Interactions among Variants in Eicosanoid Genes Increase Risk of Atherothrombotic Stroke in Chinese Populations

Xingyang Yi, MD,* Jing Lin, MD,† Chun Wang, MSc,* Ruyue Huang, MD,† and Yingying Liu, MD*

Background: Eicosanoids are lipid mediators that may play a role in ischemic stroke (IS). However, the association of variants in eicosanoid genes and these interactions with IS risk has not been investigated. The aim of the present study was to investigate the association of 11 variants in eicosanoid genes with IS and to determine whether these gene–gene interactions increase the risk of IS. *Methods:* Eleven variants in prostaglandin H synthase-1 (*PTGS1*), *PTGS2*, thromboxane A2 synthase (*TBXAS1*), prostacyclin synthase (*PTGIS*), and prostaglandin E synthase (*PTGES*) genes were examined using mass spectrometry method in 297 patients with atherothrombotic stroke and 291 controls. Gene–gene interactions were analyzed using generalized multifactor dimensionality reduction (GMDR) method. Platelet aggregation and platelet–leukocyte aggregates were measured on admission. *Results:* There were no significant differences in the genotype distributions of the 11 variants between patients and controls. However, GMDR analysis showed a significant gene–gene interaction among rs20417, rs5602, and rs41708, which scored 10 for cross-validation consistency and 9 for the sign test ($P = .014$). Logistic regression analysis showed that high-risk interaction among rs20417, rs5602, and rs41708 was an independent risk factor for atherothrombotic stroke ($OR = 2.45$, 95% *CI:* 1.33-3.27, $P = .019$). The high-risk interactive genotypes were associated with higher platelet aggregation and platelet–leukocyte aggregates. *Conclusions: PTGS2* rs20417, *PTGIS* rs5602, and *TBXAS1* rs41708 three-locus interactions may confer a higher risk for atherothrombotic stroke. The combinatorial analysis used in this study may be helpful to elucidate complex genetic risk for IS. **Key Words:** Ischemic stroke—genetic polymorphism—eicosanoic acids—genetic variation—generalized multifactor dimensionality reduction.

Introduction

Stroke is the leading cause of death and disability in developed countries.[1-3] Ischemic stroke (IS) is a polygenic, complex disease resulting from combination of vascular, environmental, and genetic factors.[4] Although the mechanisms remain unclear and few single-gene variants have been identified as causing IS, genetic predisposition has been suggested to be a critical player in the pathogenesis of this disease.[5] Therefore, a detailed understanding of genetic factors involved in IS could provide valuable insights into the pathogenesis of this disease as well as contribute to its prevention and treatment.

Towards this aim, many genetic studies, especially genome-wide association studies, have been conducted on IS patients in the last decade.[5,6] Indeed, IS appears to be a disease that does not follow the Mendelian pattern

From the *Department of Neurology, People's Hospital of Deyang City, Deyang, Sichuan, China; and †Department of Neurology, The Third Affiliated Hospital of Wenzhou Medical University, Wenzhou, Zhejiang, China.

Received February 17, 2017; revision received March 27, 2017; accepted April 2, 2017.

This study was supported by the Deyang City Science and Technology Research Foundation (Grant No. 2015SZ064).

Address correspondence to Jing Lin, MD, Department of Neurology, The Third Affiliated Hospital of Wenzhou Medical College, 108 Wanson Road, Ruan City, Wenzhou, Zhejiang 325200, China. E-mail: 22350277@qq.com.; Address correspondence to Chun Wang, MSc, Department of Neurology, People's Hospital of Deyang City, No. 173, North Taishan Road, Deyang, Sichuan 618000, China. E-mail: 1842942576@qq.com.

1052-3057/$ - see front matter

http://dx.doi.org/10.1016/j.jstrokecerebrovasdis.2017.04.005

of inheritance, suggesting that single-locus analysis may not be appropriate to investigate the genetic risk factors of IS.[5] Additionally, single-locus analysis may fail to detect significant variants that may exert significant influence on the pathogenesis of IS via synergistic interactions with other locus variants.[7] Thus, the search for gene variants linked to IS risk may be significantly enhanced by thoroughly investigating gene–gene interactions via alternative analytical methods, such as the generalized multifactor dimensionality reduction (GMDR) approach .[8]

Eicosanoids are lipid mediators. The main eicosanoids include prostaglandins, prostacyclins (PGI), and thromboxanes (TXA). PGI_2 and TXA_2 have opposite effects on blood flow and platelet activity and may play a key role in acute coronary syndromes and atherosclerosis.[9] Prostaglandin E also affects platelet activity.[10] Arachidonic acid (AA) is a precursor of vasoactive substances in humans and is metabolized by prostaglandin H synthase-1 and synthase-2 (more commonly described as cyclooxygenase [COX]-1 and COX-2, respectively) to prostaglandin H.[11] Some studies have shown that single nucleotide polymorphisms (SNPs) in the prostaglandin H synthase-1 gene (*PTGS1*) and *PTGS2* are associated with cardiovascular disease.[12,13] However, the relationship between these genetic variants and IS risk has not been well addressed. Prostaglandin H is in turn metabolized to TXA_2 by thromboxane synthase in platelets, to PGI_2 by prostacyclin synthase in endothelial cells, and to prostaglandin E by prostaglandin E synthase in many different tissues.[11] Possible role of genetic variants in these synthases in relation to IS has received limited attention.

Despite some previous single-locus studies of eicosanoid genes in cardiovascular disease, currently there are no reports on the potential effects of gene–gene interactions among eicosanoid genes on IS risk. Therefore, we hypothesized that the interaction of variants in multiple genes might confer a higher IS risk than a single variant in 1 gene. In this study, we examined the association of genetic variants in *PTGS1*, *PTGS2*, thromboxane A2 synthase (*TBXAS1*), prostacyclin synthase (*PTGIS*), and prostaglandin E synthase (*PTGES*) with the risks of IS. In addition, we investigated whether these gene–gene interactions increase the risk of IS in Chinese populations.

Materials and Methods

Study Populations

This study protocol was reviewed and approved by the Ethics Committee of the People's Hospital of Deyang City. Each of the participants provided informed consent before participating in this study.

The study population comprised 297 IS patients with atherothrombotic stroke and 291 controls. Patients who had their first strokes were admitted to our hospitals within 72 hours of the onset of stroke between March 2012 and June 2014. All patients were subjected to computed tomographic angiography or magnetic resonance angiography of the brain, as well as color duplex ultrasound investigation of the carotid arteries. Common electrocardiogram (ECG), 24-hour Holter ECG (type 3000, Kangtuo Sheehan International Trade (Beijing) Co., Ltd, Beijing, China), and echocardiogram were performed to reveal any possible cardioembolic stroke. The inclusion criteria were as follows: (1) age 40 years or more; (2) diagnosis of IS based on both clinical findings and the results of brain magnetic resonance imaging; and (3) IS in all cases was due to atherothrombotic stroke, according to the Trial of ORG 10172 in the Acute Stroke Treatment classification system.[14] The exclusion criteria were as follows: (1) small artery disease, cardiac, other determined or undetermined etiology of stroke; and (2) individuals declined to participate in the study.

The healthy volunteers who served as controls were selected from outpatients with no history of stroke as confirmed by medical history as well as physical and laboratory examinations at our center. They had no family history of stroke and were not genetically related to the cerebral infarction patients.

A detailed medical history and information on stroke risk factors was obtained from each participant, including age, gender, body mass index, hypertension, diabetes mellitus (DM), current smoking, total plasma cholesterol, triglycerides, low-density lipoprotein cholesterol, and high-density lipoprotein cholesterol.

Blood Collection and Genotyping

Whole blood (3 mL) was drawn from an arm vein into a sterile tube containing ethylenediaminetetraacetic acid and stored at −80°C until genotype analysis was performed. The SNPs of the eicosanoid genes were selected from the NCBI database (http://www.ncbi.nlm.nih.gov/SNP) according to the following criteria: (1) the SNPs had been examined in previous studies[12,13,15]; (2) the SNPs with minor allele frequency greater than .05; and (3) the SNPs lead to amino acid changes. According to the criteria, 11 variants in 5 genes were examined, including *PTGS1* (rs1236913, rs3842787), *PTGS2* (rs689466, rs20417), *TXAS1* (rs194149, rs2267679, rs41708), *PTGIS* (rs45498106, rs5602, rs5629), and *PTGES* (rs6478818).

The genotypes of 11 variants were examined using matrix-assisted laser desorption ionization time-of-flight mass spectrometry methods according to our previous study.[16,17] In brief, each SNP was amplified using 2 specific polymerase chain reaction primers and 1 extension primer. Once the primer extension reaction was completed, the samples were spotted onto a 384-well spectroCHIP (Sequenom Inc., San Diego, CA) using a MassARRAY Nanodispenser (Sequenom Inc.) and genotyped using the mass spectrometer. Genotyping was performed in real time with MassARRAY RT software, version 3.0.0.4, and analyzed using the MassARRAY Typer software, version 3.4 (Sequenom Inc.).