOX5AP [5], and SAA1 gene [6].

CYP2J2 is a human CYP epoxygenase expressed predominantly in vascular endothelial cells and heart tissue that metabolizes arachidonic acid into all four EET regioisomers [7]. Recently, the G-50T promoter polymorphism in the CYP2J2 gene was found to be independently associated with an increased risk of coronary artery disease, and plasma concentrations of EETs were shown to be significantly lower in individuals with the CYP2J2-50TT genotype than in those with the CYP2J2-50GG genotype [8]. Although CYP2J2 gene has been reported to be associated with stroke [9-11], the results were inclusive.

In the present study, we aim to investigate the relation between CYP2J2 gene and stroke in a Chinese population.

Subjects and methods

Ethics

The present study was approved by the Ethics Committee of the First Affiliated Hospital of Xinxiang Medical University and was conducted according to the standards of the Declaration of Helsinki. Written informed consent was obtained from the participants.

Subjects

A total of 300 hospitalized ischemic stroke patients (181 of male and 119 of female) in the Department of Neurology, First Affiliated Hospital of Xinxiang Medical University were enrolled from September 2011 to Oct. 2014. The average age is (56.1 ± 13.1) years. All these stroke patients were unrelated Han Chinese people. The patients were diagnosed stroke according to the criteria of the 9th Edition of International Classification of Diseases (ICD9), and all the stroke patients were performed the head MRI or CT to confirm the diagnosis. The patients who with cerebral hemorrhage, sub-arachnoid hemorrhage, collagen disease, cerebral infarction caused by vascular inflammation, amyloidosis, arteriovenous malformations and other diseases were excluded from this study.

Table 1. Primers and PCR conditions of G-50T locus

SNPs	Primers	Endonuclease	Tm (°C)
G-50T	F: 5'-TTTCTGAGACCGGTGCGTG-3' R: 5'-CAGGTGCGACTGCTCGAAG-3'	Alu I	62

at 37°C for 2-3 h and digested products were separated on 1.5% agarose gels containing ethidium bromide.

Statistical analysis

During the same period, 300 healthy persons in medical center of the same hospital were selected as the control group. All these control subjects were unrelated Han people whose age and sex were matched with the patient group. There were 180 male and 120 female who were aged from 38 to 84 (57.1 ± 12.4) years old. The subjects with cerebrovascular disease, neurological diseases, kidney disease, blood disorders, cancer, peripheral vascular disease, and autoimmune diseases were excluded from the control group. These control subjects have not the history of cerebrovascular disease, and have not sign of cerebrovascular disease by scanning of CT or MRI. The clinically characteristics including age, gender, height, weight, blood pressure, lipids profiles, fasting glucose, past medical history, drug history, smoking history, and alcohol history were collected.

DNA extraction

The genomic DNA extraction kit (Promega Corporation, United States) was used for DNA extraction from blood samples of the subjects according the protocol of the kit.

Genotyping

Genotyping was performed by a polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) technique. A 373-bp fragment of CYP2J2 G-50T (Genbank sequence AF272142, nucleotides 5856-6228, rs89029) was amplified with the primer pairs as shown in **Table 1**. The reaction was carried out in a 25- μ l volume that included 50 ng of genomic DNA, 200 μ mol/L dNTP mixture, 0.2 μ mol/L of each primer, 2 \times GC buffer and 1 U of TaKaRa LA Taq DNA Polymerase (TaKaRa Biomedicals, Dalian, Liaoning, China). PCR was performed in an Applied Biosystems (Foster City, California, USA) 2720 Thermocycler with an initial denaturation at 95°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 62°C for 30 s and extension at 72°C for 30 s. A final extension at 72°C for 5 min was performed. The PCR products were digested with Alu I (Fermentas, Burlington, Ontario, Canada)

We utilized the SPSS 18.0 statistical software (Chicago, IL, USA) to perform the data analyses. Hardy-Weinberg (H-W) equilibrium test was carried out by χ^2 test. The continuous data were compared using *t* test, and categorical data were compared using the χ^2 test. Genotype and allele frequencies were compared using the χ^2 test. The non-conditional Logistic regression was used to adjust the traditional risk factors for stroke such as gender, age, body mass index, blood pressure, blood lipids, blood glucose, smoking history, history of alcohol and other confounding factors. The odds ratio (OR) and 95% CI (confidence interval, CI) were calculated before adjustment and after adjustment. $P < 0.05$ was consider significant.

Results

The characteristics of participants

As shown in **Table 2**, there were not significantly different between the stroke and the control subjects in age, BMI, HDL-C and LDL-C. However, there were significant differences between stroke group and control group in hypertension, diabetes, smoking history, GLU, TG, and TC concentration.

Hardy-Weinberg equilibrium

The genotype distribution in G-50T was in line with Hardy-Weinberg genetic equilibrium in both stroke group and the control group ($P > 0.05$, data not shown).

Genotype and allele frequencies

We found GT genotype frequency of G-50T was significantly higher in the stroke patients than that in the control group ($P = 0.037$). The T allele frequency was also significantly higher than that in control group ($P = 0.015$, **Table 3**).

Logistic regression analysis showed that after adjustment of the conventional risk factors such as hypertension, diabetes, hyperlipidemia and smoking, the difference of GT genotype fre-

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Table 2. The characteristics of participants

Groups	N	Age (years)	BMI Kg/m ²	Hypertension (n, %)	Diabetes (n, %)	Smoking (n, %)	GLU (mmol/L)	TG (mmol/L)	TC (mmol/L)	HDL-C (mmol/L)	LDL-C (mmol/L)
Stroke group	300	56.1 ± 13.1	24.6 ± 3.2	104 (34.7)	89 (29.7)	97(32.3)	7.3 ± 3.1	3.1 ± 1.0	5.2 ± 2.1	1.1 ± 0.8	2.8 ± 1.4
Control group	300	57.1 ± 12.4	24.2 ± 3.1	54 (18.0)	12 (4.0)	52 (17.3)	5.0 ± 1.1	1.5 ± 1.3	4.4 ± 1.0	1.3 ± 0.6	2.3 ± 1.1
<i>P</i>		0.181	0.353	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.228	0.5

Table 3. Distribution of CYP2J2 genotypes and allele

SNPs	Alleles	Groups	n	Genotypes (n, %)		P value	MAF	P value
				GG	GT			
G-50T	G/T	Stroke	300	259 (86.3)	41 (13.7)	0.037	0.068	0.015
		Control	300	277 (92.3)	23 (7.7)			

Table 4. Logistic analysis results

	OR	95% CI	P
GT genotype	1.890	1.042-3.011	0.002
Diabetes	2.457	1.769-4.761	0.009
Smoking	3.780	1.396-10.24	0.013
Hypertension	2.620	1.970-5.073	0.037

quency in stroke patients and control group remained statistically significant (OR = 1.89, 95% CI: 1.04-3.01, P = 0.018, **Table 4**).

Discussion

In this study, we found T allele carriers of G-50T locus have higher risk for stroke in Han Chinese population. Stroke is caused by the interaction between environmental and genetic factors. In this study, an important human metabolic enzyme P450 gene family member-CYP2J2 gene was selected as the candidate gene to perform the case-control study, and we not only found that the GT genotype of G-50T in CYP2J2 gene was significantly higher in stroke patient than that in control group, but also found so did T allele. Our results were not consistent with the results of previous studies [9-11]. Fava et al.'s [9] findings did not support a major role for the CYP2J2 -50G > T variant in determining BP level and incident ischemic events. Marciante et al. [10] used publicly available single nucleotide polymorphism discovery data from a mixed race panel of 90 individuals to select 30 tag-single nucleotide polymorphisms that were genotyped in 856 myocardial infarction cases, 368 stroke cases and 2688 controls. The authors found common variation in CYP2J2 is associated with the risk of myocardial infarction but not with stroke. Zhang et al. [11] also reported that G-50T polymorphism in CYP2J2 gene was not associated with stroke.

The G-50T variant has previously been described by King et al. [12] with variable frequency in different racial groups. There are 4 putative Sp1 binding sites in the proximal CYP2J2 promoter, with their 5-ends in positions

-84, -72, -50, and -45. The loss of an SP1 binding site in G-50T variants significantly reduces binding of Sp1. [13-16]. Although our data do not prove a causal relationship, they are certainly consistent

with a vascular protective role of products of this gene in humans. Strong constitutive expression of CYP2J2 would contribute to the biosynthesis of EETs, leading to protective vascular effects. Individuals with the G-50T polymorphism would have considerably less basal transcriptional activity of CYP2J2, which might result in reduced vascular protection. Given the multifactorial nature of atherosclerosis, it is unlikely that a polymorphism in a single gene will have a profound effect on the risk of atherosclerotic diseases.

In conclusion, this study showed that the GT genotype of G-50T in CYP2J2 gene was associated with stroke in Han Chinese population.

Disclosure of conflict of interest

None.

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