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Original Article

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

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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'-CTTCCTAGGCTG GTCCTTACTGA-3', and reverse, 5'-CTGAGACCTGAAGA CCTAAAGAGCT-3', for amplification of *MMP-2* -1306C/T; forward, 5'-GGATT CTTGGCTTGGCGCAGGA-3', and reverse, 5'-GGGGGCTGGGTAAAATGAGG CTG-3', for amplification of *MMP-2* -735C/T; and forward, 5'-GCCT GGCACATAGTAGGCCC-3', and reverse, 5'-CTTCCTAGCCAGCCGGCATC-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; χ^2 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

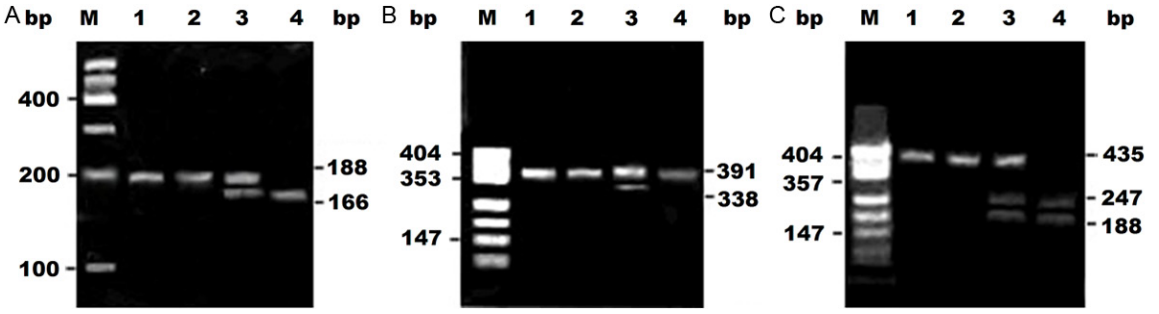

Figure 1. Electrophoretograms of amplified and digested PCR products of A: *MMP-2* -1306C/T, B: *MMP-2* -735C/T, and C: *MMP-9* -1562C/T. PCR products less than 100 bp were not visualized. (M: Marker; 1: PCR amplified product; 2: CC genotype; 3: CT genotype; 3: TT genotype).

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

Polymorphism	Group	*n*	Genotype [*n* (%)]			Allele [*n* (%)]	
			CC	CT	TT	C	T
MMP-2 -1306C/T	IS	396	315 (79.5)	51 (12.9)	30 (7.6)	681 (86.0)	111 (14.0)
	Control	400	306 (76.5)	55 (13.8)	39 (9.8)	667 (83.4)	133 (16.6)
	χ^2			1.435		2.089	
	P			0.488		0.148	
MMP-2 -735C/T	IS	396	275 (69.4)	107 (27.0)	14 (3.5)	657 (83.0)	135 (17.0)
	Control	400	231 (57.8)	148 (37.0)	21 (5.3)	610 (76.3)	190 (23.8)
	χ^2			11.798		11.011	
	P			0.003		0.001	
MMP-9 -1562C/T	IS	396	305 (77.0)	62 (15.7)	29 (7.3)	672 (84.8)	120 (15.2)
	Control	400	338 (84.5)	41 (10.3)	21 (5.3)	717 (89.6)	83 (10.4)
	χ^2			7.235		8.162	
	P			0.027		0.004	

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

Allele	IS [*n* (%)]	Control [*n* (%)]	*OR*	95% *CI*	*P*
MMP-2 -1306T	111 (14.0)	133 (16.6)	Reference		
MMP-2 -1306C	681 (86.0)	667 (83.4)	1.223	0.930-1.608	0.149
MMP-2 -735T	135 (17.0)	190 (23.8)	Reference		
MMP-2 -735C	657 (83.0)	610 (76.3)	1.516	1.185-1.940	0.001
MMP-9 -1562T	120 (15.2)	83 (10.4)	Reference		
MMP-9 -1562C	672 (84.8)	717 (89.6)	1.543	1.144-2.080	0.004

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

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.

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