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*Original Article*

# Relationship between adiponectin receptor 1 gene polymorphisms and ischemic stroke

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**Abstract:** Objective: Previous study suggested adiponectin receptor 2 (ADIPOR2) genetic polymorphisms were associated with the risk of ischemic stroke. However, the relation between adiponectin receptor 1 (ADIPOR1) gene polymorphism and stroke remains unclear. Methods: In the present study, we utilized the polymerase chain reaction-sequencing method to detect rs2275737 and s1342387 genotypes of ADIPOR1 gene in 300 cases of ischemic stroke patients and 300 age- and sex- matched healthy controls. Results: For rs2275737, we found A allele carriers have increased risk to ischemic stroke (OR=2.570, 95% CI: 1.999-3.305); also, we found rs1342387 A allele was associated with the risk for stroke (OR=1.351, 95% CI: 1.074-1.699). After adjusted for confounders such as hypertension, diabetes, and smoking, we found the association remains significant. Conclusion: ADIPOR1 genetic polymorphism may increase the risk of ischemic stroke.

**Keywords:** Ischemic stroke, adiponectin receptor 1 gene, single nucleotide polymorphisms

## Introduction

Adiponectin is an adipocytokine that is involved in lipid and glucose metabolism, energy homeostasis, and inflammatory pathways [1-3]. Circulating concentrations of adiponectin are reduced in subjects with type 2 diabetes [4-7]. Ischemic stroke is a multifactorial disease related to infarction, hypertension and diabetes. Recently, two receptors, adiponectin receptor 1 (ADIPOR1) and adiponectin receptor 2 (ADIPOR2), were cloned, each consisting of eight exons that are located on chromosomes 1q32.1 and 12p13.33, respectively [8]. In humans, ADIPOR1 is ubiquitously expressed, with highest levels in the skeletal muscle, while ADIPOR2 is predominantly expressed in skeletal muscle and liver [9]. Previous study indicated that adiponectin receptor 2 (ADIPOR2) genetic polymorphisms were associated with the risk of ischemic stroke [10]. However, the relation between adiponectin receptor 1 (ADIPOR1) gene polymorphism and stroke remains unclear.

In the present study, we selected two SNPs (rs2275737 and rs1342387) of ADIPOR1 gene to explore its association with the risk of ischemic stroke in a Chinese population.

## Materials and methods

### *Subjects*

We enrolled 300 patients with ischemic stroke who were treated in the Department of Internal Neurology, Tianjin 4th Central Hospital from September 2010 to May 2014. All the patients were confirmed by brain MRI and/or head CT. The patients with cerebral embolism, arterial inflammation, malignancy, trauma, drugs, blood disease, cerebral infarction caused by vascular malformation or aneurysm, liver and kidney disease, and thyroid disease were excluded from the study. We also included 300 age- and sex-matched control subjects. All these control subjects were unrelated Han people. The subjects with cerebrovascular disease, neurological diseases, kidney disease, blood disorders, cancer,

**Table 1.** The characteristics of participants

| Characteristics | Ischemic stroke group | Control group | *P* value |
|---|---|---|---|
| Age (M ± SD, y) | 64.3 ± 11.0 | 64.1 ± 11.5 | 0.543 |
| Gender (M/F, n) | 188/112 | 190/110 | 0.941 |
| BMI (M ± SD, kg/m$^2$) | 23.13 ± 3.01 | 23.44 ± 4.01 | 0.220 |
| Smoking (n, %) | 121 (40.3%) | 61 (20.3%) | <0.001 |
| Alcohol drinking (n, %) | 129 (43.0%) | 70 (23.3%) | <0.001 |
| Hypertension (n, %) | 212 (79.7%) | 109 (36.3%) | <0.001 |
| Diabetes (n, %) | 143 (47.7%) | 73 (24.3%) | <0.001 |
| Hyperlipidemia (n, %) | 101 (33.7%) | 35 (11.7%) | <0.001 |

**Table 2.** The primer sequences of the two SNPs

| SNP | Sense primer | Antisense primer |
|---|---|---|
| rs2275737 | 5'CATAGGGACTTGGATAGAG3' | 5'AGTGAGGTTCTGGGTAAA3' |
| rs1342387 | 5'ACCATATCCATGCCAACA3' | 5'GAGAAACAGCACGAAACC3' |

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 (**Table 1**). All study subjects signed informed consents before the study.

*Blood collection and DNA extraction*

2 mL of fasting venous blood was taken from antecubital vein and placed in EDTA-containing tubes. The genomic DNA extraction kit (Promega Corporation, United States) was used for DNA extraction from blood samples of the subjects according the protocol provided by the company.

*Primer design*

The primers were designed using of Primer 5.0 software according to the primer design principles. The primer sequences were shown in **Table 2**.

*Genotyping*

The PCR-sequencing method was used to perform the genotyping. PCR amplification was performed as follows: totally, 25 μL of reaction system, including 1 μL of template DNA (200 ng/μL), 0.5 μL of upstream and downstream primers (20 μmol/L), 2 μL of dNTP mixture, 0.125 μL of Taq polymerase (Takara Company, China), 2.5 μL of 10 × PCR buffer ($Mg^{2+}$ Plus), and 18.375 μL of $ddH_2O$. The PCR procedure was 94°C for 5 min, 94°C denaturation for 30 s, 55°C refolding for 30 s, 72°C extension for 1 min, after 35 cycles, 72°C extension for 10 min, and store at 4°C. The PCR products were sequenced by Beijing Sanboyuanzhi Biotech Company (Beijing, China).

*Statistical analysis*

We utilized SPSS 17.0 software to analyze the data. Hardy-Weinberg (H-W) equilibrium test was carried out by $X^2$ test. The continuous data were compared using *t* test, and categorical data were compared using the $X^2$ test. Genotype and allele frequencies were compared using the $X^2$ 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

*Hardy-Weinberg equilibrium*

The genotype and allele distributions were in line with Hardy-Weinberg equilibrium in both ischemic patients group (data not shown).

*Clinical characteristics*

The age and sex were matched in this case-control study. The body weight index (BMI) showed no significant difference between these two groups (*P*>0.05). However, the tradition risk factors of ischemic stroke such as the incidence of smoking, drinking, hypertension, diabetes, hyperlipidemia in ischemic stroke group was significantly higher than that in the control group (all *P*<0.05) (**Table 1**).

**Table 3.** The distribution of genotype and alleles between Ischemic stroke group and control group

| SNP | | Ischemic stroke group | Control group | OR (95% CI) | P |
|---|---|---|---|---|---|
| rs2275737 | | | | | |
| Genotype | CC | 97 (32.4%) | 177 (41.0%) | 1 | |
| | AC | 151 (50.3%) | 112 (37.3%) | 1.626 (1.131-2.339) | 0.008 |
| | AA | 52 (17.3%) | 11 (3.7%) | 5.702 (2.820-11.527) | <0.001 |
| Allele | A | 0.425 | 0.223 | 2.570 (1.999-3.305) | <0.001 |
| | C | 0.575 | 0.777 | | |
| rs1342387 | | | | | |
| Genotype | GG | 90 (30.0%) | 125 (41.7%) | 1 | |
| | GA | 148 (49.3%) | 118 (39.3%) | 1.742 (1.211-2.505) | <0.001 |
| | AA | 62 (20.7%) | 57 (19.0%) | 1.510 (0.963-2.370) | 0.075 |
| Allele | G | 0.547 | 0.613 | 1.351 (1.074-1.699) | 0.012 |
| | A | 0.453 | 0.387 | | |

**Table 4.** Logistic analysis results

| | OR | 95% CI | P |
|---|---|---|---|
| rs2275737 | 2.271 | 1.122-4.021 | 0.005 |
| rs1342387 | 1.501 | 1.044-2.893 | 0.015 |
| Diabetes | 2.113 | 1.013-4.312 | 0.001 |
| Smoking | 2.124 | 1.881-5.242 | 0.030 |
| Hypertension | 2.103 | 1.122-3.013 | 0.021 |

*Genotype and allele frequency distribution*

The genotypes and alleles frequencies distribution in rs2275737 and rs1342387 was significantly different ($P<0.05$) between these two groups. AA genotype and A allele frequencies of rs2275737 in case group were significantly higher than that in control group ($P<0.001$); also, the GA genotype and G allele were common in the ischemic stroke patients group (**Table 3**).

*Risk analysis of rs1342387 and rs2275737 and ischemic stroke*

For rs2275737, compared to CC genotype, the ischemic stroke risk of AC genotype carriers was 1.626-fold increased (OR=1.626, 95% CI: 1.131-2.339; P=0.008). However, AA genotype carriers increased 5.702 folds risk to ischemic stroke (OR=5.702; 95% CI: 2.820-11.527). Also, we found rs1342387 was associated with ischemic stroke risk. After adjustment of gender, age, BMI, smoking history, alcohol consumption, hypertension, diabetes and hyperlipidemia and other confounding factors, the differences remain significant (**Table 4**).

## Discussion

In the present study, we found genetic polymorphisms of ADIPOR1 were associated with ischemic stroke in a Chinese population. This is the first study to reveal the relation between ADIPOR1 genetic polymorphism and ischemic stroke in Chinese population.

Adiponectin is a fat factor secreted by adipocytes. It has anti-atherosclerotic, anti-inflammatory, insulin sensitivity increasing, energy balance, glucose and lipid metabolism maintaining effects and other important biological effects [11, 12]. Although in a recent report, Wang et al. [13] screened ADIPOR1 (but not ADIPOR2) for sequence variation in Caucasian and African-American subjects but found no association between the SNPs they detected (including the five SNPs we examined) and type 2 diabetes, insulin sensitivity, or insulin secretion, Damcott et al. found ADIPOR1 SNPs were associated with type 2 diabetes.

Studies showed that patients with ischemic stroke had decreased plasma adiponectin levels [12]. ADIPOR1 were mainly expressed in the skeletal muscle, also abundantly expressed in hypothalamus and cerebral vascular endothelial cells [14]. Studies showed that adiponectin receptors were closely associated with diabetes [15], metabolic syndrome [16], and cardiovascular disease [17]. They can affect cell metabolism and function and become one of the important mechanisms in the promotion and development of disease [18].

Both rs2275737 and rs2275737 were located in the promoter region of ADIPOR1 genes. In this study, we found that AA genotype and A allele of rs2275737 carriers have increased risk of ischemic stroke in Chinese population. The mechanism of the association between ADIPOR1gene polymorphism and the incidence of ischemic stroke was unclear. Both these two SNPs were located in the promoter region of ADIPOR1 gene. The genetic polymorphisms

may affect the transcript activity of the promoter, which may affect ADIPOR1 receptor expression and function through the above mechanism. Furthermore, these loci may be associated with other SNPs in linkage disequilibrium and cause changes in receptor activity. The results also needed to be confirmed by a larger sample size study, the molecular mechanism of mutations causing the increased risk of ischemic stroke need further in-depth research, such as whether mutations can lead to changes in gene expression. This will further strengthen the awareness of the pathogenesis of ischemic stroke which may lead to better disease prevention.

## Disclosure of conflict of interest

None.

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