The Association of Growth Differentiation Factor-15 Gene Polymorphisms with Growth Differentiation Factor-15 Serum Levels and Risk of Ischemic Stroke

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Background: Current evidence shows that growth differentiation factor-15 (GDF-15) plays an important role in the progression of ischemic stroke (IS). The aim of this study was to investigate the association between 3 single-nucleotide polymorphisms of the GDF-15 gene and IS susceptibility in the Chinese population. *Materials and Methods:* The study subjects comprised 601 Chinese individuals, including 298 stroke patients and 303 age- and gender-matched healthy controls. The polymorphisms were measured using snapshot single-nucleotide polymorphism genotyping assays and confirmed by sequencing. Serum GDF-15 (sGDF-15) levels were measured by enzyme-linked immunosorbent assay. *Results:* The distribution of rs1804826G/T polymorphism was significant different between the 2 groups ($P < .05$). Compared with the rs1804826 G allele, the rs1804826 T allele was significantly associated with an increased risk of IS ($P < .05$). Haplotype analyses showed that the T-T-G haplotype was significantly associated with an increased risk of IS (odds ratio = 1.671, 95% confidence interval = 1.231-2.268; $P = .001$). Compared with the normal controls, the sGDF-15 levels were significantly increased in stroke patients ($P < .001$). Besides, patients carrying rs1804826 GT/TT genotypes had higher sGDF-15 levels compared with those carrying GG genotypes ($P < .05$). *Conclusions:* The GDF-15 gene rs1804826G/T polymorphism and sGDF-15 levels are associated with IS in the Chinese population. Our data indicate that the GDF-15 gene may play a role in the development of IS. **Key Words:** Ischemic stroke—GDF-15—gene—single-nucleotide polymorphisms.

Introduction

Ischemic stroke (IS) is a common cerebral blood circulation disorder. It is a serious threat to human health. In many developed and developing countries, IS ranked among the top causes of death.[1] Up to now, the exact causes of this disease still have not been figured out. Existing studies have shown that the etiology and pathogenesis of IS include genetic and environmental factors and their interaction with each other. Common risk factors, such as obesity, smoking, hypertension, and abnormal lipid metabolism, account for a part of the prevalence of IS[2-4]; genetic factors have been defined as novel and important risk contributors to the pathogenesis of IS.[5-8] This therefore has led to a growing interest in finding novel measurable soluble biomarkers and genetic markers to improve global cerebrovascular prevention and therapeutic decision making.

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Growth differentiation factor-15 (GDF-15), a distant member of the transforming growth factor-β cytokine super family, has been increasingly investigated as a prognostic biomarker in patients with cardiac–cerebral vascular diseases.[9,10] Under physiological conditions, GDF-15 is weakly expressed in most tissues, including the central nervous system,[11] whereas in inflammation or tissue injury, the expression of GDF-15 may increase significantly.[12,13] Increased serum GDF-15 (sGDF-15) levels have been detected in atherosclerosis-related diseases, such as coronary artery disease (CAD), IS, atrial fibrillation, and acute myocardial infarction.[14-18] GDF-15 has been shown to predict adverse functional outcome and total mortality beyond established risk factors in IS patients.[19] In a mouse model of cerebral ischemia research, Schindowski et al[20] substantiate the idea that GDF-15 may be involved in regulating the postlesional responses of ischemic insult.

The gene encoding GDF-15 is located on chromosome 19p13.11 in humans. Recently, a number of polymorphisms in the GDF-15 gene have been identified in atherosclerosis-related diseases.[21-24] However, the association between GDF-15 gene polymorphism and IS susceptibility in the Chinese population remains unclear. Besides, little is known about the relationship between GDF-15 gene single-nucleotide polymorphisms (SNPs) and the expression of sGDF-15. Therefore, the aim of the present study was to analyze, for the first time, the association between 3 GDF-15 SNPs (rs1804826G/T, rs3787023C/T, and rs1055150C/G) and IS susceptibility in the Chinese population, and to assess the relationship between GDF-15 gene polymorphisms and the sGDF-15 level.

Materials and Methods

Study Subjects

Our study was designed as a retrospective study. The study consisted of 298 patients with IS (175 men aged 31-84 years and 123 women aged 35-86 years). All IS patients were consecutively selected. The patients were recruited from the Department of Neurology, Affiliated Hospital of Youjiang Medical University for Nationalities, Guangxi, China, between May 2014 and September 2015. The 303 control subjects were matched to the patients on the basis of age and gender (181 men aged 28-79 years and 122 women aged 25-80 years). The control subjects underwent a routine medical checkup in the outpatient clinic of the Department of Internal Medicine, Affiliated Hospital of Youjiang Medical University for Nationalities, Guangxi, China, in the same period. According to thorough clinical and laboratory evaluation, none of the subjects were found to have any medical condition other than hypertension, hypercholesterolemia, or hypertriglyceridemia. All study subjects were Chinese and resided in the same geographic area in Guangxi, China.

IS was defined as an acute-onset focal neurological deficit combined with neuroimaging evidence of cerebral infarction by cranial computed tomography or magnetic resonance imaging. The subtype classification for the etiology of IS was based on the Trial of Org 10172 in Acute Stroke Treatment (TOAST) classification system. In the present study, the IS subtypes were classified as large-artery atherosclerosis (LA) or small-artery occlusion (SA) based on the clinical findings, magnetic resonance imaging, computed tomography, results of diagnostic studies such as cardiac imaging, duplex imaging of extracranial arteries, and laboratory evaluations. The case group comprised 298 stroke patients, including 210 LA patients and 88 SA patients.

At the time of enrollment, data were collected from each subject, including a complete survey of IS traditional risk factors, such as hypertension, type 2 diabetes mellitus, lipid metabolism, and smoking status. Anthropometric measurements and blood pressure determination were performed according to standard protocols. The study was performed with the approval of the ethics committee of the Affiliated Hospital of Youjiang Medical University for Nationalities, and written informed consent was obtained from all the subjects.

SNP Selections

We chose these SNPs according to the following standards. First, the minor allele frequency was greater than 10% (according to dbSNP from 1000 Genomes Project, https://ncbi.nlm.nih.gov/ variation/view/). Second, based on genotyping putative functional SNPs, we selected 3 SNPs with different putative functions (rs1804826G/T, synonymous; rs3787023C/T, intron variant; and rs1055150C/G, 3 prime untranslated region variant) to study.

DNA Extraction

Genomic DNA was extracted from blood leukocytes by using a whole-blood genome DNA extraction reagent kit (Axygene Biotechnology [Hangzhou] Limited, Hangzhou City, China), following the manufacturer's instructions.

Determination of the GDF-15 Genotype

The method of snapshot SNP genotyping assay was taken to detect the allele and genotype frequencies. To confirm the genotyping results, polymerase chain reaction (PCR)-amplified DNA samples were examined by DNA sequencing and the results were 100% concordant. The PCR primers were designed based on the GenBank reference sequence (accession no. NC_000019.10) and are shown in Table 1.

sGDF-15 Determinations

In all patients, plasma samples were collected within 12 hours after symptom onset. After an overnight fasting, venous blood samples of the control population were collected. Plasma samples from the patients and healthy