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Matrix metalloproteinase-2 gene polymorphisms are associated with ischemic stroke in a Hainan population

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Abstract

Ischemic stroke is a complex vascular disease, which has become 1 of the major causes of morbidity and mortality worldwide. More and more data showed that matrix metalloproteinases (MMPs), in particular, MMP-2 are deleterious after ischaemic stroke. This study investigated the relationship between MMP-2 and stroke risk in the Southern Chinese population.

We evaluated single nucleotide polymorphisms (SNP) of MMP-2 in stroke patients in an association study using a case-control design. Six SNPs of MMP2 were selected and genotyped by Agena MassARRAY. SNPStats, Haploview was used to analyze genetic data.

Two SNPs in the MMP-2 gene were significantly associated with stroke risk.

For rs1132896 (C versus G allele), the C allele was significantly reduced stroke risk (OR = 0.56, 95% confidence intervals [95% CI] = 0.39–0.81, $P = .002$). The effect of the T allele of rs243849 was IS risk according to an additive genetic model (OR = 0.67, 95% CI = 0.47–0.96, $P = .028$). We did not found any strong linkage between the six SNPs (rs1132896, rs1053605, rs243849, rs243847, rs243832, rs7201)

The results presented strongly indicate that MMP-2 genetic variants are an important mediator of stroke risk.

Abbreviations: 95% CI = 95% confidence intervals, HWE = Hardy–Weinberg equilibrium, IS = ischemic stroke, LD = linkage disequilibrium, MMPs = matrix metalloproteinases, ORs = odds ratios, SNP = single nucleotide polymorphism.

Keywords: case-control study, Hainan population, ischemic stroke, MMP

1. Introduction

Ischemic stroke (IS) is a complex vascular disease, which has become 1 of the major causes of morbidity and mortality worldwide.[1] The etiology of IS is not clear, and the process of IS is the result of multiple environmental factors, such as hypertension, diabetes, hyperlipidemia, atrial fibrillation, asymptomatic carotid stenosis, drinking and smoking.[2]

However, clinical, environmental, and demographic risk factors do not fully explain disparities in IS disease progression.[3] As with many other diseases, it is the interaction between individual gene composition and environmental exposure.[4] Not all of the individuals who exposed to similar environmental factors would suffer from IS, suggesting that genetic factors play a major role in susceptibility to IS. Currently, some studies reveal that stroke is related to the inflammatory response.[4,5] Inflammatory proteins might play a significant role in the pathogenesis of stroke.[6] Intuitively, it seems that inflammation mediators may play an important role in atherothrombotic stroke. Numerous evidence suggested that matrix metalloproteinases (MMPs) are fundamental players in stroke recovery.[7] The MMP is a family of more than 20 proteinases widely distributed in human tissues. They can be able to degrade almost all of the extracellular matrix proteins, and are essential for cell migration, development, healing processes, scar formation and other tissue changes.[8,9] Besides their functions in healing processes and matrix buildup, Many data suggest that serum levels of MMP2 increase in the stroke.[8,10] There is established that MMPs increases in the human brain after stroke.[11] In experimental models of brain injury, some MMPs are unregulated after ischemia,[12,13] hemorrhage[14] and trauma.[15] Accumulating evidence that MMPs plays an important role in acute brain injury.[16] It has, therefore, become clear that genetic factors assist in the development of IS. MMP polymorphisms have been carried out to establish the IS relationship. MMP polymorphisms have been evaluated in various diseases. The studies have been found in cancer incidence,[17] coronary artery disease,[18] and glaucoma.[19] Not surprisingly, burgeoning research in MMP polymorphisms for various types of populations have been conducted in IS as well. This review will evaluate MMP polymorphisms and provide some insight into their roles in IS incidence and clinical outcome. There are studies that evaluated the association of

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Written informed consent was obtained from all the subjects who participated in this study, and the study protocol was approved by the Haikou People's Hospital and Northwest University.

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MMP2 gene polymorphism with susceptibility to stroke. Still, differences in the incidence, pathogenesis, and clinical outcome have long been noted when comparing IS among patients with different racial and ethnic backgrounds. The aim of this study was to assess the association between the MMP-2 gene polymorphism and risk of stroke in Chinese Han population.

2. Materials and methods

2.1. Subjects

The study participants comprised 250 unrelated patients who were admitted to the Haikou People's Hospital. Cases were patients who had a first-event myocardial infarction (MI) or IS. Patients were diagnosed to be IS 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 computed tomography (CT) or Magnetic Resonance Imaging (MRI) according to the diagnostic criteria of IS from World Health Organization. Controls (n = 250) were matched for age and gender, who had no history of IS. Written informed consent was obtained from all the subjects who participated in this study, and the study protocol was approved by the Haikou People's Hospital and Northwest University.

2.2. Genotyping

For the present study, 500 samples with relevant clinical data and a DNA sample were available. DNA was isolated from whole blood were used the GoldMag-Mini Whole Blood Genomic DNA Purification Kit (GoldMag Co. Ltd. Xi'an City, China) extracted. The primer is listed in Table 1. 6 single nucleotide polymorphisms (SNPs) in MMP-2 were genotyped using the Agena iPLEX assays with allele detection by mass spectroscopy, using Sequenom MassARRAY technology (Agena Bioscience, San Diego, CA) and following the manufacturer's protocol.[20] In this study, we used Agena MassARRAY Assay Design 3.0 Software to design a Multiplexed SNP MassEXTEND assay. The PCR primers for each SNP are shown in Table 1. Data management and analysis were performed using the Agena Typer 4.0 Software (Agena Bioscience, San Diego, CA).

2.3. Statistical analysis

Statistical analysis was done with the variety of statistical software. Hardy–Weinberg equilibrium (HWE) was calculated using a Chi-squared test. Allele frequencies, odds ratios (ORs) and their 95% confidence intervals (95% CI) were calculated.[21] Comparison of allele frequencies was done using contingency table with a chi-square test. To determine whether SNPs of MMP2 were associated with susceptibility to stroke, multiple logistic regression analysis while adjusting for age and gender was conducted. Multiple logistic regression models (codominant models,

Dominant models, recessive models and additive model) were conducted to determine the OR, 95% CI, and P value, while controlling for age and gender as covariables. Haploview version 4.2 was used to identify the linkage disequilibrium (LD) block and haplotypes.[22] The significance level for all statistical analyses was 0.05.

Table 2

Demographic characteristics of patients with ischemic stroke and control subjects.

	Case	Control	*P*
	250	250	
Gender			.193
Male	167	152	
Female	83	98	
Age	64.13.27 ± 10.98	48.31 ± 12.31	.063

P value < .05 indicates statistical significance.

3. Results

This study consisted of 500 subjects, with 250 cases and 250 controls. The case group included 167 males and 83 females with the sex ratio of 2.01:1 and the mean age was 63.56 ± 9.83. The control group included 152 males and 98 females, in which the sex ratio was 1.55:1 and the mean age was 64.13 ± 10.22. The demographic and clinical characteristics of all studied subjects are summarized in Table 2. The age and gender between 2 groups had no statistically significant difference ($P > .05$). The prevalence of MMP2 SNPs genotype and allele frequencies in stroke patients and controls are presented in Table 3. The genotype distribution among the controls

were in HWE ($P > .05$). For rs1132896 (C versus G allele), the C allele was significantly reduced stroke risk (OR 0.56, 95% CI = 0.39–0.81, $P = .002$). In Table 4, the inverse association C allele of the rs1132896 polymorphism might decrease risk of stroke. Compared with control group, the decrease was 0.57-fold for stroke patients carrying GG genotype in codominant model and 0.53-fold in dominant model. The effect of the T allele of rs243849 was IS risk according to an additive genetic model. In codominant model, CT versus CC in the rs243849 was associated with a greater reduce the risk of IS (OR = 0.56, 95% CI = 0.35–0.89, $P = .042$).CT/TT versus CC was decreased the IS risk in dominant model (OR = 0.57, 95% CI = 0.37–0.89, $P = .012$). Six SNPs were analyzed for LD and haplotypes using Haploview 4.2. There is no strong LD block was constructed among 6 SNPs

Table 1

Primers used for this study.

SNP	Band	Alleles A/B	Gene (s)	1st-PCR	2nd-PCR	UEP-SEQ
rs243849	16q12.2	C/G	MMP2	ACGTTGGATGTACCTTGGTCAGGGCAGAAG	ACGTTGGATGAGTGACGGAAAGATGTGGTG	ACAGCCAACTACGATGA
rs1132896	16q12.2	T/C	MMP2	ACGTTGGATGTCAGTGCAGCTGTTGTACTC	ACGTTGGATGTCACTCTTTAGTGGTCCGTG	TTAGTGGTCCGTGTGAAGTATGG
rs7201	16q12.2	T/C	MMP2	ACGTTGGATGTCCAATCCCACCAACCCTCA	ACGTTGGATGGCAGGGCTGCGTTGAAAATA	aAGGGCTGCGTTGAAAATATCAAAG
rs1053605	16q12.2	C/T	MMP2	ACGTTGGATGCTCAAAGTTGTAGGTGGTGG	ACGTTGGATGAAGGAGTACAACAGCTGCAC	AACAGCTGCACTGATAC
rs243847	16q12.2	C/G	MMP2	ACGTTGGATGAAGACAAGAGCAGTGACCCC	ACGTTGGATGCCAAAATCAGACCCTGGTAG	ccTGCTGCTACTCACCTCC
rs243832	16q12.2	C/A	MMP2	ACGTTGGATGCCTATGCCAGGCAGAAATTC	ACGTTGGATGGAGAAAGAAGAGACCGTGAC	ACATTCTGGCACACAGAAG

UEP-SEQ = single nucleotide primer extension.

Table 3

Basic information of SNPs in this study.

SNP	Band	Alleles A/B	Gene (s)	MAF (Case)	MAF (Control)	HWE-P	OR	95%CI	*P*
rs1132896	16q12.2	C/G	MMP2	0.108	0.177	0.08	0.56	0.39–0.81	.002
rs1053605	16q12.2	T/C	MMP2	0.153	0.137	1.00	1.13	0.80–1.61	.487
rs243849	16q12.2	T/C	MMP2	0.228	0.255	0.32	0.86	0.65–1.15	.319
rs243847	16q12.2	C/T	MMP2	0.418	0.367	0.79	1.24	0.96–1.59	.102
rs243832	16q12.2	C/G	MMP2	0.366	0.349	1.00	1.07	0.83–1.39	.584
rs7201	16q12.2	C/A	MMP2	0.232	0.233	0.10	0.99	0.74–1.33	.952

P value was calculated by Pearson χ^2 test; *P* value < .05 indicates statistical significance.
CI = confidence interval, HWE = Hardy–Weinberg equilibrium, MAF = minor allele frequency, OR = odds ratio, SNPs = single nucleotide polymorphisms.

Table 4

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

SNP	Model	Genotype	control	case	OR (95% CI)	*P* value	AIC	BIC
rs1132896	Codominant	G/G	173 (69.5%)	199 (79.6%)	1	.028	514.8	535.8
		C/G	64 (25.7%)	48 (19.2%)	0.57 (0.34–0.95)			
		C/C	12 (4.8%)	3 (1.2%)	0.29 (0.06–1.30)			
	Dominant	G/G	173 (69.5%)	199 (79.6%)	1	.012	513.5	530.4
		C/G-C/C	76 (30.5%)	51 (20.4%)	0.53 (0.32–0.87)			
	Recessive	G/G-C/G	237 (95.2%)	247 (98.8%)	1	.11	517.4	534.3
		C/C	12 (4.8%)	3 (1.2%)	0.32 (0.07–1.46)			
	Log-additive	—	—	—	0.56 (0.36–0.86)	.0075	512.8	529.6
rs243849	Codominant	C/C	135 (54.2%)	154 (61.6%)	1	.042	515.6	536.6
		C/T	101 (40.6%)	78 (31.2%)	0.56 (0.35–0.89)			
		T/T	13 (5.2%)	18 (7.2%)	0.65 (0.26–1.63)			
	Dominant	C/C	135 (54.2%)	154 (61.6%)	1	.012	513.7	530.5
		C/T-T/T	114 (45.8%)	96 (38.4%)	0.57 (0.37–0.89)			
	Recessive	C/C-C/T	236 (94.8%)	232 (92.8%)	1	.64	519.7	536.6
		T/T	13 (5.2%)	18 (7.2%)	0.81 (0.33–1.98)			
	Log-additive	—	—	—	0.67 (0.47–0.96)	.028	515.1	531.9

P value < .05 indicates statistical significance.
AIC = Akaike information criterion, BIC = Bayesian information criterions, CI = confidence interval, OR = odds ratio, SNPs = single nucleotide polymorphisms.

4. Discussion

In the present study, we identified an association between genetic polymorphism in MMP2 gene and the risk of IS. The main finding is that the C allele of the rs1132896 and the T allele of rs243849 polymorphism might decrease risk of IS.

MMPs belong to the family of zinc-binding proteolytic enzymes, which normally remodel the extracellular matrix. MMPs involve in many physiological processes, such as cell growth, proliferation, differentiation, migration, apoptosis, and cell interactions.[23,24] MMPs are secreted in the form of inactive proenzymes and obtain activity when they are cracked by extracellular proteinases.[25,26] A large number of studies have verified that the genetic polymorphisms within the MMP genes that alter expression levels of the enzymes, implying a possible role in IS development. In particular, MMP-2 is an important member in MMPs family encoded by *MMP-2* gene and plays a leading role in the lesion of blood-brain barriers.[27] A number of studies have shown that *MMP-2* is involved in the formation, migration, fracture collapse of atheromatous plaque, and cerebral ischemia-reperfusion, hemorrhagic transformation, and neuron apoptosis through degrading ECM,[28,29] which may be closely related to the occurrence and development of stroke.

Currently, some studies reveal that MMP2 polymorphism is correlated with an elevated risk of IS, and most study focuses on the rs243865, rs243864.[30–33] In our study we selected rs1132896, rs1053605, rs243849, rs243847, rs243832, rs7201 in MMP2 gene. We found 2 SNPs (rs1132896, rs243849) were associated with IS, similar results were found in Manso et al[34] study that rs243849 was significantly associated with stroke outcome in a Portuguese population sample. Marc Fatar et al[35] research found rs7201were associated with IS risk in Caucasian, however, our results did not find significant differences. For rs1132896, rs1053605, rs243847, rs243832 we did not found any significant results, and other study other studies have found no correlation between these SNPS and stroke risk. Further studies on these SNPs were related to obesity,[36] high myopia,[37] New-Onset Diabetes et al.[38]

There were 2 limitations in our study. First, some other genetic polymorphisms may have a role in the development of ischaemic stroke, but our study only investigated the association of MMP2 gene polymorphisms with the development of IS. Second, the sample size is relatively small, which may limit the statistical power to find the differences between groups.

In conclusion, our study suggests that MMP2 polymorphism is correlated with an elevated risk of IS in Southern Chinese population. Future studies with larger sample size may contribute to elucidate the impact of these polymorphisms on the risk of IS.

Author contributions

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