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Clinical Study

The role of fibroblast growth factor receptor 4 polymorphisms in the susceptibility and clinical features of ischemic stroke

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ABSTRACT

Some polymorphisms in the fibroblast growth factor receptor 4 gene (FGFR-4) have been correlated with coronary artery disease, however, the role of polymorphisms in the FGFR-4 gene in ischemic stroke remain unknown. A total of 270 patients with ischemic stroke and 297 controls were recruited. Stroke subtype was classified and clinical severity of stroke in patients was evaluated. The polymorphisms in the FGFR-4 were genotyped. There were no significant differences of genotype distributions and allele frequencies of rs145302848C/G and rs147603016G/A between stroke patients and controls (all $p > 0.05$). However, genotype frequencies and allele frequencies at rs351855G/A (Gly388Arg) were significantly different between stroke patients and controls (both $p < 0.001$). With the rs351855GG genotype as a reference, the presence of rs351855AA homozygote had a significantly increased risk for stroke (adjusted odds ratio 2.663; 95% confidence interval 1.673–4.229, $p < 0.001$). The polymorphisms at rs145302848C/G and rs147603016G/A did not influence the susceptibility of stroke in this study. All FGFR-4 polymorphisms were not associated with clinical features such as Trial of Org 10172 in Acute Stroke Treatment subtype or stroke severity as indicated by mean National Institutes of Health Stroke Scale scores. Our study suggests a positive association between FGFR-4 gene polymorphism at rs351855G/A and susceptibility to ischemic stroke.

1. Introduction

Stroke is the one of the leading causes of morbidity and mortality in adults in developing countries.[1,2] The etiology of ischemic stroke (IS) includes age, hypertension, diabetes mellitus (DM), smoking, hyperlipidemia, and cardiac disease.[3,4] However, about one-third of stroke patients have no established etiology. Therefore, genetic and environmental factors are likely to be involved in the occurrence of IS.[5,6] Several candidate genes have been reported to be associated with IS risk.[7–9] However, no candidate genes proposed to determine IS risk have been confirmed with satisfying accuracy.

Fibroblast growth factor receptor 4 (FGFR-4) belongs to the tyrosine kinase receptor family, and its activation mediates cell proliferation, survival, migration, and resistance to apoptosis.[10,11] FGFR-4 expresses differently in each tissue or cell type and has distinct differences in receptor functions.[12,13] Recently, high levels of expression of FGFR-4 in coronary arteries was reported and FGFR-4 is believed to play an important role in atherosclerosis (AS) and restenosis in coronary arteries.[14,15] However, to our knowledge, there is no study reporting the expression of FGFR-4 in IS.

Several genetic polymorphisms have been identified in the FGFR-4 gene. Some polymorphisms in the FGFR-4 gene, such as rs351855G/A (Gly388Arg), rs145302848C/G and rs147603016G/A, have been correlated with coronary artery disease (CAD) in Chinese populations from different areas.[16–18] Therefore, the role of polymorphisms in the FGFR-4 gene in IS in a Chinese population aroused our interest. In the present study, we enrolled IS patients and controls to investigate the association between FGFR-4 genetic polymorphisms and susceptibility to and clinical features of stroke.

2. Methods

2.1. Recruitment

A total of 345 patients presenting with stroke symptoms were screened. Patients with a history of transient ischemic attack, fever, rheumatologic disease, autoimmune disease, any acute or chronic infection, CT scan or MRI proven hemorrhagic stroke, or a history of regular immunosuppressive or analgesic therapies were excluded. Thus, a total of 270 patients were recruited for the study

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after radiologic confirmation of IS by CT scans or MRI of the brain. All patients were recruited within 24 hours of stroke symptom onset. All patients had clinical signs consistent with the World Health Organization definition of stroke. A control group comprised of 297 age- and sex-matched healthy individuals was recruited from volunteers. Informed consent was obtained from all patients and participants before the collection of information. The study was approved by the Institutional Ethical Committee of our hospital.

2.2. Clinical examination and laboratory data

Stroke subtype was classified by two neurologists blinded to this study according to the Trial of Org 10172 in Acute Stroke Treatment (TOAST) criteria.[19] The National Institutes of Health Stroke Scale (NIHSS) score was used to determine the clinical severity of stroke.[20] A history of hypertension and diabetes, as well as smoking status, and body mass index (BMI) were obtained. The total cholesterol (TC), triglyceride (TG), low density lipoprotein-cholesterol (LDL-C) and high density lipoprotein-cholesterol (HDL-C) levels were measured, as was serum C reaction protein (CRP).

2.2.1. Genotyping analyses

Genomic DNA was extracted from peripheral blood lymphocytes. The primers were as follows: rs351855: Forward [F]: 5′-GACCGCAGCAGCGCCCGAGGCCAG-3′, Reverse [R]: 5′-AGAGGGAAGAGGGAGAGCTTCTG-3′; FGFR-4 rs145302848: F: 5′-CAGAGGAGGACCCCACATG-3′, R: 5′-TGGAGTCAGGCTGTCACATG-3′; and rs147603016: F: 5′-CCGCAGCAGCGCCCGAGGCC-3′, R: '-GAAGCGGGAGAGCTTCTGC-3′. Polymerase chain reaction (PCR) products containing the three polymorphic sites were then digested with the restriction enzymes BstNI, BsAJI and Cac8I (New England Biolabs, Beverly, MA, USA), as recommended in the manufacturer's instructions. The digested PCR products were fractionated on 3% agarose Tris-borate-EDTA gel (Agarose 1000; Gibco BRL, Rockville, MD, USA) and stained with ethidium bromide (product size after digestion shown in Table 1). To confirm the genotyping results, more than 10% of PCR-amplified DNA samples were examined by DNA sequencing. Results between PCR and DNA sequencing analysis were 100% concordant.

Table 1
General characteristics of ischemic stroke patients and controls from a Chinese population

Characteristic	IS (n = 270)	Control (n = 297)	*p* Value
Age (years)	58.2 ± 7.1	58.1 ± 6.9	NS
Sex			0.406
Male	150 (55.6%)	161 (54.2%)	
Female	120 (44.4%)	136 (45.8%)	
BMI (kg/m^2)	23.6 ± 9.1	23.4 ± 9.7	NS
Smoking, n (%)			0.025
Ever	101 (37.4%)	87 (29.3%)	
Never	169 (62.9%)	210 (70.7%)	
Hypertension, n (%)			<0.001
Yes	166 (61.5%)	110 (37.0%)	
No	104 (38.5%)	187 (63.0%)	
Diabetes, n (%)			0.028
Yes	79 (29.3%)	65 (21.9%)	
No	191 (70.7%)	232 (78.1%)	
TG (mmol/l)	1.92 ± 0.38	1.91 ± 0.76	0.45
TC (mmol/l)	4.51 ± 0.79	4.54 ± 0.36	0.61
HDL-C (mmol/l)	1.18 ± 0.58	1.26 ± 0.52	0.052
LDL-C (mmol/l)	2.67 ± 0.46	2.14 ± 0.76	<0.001

Data are presented as mean ± standard deviation or count (percentage).
BMI = body mass index, HDL-C = high density lipoprotein-cholesterol, IS = ischemic stroke, LDL-C = low density lipoprotein-cholesterol, NS = not significant, TC = total cholesterol, TG = triglyceride.

2.3. Statistical analysis

Differences in demographic characteristics and vascular risk factors between patients and controls were compared using Student's *t*-test or analysis of variance for continuous variables and the chi-squared (χ^2) test for all categorical variables. Tests for Hardy–Weinberg equilibrium were conducted using χ^2 tests. Genotypes and allele frequencies were compared by χ^2 analysis or Fisher's exact test. Multivariate logistic regression analysis was used to determine the influence of FGFR-4 polymorphisms on stroke risk, controlling potential confounding risk variables including age, sex, and other conventional risk factors. A forward stepwise (likelihood ratio) procedure was used for multivariable analysis. Data were analyzed with the Statistical Package for the Social Sciences version 16.0 (SPSS, Chicago, IL, USA) and results were considered statistically significant at $p < 0.01$ using a two-tailed test.

3. Results

Demographic data and risk factor profiles of patients and controls are presented in Table 1. The age, gender, BMI, TC and TG levels were similar between IS cases and controls. However, smoking, hypertension and diabetes were more common in IS subjects than controls. In addition, the serum CRP level and LDL-C were higher in IS patients than that in controls.

Table 2 described the genotype distributions and an allele frequency of FGFR-4 gene polymorphisms in IS and control subjects. The genotype frequencies for all polymorphisms in controls did not differ significantly from those expected under Hardy–Weinberg equilibrium (all $p > 0.05$).There were no significant differences of genotype distributions and alleles frequencies of rs145302848C/G and rs147603016G/A between stroke subjects and control subjects (all $p > 0.05$, Table 2). However, genotype frequencies and allele frequencies at rs351855G/A (Gly388Arg) were significantly differed between stroke subjects and control subjects (both $p < 0.001$). The presence of AA was significantly higher in stroke subjects than in controls (38.15% vs. 23.91%). Similarly, the A allele carriage in IS was significantly higher than controls (60.19% vs. 47.14%, $p < 0.001$). To determine the independent risk factors for stroke, we performed a multivariate logistic regression analyses. The forward stepwise model was used. The presence of IS was considered as the outcome variable and the following variables were used as covariates: age, sex, TG, HDL-C, LDL-C, hypertension, smoking, BMI, and DM status. With the rs351855GG genotype as reference, the rs351855GA heterozygote alone did not significantly increased stroke risk (adjusted OR = 1.581; 95% CI: 1.031–2.427, $p = 0.056$), however, the presence of rs351855AA homozygote had a significantly increased risk for stroke (adjusted OR: 2.663; 95% CI: 1.673–4.229, $p < 0.001$, Table 3). The polymorphisms at rs145302848C/G and rs147603016G/A did not influence the susceptibility of stroke in this study. The other independent risk factors included LDL (adjusted OR: 1.852; 95% CI: 1.292–3.242 $p = 0.023$), hypertension (adjusted OR: 2.352; 95% CI: 1.713–2.954, $p = 0.016$), and smoking (adjusted OR: 1.937; 95% CI: 1.476–3.344, $p = 0.024$).

We also analyzed the association between stroke subtypes by TOAST criteria and the FGFR-4 polymorphisms (Table 4). We did not find any specific association between TOAST subtype and the presence of genotypes (all $p > 0.05$). We next analyzed the association between IS severity and the FGFR-4 polymorphisms. We found the mean NIHSS scores were similar among the genotype carriers of FGER-4 gene (all $p > 0.05$, Fig. 1).