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Associations between matrix metalloproteinase gene polymorphisms and the development of cerebral infarction

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ABSTRACT. The aim of this study was to investigate the association between MMP3 rs3025058 and MMP9 rs3918242 polymorphisms and the development of ischemic stroke in a Chinese population. Between May 2013 and January 2015, 335 patients with ischemic stroke and 335 health control subjects were enrolled in this study. The MMP3 rs3025058 and MMP9 rs3918242 polymorphisms were analyzed using polymerase chain reaction coupled with restriction fragment length polymorphism. By multivariate logistic regression analysis, the CC genotype of MMP9 rs3918242 was shown to be associated with a significantly increased risk of ischemic stroke when compared with the TT genotype [OR (95%CI) = 5.47 (2.64-12.38)]. The TC+CC genotype of MMP9 rs3918242 was furthermore found to be associated with an elevated risk of ischemic stroke in higher BMI individuals [OR (95%CI) = 1.81 (1.03-3.22)]. The findings of this study suggest that the MMP9 rs3918242 polymorphism is associated with an elevated risk of ischemic stroke and that this gene polymorphism interacts with BMI in the risk of ischemic stroke.

Key words: Matrix metalloproteinases; Polymorphism; Cerebral infarction

INTRODUCTION

Ischemic stroke is a complex and devastating vascular disease that has become one of the leading causes of disability and mortality worldwide. The etiology of ischemic stroke is not well understood; however, ischemic stroke is known to be caused by a large array of biological and lifestyle factors including hypertension, diabetes, drinking, and smoking (Goldstein et al., 2011). Moreover, genetic factors are involved in the development of ischemic stroke since not all individuals would develop ischemic stroke.

Increasing evidence has reported that inflammation has an important role in ischemic stroke susceptibility. Matrix metalloproteinases (MMPs) are an inflammatory mediator that belong to a family of zinc-dependent proteolytic enzymes, and are involved in degrading the extracellular matrix (ECM). The MMPs function in the development of atherosclerosis through activation of migration and proliferation of smooth muscle cells and inducing the artherosclerotic plaques (Galis and Khatri, 2002; Katakami et al., 2010). Previous studies have reported that alteration of MMP activity are observed in the cardiovascular and cerebrovascular diseases (Siefert and Sarkar, 2012; Chen et al., 2013).

Expression of MMP3, and MMP9 has previously been reported to be associated with risk of ischemic stroke (Moniche et al., 2014; Zhang et al., 2015). Only little research has, however, been done on the role of MMP3 and MMP9 polymorphisms in the susceptibility to ischemic stroke, and the results of such research have been inconclusive (Fatar et al., 2008; Kaplan et al., 2008). In this study, we therefore assessed the association between MMP3 rs3025058 and MMP9 rs3918242 polymorphisms and the susceptibility of ischemic stroke in a Chinese population.

MATERIAL AND METHODS

Study participants

This study was a case-control study in which a total of 335 patients from the First Affiliated Hospital of Xinxiang Medical University with proved ischemic stroke were enrolled between May 2013 and January 2015. Patients were confirmed with ischemic stroke by computed tomography (CT) or magnetic resonance imaging (MRI) based on the diagnostic criteria of ischemic stroke from World Health Organization. Patients who had intracranial hemorrhage, brain tumors, and brain trauma were excluded from the present study.

Three hundred and thirty-five healthy control subjects were randomly selected from the outpatients who visited the hospital for health check-ups. The control subjects were age- and gender-matched with the patients. Controls were free of ischemic stroke.

The clinical and demographic characteristics of patients with ischemic stroke and control subjects were interviewed using a standardized questionnaire, which included questions regarding their socio-demographic characteristics, including age, gender, body mass index, tobacco smoking, and alcohol drinking. Detailed clinical data, including hypertension, diabetes mellitus, total cholesterol, triglyceraldehydes, high-density lipoprotein and low-density lipoprotein, of patients with ischemic stroke were collected from their medical records.

Blood samples and signed written informed consents were obtained from each ischemic stroke patient and control subject prior to enrollment in the study. The study was approved by the Ethics Committee of the First Affiliated Hospital of Xinxiang Medical University.

Genotyping assays

The blood samples (5 mL) were collected in EDTA-containing tubes and stored at -20°C until use. Genomic DNA was isolated from the blood sample using the TIANamp Blood DNA Kit (Tiangen, Beijing, China). The MMP3 rs3025058 and MMP9 rs3918242 polymorphisms were done by polymerase chain reaction (PCR) coupled with restriction fragment length polymorphism (RFLP). The forward and reverse primers, respectively, were 5'-ACGTTGGATCATATCTCCGG-3' and 5'-ACGTTCCATGCTATCTCCAT-3' for MMP3 rs3025058; and 5'-CCGTGGCACACTATGCCCGG-3' and 5'-CTTCCTAGCCAGGGCCGATC-3' for MMP9 rs3918242. The PCRs were set as follows: 95°C for 5 min for initial denaturation, and 30 cycles of denaturation at 95°C for 30 s, annealing at 59°C for 45 s, extension at 72°C for 30 s, and a final extension at 72°C for 5 min. PCR products of the MMP3 rs3025058 and MMP9 rs3918242 were digested by *Pfl*F1 and *Sph*I restriction enzymes, and resulting fragments were separated on a 2% agarose gel. The fragment sizes were 187 bp for the 6A allele of rs3025058, and 157 and 30 bp for the 5A allele. The fragment sizes were 435 bp for the C allele of rs3918242, and 247 and 188 bp for the T allele.

Statistical analysis

All statistical analysis was done using the SPSS software for Windows version 20.0 (SPSS Inc., Chicago, IL, USA). Statistically significant differences between cases and controls for demographic and clinical data were compared by the independent sample *t*-test and the χ^2 test. Fisher exact tests were taken to analyze whether MMP3 rs3025058 and MMP9 rs3918242 genotypes were in line with the Hardy-Weinberg equilibrium in controls. The associations between the MMP3 rs3025058 and MMP9 rs3918242 polymorphisms and ischemic stroke risk were determined by estimating the odds ratio (OR) and 95% confidence interval (95%CI) by logistic regression. The major homozygous genotype of the MMP3 rs3025058 and MMP9 rs3918242 was used as a reference. Differences with P values <0.05 were considered to be statistically significant.

RESULTS

The mean ages of patients with ischemic stroke and of control subjects were 63.65 ± 9.40 and 64.45 ± 9.15 years, respectively. Both the ischemic stroke group and the healthy control group consisted of 141 females and 194 males. No significant difference was found between the patients and control subjects in terms of gender and age. Compared with the control subjects, the patients with ischemic stroke were more likely to have higher BMI (t = 5.09, P < 0.001), hypertension (χ^2 = 8.20, P = 0.004), total cholesterol (t = 41.49, P < 0.001), triglyceraldehydes (t = 4.29, P < 0.001), high-density lipoprotein (t = 68.90, P < 0.001) and low-density lipoprotein (t = 151.12, P < 0.001), and diabetes mellitus (χ^2 = 29.99, P < 0.001), and were more likely to smoke tobacco (χ^2 = 4.90, P < 0.001) and drink alcohol (χ^2 = 4.07, P < 0.001) (Table 1).

The genotype distributions of MMP3 rs3025058 and MMP9 rs3918242 are shown in Table 2. The observed genotype frequencies of MMP9 rs3918242 in controls were in line with the Hardy-Weinberg equilibrium (P for HWE = 0.08), while genotype distributions of MMP3 rs3025058 were not (P < 0.001). By the chi-squire test, a significant difference was observed in the genotype

frequencies of MMP9 rs3918242 between the ischemic stroke group and the control group (χ^2 = 27.27, P < 0.05), while no significant difference was found in the genotype frequencies of MMP3 rs3025058 (χ^2 = 0.79, P > 0.05). By multivariate logistic regression analysis, the presence of the CC genotype of MMP9 rs3918242 was correlated with a significantly increased risk of ischemic stroke compared to the TT genotype, with an OR (95%CI) of 5.47 (2.64-12.38).

Table 1. Demographic characteristics of patients with ischemic stroke and control subjects.

Variables	Patients (N = 335)	%	Controls (N = 335)	%	χ^2 or *t*-test	P value
Age [years (mean ± SD)]	63.65 ± 9.40		64.45 ± 9.15		1.12	0.13
≤60	156	46.57	158	47.16		
>60	179	53.43	177	52.84	0.02	0.88
Gender						
Females	141	42.09	141	42.09		
Males	194	57.91	194	57.91	0.00	1.00
Body mass index (kg/m^2)	24.8 ± 3.2		23.6 ± 2.9		5.09	<0.001
Hypertension						
No	196	58.51	159	47.46		
Yes	139	41.49	176	52.54	8.20	0.004
Diabetes mellitus						
No	249	74.33	303	90.45		
Yes	86	25.67	32	9.55	29.99	<0.001
Tobacco smoking						
Never	189	56.42	217	64.78		
Yes	146	43.58	118	35.22	4.90	0.03
Alcohol intake						
Never	170	50.75	196	58.51		
Yes	165	49.25	139	41.49	4.07	0.04
Total cholesterol (mg/dL)	227.30 ± 22.64		162.50 ± 17.45		41.49	<0.001
Triglyceraldehydes (mg/dL)	106.42 ± 26.16		97.32 ± 28.66		4.29	<0.001
High-density lipoprotein (mg/dL)	176.35 ± 10.67		104.30 ± 15.89		68.90	<0.001
Low-density lipoprotein (mg/dL)	179.70 ± 10.61		87.40 ± 3.52		151.12	<0.001

Table 2. Associations between MMP polymorphisms (MMP3 rs3025058 and MMP9 rs3918242) and ischemic stroke risk.

Gene	Patients (N = 335)	%	Controls (N = 335)	%	χ^2 test	P value	P for Hardy-Weinberg equilibrium	OR (95%CI)[1]	P value
MMP3 rs3025058									
5A/5A	232	69.25	243	72.54				1.0 (Ref.)	-
5A/6A	53	15.82	49	14.63				1.13 (0.72-1.78)	0.57
6A/6A	50	14.93	44	13.13	0.79	0.67	<0.001	1.19 (0.75-1.90)	0.44
MMP9 rs3918242									
TT	223	66.57	254	75.82				1.0 (Ref.)	-
TC	64	19.10	71	21.19				1.03 (0.69-1.53)	0.89
CC	48	14.33	10	2.99	27.27	<0.001	0.08	5.47 (2.64-12.38)	<0.001

[1]Adjusted for body mass index, hypertension, diabetes mellitus, tobacco smoking, alcohol drinking, total cholesterol, triglyceraldehydes, high-density lipoprotein, and low-density lipoprotein.

Further analysis was carried out for the association between MMP9 rs3918242 and ischemic stroke risk stratified by demographic characteristics including BMI, hypertension, diabetes mellitus, tobacco smoking, and alcohol intake (Table 3). By logistic regression analysis, the TC+CC genotype of MMP9 rs3918242 was observed to be correlated with an elevated risk of ischemic stroke in higher BMI individuals, with an OR (95%CI) of 1.81 (1.03-3.22).

Table 3. Association between MMP9 rs3918242 and risk of ischemic stroke stratified by demographic characteristics.

Variables	Case		Control		OR (95%CI)	P value
	TT	TC+CC	TT	TC+CC		
Body mass index						
≤24	92	49	160	56	1.52 (0.93-2.48)	0.07
>24	131	63	94	25	1.81 (1.03-3.22)	0.03
Hypertension						
No	129	67	119	40	1.55 (0.95-2.53)	0.07
Yes	94	45	135	41	1.58 (0.93-2.68)	0.07
Diabetes mellitus						
No	171	78	229	74	1.41 (0.95-2.09)	0.07
Yes	52	34	25	7	2.34 (0.85-7.08)	0.07
Tobacco smoking						
Never	129	60	165	52	1.48 (0.93-2.34)	0.08
Yes	94	52	89	29	1.70 (0.96-3.03)	0.05
Alcohol drinking						
Never	113	57	147	49	1.51 (0.94-2.45)	0.07
Yes	111	54	107	32	1.63 (0.95-2.81)	0.06

DISCUSSION

In the present study, the roles of two important polymorphisms of the MMP3 rs3025058 and MMP9 rs3918242 in ischemic stroke risk in a sample of Chinese population were assessed. The results of this study revealed that the MMP9 rs3918242 polymorphism is associated with an increased risk of ischemic stroke, so that the CC genotype of MMP9 rs3918242 increased the risk of ischemic stroke in comparison with the TT genotype. However, no significant association was observed between MMP3 rs3025058 variant and ischemic stroke risk. Furthermore, subjects carrying the TC+CC genotype interact with BMI in the development of ischemic stroke.

Several studies have reported a significant association between expression of MMP9 and the ischemic stroke risk (Nie et al., 2014; Zhang et al., 2015). Zhang et al. (2015) conducted a study with 222 Chinese patients with ischemic stroke, and reported that the CT+TT genotype of MMP9 rs3918242 was correlated with an increased risk of ischemic stroke. Our results are not consistent with a previous study performed in 396 Chinese patients with ischemic stroke, and they found that the TT genotype and T allele frequencies of MMP9 rs3918242 polymorphism were associated with increased risk of ischemic stroke (Nie et al., 2014). Other studies, however, reported no association between MMP9 rs3918242 polymorphism and ischemic stroke risk (Montaner et al., 2003; Manso et al., 2010; Szczudlik et al., 2010). Montaner et al. (2003) conducted a study in a Spanish population and found no association between MMP9 rs3918242 polymorphism and the development of stroke. In another study in a Polish population, Szczudlik et al. (2010) showed no significant association between the MMP9 rs3918242 polymorphism and increased risk of ischemic stroke. Since stroke is a complex disease and caused by many risk factors, MMP9 rs3918242 polymorphism could not be responsible for all the impacts for the development of ischemic stroke. Therefore, the discrepancies of these studies might be explained by the ethnic variations, differences in the source of patients and controls and sample size.

In the present study, the MMP9 rs3918242 polymorphism was shown to interact with higher BMI in the development of ischemic stroke. The MMP9 rs3918242 polymorphism has previously also been shown to be associated with high BMI-related diseases such as type 2 diabetes, coronary heart disease, and myocardial infarction (Ahluwalia et al., 2009; Wang et al., 2011; Li et al., 2013). There is a great need for further studies to confirm these findings reported here.

There were some limitations to our study. First, control subjects are free of ischemic stroke by medical history, lack of examination by CT or MRI. Without confirmation of imaging examinations, some control subjects may have been affected by silent stroke, which may reduce the statistical power to find difference between groups. Second, some other genetic polymorphisms may contribute to the development of ischemic stroke, and have interaction with MMP-9 polymorphism, but our study did not consider other genes. Third, the study sample size was relatively small, which may limit the statistical power for the identification of differences between groups.

The findings of our study suggest that the MMP9 rs3918242 polymorphism plays a critical role in the pathogenesis of ischemic stroke, and that this gene polymorphism interacts with BMI in the risk of ischemic stroke. Our study provided new clinically information for impact of MMP3 and MMP9 gene polymorphisms on the susceptibility to stroke.

Conflicts of interest

The authors declare no conflict of interest.

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