Original Article

Correlations between MMP-2/MMP-9 promoter polymorphisms and ischemic stroke

Shu-Wei Nie¹, Xiao-Feng Wang², Zong-Chun Tang²

¹Department of Emergency, The Fourth Peoples Hospital in Shaanxi Province, Xi’an 710043; ²Department of Neurosurgery, The Third Hospital of Chinese Peoples Liberation Army, Baoji 721004, China

Received July 15, 2013; Accepted December 8, 2013; Epub February 15, 2014; Published February 28, 2014

Abstract: Objective: Ischemic stroke (IS) results from interrupted blood flow to the brain and can be caused by formation of atherosclerotic plaques in blood vessels. Previous studies have suggested a contribution of matrix metalloproteinases (MMP) to plaque formation and stroke risk. Here, we explored associations between two MMPs, MMP-2 and MMP-9, and IS. Methods: There were 396 IS patients and 400 control individuals involved in this study. The MMP-2 -1306C/T and -735C/T polymorphisms and MMP-9 -1562C/T polymorphism were analyzed by restriction fragment length polymorphism (RFLP) from patient blood samples, and the relationships to IS were analyzed by logistic regression. Results: Genotype and allele frequencies of MMP-2 -1306C/T did not differ statistically (P>0.05), but those of MMP-2 -735C/T and MMP-9 -1562C/T did differ between IS and control samples (P<0.05). While MMP-2 -1306C/T polymorphism was not associated with increased risk of IS, MMP-2 -735C allele was associated with a 1.5-fold increase in IS incidence (OR=1.516, 95% CI=1.185-1.940, P=0.001), and MMP-9 -1562T allele was associated with a 1.5-fold increase in IS incidence (OR=1.543, 95% CI=1.144-2.080, P=0.004). Conclusion: MMP-2 -735C and MMP-9 -1562T may act as IS susceptibility alleles.

Keywords: MMP-2, MMP-9, gene polymorphism, ischemic stroke

Introduction

Ischemic stroke (IS), a major cause of morbidity and mortality worldwide, is characterized by the disintegration and destruction of local brain tissues caused by sudden reduction in or interruption of arterial blood perfusion. A major cause of IS is the formation, rupture, and shedding of carotid atherosclerotic plaques [1]. Plaque formation involves remodeling and conversion of the extracellular matrix (ECM), the supportive material between cells that forms the basis of connective tissue [2]. Remodeling of ECM occurs with aging, injury, or disease and involves increased expression of a family of proteins called matrix metalloproteinases (MMPs), which digest ECM components [2, 3].

In particular, two MMPs, MMP-2 and MMP-9, exhibit increased expression levels in serum [4] and in brain tissues [5] of IS patients, suggesting that MMP-2 and MMP-9 may play important roles in the pathology of IS. Previous studies have identified polymorphisms -1306C/T and -735C/T within the MMP-2 promoter and -1562C/T within the MMP-9 promoter; these polymorphisms can affect promoter activity [6, 7]. To better understand the importance of these observations on the pathology of IS, we directly investigated the relationship between polymorphisms of MMP-2 and MMP-9 and IS.

Materials and methods

Participants

This study recruited 396 IS patients who were treated Department of Neurosurgery, the Third Hospital of the Chinese Peoples Liberation Army (Baoji, Shaanxi Province, China), including 279 males and 117 females aged 45 to 84 years (mean age 64.9±8.9 years). All patients met the 1989 WHO Diagnostic Criteria for Stroke [8] by clinical diagnosis, radiological examination, cardiac function test, and ultrasonic inspection. Patients with history of diabetes, liver and kidney dysfunction, myocardial infarction, asthma, cancer, peripheral vascular...
disease, or cardiovascular surgery were excluded. An additional 400 healthy individuals undergoing physical examination during the same time period in the Third Hospital of the Chinese Peoples Liberation Army (Baoji, Shaanxi Province, China) were selected as controls, including 276 males and 124 females aged 43 to 85 years (mean age 64.1±9.7 years). Control individuals excluded those with history of diabetes, kidney disease, asthma, or cerebrovascular disease. The study was approved by the ethics committee of School of Medicine Anhui University of Science & Technology, and all patients provided informed consent.

**DNA extraction**

Venous blood (3 mL) was collected from each fasting patient and placed in a 2.5% EDTA anticoagulant tube. DNA was extracted with the Genomic DNA Isolation Kit (Takara) per manufacturer's instructions and stored at -70°C.

**Restriction fragment length polymorphism (RFLP) analysis of MMP-2 and MMP-9 promoters**

Polymerase chain reaction (PCR) primers were designed to amplify the promoter regions of MMP-2 and MMP-9 using primer design software and were synthesized by Sangon Biological Engineering (Shanghai). Sequences were as follows: forward, 5’-CTTCCTAGGGCT GTCCATTACT-GA-3’, and reverse, 5’-CTGAGACCTGAAGACTTCAA-3’, for amplification of MMP-2 -1306C/T; forward, 5’-GGATTCTTTGGCTTGGCGCAGGA-3’, and reverse, 5’-GGGGGCTGGGTAAATGAGGCTG-3’, for amplification of MMP-2 -735C/T; and forward, 5’-GCTGCATCGCATCAG-3’, and reverse, 5’-CTTCTAGCCAGCGGCATC-3’, for amplification of MMP-9 -1562C/T. PCR reaction mix (25 μL) contained 100 ng template DNA, 1.0 U Taq polymerase (Takara, Dalian, China), and 2 μL forward and reverse primers in 2× PCR buffer. Cycling parameters were as follows: 94°C for 5 minutes; 30 cycles of 94°C for 30 seconds, 58°C (MMP-2 -1306C/T), 62°C (MMP-2 -735C/T), or 65°C (MMP-9 -1562C/T) for 45 seconds, and 72°C for 45 seconds; 72°C for 10 minutes. Polymorphisms within amplified PCR products were detected by digestion with XspI (MMP-2 -1306C/T), HinfI (MMP-2 -735C/T), or PaeI (MMP-9 -1562C/T) restriction endonucleases, and digestion products were separated by 2% agarose gel electrophoresis with ethidium bromide staining.

**Statistical analysis**

SPSS17.0 for windows statistical software was applied for statistical analysis. Two-sample t-test was used to compare age difference between IS and control groups; χ² test was used to compare gender distribution, genotype, and allele frequencies between the two groups. The relationship between gene polymorphism and IS was analyzed by odds ratio (OR) and 95% confidence intervals (CI) generated by logistic regression. All analyses were two-sided with an α level of 0.05 and P<0.05 considered statistically significant.

**Results**

**Prevalence of MMP-2 and MMP-9 promoter polymorphisms**

Age and gender did not statistically differ (P>0.05) between IS and control groups. Digestion of MMP-2 -1306C/T amplicons (193 bp) yielded 88-bp and 5-bp fragments for CC genotype; 188-bp, 162-bp, 5-bp, and 26-bp fragments for CT genotype; and 162-bp, 26-bp, and 5-bp fragments for TT genotype (Figure 1). Digestion of MMP-2 -735C/T amplicons (391 bp) yielded a 39-bp fragment for CC genotype; 391-bp, 338-bp, and 53-bp fragments for CT genotype; and 338-bp and 53-bp fragments for TT genotype (Figure 1). Digestion of MMP-9 -1562C/T amplicons (435 bp) yielded a 435-bp fragment for CC genotype; 435-bp, 247-bp and 188-bp fragments for CT genotype; and 247-bp and 188-bp fragments for TT genotype (Figure 1). Genotype distributions are summarized in Table 1.

There was no significant difference in MMP-2 -1306C/T genotype and allele frequency between IS and control samples (P>0.05). Genotypes and allele frequencies of MMP-2 -735C/T and MMP-9 -1562C/T significantly differed between IS and control samples (P<0.05) (Table 1).

**Relationship between genotype and IS**

Logistic regression analysis showed that IS risk was not significantly associated with MMP-2-1306C/T polymorphisms (P>0.05) (Table 2). However, MMP-2 -735C and MMP-9 -1562T
MMP-2/MMP-9 polymorphisms and IS

polymorphisms were associated with 1.5-fold increased risk of IS (OR=1.516, 95% CI=1.185-1.940, P=0.001; OR=1.543, 95% CI=1.144-2.080, P=0.004, respectively) (Table 2).

Discussion

MMP-2 has been shown to contain multiple single nucleotide polymorphisms [9, 10]. The promoter region contains -1306C/T and -735C/T polymorphisms; for both, C allele transcriptional activity is significantly higher than for the T allele [11, 12]. This study identified no significant change in CC genotype and C allele frequencies for MMP-2 -1306C/T in IS patients compared to healthy controls, but CC genotype and C allele frequency of MMP-2 -735C/T were significantly increased in IS patients. Therefore, IS incidence was not significantly correlated with -1306C/T polymorphism, but was increased 1.5-fold in patients carrying the -735C allele. This may be due to increased MMP-2 transcription levels from the C allele, resulting in increased MMP-2 expression within infarcted brain tissues. MMPs can degrade ECM and contribute to

Table 1. Comparison of genotype and allele frequencies of MMP-2 and MMP-9 polymorphisms in IS

<table>
<thead>
<tr>
<th>Polymorphism</th>
<th>Group</th>
<th>n</th>
<th>Genotype [n (%)]</th>
<th>Allele [n (%)]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>CC</td>
<td>CT</td>
</tr>
<tr>
<td>MMP-2 -1306C/T</td>
<td>IS</td>
<td>396</td>
<td>315 (79.5)</td>
<td>51 (12.9)</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>400</td>
<td>306 (76.5)</td>
<td>55 (13.8)</td>
</tr>
<tr>
<td>χ²</td>
<td></td>
<td></td>
<td></td>
<td>1.435</td>
</tr>
<tr>
<td>P</td>
<td></td>
<td></td>
<td></td>
<td>0.488</td>
</tr>
<tr>
<td>MMP-2 -735C/T</td>
<td>IS</td>
<td>396</td>
<td>275 (69.4)</td>
<td>107 (27.0)</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>400</td>
<td>231 (57.8)</td>
<td>148 (37.0)</td>
</tr>
<tr>
<td>χ²</td>
<td></td>
<td></td>
<td></td>
<td>11.798</td>
</tr>
<tr>
<td>P</td>
<td></td>
<td></td>
<td></td>
<td>0.003</td>
</tr>
<tr>
<td>MMP-9 -1562C/T</td>
<td>IS</td>
<td>396</td>
<td>305 (77.0)</td>
<td>62 (15.7)</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>400</td>
<td>338 (84.5)</td>
<td>41 (10.3)</td>
</tr>
<tr>
<td>χ²</td>
<td></td>
<td></td>
<td></td>
<td>7.235</td>
</tr>
<tr>
<td>P</td>
<td></td>
<td></td>
<td></td>
<td>0.027</td>
</tr>
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</table>

Table 2. Association between MMP-2/MMP-9 polymorphisms and IS

<table>
<thead>
<tr>
<th>Allele</th>
<th>IS [n (%)]</th>
<th>Control [n (%)]</th>
<th>OR</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMP-2 -1306T</td>
<td>111 (14.0)</td>
<td>133 (16.6)</td>
<td>Reference</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MMP-2 -1306C</td>
<td>681 (86.0)</td>
<td>667 (83.4)</td>
<td>1.223</td>
<td>0.930-1.608</td>
<td>0.149</td>
</tr>
<tr>
<td>MMP-2 -735T</td>
<td>135 (17.0)</td>
<td>190 (23.8)</td>
<td>Reference</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MMP-2 -735C</td>
<td>657 (83.0)</td>
<td>610 (76.3)</td>
<td>1.516</td>
<td>1.185-1.940</td>
<td>0.001</td>
</tr>
<tr>
<td>MMP-9 -1562T</td>
<td>120 (15.2)</td>
<td>83 (10.4)</td>
<td>Reference</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MMP-9 -1562C</td>
<td>672 (84.8)</td>
<td>717 (89.6)</td>
<td>1.543</td>
<td>1.144-2.080</td>
<td>0.004</td>
</tr>
</tbody>
</table>
opening of the blood-brain barrier, further resulting in brain damage secondary to ischemia [13, 14].

MMP-9 also contains single nucleotide polymorphisms [15, 16]. The promoter region contains a -1562C/T polymorphism; transcriptional activity of the T allele is significantly higher than the C allele [17]. Here, we found that the TT genotype and T allele frequencies of MMP-9 -1562C/T were significantly increased in IS patients. IS incidence was increased 1.5-fold in patients carrying the -1562T allele. As with MMP-2, this may be due to increased MMP-9 transcription levels from the T allele. Increased MMP expression may also increase plaque instability to promote IS. Furthermore, brain damage secondary to infarction increases risk of IS [18, 19], indicating that MMP-9 -1562T is correlated with IS incidence.

In summary, single nucleotide polymorphisms of MMP-2 and MMP-9 are correlated with IS. The C allele of MMP-2 -735C/T and the T allele of MMP-9 -1562C/T may be risk factors for IS. Further investigation of genetic polymorphisms can contribute to better understanding of IS and prediction of at-risk individuals.

Disclosure of conflict of interest

None.

Address correspondence to: Zong-Chun Tang, Department of Neurosurgery, The Third Hospital of The Chinese Peoples Liberation Army, Baoji 721004, Shaanxi Province, China. Tel: +86-29-83283229 (0); +86-13892895427; E-mail: chinjna@fmmu.edu.cn; neitangzc@163.com

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