Relationship between nerve injury-induced protein gene 2 polymorphism and stroke in Chinese Han population

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Abstract

The aim of present study was to investigate the relationship between nerve injury-induced protein 2 (\textit{NINJ2}) gene polymorphism and stroke in Chinese Han population. Fifty-two patients with large-artery atherosclerosis (LAA) infarction, 85 patients with small-artery occlusion lacunar (SAO) infarction, 50 patients with intracerebral hemorrhage (ICH) and 66 controls were included. Genotypes and alleles frequencies of the two single nucleotide polymorphisms (SNPs) of \textit{NINJ2} among different groups were analyzed and compared. In regard to \textit{rs}12425791, the frequencies of the \textit{AG} and \textit{AA+AG} genotypes of the LAA and SAO groups were significantly higher than those in the control group; the frequency of the \textit{A} allele of the SAO group was significantly higher than that of the control group. In regard to \textit{rs}11833579, there were not any significant differences between the case and the control groups. The SNP \textit{rs}12425791 is significantly associated with ischemic stroke, and the \textit{A} allele increases the susceptibility to stroke. The SNP \textit{rs}11833579 is not significantly associated with stroke.

Keywords: nerve injury-induced protein 2 (\textit{NINJ2}), single nucleotide polymorphism (SNP), stroke, cell adhesion molecule

INTRODUCTION

Stroke is a disease with high prevalence, and is especially notable for its high mortality and disability rate, inflicting a heavy socioeconomic burden. Many risk factors, such as hypertension, diabetes, hyperlipidemia, smoking and heart disease have been identified nowadays, but the genetic background of stroke still remains unclear.

A prospective cohort study found that two single nucleotide polymorphisms (SNPs) of the nerve injury-induced protein 2 (\textit{NINJ2}) gene were significantly associated with stroke, and the two minor alleles of this gene increased the susceptibility to stroke, especially to the atherosclerotic thrombotic subtype\textsuperscript{[1]}. The two SNPs were close to the 5\textsuperscript{'} end of the \textit{NINJ2} gene, indicating that \textit{NINJ2} may play an important role in stroke. But the study was only carried out in the Caucasian and Black population, while the relationship between the two SNPs and stroke in other populations is still unknown. Heve, we carried out a case-control study to investigate the relationship between the two SNPs and stroke in Chinese Han population. Fifty-two patients with large-artery atherosclerosis (LAA) infarction, 85 patients with small-artery occlusion lacunar (SAO) infarction, 50 patients with intracerebral hemorrhage (ICH) and 66 controls were included in
this study. Genotypes of the two SNP sites among different groups were determined by PCR-restriction fragment length polymorphism (RFLP).

**MATERIALS AND METHODS**

**Subjects**

All the cases of this study were patients with stroke admitted to the Neurology Department of Nanjing Brain Hospital from 2009 to 2010, they were all Han Chinese, and were classified into four groups: 52 patients with LAA and 85 patients with SAO according to the TOAST typing, 50 patients with ICH, and 66 healthy people as control. The inclusion criteria were as follows: all the subjects of case groups were 50 to 80 years old, either male or female, with new-onset or recurrent stroke. The diagnoses were based on clinical history, physical examination and CT or MRI imaging. The subjects of the control group were healthy people of the same age range, without stroke or other diseases. The following data of all the subjects were recorded: age, gender, weight, body mass index (BMI), history of smoking, alcohol, hypertension, diabetes and heart disease, the levels of triglyceride (TG), cholesterol (CHO), high density lipoprotein (HDL), low density lipoprotein (LDL), apolipoprotein A (apoA), apolipoprotein B (apoB), and lipoprotein (a) [Lp(a)] (Table 1). All the study participants provided informed consents.

**Table 1 Demographic characteristics of the subjects**

| Variable        | LAA (n = 52) | SAO (n = 85) | ICH (n = 50) | Control (n = 66) | χ²/F | p    |
|-----------------|--------------|--------------|--------------|-----------------|------|------|
| Women (%)       | 34.6         | 40.0         | 32.0         | 47.0            | 3.245 | 0.355|
| Age (y)         | 64.0±8.2<sup>*</sup> | 66.8±7.6<sup>*</sup> | 62.8±7.8<sup>*</sup> | 58.7±6.1        | 14.520 | <0.001|
| BMI (kg/m²)     | 24.4±4.1     | 24.6±4.5     | 24.3±4.1     | 23.9±3.3        | 0.290  | 0.833|
| Smoking (%)     | 30.8         | 22.4         | 18.0         | 18.2            | 3.680  | 0.298|
| Drinking (%)    | 11.5         | 10.6         | 18.0         | 7.6             | 3.201  | 0.362|
| Hypertension (%)| 65.4<sup>+</sup> | 59.3<sup>+</sup> | 64.0<sup>+</sup> | 25.8            | 30.630 | <0.001|
| Diabetes (%)    | 34.6<sup>+</sup> | 27.1<sup>+</sup> | 6.0          | 6.1             | 27.490 | <0.001|
| Heart disease (%)| 9.6<sup>+</sup> | 7.1<sup>+</sup> | 4.0<sup>+</sup> | 0              | 9.638  | 0.022|
| TG (mmol/L)     | 1.69±0.88    | 1.49±0.94    | 1.51±0.78    | 1.56±1.04       | 0.460  | 0.708|
| CHO (mmol/L)    | 4.71±1.15    | 4.51±1.13    | 4.60±0.82    | 4.71±0.80       | 0.590  | 0.620|
| HDL (mmol/L)    | 0.99±0.31<sup>+</sup> | 1.06±0.30     | 1.07±0.30    | 1.16±0.34       | 2.610  | 0.052|
| LDL (mmol/L)    | 2.70±0.86    | 2.60±0.92    | 2.51±0.65    | 2.59±0.64       | 0.420  | 0.739|
| ApoA (mmol/L)   | 0.98±0.24<sup>+</sup> | 1.03±0.21<sup>+</sup> | 1.06±0.23    | 1.13±0.26       | 3.600  | 0.013|
| ApoB (mmol/L)   | 0.98±0.26<sup>+</sup> | 0.89±0.21<sup>+</sup> | 0.92±0.17    | 0.93±0.14       | 2.090  | 0.103|
| Lp(a) (mmol/L)  | 349.21±391.23<sup>+</sup> | 261.09±260.57 | 283.79±297.60 | 203.91±163.46  | 1.960  | 0.121|

<sup>*</sup>Compared with the control group, P < 0.05. ApoA: apolipoprotein A; ApoB: apolipoprotein B; BMI: body mass index; CHO: cholesterol; HDL: high density lipoprotein; ICH: intracranial hemorrhage stroke; LAA: large-artery atherosclerotic stroke; LDL: low density lipoprotein; Lp(a): lipoprotein (a); SAO: small-artery occlusion lacunar stroke; TG: triglyceride.

**SNP selection and genotyping**

Five mL venous blood samples were drawn from all the subjects after fasting for at least 8 h. Genomic DNA was extracted (TIANamp, Tiangen). The primers were synthesized by Sangong Bioengineer Ltd, Shanghai. The sequences of primers for rs12425791 were: forward 5'-GGCGAGCTGCTGTTTTTAG-3', reverse 5'-TGTCAGAGGAACCGAGGAAC-3'; for rs11833579: forward 5'-AGGTGGAGGATTGCTTG-3', reverse 5'-TTTCCCTTATTCGCA-GAT-3'. The premix 2× Taq PCR mastermix (Bioedyfi, Nanjing, China) was used in the PCR assay. The assay mix of rs12425791 contained in a volume of 50 µL, 25 µL 2× Taq PCR mastermix, 5 µL genomic DNA and 0.25 µL (100 µmol/L) each primer; the thermal cycling conditions were as follows: an initial denaturation at 94°C for 5 min, 35 cycles of 94°C for 30 s, 55°C for 30 s and 72°C for 30 s, and a final extension at 72°C for 5 min. The assay mix of rs11833579 contained in a volume of 50 µL, 25 µL
Fig. 1 Electrophoretogram of the typical PCR products of rs12425791 (A) and rs11833579 (B) after digestion. A: Lane 1 is molecular weight marker, Lane 2, 3, 4 is the AG, AA, GG genotype, respectively, and Lane 5 is the blank control (17 bp are not visible). B: Lane 1 is molecular weight marker, Lane 2, 3 is the AA, AG genotype, respectively, Lane 4 is the GG genotype (lighter than others ), and Lane 5 is the blank control.

Statistical analysis

The background data of the subjects were compared using χ²-test or variance analysis. Hardy-Weinberg equilibrium was performed using goodness-of-fit χ²-test. The allele and genotype frequencies between the case and control groups were compared using χ²-test or Fisher exact test. Multinomial logistic regression model was used to calculate the initial odds ratio (OR) of risk factors and genotypes, and 95% confidential intervals (95%CI) were given; if the P-value of some initial OR was less than 0.25, the corresponding risk factors or genotypes would be subjected to the model until all the P values < 0.05, and the adjusted OR and 95%CI were given. A value of P < 0.05 was considered as significant. All the statistical analyses were performed using the Stata10.0 package.

RESULTS

The genotype counts were in Hardy-Weinberg equilibrium in both the control and case groups at the two polymorphic loci (P = 0.15 for the control group, 0.13 for the LAA group, 0.20 for the SAO group, 0.85 for the ICH group). The genotype frequencies and allele frequencies of the two SNPs in the different groups were shown in Table 2. The genotype frequencies of rs12425791 in the LAA group and the SAO group were significantly different from that of the control group, whereas these differences were not found in the ICH group. The allele frequencies of rs12425791 in the SAO group were significantly different from those of the control group. In regard to rs11833579, there was not any significant difference in genotype or allele frequencies.

Multinomial logistic regression was used to calculate the OR values of the genotypes of the two SNPs, and the results are shown in Table 3. Before other risk factors were adjusted, the AG and AA+AG genotype in the LAA group and SAO group were significantly associated with stroke, while this was not found in the ICH group. After adjustment of other risk factors such as age, smoking, hypertension, diabetes, TG, CHO, LDL, HDL, apoA, apoB and Lp(a) the AG genotype of the LAA group and the AG and AA+AG genotypes of the SAO group were still significantly associated with stroke, while there was not significant association between the AA+AG genotype and stroke in the LAA group after adjustment of other risk factors mentioned above, the AG and AA+AG genotypes of the ICH group were not significantly associated with stroke even after adjustment other risk factors mentioned as above. All genotypes of rs12425791 were not associated with stroke, no matter before or after adjustment.
The molecules coded by the two genes - Allele - are membrane protein, which could mediate cell adhesion. Thus, the original NINJ1 gene was first found in the study on the peripheral nerve regener and then its homologue was subsequently found[4,5]. Thus, the original NINJ1 was renamed the NINJ1 gene, and its homologues was named the NINJ2 gene. The molecules coded by the two genes are membrane protein, which could mediate cell adhesion[4,5]. Ninjurin plays an important role in the peripheral nerve injury and regeneration process[6]. The two molecules can also promote axonal growth[6]. So far, no studies have shown an association between the NINJ2 gene with stroke. The two SNPs in this study are close to the 5’ end of the NINJ2 gene, indicating that NINJ2 may play an important role in stroke.

Table 2: The genotypes and allele frequencies in each group [n(%)]

| Group    | Genotype | G | A |
|----------|----------|---|---|
| rs12425791 | GG       | 112(34.8) | 20(15.2) |
|          | AG       | 79(26.0)  | 25(19.0) |
|          | AA+AG    | 36(11.2)  | 14(10.4) |
| LAA      | GG       | 49(74.2)  | 14(21.2) |
|          | AG       | 23(44.2)  | 1(1.9)   |
|          | AA+AG    | 1(11.1)   | 1(1.9)   |
| SAO      | GG       | 46(54.1)  | 36(42.4) |
|          | AG       | 3(3.5)    | 39(45.9) |
|          | AA+AG    | 1(12.8)   | 49(57.6) |
| ICH      | GG       | 33(66.0)  | 15(30.0) |
|          | AG       | 2(4.0)    | 31(62.0) |
|          | AA+AG    | 3(6.2)    | 38(76.0) |

Table 3: Initial and adjusted OR values of genotypes in each case group

| Group    | Genotype | GG | AG | AA+AG |
|----------|----------|----|----|-------|
| rs12425791 | LAA      | 1.00 | 2.375 (1.278-4.636) | 4.296 (1.430-12.922) |
|          | Corrected OR(95% CI) | 1.00 | 4.296 (1.430-12.922) | 2.679 (0.944-7.598) |
|          | SAO      | 1.00 | 2.739 (1.311-5.723) | 3.923 (1.417-10.860) |
|          | Corrected OR(95% CI) | 1.00 | 3.923 (1.417-10.860) | 2.937 (1.119-7.710) |
|          | ICH      | 1.00 | 1.591 (0.679-3.728) | 2.296 (0.773-6.818) |
|          | Corrected OR(95% CI) | 1.00 | 1.591 (0.679-3.728) | 1.485 (0.664-3.319) |
| rs11833579 | LAA      | 1.00 | 1.823 (0.832-3.995) | 1.806 (0.863-3.782) |
|          | Corrected OR(95% CI) | 1.00 | 1.823 (0.832-3.995) | 1.814 (0.701-4.692) |
|          | SAO      | 1.00 | 1.539 (0.771-3.072) | 1.537 (0.805-2.935) |
|          | Corrected OR(95% CI) | 1.00 | 1.539 (0.771-3.072) | 1.576 (0.671-3.706) |
|          | ICH      | 1.00 | 1.155 (0.521-2.557) | 1.223 (0.586-2.553) |
|          | Corrected OR(95% CI) | 1.00 | 1.155 (0.521-2.557) | 1.180 (0.468-2.979) |

As the samples of the AA genotype of the two SNP sites in all groups were relatively few, the AA genotype was not subjected to multinomial logistic regression analysis.

DISCUSSION

The NINJ gene was first found in the study on the gene expression of Schwann cell during peripheral nerve regener and then its homologue was subsequently found[3,4]. Thus, the original NINJ was renamed the NINJ1 genes, and its homologues was named the NINJ2 gene. The molecules coded by the two genes are membrane protein, which could mediate cell adhesion[3,4]. Ninjurin plays an important role in the peripheral nerve injury and regeneration process[5]. The two molecules can also promote axonal growth[6]. So far, no studies have shown an association between the NINJ2 gene with stroke. The two SNPs in this study are close to the 5’ end of the NINJ2 gene, indicating that NINJ2 may play an important role in stroke.

Besides stroke, the NINJ gene was believed to play an important role in many other neurological diseases, such as multiple sclerosis, Alzheimer’s disease, leprosy, hereditary sensory neuropathy and Charcot-Marie-Tooth disease[6-11]. Possible mechanisms included homologue assembly, Wnt signaling pathway, NF-κB signaling pathway and P53 signaling pathway[12-17].

Our study showed that the minor allele (A) of SNP rs12425791 in the Chinese Han population would increase the susceptibility to ischemic stroke. This was the same as the conclusion from other studies on other racial groups[11], indicating that the association between SNP rs12425791 and ischemic stroke might be general in different races. This would help us further explore the underlying mechanism of this association. However, we failed to observe any association between SNP rs11833579 and stroke, which was contradictory to previous report[11]. We presumed that the association between SNP rs11833579 and ischemic stroke may be related to racial differences, because a previous
study also failed to find an association between SNP rs11833579 and stroke in the Black population[1].

The sample size of this study was relatively small, and it is a case-control study limiting the conclusion of the study. The study provided the preliminary data on the genotype distribution of the two SNP loci in Chinese Han population and the relationship between them and stroke. Prospective studies with a larger sample population are further being explored.

In conclusion, the SNP rs12425791 is significantly associated with ischemic stroke in Chinese Han population, and the A allele increases the risk of susceptibility to stroke. The SNP rs11833579 is not significantly associated with stroke in Chinese Han population.

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