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Original Article

Association between matrix metalloproteinase gene polymorphisms and development of ischemic stroke

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Abstract: We investigated the association between MMP2 rs243865, MMP3 rs3025058 and MMP9 rs3918242 polymorphisms and development of ischemic stroke in a Chinese population. Between January 2012 and May 2014, a total of 317 patients with ischemic stroke and 317 health control subjects were enrolled into our study. The MMP2 rs243865, MMP3 rs3025058 and MMP9 rs3918242 polymorphisms were analyzed using polymerase chain reaction (PCR) coupled with restriction fragment length polymorphism (RFLP). By multivariate logistic regression analysis, we found that individuals carrying with the CC genotype and the TC+CC genotype of MMP9 rs3918242 were associated with a significantly increased risk of ischemic stroke when compared with the TT genotype, and the ORs (95% CI) was 5.47 (2.64-12.38) and 1.55 (1.08-2.24), respectively. The TC+CC genotype of MMP9 rs3918242 was associated with an elevated risk of ischemic stroke in tobacco smokers, and the OR (95% CI) was 2.03 (1.11-3.74). In conclusion, our study suggests that MMP9 rs3918242 polymorphism is correlated with an elevated risk of ischemic stroke, and this gene polymorphism has interaction with tobacco smoking in the risk of ischemic stroke.

Keywords: Matrix metalloproteinases, polymorphism, ischemic stroke

Introduction

Ischemic stroke is a complex vascular disease, and this disease has become one of the leading causes of morbidity and mortality worldwide. The etiology of ischemic stroke is not well understood, and the process of ischemic stroke is caused by many environmental factors, such as hypertension, diabetes, dyslipidemia, atrial fibrillation, asymptomatic carotid stenosis, drinking and smoking [1]. However, not all of the individuals who exposed to similar environmental factors would suffer from ischemic stroke, which suggests that genetic factors have an important role in the susceptibility to ischemic stroke.

Matrix metalloproteinases (MMPs) belong to a family of zinc-dependent proteolytic enzymes, and play a central pathologic role in degrading the extracellular matrix (ECM). MMPs have function in physiological degradation of the ECM in angiogenesis, tissue repair, and tissue morphogenesis [2]. Previous studies have reported that degradation of ECM could cause the loss of ECM components, make the plaque more unstable and cause the obstruction of the distal vasculature [3, 4]. MMP2, MMP3 and MMP9 are three important members of the MMP family, and previous studies have reported that the expression of MMP2, MMP3 and MMP9 are associated with risk of ischemic stroke [5, 6].

However, few studies investigated the role of MMP2, MMP3 and MMP9 polymorphisms in the susceptibility to ischemic stroke, and the results were inconsistent [7, 8]. Therefore, we conducted a case-control study to investigate the association between MMP2 rs243865, MMP3 rs3025058 and MMP9 rs3918242 polymorphisms and development of ischemic stroke in a Chinese population.

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

Variables	Patients (N=317)	%	Controls (N=317)	%	χ^2 or *t* test	*P* value
Age, years	62.13±10.25		62.50±9.85		0.46	0.32
Mean ± SD						
≤60	145	45.74	149	47.00		
>60	172	54.26	168	53.00	0.1	0.75
Sex						
Females	137	43.22	137	43.22		
Males	180	56.78	180	56.78	0	1
Body mass index (BMI), kg/m^2	25.2±3.1		23.2±2.8		8.52	<0.001
≤24	123	38.80	198	62.46		
>24	194	61.20	119	37.54		
Hypertension						
No	192	60.57	226	71.29		
Yes	125	39.43	91	28.71	8.12	0.004
Diabetes mellitus						
No	248	78.23	289	91.17		
Yes	69	21.77	28	8.83	20.46	<0.001
Tobacco smoking						
Never	172	54.26	211	66.56		
Ever	145	45.74	106	33.44	10.03	0.002
Alcohol drinking						
Never	151	47.63	188	59.31		
Ever	166	52.37	129	40.69	8.68	0.003

Materials and methods

Study subjects

A hospital-based case-control study was taken in our study. Between January 2012 and May 2014, a total of 344 patients with proved ischemic stroke were enrolled into our study. Patients were diagnosed to be ischemic stroke if they had rapid developing clinical signs of focal or global disturbance of cerebral function lasting more than 24 hours without apparent cause but vascular origin, and the patients were confirmed by CT or MRI according to the diagnostic criteria of ischemic stroke from world Health Organization. The exclusion criteria of patients with ischemic stroke were those who had transient ischemic attacks, intracranial hemorrhage and brain tumors as well as brain trauma. Finally, 317 patients were enrolled in our study, and the participation rate was 92.15%.

For the frequency-matched controls on age and sex, 317 health control subjects were randomly collected from individuals who came to our hospital for health check-up. Controls that had a history of ischemic stroke were excluded from our study.

Demographic characteristics were interviewed using a standardized questionnaire, including age, sex, Body mass index (BMI), tobacco smoking and alcohol drinking as well as hypertension. All patients with ischemic stroke and control subjects signed written informed consents. The signed written informed consents were obtained from patients with ischemic stroke and controls. Our study was approved by the ethics committee of our hospital.

Genotyping assays

All the patients with ischemic stroke and control subjects were required to provide 5 ml blood sample after enrolling into this study, and the blood samples were kept in -20°C until use. Genomic DNA was isolated from the peripheral blood sample using the TIANamp Blood DNA Kit (Tiangen, Beijing, China). The MMP2 rs243865, MMP3 rs3025058 and MMP9 rs3918242 polymorphisms were analyzed using polymerase chain reaction (PCR) coupled with restriction fragment length polymorphism

Table 2. Association between MMP2 rs243865, MMP3 rs3025058 and MMP9 rs3918242 and risk of ischemic stroke

Genes	Patients N=317	%	Controls N=317	%	OR (95% CI)[1]	*P* value
MMP2 rs243865						
CC	171	53.94	182	57.41	1.0 (Ref.)	-
CT	107	33.75	101	31.86	1.13 (0.79-1.61)	0.49
TT	39	12.30	34	10.73	1.22 (0.71-2.09)	0.44
CT+TT	146	46.05	135	42.59	1.15 (0.83-1.59)	0.38
MMP3 rs3025058						
5A/5A	223	70.35	235	74.13	1.0 (Ref.)	-
5A/6A	48	15.14	42	13.25	1.20 (0.75-1.95)	0.42
6A/6A	46	14.51	40	12.62	1.21 (0.74-1.98)	0.41
5A/6A+6A/6A	94	29.65	82	25.87	1.21 (0.84-1.74)	0.29
MMP9 rs3918242						
TT	214	67.51	242	76.34	1.0 (Ref.)	-
TC	59	18.61	66	20.82	1.01 (0.67-1.53)	0.96
CC	44	13.88	9	2.84	5.53 (2.58-13.15)	<0.001
TC+CC	103	32.49	75	23.66	1.55 (1.08-2.24)	0.01

(RFLP). The forward and reverse primers for MMP2 rs243865 were 5'-CTGACCGGAGTGGTATCTGCC-3' and 5'-TGTTGCCTTCGCCTGACAACAG-3', respectively. The forward and reverse primers for MMP3 rs3025058 were 5'-ACGTTGGATCATATCTTAGACCGG-3' and 5'-ACGTTCCATGCTATCCAACTCCAT-3', respectively. The forward and reverse primers for MMP9 rs3918242 were 5'-GCCTGGCACTATGTACCGGG-3' and 5'-TTAATAGGGAGGGCCCATC-3', respectively. The reaction was conducted at 95°C for 5 min for the initial denaturation, following 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 final extension at 72°C for 5 mins. PCR products were verified 2% agarose gel stained with ethidium bromide and ultraviolet light.

Statistical analysis

Statistically significant differences between patients with ischemic stroke and controls for demographic characteristics were assessed by χ^2 test or *t* test. Departures from Hardy-Weinberg equilibrium for MMP2 rs243865, MMP3 rs3025058 and MMP9 rs3918242, genotype were evaluated by comparing the expected frequencies to observed genotype frequencies using χ^2 tests. The association between the MMP2 rs243865, MMP3 rs3025058 and MMP9 rs3918242 polymorphisms and risk of ischemic stroke was analyzed by calculating odds ratios (ORs) and 95% confidence intervals (95% CI) as well as their corresponding *P*-values. Analysis for gene-environment interaction was carried out stratifying the demographic and lifestyle factors. A *P*-value of less than 0.05 was considered to be statistically significant. All statistical tests were conducted using SPSS software for Windows version 16.0 (SPSS Inc., Chicago, IL).

Results

The distributions of demographic characteristics were summarized in **Table 1**. The mean age of patients with ischemic stroke and control subjects were 62.13±10.25 years and 62.50±9.85 years, respectively. There were 137 females and 180 males in patients with ischemic stroke and control subjects, respectively. There were no significant differences in sex and age between patients with ischemic stroke and control subjects. By comparing with control subjects, patients with ischemic stroke were more likely to have higher BMI, hypertension, diabetes mellitus, tobacco smoking and alcohol drinking (*P*<0.01).

Genotype distributions of MMP2 rs243865, MMP3 rs3025058 and MMP9 rs3918242 were shown in **Table 2**. The observed genotype frequencies of MMP9 rs3918242 in controls confirmed with Hardy-Weinberg equilibrium (*P* for HWE=0.08), while genotype distributions of MMP2 rs243865 and MMP3 rs3025058 were not (*P*<0.05). The genotype frequencies were not significantly different in frequencies of the MMP2 rs243865 and MMP3 rs3025058 between the cases and controls (*P*>0.05), and significant difference was found in genotype frequencies of MMP9 rs3918242 (χ^2=25.22, P<0.05). By multivariate logistic regression analysis, we found that individuals carrying with the CC genotype and TC+CC genotype of MMP9 rs3918242 were associated with a significantly increased risk of ischemic stroke

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 (BMI)						
≤24	79	44	148	50	1.65 (0.97-2.76)	0.06
>24	135	59	94	25	1.64 (0.93-2.94)	0.07
Hypertension						
No	121	71	165	61	1.42 (0.87-2.35)	0.14
Yes	93	32	77	14	1.65 (0.94-2.90)	0.06
Diabetes mellitus						
No	178	70	223	66	1.33 (0.88-2.00)	0.15
Yes	36	33	19	9	1.94 (0.71-5.54)	0.16
Tobacco smoking						
Never	123	49	160	51	1.25 (0.77-2.03)	0.34
Ever	91	54	82	24	2.03 (1.11-3.74)	0.01
Alcohol drinking						
Never	98	53	140	48	1.58 (0.96-2.59)	0.06
Ever	116	50	102	27	1.63 (0.92-2.91)	0.07

when compared with the TT genotype, and the ORs (95% CI) was 5.53 (2.58-13.15) and 1.55 (1.08-2.24), respectively. However, no significant association was found between MMP2 rs243865 and rs3025058 polymorphisms and risk of ischemic stroke ($P>0.05$).

We further analyzed the association between MMP9 rs3918242 and risk of ischemic stroke stratified by demographic characteristics, including BMI, hypertension, diabetes mellitus, tobacco smoking and alcohol drinking (**Table 3**). By logistic regression analysis, we found that the TC+CC genotype of MMP9 rs3918242 was associated with and an elevated risk of ischemic stroke in tobacco smokers, and the OR (95% CI) was 2.03 (1.11-3.74). However, no significant interaction was found in MMP9 rs3918242 polymorphism and hypertension, diabetes mellitus, tobacco smoking and alcohol drinking in the risk of ischemic stroke.

Discussion

In this hospital-based case-control study, we investigated the role of three important polymorphisms of the MMP gene, including rs243865, rs3025058 and rs3918242, in the risk of ischemic stroke, and their interaction with environmental factors in the development of ischemic stroke. In our study, we found that MMP9 rs3918242 polymorphism was associated with an increased risk of ischemic stroke, and had interaction with tobacco smoking in the risk of ischemic stroke.

The encoding gene of MMP9 rs3918242 is located on chromosome 20q12.2-13.1 at the position 1562 bp upstream of the transcriptional start site, and this gene contains either C or T and influences the transcriptional activity of the MMP9 rs3918242. A previous experimental study reported that the T allele of MMP9 rs3918242 had a higher promoter activity compared to the C allele due to the binding of a transcriptional repressor [9]. Several previous studies reported the association between MMP9 rs3918242 and development of ischemic stroke, but the results are inconsistent [6, 10-13]. Nie et al. conducted a study in a Chinese population, and they found that MMP9 rs3918242 polymorphism may contribute to the development of ischemic stroke [13]. Zhang et al. conducted another study in a Chinese population, and they reported that the C allele of MMP9 rs39-18242 was correlated with an increased risk of ischemic stroke [6]. However, some studies reported no association between MMP9 rs3918242 polymorphism and risk of ischemic stroke. Montaner et al. conducted a study in a Spanish population, and they found that no association of MMP9 rs3918242 polymorphism with the development of stroke [10]. Another study was conducted in Portuguese population, and they reported that MMP9 genetic variants were not genetic mediator for the functional outcome after stroke [11]. Szczudik et al. conducted a case-control study in a Polish population, and they found no association between MMP9 rs3918242 polymorphism and ischemic stroke [12]. The discrepancies of these results may be caused by differences in ethnicities, study design, and sample size as well as by chance.

Moreover, our study found that MMP9 rs3918242 polymorphism had interaction with

tobacco smoking in the development of ischemic stroke. Previous studies have reported that MMP9 rs3918242 polymorphism is associated with tobacco smoking related diseases, such as coronary heart disease and myocardial infarction [14, 15]. Further studies are greatly needed to confirm the finding of our study.

There were two limitations in our study. First, patients and controls were selected from one single hospital in China, which could not be representative of the general population and selection bias may exist in our study. Second, some other genetic polymorphisms may have a role in the development of ischaemic stroke, but our study only investigated the association of MMP2, MMP3 and MMP9 gene polymorphisms with the development of ischemic stroke. Third, the sample size is relatively small, which may limit the statistical power to find the differences between groups.

In conclusion, our study suggests that MMP9 rs3918242 polymorphism is correlated with an elevated risk of ischemic stroke, and this gene polymorphism has interaction with tobacco smoking in the risk of ischemic stroke. Future studies with larger sample size may contribute to elucidate the impact of these polymorphisms on the risk of ischemic stroke.

Disclosure of conflict of interest

None.

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