Variants in *COX-2*, *PTGIS*, and *TBXAS1* Are Associated with Carotid Artery or Intracranial Arterial Stenosis and Neurologic Deterioration in Ischemic Stroke Patients

Xingyang Yi, MD,* Bing Ming, MD,† Chun Wang, MSc,* Hong Chen, MD,* and Chun Ma, MD†

Background: Eicosanoids may play a role in ischemic stroke (IS). However, the association of variants in eicosanoid genes with symptomatic carotid artery or intracranial arterial stenosis and neurologic deterioration (ND) is not fully understood. The aim of the present study was to investigate the association of 11 variants in eicosanoid genes with symptomatic carotid artery or intracranial arterial stenosis and ND. *Methods:* Eleven variants in eicosanoid genes were examined using mass spectrometry method in 297 IS patients. The symptomatic carotid artery or intracranial arterial stenosis was assessed by computed tomographic angiography. Platelet aggregation and platelet–leukocyte aggregates were measured. The primary outcome was ND within 10 days of admission. ND was defined as an increase of 2 or more points in National Institutes of Health Stroke Scale score. *Results:* Among 297 IS patients, 182 (61.3%) cases had symptomatic carotid artery or intracranial arterial stenosis, and 88 (29.6%) patients experienced ND within 10 days after admission. Symptomatic carotid artery or intracranial arterial stenosis was significantly associated with higher ND ($P < .001$). Rs20417CC, rs41708TT, and rs5629CC were independent risk factors for symptomatic carotid artery or intracranial arterial stenosis and ND, and associated with higher platelet aggregation and platelet–leukocyte aggregates. *Conclusions:* Symptomatic carotid artery or intracranial arterial stenosis was associated with higher ND. Rs20417CC, rs41708TT, and rs5629CC were not only independent risk factors for symptomatic carotid artery or intracranial arterial stenosis, but also independent risk predictors for ND. **Key Words:** Ischemic stroke—carotid stenosis—intracranial arterial stenosis—neurological deterioration—single nucleotide polymorphisms—eicosanoic acids.

Introduction

Stroke is the leading cause of mortality and disability in the world and in China as well.[1] Severe internal carotid arterial (ICA) stenosis or intracranial arterial (IA) stenosis could induce thrombosis formation, which has been demonstrated to confer an increased risk of developing stroke.[2] It is critical to better understand the etiology of these arterial stenoses within the context of stroke, including the genetic etiology that can provide valuable insights into the pathogenesis of this disease, which has potential implication for the prevention of stroke eventually. However, to date this has not been satisfactorily understood.

From the *Department of Neurology, People's Hospital of Deyang City, Deyang, Sichuan 618000, China; and †Department of Radiology, People's Hospital of Deyang City, Deyang, Sichuan 618000, China.

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Address correspondence to Bing Ming, MD, Department of Radiology, People's Hospital of Deyang City, No 173, North Taishan Road, Deyang, Sichuan 618000, China. E-mail: 1842942576@qq.com.

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Neurologic deterioration (ND) occurs in 20% to 40% of patients with acute ischemic stroke (IS),[3,4] and is associated with increased morbidity and mortality.[5] Some studies have reported that the presence of an ICA or middle cerebral artery (MCA) occlusion and degree of carotid stenosis are associated with ND in European population.[6,7] However, this association is not clear in Asians. Once deterioration has occurred, a large proportion of these patients did not recover back to predeterioration deficits.[8] Thus, it is very important to understand the mechanisms of ND for target therapies to reverse, halt, or even prevent deterioration in patients with acute IS.

Eicosanoids are lipid mediators that may play a role in atherosclerosis.[9] 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.[10] Prostaglandin E also affects platelet activity.[11] Arachidonic acid (AA) is a precursor of vasoactive substances in humans and is metabolized by cyclooxygenase (COX)-1 and COX-2 to prostaglandin H. Prostaglandin H is in turn metabolized to TXA_2 by thromboxane synthase (TBXAS) in platelets, to PGI_2 by prostacyclin synthase (PTGIS) in endothelial cells, and to prostaglandin E by prostaglandin E synthase (PTGES) in many different tissues.[12] Some studies have shown that single-nucleotide polymorphisms (SNPs) in *COX-1* and *COX-2* gene are associated with cardiovascular disease.[13,14] And variation in *TBXAS1* and *PTGIS* may influence myocardial infarction (MI) risk.[15] Our previous study has demonstrated that platelet activation markers (platelet aggregation and platelet-leukocyte aggregates) may play a key role in the pathogenesis of atherosclerosis and ND.[16] However, the association of these genetic variants with ICA or IA stenosis and ND has not been well addressed. Therefore, we hypothesized that variants in eicosanoid genes might influence platelet activation, and associate with ICA or IA stenosis and ND. In this study, we examined 11 variants in *COX-1*, *COX-2*, *PTGES*, *TBXAS1*, and *PTGIS* in acute IS patients, and investigated the association of these genetic variants with ICA or IA stenosis, ND, and platelet activation in Chinese population.

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.

We consecutively enrolled 297 IS patients who had their first strokes and were admitted to our hospital within 48 hours of the onset of stroke between March 2012 and June 2014. IS was confirmed based on both clinical findings and the results of brain magnetic resonance imaging. Vascular imaging of the brain and carotid artery was performed to identify more than 50% stenosis in the carotid, internal carotid, vertebrobasilar, middle cerebral, anterior cerebral, and posterior cerebral arteries by 128-row computed tomographic angiography (CTA), according to previous method.[17] Common electrocardiogram, 24-hour Holter electrocardiogram, and echocardiogram were performed to reveal any possible cardioembolic stroke. The inclusion criteria were (1) age ≥40 years old and (2) carotid artery territory IS. Exclusion criteria were the following: (1) small artery disease, cardiac, other determined or undetermined etiology of stroke; (2) asymptomatic ICA or IA stenosis; (3) vertebrobasilar territory IS; (4) history of carotid endoartectomy or carotid stent therapy; (5) intravenous thrombolytic therapy; (6) hemorrhagic transformation of infarct or a new infarct in another vascular territory within 10 days after admission; (7) usage of warfarin or heparin in the preceding 2 weeks or within 10 days after admission; (8) fever, infection, hypoxia, or any relevant hemodynamic compromise at admission or within 10 days after admission; (9) other conditions, such as asthma or severe cardiovascular, liver, renal disease, or peripheral artery occlusion; (10) National Institutes of Health Stroke Scale (NIHSS) score >15 at admission; (11) individuals declined to participate in the study. According to the results of brain and carotid artery CTA, the enrolled patients were divided into patients with or without symptomatic ICA or IA stenosis. Patients with symptomatic ICA or IA stenosis were defined as stenosis ≥50%, and experienced an ipsilateral (carotid territory) IS. Patients without symptomatic ICA or IA stenosis were defined as no stenosis or <50% stenosis, and experienced an ipsilateral IS.[18] The patients with ICA or IA stenosis ≥50%, but with non–infarct-related artery, were also excluded.

To assess the reproducibility of the measurement of the degree of stenosis by CTA in the current study, for 28 randomly selected patients, the analyses of the degree of stenosis were performed twice by an investigator (B.M.), and subsequently by another investigator (C.M.), after which the consistency was determined. The coefficients of intraobserver and interobserver variations for the degree of stenosis were 7.6% and 7.7%, respectively, suggesting relatively reliable measurements in the current study. One of the investigators (C.M.) then assessed all patients. These CTA-based stenosis groups were also compared with 60 carotid arteries measured blindly on conventional angiographies by the North American Symptomatic Carotid Endarterectomy Trial criteria.[19] Exact agreement was found in 89% of the cases, and there were no major disagreements.

Data on various risk factors, including age, gender, current smoking, history of diabetes mellitus (DM), and hypertension, were recorded. Hypertension was defined as the mean of 3 independent measures of BP ≥140/