Association of Matrix Metalloproteinase-1 and Matrix Metalloproteinase-3 Gene Variants with Ischemic Stroke and Its Subtype

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Background: Genetic variations in the genes of matrix metalloproteinases (MMPs) may play an important role in the pathogenesis of ischemic stroke (IS). Here we investigate the association between *MMP-1* –1607 1G/2G and *MMP-3* –1171 5A/6A genetic polymorphisms and etiological subtypes of IS in the Han Chinese population. *Methods:* A total of 640 eligible patients with IS and 637 age- and gender-matched apparently healthy volunteers were enrolled. Subtypes of IS were classified by Trial of Org 10172 in Acute Stroke Treatment criteria. *MMP-1* (–1607 1G/2G) and *MMP-3* (–1171 5A/6A) polymorphisms were evaluated using polymerase chain reaction-restriction fragment length polymorphism. *Results:* The frequencies of the 5A/6A + 5A/5A genotypes and 5A allele were significantly higher in patients with IS than in controls ($P < .001$, $P < .001$, respectively). No association was found between *MMP-1* 1G/2G polymorphism and overall IS. In subgroup analyses, *MMP-1* 1G/2G and 2G/2G genotypes increased the risk of small-artery occlusion (SAO) subtype (multivariate-adjusted, $P < .001$, $P = .002$, respectively), and *MMP-3* 5A/6A + 5A/5A genotypes were related with large-artery atherosclerosis (LAA) subtype (multivariate-adjusted, $P < .001$). Haplotype analyses indicated that 2G-6A and 1G-5A increased the risk of SAO (multivariate-adjusted, $P = .029$) and LAA (multivariate-adjusted, $P < .001$), respectively. *Conclusions:* *MMP-1* (–1607 1G/2G) and *MMP-3* (–1171 5A/6A) polymorphisms may contribute to different subtypes of IS susceptibility. **Key Words:** Ischemic stroke—matrix metalloproteinase—polymorphism—stroke subtype.

Introduction

Ischemic stroke (IS) is an acute neurological event that leads to morbidity and mortality worldwide. It is a multifactorial disease attributed to both genetic and environmental components.[1] To date, several candidate genes have been shown to influence the stroke risk in different populations[2-4]; however, its genetic basis remains incompletely understood. Extracellular matrix (ECM) remodeling is an essential process in the pathogenesis of atherosclerosis and IS. Matrix metalloproteinases (MMPs) are a family of protein-digesting enzymes that are fundamental mediators of matrix turnover and play an important role in catalyzing the breakdown of major ECM components.[5,6] On activation by proteolytic cleavage, MMPs

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are capable of degrading many ECM components, resulting in weakening of the fibrous cap and predisposing the atherosclerotic plaques to disruption and embolic events.[7] MMP expression can vary among individuals owing to genetic diversity, and derangement of MMP regulation is considered to be a critical factor in atherosclerosis and restenosis.[8,9]

MMP-1 is the only MMP that can cleave native collagen types I and III, which are major structural components of the fibrous plaque cap. A single-guanine polymorphism located at the *MMP-1* promoter region (*MMP-1* –1607 1G/2G, rs1799750) that affects the transcription level of the gene has been identified. Experiments demonstrated that the promoter comprising the 2G allele has significantly greater transcriptional activity than the 1G allele, because the 2G allele creates an Ets (E-26 virus transcription site) transcription factor binding site and increases transcription capacity.[10] MMP-3 degrades all noncollagenous matrix components of ECM and therefore plays an important role in atherosclerosis. In *MMP-3*, an inhibitory element binds the 6A sequence 1171 bases upstream from the transcription site, therefore making the 5A allele resistant to inhibition. In vitro assays of promoter activity revealed that the 5A allele had 2-fold higher promoter activity than the 6A allele.[11,12]

Considering the role of MMP-1 and MMP-3 in the pathophysiology of IS, we hypothesize here that there might be a possible association between the polymorphisms of these 2 genes and the subtypes of IS in the Han Chinese population. The present study was carried out with an aim to investigate whether *MMP-1* –1607 1G/2G or *MMP-3* –1171 5A/6A (rs35068180) promoter polymorphisms are associated with the subtypes of IS in the Han Chinese population, with adjustment for known risk factors.

Methods

Subjects

From January 2010 to January 2014, we recruited 647 unrelated patients with acute first-ever IS who were admitted to the study hospital. Inclusion criteria were IS evaluated within 7 days and age 18 years or older. Exclusion criteria included having a history of IS or transient ischemic attack; diagnosed with malignancies; severe kidney or liver diseases; and inflammatory or autoimmune diseases. All the patients were diagnosed by at least 2 independent neurologists. The diagnosis of IS was confirmed based on strict neurological examination, computed tomography, or magnetic resonance imaging. As a control group, we recruited 642 unrelated age- and gender-matched volunteers who came from the medical center of the study hospital at the same period. Controls were participants examined through an annual health checkup program. They were all free of clinically detectable cerebrovascular disease and without any stroke history, and were applied the same exclusion criteria as the patients with IS. All participants were of Han ethnic origin and were from the same demographic area. Controls had no relationship with cases.

According to the criteria of the Trial of Org 10172 in Acute Stroke Treatment,[13] patients with IS were divided into large-artery atherosclerosis (LAA), small-artery occlusion (SAO), cardioembolism (CE), stroke of other determined etiology, and stroke of undetermined etiology. Adjudication of subtype was performed by 2 neurologists who were blinded to genotype. Disagreements between the 2 neurologists were resolved by a third reviewer to reach a consensus.

Written informed consent was obtained from all participants. The study was approved by the ethical committee of the study hospital.

Data Collection

All participants underwent a strict protocol including complete medical history, physical examination, and clinical chemistry analysis before enrollment. Information on demographic studies, current medication, and risk factors was collected by using a structured questionnaire. The definitions of hypertension, diabetes mellitus, and hypercholesterolemia have been described before.[14] Smoking habits were defined as current smokers of ≥1 cigarette per day, former smokers, or nonsmokers. For statistical analysis, "current" and "former" smokers were pooled together. Alcohol consumption was categorized into >2 units per day (drinker) or ≤2 units per day (nondrinker).

Genotyping

A total of 5 mL venous blood was collected in ethylenediaminetetraacetic acid (disodium salt, 50 mmol/L) tubes, and genomic DNA was isolated with a DNA extraction kit (TaKaRa Biotechnology Co., Ltd, Dalian, China) according to the manufacturer's instructions. Genotyping was performed by laboratory personnel blinded to case-control status. The polymerase chain reaction method was used to amplify fragments containing the *MMP-1* –1607 1G/2G and *MMP-3* –1171 5A/6A polymorphisms, using the primers and conditions described by Dunleavey et al.[15,16] The polymerase chain reaction products were then subjected to digestion by the restriction endonuclease AluI and PsyI respectively, following the manufacturer's instructions (Ferments, Glen Burnie, MD). The digested fragments were electrophoresed on a 2.5% agarose gel and visualized with silver nitrate staining. Genotyping for the 2 polymorphisms was successfully performed in 640 patients and 637 controls. The results were confirmed by repeat genotyping of randomly selected 10% samples, which obtained a 100% concordance.