International Journal of General Medicine

Dovepress

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ORIGINAL RESEARCH

Polymorphisms of the Matrix Metalloproteinase Genes are Associated with Acute Ischemic Stroke in Chinese Han Population

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Background and Purpose: Studies have shown that matrix metalloproteinase (MMP-2,3,9) plays an important role in the pathologic process of ischemic stroke (IS). The aim of this study was to investigate the relationship between C1306T, 1612–5A/6A, C-1562T polymorphisms of *MMP*-2,3,9 genes and IS in Chinese Han population.

Methods: The polymorphisms of *MMP*-2(C1306T), −3(1612–5A/6A), −9(C-1562T) gene were detected by PCR-RFLP and SNaPshot sequencing. Then, stratified analysis was used to study the relationship between IS subtypes and *MMP-2,3,9* polymorphisms.

Results: For the *MMP-2* gene C1306T polymorphism, TT genotype and T allele were significantly associated with a reduced risk of IS (P = 0.015, P = 0.003, respectively). T allele was significantly associated with a reduced risk of small artery occlusion (SAO) subtype compared with the control group (P = 0.012, OR = 0.550, 95% CI = 0.065–1.291). For the *MMP-3* gene-1612 (5A/6A) polymorphism, 5A/5A genotype was significantly increased in the IS group (P = 0.011, OR = 0.370, 95% CI = 0.168–0.814), especially in the large-artery atherosclerosis (LAA) subtype (P = 0.001, OR = 2.345) as compared to the control group.

Conclusion: Our study suggested that the T allele of *MMP*-2 may be a protective factor of IS, especially in SAO subtype, while the 5A/5A gene of *MMP*-3 may increase the risk of IS, especially in LAA subtype in Chinese Han population.

Keywords: ischemic stroke, matrix metalloproteinase, polymorphism, association study

Introduction

Epidemiological data show that the incidence of stroke in China is 170.3/100 000, of which 80% is ischemic stroke (IS).[1] IS is a multifactorial disease characterized by cerebral artery stenosis or occlusion, which can lead to cerebral ischemia, hypoxia or necrosis. IS is widely concerned because of its high morbidity, disability and mortality,[2] which seriously threatens human health. Therefore, it is great significance to study the molecular genetic mechanism of ischemic stroke.

Extracellular matrix (ECM) remodeling is an important mechanism in the pathogenesis of atherosclerosis and IS.[3] Matrix metalloproteinases (MMPs) belong to the family of protein digestive enzymes that are the basic mediators of matrix conversion and play an important role in catalyzing the decomposition of main components of ECM.[4] Activated by proteolytic cleavage, *MMP* can degrade many ECM components, weaken the fiber cap, and make atherosclerotic plaque prone to rupture and cause embolic events.[5]

Received: 12 November 2022
Accepted: 2 February 2023
Published: 20 February 2023

Generally, the expression of matrix metalloproteinase gene is maintained at a low level, but it will be up-regulated in the process of pathological reconstruction.[6] The expression of *MMP-2* and *MMP-9* is up-regulated in human atherosclerotic unstable plaques. In addition, the high expression of *MMP-3* can degrade extracellular matrix components, resulting in plaque instability. Inzitari et al[7] found that *MMPs*, especially *MMP-2* and *MMP-9*, play an important role in the opening of blood–brain barrier, and the destruction of blood–brain barrier will lead to IS. Thus, the high serum level of *MMP-2,9* indicates that the risk of IS would increase. According to Zhang et al,[8] in *MMP-9*, T allele could rise the promoter activity of the *MMP-9* significantly so as to further improve the expression of *MMP-9* resulting in high serum level of *MMP-9*. But in *MMP-2*, the occurrence of C allele and the T allele could decrease risk of IS. On the acute stage of IS, the expression and activity of *MMPs-2,9* increase obviously and the disordered *MMPs-2,9* would result in neurovascular disruption and brain parenchymal damage.[9] In *MMP-3*, the inhibitory element bounds to 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 has twofold higher promoter activity than the 6A allele.[10]

Considering the role of *MMP-2, MMP-3* and *MMP-9* in the pathophysiology of IS, we assumed that there may be a correlation between *MMP* gene polymorphisms and IS in Chinese Han population. Therefore, in the present study, we examined whether the gene polymorphism of *MMP −2*(C1306T), −3(1612-5A/6A), *−9* (C-1562T) are associated with IS in the Chinese Han population.

Materials and Methods

Study Population

Three hundred patients (163 males and 137 females) with IS were recruited from the Department of Neurology at the first people's Hospital of Zhengzhou City from April 2021 to July 2022. The diagnosis of IS patients was confirmed by a series of clinical inspections, including medical history, physical examination, biochemical tests, and CT or MRI, and the results accorded with the diagnostic standards of Treatment of Acute Ischemic Stroke in China (2014) issued by the Department of Cerebrovascular Diseases, Neurology Branch of Chinese Medical Association.[11] The mean age of IS patients was 59.7±12.6 years. On the basis of the TOAST stroke classification system, IS patients were divided into three categories, including large-artery atherosclerosis, LAA; small artery occlusion, SAO and stroke of other determined cause, SOE in this cohort. The patient's history and risk factors for stroke, such as hypertension, diabetes, smoking, drinking and stroke were recorded.

The control group consisted of 300 age and gender matched healthy subjects (145 men and 155 women) with no history of stroke or any other atherothrombotic disease. The mean age of control was 58.7±11.7 years. Individuals with epilepsy, cancer, thyroid dysfunction, liver and kidney dysfunction, gastric disease or megaloblastic anemia were excluded. All the participants were from Henan Han province. The study was approved by the Zhengzhou University Life Science Ethics Review Committee, and all participating individuals signed informed consent.

DNA Extraction

2 mL of peripheral venous blood samples were collected, added to an anticoagulant tube containing 2% EDTA-Na_2, mixed upside down, and stored at −20 °C. 250 μL of proteinase K was added to the blood sample, vortexed for 30 s, and then genomic DNA was extracted using the Blood DNA Extraction Kit (TIANGEN), according to the manufacturer's instructions.

SNPs Genotyping

The polymorphisms of *MMP-3* and *MMP-9* were genotyped by polymerase-chain reaction-restriction fragment length polymorphism (PCR-RFLP). The primer pairs of *MMP-3,9* were respectively 5'-TTCTCCATTCCT TTGATGGGGGAAAGA-3' and 5'-TTATCTGTTGGGCTCCACTGTTTCTTCC-3'; 5'-GCC TGG CAC ATA GTA GGC CC-3` and5'-CTT CCT AGC CAG CCG GCA TC-3'. The PCR amplification system (25 μL)

contained 2.5 μL of STR Buffer ($MgCl_2$, dNTPs and 10ラbuffer), 2.5 μL of each sense and antisense primer, 0.4μL of Taq DNA polymerase (Shanghai Bao Sheng Co. Ltd. China), and 1.5 μL of genomic DNA, and the total volume was adjusted to 25 μL using ddH_2O. Amplification was performed as follows: pre-denaturation at 95°C for 5 min; followed by 30 cycles of denaturation at 94°C for 1 min, 63°C for 1 min, and 72°C for 1 min; and extension at 72°C for 10 min. The PCR products of *MMP3,9* were digested with the restriction enzyme *HinfI* (Sangon Biotech Co., Ltd., Shanghai) and *Sph I* (Sangon Biotech Co., Ltd., Shanghai), respectively. A reaction mixture of 20 μL was prepared by mixing 6 μL of PCR product, 1 μL of *HinfI* or *Sph I*, 2.0 μL of Buffer R, and 11 μL of ddH2O. The reaction mixture was incubated at 37°C for 4 h. Then, PCR fragments were separated by electrophoresis on a 2% agarose gel for 30 min at 100 V and were visualized using an imaging spectrometer at 300 nm.

The polymorphisms of *MMP-2* were genotyped by SNaPshot sequence. The extended primer sequence of *MMP-2* gene was 5′ - AGACCTGAAGAGCTAAAGAGGT −3′. The reaction system (6 μL) contained SNaPshot Multiplex Kit 1 μL; purified PCR product 2 μL; extension primer 0.2 μL and ddH2O 2.8 μL. The reaction conditions were pre-denaturation at 96°C for 1 min, 30 cycles of denaturation at 96°C for 10 s, annealing at 52°C for 5 s and extension at 60°C for 30 s. Finally, take 1 μ L extension product, plus 9 μL HIDI enzyme, denatured at 95°C for 3 min, and immediately in ice water bath, sequenced and typed by sequencer. The primer sequences of MMP-2,3,9 and PCR conditions was shown in Table 1.

DNA Sequencing Analysis

Randomly select 10% of all genotypes for sequencing to check the accuracy of genotyping results, which was conducted by the Shang Hai Bao Sheng Company. And no discrepancies were detected.

Statistical Analysis

All statistical analyses were performed using the SPSS 21.0 package (SPSS, Chicago, IL, USA). Quantitative data are expressed as mean ± SD. Differences between the cases and controls were evaluated by using the *t*-test. Allele and genotype frequencies in both groups were calculated and compared using the χ^2 test. The Hardy-Weinberg equilibrium of *MMP-2,3,9* SNPs was detected by SHEsis software and the association of SNPs of *MMP-2,3,9* and other risk factors with cerebral infarction was examined by means of stepwise logistic regression analysis. The

Table 1 PCR Conditions and Primer Sequences

Gene	PCR Conditions	Primer Sequences*
MMP-2-1306T	Pre-denaturation at 96 °C for 1 min Denaturation at 96 °C for 10s Annealing at 52 °C for 5s Extension at 60 °C for 30s	5′- AGACCTGAAGAGCTAAAGAGGT −3′
MMP-3-1612	Pre-denaturation at 95°C for 5 min Denaturation at 94°C for 1 min, 63°C for 1 min, and 72°C for 1 min Extension at 72°C for 10 min.	F:5′-TTCTCCATTCCTTTGATGGGGGAAAGA-3′ R:5′-TTATCTGTTGGGCTCCACTGTTTCTTCC-3′
MMP-9 −1562	Pre-denaturation at 95°C for 5 min Denaturation at 94°C for 1 min, 63°C for 1 min, and 72°C for 1 min Extension at 72°C for 10 min.	F: 5′-GCC TGG CAC ATA GTA GGC CC-3′ R: 5′-CTT CCT AGC CAG CCG GCA TC-3′

Note: *The primer sequence of MMP-2-1306T is used for SNaPshot sequence and the primer sequence of MMP-3-1612 and MMP-3-1612 are used for polymerase chain reaction.
Abbreviations: PCR, polymerase chain reaction; min, minute; s, second; °C, Celsius.

results were expressed as odds ratios (OR) and 95% confidence intervals (CI). *P* less than 0.05 were considered to indicate statistical significance.

Results

Clinical Characteristics of the Subjects

The clinical characteristics of the subjects are shown in Table 2. There was no significant difference in age or gender between the two groups ($P > 0.05$). The incidence of hyper homocysteine, high-density lipoprotein (HDL), hypertension and diabetes in the patients were significantly higher than those in the controls ($P < 0.05$). See Table 2.

Results of SNPs Genotyping

The C1306T polymorphisms of *MMP-2* were performed by SNaPshot microsequencing technology. See Figure 1. While the polymorphisms of *MMP-3*(1612-5A/6A) and *MMP-9* (C-1562T) were performed by PCR-RFLP. See Figure 2.

Association Analysis of MMP-2,3,9 Polymorphisms

The genotype frequency distributions of *MMP-2* (C1306T), *MMP-3*(1612-5A/6A) and *MMP-9*(C-1562T) were consistent with Hardy-Weinberg equilibrium (HWE) in the Chinese Han cohort. After adjusting for conventional risk factors, the T allele frequency (10.8%) of *MMP-2* in the IS group was found to be significantly lower than that (16.7%) in the control group (*P*=0.003, OR=0.792, 95% CI=0.434~0.849). The homozygous TT genotype frequency (1.7%) of *MMP-2* in the IS group was significantly lower than that (5.0%) in the control group (*P*=0.015, OR=0.581, 95% CI=0.354~0.955). 5A/5A genotype frequency (3.0%) of *MMP-3* in case group was also found to be significantly lower than that (8.0%) in the control group ($P = 0.011$, OR=0.370, 95% CI=0.168~0.814) See Table 3.

Association Analysis of MMP-2,3,9 Polymorphisms in the Subtypes of IS

Correlation analysis between genotypes and the subtypes of IS was performed. The distribution of T allele (9.9%) of *MMP-2 C1306T* in SAO subgroup was significantly lower than that (16.7%) in control group. (*P*=0.012, OR=0.550, 95% CI=0.343~0.883). And the 5A/5A genotypes of *MMP-3-1612* in LAA group (63.5%) was significantly higher than (8.0%) that in control group (*P*=0.001, OR=2.345, 95% CI=1.876~2.964). See Table 4.

Table 2 Clinical Parameters of Patients and Controls

Clinical Parameters	Patients	Controls	Statistical Analysis	*P* value
Male / Female (ratio)[b]	163/137	145/155	2.162	0.142
Age(years)[a]	59.69±12.55	58.69±11.71	1.002	0.317
CHO (mmol/L, x±s)	4.47±1.15	4.42±0.84	0.638	0.524
TG (mmol/L, x±s)	1.70±1.32	1.76±1.61	0.527	0.590
Glu (mmol/L, x±s)	6.37±2.65	5.51±1.72	4.69	<0.01*
HDL (mmol/L, x±s)	1.05±0.43	1.19±0.35	4.402	<0.01*
LDL (mmol/L, x±s)	2.62±1.06	2.67±0.91	0.546	0.586
Hcy (mmol/L, x±s)	17.95±9.09	15.52±6.29	3.806	<0.01*
Number of hypertension (n,%)[b]	97 (32.3)	21 (7)	59.34	<0.01*

Notes: [a]Paired *t*-test; [b]χ^2 test. *$P < 0.05$ denotes statistical significance.
Abbreviations: TG, triglyceride; LDL, low-density lipoprotein; HDL, high-density lipoprotein; CHO, cholesterol.

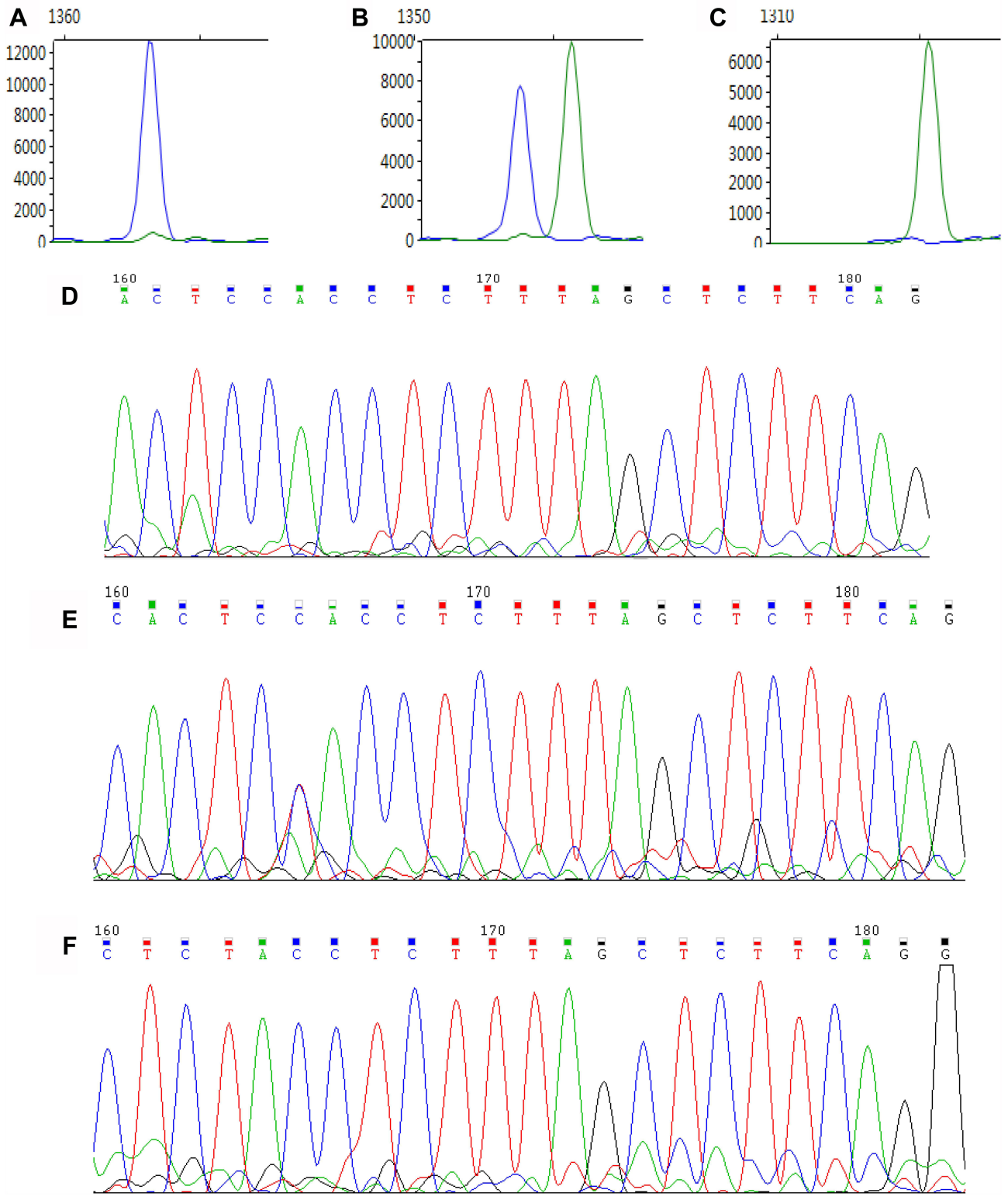

Figure 1 Results of SNaPshot microsequencing and DNA sequencing of *MMP-2*. (**A**–**C**) Results of SNaPshot microsequencing of *MMP-2*. (**A**) The results of SNaPshot microsequencing of CC genotype. (**B**) The results of SNaPshot microsequencing of CT genotype. (**C**) The results of DNA sequencing of TT genotypes. (**D**–**F**) Results of DNA sequencing of *MMP-2*. (**D**) The DNA sequencing of the CC genotype of *MMP-2*. (**E**) The DNA sequencing of the CT genotype of *MMP-2*. (**F**) The DNA sequencing of the TT genotype of *MMP-2*.

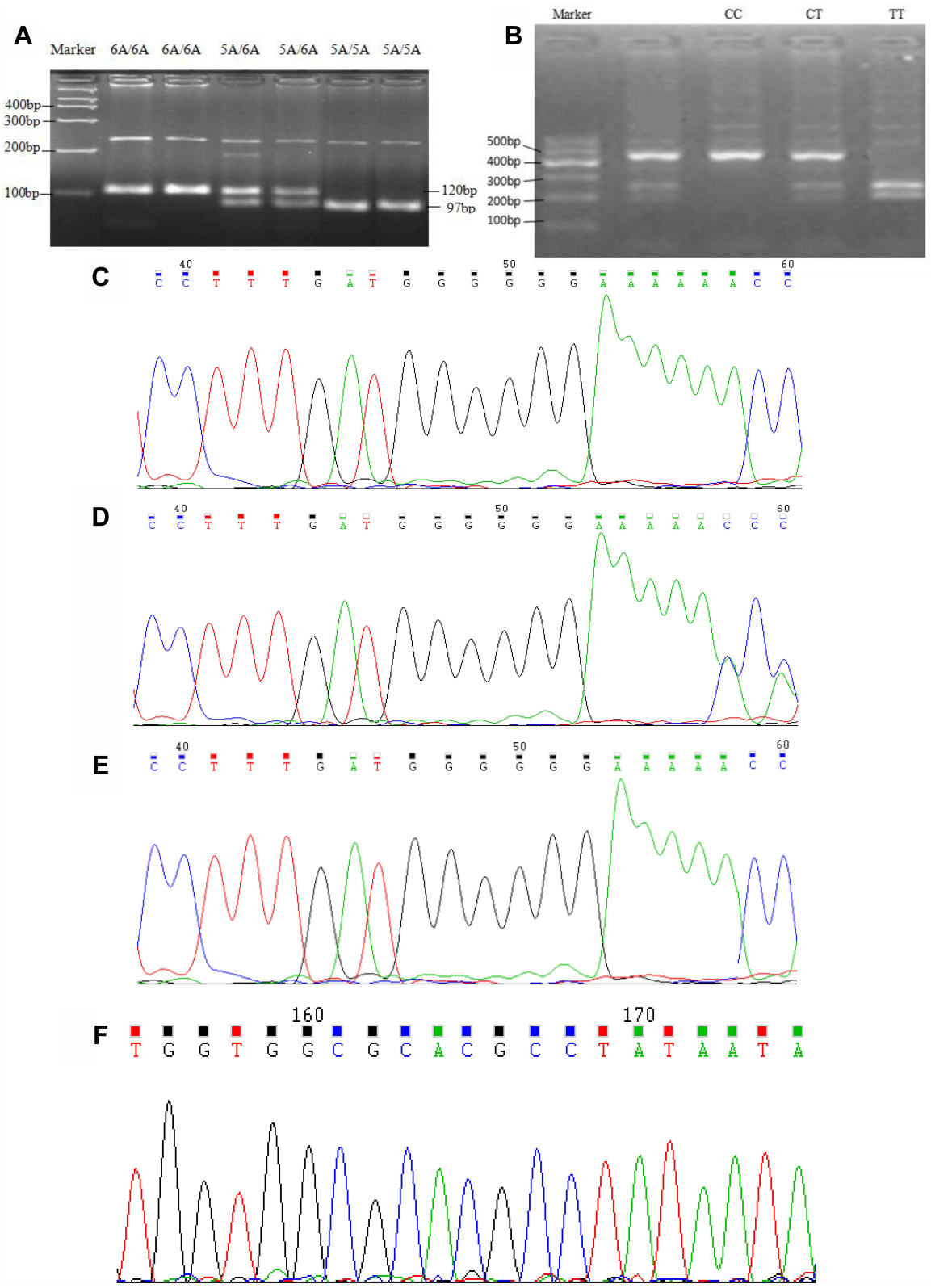

Figure 2 Continued.

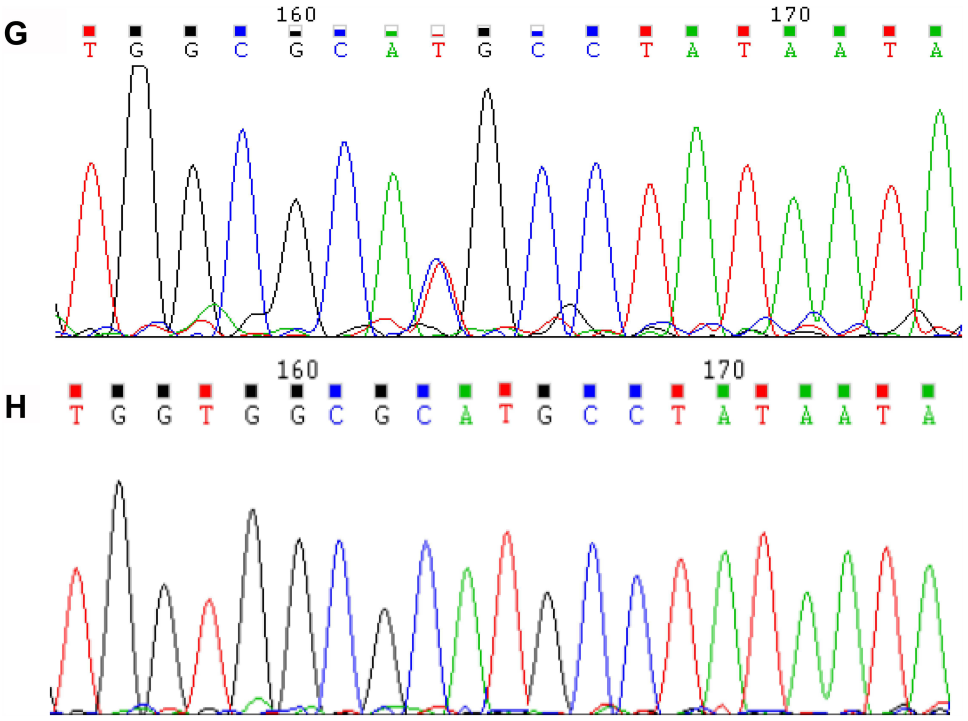

Figure 2 Results of electrophoretic map and DNA sequencing of *MMP-3,9*. (**A**) The results of electrophoretic map of *MMP-3*. (**B**) The results of electrophoretic map of *MMP-9*. (**C–E**) Results of DNA sequencing of *MMP-3*. (**C**) The results of DNA sequencing of 6A/6A genotype. (**D**) The results of DNA sequencing of 5A/6A genotype. (**E**) The results of DNA sequencing of 5A/5A genotype. (**F–H**) Results of DNA sequencing of *MMP-9*. (**F**) The results of DNA sequencing of CC genotype. (**G**) The results of DNA sequencing of CT genotype. (**H**) The results of DNA sequencing of TT genotype.

Logistic Regression Analysis

Stepwise logistic regression analysis was conducted to find the effect of the risk factors on IS. From the results of stepwise logistic regression analysis, a positive correlation was observed between cerebral infarction and *MMP-2-C1306T, MMP-3-1612* polymorphism (*P*=0.000, 0.002 95% CI=0.494–0.584, 1.260–5.591). And smoking, hypertension, diabetes, hyperlipidemia are the risk factors as well. See Table 5.

Table 3 Frequencies of MMPs-2,3,9 Genotype and Alleles in Controls and Patients

Gene	Patient (n, %)	Control (n, %)	*P* value	OR (95% CI)
MMP-2-1306T				
Genotypes				
CC	240 (80.0)	215 (71.7)		
CT	55 (18.3)	70 (23.3)	0.083	0.704 (0.473~1.048)
TT	5 (1.7)	15 (5.0)	0.015*	0.299 (0.107~0.835)
Alleles frequencies				
C	535 (89.2)	500 (83.3)		
T	65 (10.8)	100 (16.7)	0.003*	0.607 (0.434~0.849)
MMP-3-1612				
Genotypes				
6A/6A	216 (72.0)	213 (71.0)		
5A/6A	39 (25.0)	75 (21.0)	0.414	1.174 (0.799~1.725)
5A/5A	45(3.0)	12 (8.0)	0.011*	0.370 (0.168~0.814)

(*Continued*)

Table 3 (Continued).

Gene	Patient (n, %)	Control (n, %)	*P* value	OR (95% CI)
Alleles frequencies				
6A	471(84.5)	501 (81.5)		
5A	84 (15.5)	87 (18.5)	0.167	0.808 (0.597~1.093)
MMP-9 −1562				
Genotypes				
CC	201 (67.0)	221 (73.6)		
CT	95 (31.7)	76 (25.3)	0.080	1.374 (0.962~1.964)
TT	4 (1.3)	3 (1.0)	0.617	1.466 (0.324~6.630)
Alleles frequencies				
C	497 (82.8)	518 (86.3)		
T	103 (17.2)	82 (13.7)	0.093	1.309 (0.955~1.794)

Note: *$P < 0.05$ denotes statistical significance.
Abbreviation: OR (95% CI), 95% confidence interval of OR value.

Discussion

Matrix metalloproteinases (MMPs) are a group of neutral proteases derived from the same source and dependent on Zn^{2+}, which can degrade the components of extracellular matrix.[12] While many complicated elements of extracellular matrix involve in the process of vascular remodeling, and the disorder of MMPs expression results in imbalanced of components of extracellular matrix, which leads to vascular remodeling and dysfunction.[13] When arterial endothelium dysfunction, especially in cerebral and carotid artery, it may lead to vascular stenosis affecting normal bloodstream then induce cerebral ischemia and ischemic stroke.[14]

In the present study, the gene polymorphism of *MMP −2*(C1306T), −3(1612–5A/6A), *−9* (C-1562T) were analyzed between IS group and control group. The results showed that TT genotype and T allele of *MMP-2 C1306T* site was negatively correlated with the risk of IS ($P<0.05$). Multivariate logistic regression analysis showed that the T allele was associated with a 0.652-fold increased risk of IS after adjusting for conventional risk factors (95% CI=0.494~0.584; $P=0.000$). This means that individuals with TT genotype or T allele in C1306T of *MMP*-2 have a reduced risk of IS, and the T allele of *MMP*-2 may be a protective factor of IS. Lorenzano et al[15] also reported that T allele in *MMP*-2 have lower risk of IS than those people who without T allele. In addition, Carcel-Marquez et al[16] have also reported that C allele caused up-regulation of *MMP-2*, which led to cerebral arteriosclerosis and caused IS, while T allele of *MMP-2* may contribute to the reduction of cerebral hemorrhage associated with thrombolysis and endovascular treatment.[17] As for *MMP-3*, we found that the 5A/5A genotype was positively correlated with the risk of IS ($P<0.05$), and the results of logistic regression analysis showed that there was remained an association between 5A/5A genotype and increased risk of IS after adjusting for conventional risk factors ($P=0.002$, 95% CI=1.260~5.591). We suggested that 5A/5A of *MMP-3* may increase the risk of IS, which was consistent with the conclusion reported by Feng et al.[18] They found that 5A allele has higher promoter and transcriptional activity than 6A allele, thus 5A homozygous will lead to an increased expression of *MMP-3* and participate in the pathological process of IS. While, Johnston et al[19] concluded that homozygosity for the 6A allele of *MMP-3* promoter will lead to acute ischemic stroke, which was different from our results. The difference may be caused by the small sample size and different populations, so it is necessary to increase the sample size to conduct further research on different populations.[20]

According to TOAST classification standard, IS group was divided into LAA, SAO and SOE types.[21] We performed a stratified analysis to find the association between the types and *MMP-2,3,9* gene polymorphisms. It was found that the distribution of the T allele of *MMP-2 C1306T* was significantly different between the SAO subgroup and the controls ($P=0.012$ OR=0.550 95% CI=0.343~0.883). T allele of *MMP-2* was associated with IS, especially with SAO subtype. However, there are few studies about the relationship between T allele of *MMP-2*

Table 4 Compare of MMP-2,3,9 Polymorphism Genotype and Subtype of Cerebral Infarction

Genotype / Alleles	Control (n, %)	LAA			SAO			SOE		
		n=117	*P* value	*OR (95% CI)*	n=168	*P* value	OR (95% CI)	n=15	*P* value	OR (95% CI)
MMP-2-1306T										
CC	215 (71.7)	91 (78.3)			137 (81.8)			12 (81.5)		
CT	70 (23.3)	24 (19.7)			28 (16.5)			3 (18.5)		
TT	15 (5.0)	2 (1.2)			3 (1.7)			0 (0)		
CT vs CC	1.00		0.298	0.774 (0.478~1.255)		0.088	0.620 (0.358~1.077)		0.482	0.698 (0.255~1.912)
TT vs CC	1.00		0.100	0.361 (0.103~1.273)		0.085	0.290 (0.065~1.291)		0.217	0.309 (0.018~8.338)
T alleles (%)	16.7	11.8	0.055	0.672 (0.446~1.011)	9.9	0.012*	0.550 (0.343~0.883)	9.3	0.156	0.510 (0.198~1.312)
MMP-3 −1612										
6A/6A	213 (71.0)	88 (75.2)			119 (70.8)			9 (60.0)		
5A/6A	75 (21.0)	11 (20.5)			15 (27.4)			13 (33.3)		
5A/5A	12 (8.0)	12 (4.3)			18 (1.8)			15 (6.7)		
5A/6A vs 6A/6A	1.00		0.345	0.234 (0.145 ~0.569)		0.234	0.356(0.161 ~0.682)		0.767	0.798 (0.207 ~1.307)
5A/5A vs 6A/6A	1.00		0.001*	2.345 (1.876~2.964)		0.560	0.224 (0.066~0.759)		0.990	0.986 (0.120~8.123)
MMP-9 −1562										
CC	221 (73.6)	78 (66.7)			111 (66.1)			12 (80.0)		
CT	76 (25.3)	38 (32.5)			54 (32.1)			3 (20.0)		
TT	3 (1.0)	1 (0.8)			3 (1.8)			0 (0.0)		
CT vs CC	1.00		0.143	1.417 (0.888~2.260)		0.102	1.415 (0.933~2.146)		0.627	0.727 (0.200~2.645)
TT vs CC	1.00		0.961	0.944 (0.097~9.214)		0.395	1.991 (0.395~10.025)		0.687	2.531 (0.124~51.741)
T alleles (%)	13.7	17.1	0.208	1.302 (0.862~1.967)	17.9	0.086	1.373 (0.955~1.975)	10.0	0.788	0.702 (0.208~2.366)

Note: *$P < 0.05$ denotes statistical significance.

Abbreviation: OR (95% CI), 95% confidence interval of OR value.

https://doi.org/10.2147/IJGM.S395416 DovePress

Table 5 Effects of Risk Factors of Cerebral Infarction by Stepwise Logistic Regression Analysis

Risk Factors	B	SE	Waldχ^2	*P* value	Exp(B)	95% CI
MMP-2-C1306T	0.375	0.085	17.256	0.000	0. 638	0.494–0. 864
MMP-3-1612	0.034	0.109	0.1	0.002	1.035	0.835–1.280
MMP-9-C1562T	−0.325	0.193	2.82	0.093	0.723	0.494–1.054
Total cholesterol	−0.364	0.091	16.127	0.000	0.695	0.581–0.831
Blood sugar	0.239	0.048	25.087	0.000	1.27	1.156–1.395
Hcy	0.059	0.015	16.029	0.000	1.061	1.030–1.092
Hypertension	1.844	0.2691	47.112	0.000	6.325	3.731–10.711

Abbreviations: B, regression coefficient; SE, standard error; Waldχ^2, χ^2 value of regression coefficient Wald test; Exp(B), estimates of odds ratio (OR); 95% CI, 95% confidence interval.

and SAO subtype, so it is necessary to verify this conclusion with a large sample size. About *MMP-3*, our results of association analysis of the subtypes of IS indicated that 5A/5A genotype of cerebral infarction occurred in LAA subtype (63.5%) was significantly higher than that (8.0%) in controls (*P*=0.001 OR=2.345 95% CI=1.876~2.964). This was consistent with the result of Hafez et al,[22] which showed that in LAA stroke acute phase *MMP - 3-1612* increased significantly due to the expression of 5A allele, speculating that *MMP-3-1612* gene polymorphism was associated with stroke susceptibility LAA type.[23]

Several limitations of our study need to be addressed. First, the sample size of the study was not sufficiently large and there was potential selection bias because the cases and controls were recruited from hospital. Next, we did not test the serum level of *MMPs-2,3,9* and we did not perform the functional characterize action of *MMP*-2,3,9 polymorphism.

In conclusion, a case-control study was designed to find the relationship between polymorphisms of the matrix metalloproteinase gene and acute ischemic stroke. The TT genotype and T allele of *MMP*-2, the 5A/5A genotype of *MMP-3* have significant correlation with AIS. Moreover, *C1306T* of *MMP-2* was associated with the SAO subtype and 1612-5A/6A of *MMP-3* was associated with the LAA subtype respectively. Further studies are needed to identify other potentially causative polymorphisms and gene-gene interactions involved in the matrix metalloproteinase to fully understand the genetics of stroke susceptibility. Moreover, studies with functional evaluation are warranted to confirm our findings.

Abbreviations

95% CI, 95% confidence intervals; HWE, Hardy–Weinberg equilibrium; AIS, acute ischemic stroke; LAA, large-artery atherosclerosis; SAO, small artery occlusion; SOE, stroke of other determined cause; MMPs, matrix metalloproteinases; ORs, odds ratios; SNP, single nucleotide polymorphism.

Ethics Approval and Consent to Participate

The study protocols were in line with the tenets of the Declaration of Helsinki and approved by the Ethics Committee on Human Research of Zhengzhou University and informed written consent was obtained from each participant. All experiments were performed in accordance with relevant guidelines and regulations.

Acknowledgments

We acknowledge the technical assistance of staff members of the department of Neurology of First People's Hospital of Zhengzhou. We also thank all patients and controls for providing blood samples.

Funding

The research reported in this publication was supported by the National Natural Science Foundation of China (grant no. U2004114).

Disclosure

The authors report no conflicts of interest in this work.

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