Original Article

Association of MMP-9 gene polymorphisms with acute coronary syndrome in the Uygur population of China

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BACKGROUND: Matrix metalloproteinase-9 (MMP-9) plays a pivotal role in early atherosclerosis, vascular remodeling and development of atherosclerotic lesion. The potentially functional MMP-9 gene polymorphism may contribute to the susceptibility of acute coronary syndrome (ACS). This study aimed to investigate the association between two single nucleotide polymorphisms (−1562C>T, R279Q) of the MMP-9 gene in patients with ACS in the Uygur population of China.

METHODS: This case-control study was composed of 361 ACS patients and 432 control subjects, who had undergone coronary angiography. Among the ACS patients, 162 (44.9%) had single-vessel disease, 145 (40.2%) had two-vessel disease, and 54 (14.9%) had three-vessel disease. The genotypes of the two selected SNPs were determined by the method of polymerase chain reaction and restriction fragment length polymorphism (RFLP-PCR). The relationship between the polymorphism of the MMP-9 gene and the severity of coronary arterial stenosis was analyzed.

RESULTS: Analysis of the two SNPs showed that the frequency of CT and TT genotypes in patients with ACS was significantly higher than that in the control group (ACS vs. controls; CT+TT: 25.5% vs. 15.8%, $P=0.001$). And the −1562 gene allele (C/T) was significantly associated with acute coronary syndrome (ACS vs. controls; C allele: 85.7% vs. 91.5%, T allele: 14.3% vs. 8.5%, $P<0.001$). But the frequencies of CT+TT and CC genotypes were not statistically different among ACS patients with one, two and three or more significantly diseased vessels ($P=0.55$). The R279Q polymorphism site with regard to the association with ACS was not significant ($P>0.05$). The presence of CT or TT genotypes, assuming codominant effect of the T allele, was independently associated with increased risk of coronary artery disease when adjustment was made for age, body mass index, smoking, hypertension and diabetes mellitus [odds ratio=1.737 (95% confidence interval, 1.337-2.257), $P=0.018$].

CONCLUSIONS: MMP-9-1562C>T polymorphism is associated with the susceptibility to ACS in the Uygur population of China. However, this mutation apparently is not related to the severity of coronary arterial stenosis. Another SNP (R279Q) polymorphism of MMP-9 is not significantly associated with the risk of ACS.

KEY WORDS: Matrix metalloproteinase-9; Acute coronary syndrome; Uygur; Gene polymorphism

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INTRODUCTION

Acute coronary syndrome (ACS) consists of unstable angina pectoris, non-ST segment elevation myocardial infarction and ST segment elevation myocardial infarction. The pathogenic mechanism of ACS is

most often based on thrombosis secondary to plaque rupture in atherosclerosis (AS).^[1] In many countries including China, ACS is a main cause of mortality and morbidity. ACS is mainly caused by coronary atherosclerotic plaque rupture or erosion and subsequent

intracoronary thrombus formation.^[2,3] Age, gender, smoking, hypertension, hypercholesterolemia, diabetes mellitus, obesity and sedentary lifestyle are reported to be associated with ACS,^[4-6] but the exact mechanism of ACS is still not clear. There is a genetic association between polymorphic variants in candidate genes and atherosclerosis. The matrix metalloproteinase (MMP) family is one of the potential candidate gene systems.

Proteins of the matrix metalloproteinase (MMP) family are involved in the breakdown of the extracellular matrix in normal physiological processes, such as embryonic development, reproduction, and tissue remodeling, as well as in disease processes, such as arthritis and metastasis.^[7] Most MMPs are secreted as inactive proproteins which are activated when cleaved by extracellular proteinases. Henney et al^[8] reported that genetic change which affects the expression of MMPs may contribute to the occurrence of cardiovascular disease. Matrix metalloproteinase 9 (MMP-9), also known as 92 kDa type IV collagenase, 92 kDa gelatinase or gelatinase B (GELB), is an enzyme that in humans is encoded by the MMP9 gene. MMP-9 is highly expressed in the vulnerable regions of atherosclerotic plaques. For this reason, MMP-9 plays a key role in vascular remodeling and development of atherosclerotic lesion, and plays a potential role in arterial plaque rupture.^[9,10] Epidemiological studies^[11,12] showed that MMP-9 levels increased in the circulation of patients with ACS. The potentially functional MMP-9 gene polymorphisms may contribute to the susceptibility of ACS. Two polymorphisms of MMP-9 (-1562C>T, R279Q) were included in this study. The study aimed to investigate the association between MMP-9 gene polymorphism and ACS in a Uygur population of Xinjiang, China.

METHODS

Study population

The study was designed as a case-control study. A total of 793 Uygur patients were recruited from January 2006 to March 2010 in the First Hospital of Xinjiang Medical University, Urumqi, China. The study group consisted of 361 patients with ACS (106 females and 255 males) confirmed by coronary arteriography, with a mean age of 61.53 ± 10.47 years. All the patients were diagnosed according to their medical history, clinical symptoms, 12-lead electrocardiogram, and laboratory examinations, and were confirmed by coronary arteriography ($\geq 50\%$ stenosis affected at least one major

coronary vessel). Recorded were the data including lipid profile, smoking habit, blood glucose, body mass index, history of hypertension (systolic blood pressure ≥ 140 mmHg, diastolic blood pressure ≥ 90 mmHg, or both), diabetes mellitus and family history of CAD.

Totally 432 patients (170 females and 262 males) served as a control group, with a mean age of 59.56 ± 9.52 years. They had normal electrocardiograph, normal blood chemistry values, normal coronary arteriography, no history of heart disease, and no chest pain. All patients were genetically-unrelated ethnic Uygur people from Xinjiang Uygur Autonomous Region. Each patient was interviewed after informed consent was obtained.

The study was approved by the Ethics Committee of the First Hospital Affiliated to Xinjiang Medical University. Neither the patients nor the controls had congenital heart disease, rheumatic heart disease, heart failure, multiple organ failure and other general illnesses.

Coronary angiography

All patients received routine biplane coronary angiography using the Judkins technique. Coronary angiograms were evaluated by two experienced cardiologists. Coronary artery disease (CAD) was defined as the presence of $\geq 50\%$ stenosis in at least one major coronary artery. The extent of ACS was defined according to the number of major coronary arteries affected, namely, one-vessel, two-vessel, and three-vessel disease. The number of stenotic coronary vessels was used for assessing the severity of coronary atherosclerosis.

Laboratory examination

Five milliliter venous blood samples were obtained from all patients at 12 hours after fasting, and were put into EDTA tubes and stored at -80°C for use. The serum concentrations of triglyceride (TG), total cholesterol (TC), low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C), and blood glucose were measured by the standard methods used in the clinical laboratory of the hospital.

MMP-9 genotyping

Blood samples were collected using a venipuncture technique, and were put into EDTA-containing tubes. DNA was extracted from peripheral vein blood leukocytes using a whole blood genome extraction kit (Boiteke Corporation, Beijing, China). Primers were designed by the Primer Premier 5.0 software (PREMIER Biosoft International, Vancouver, Canada). Syntheses were performed by the Shanghai Biological Engineering

Table 1. PCR primer sets and conditions for the MMP-9 gene

Polymorphism (dbSNP No.)	Primer sequence (sense/antisense)	Initial denature	Denature	Annealing	Extension	Cycles	Final incubation
-1562C>T (rs3918242)	F:5'-GCCTGGCACATAGTAGGCC-3' R:5'-CTTCCTAGCCAGCCGGCATC-3'	95 °C 5 min	94 °C 30 s	64.5 °C 30 s	72 °C 45 s	35	72 °C 10 min
R279Q (rs17576)	F:5'-ATGGGTCAAAGAACAGGA-3' R:5'-GGTAGACAGGGTGGAGG-3'	95 °C 5 min	94 °C 30 s	58 °C 30 s	72 °C 30 s	30	72 °C 7 min

PCR: polymerase chain reaction

Table 2. Restriction enzymes, conditions and product lengths for analysis of the MMP-9 gene

Polymorphism	Restriction enzyme	Conditions	Fragment length (bp)
-1562C>T (rs3918242)	SphI	37 °C for 16 h: PCR product 8 µL, 10×buffer 2 µL, H ₂ O ₂ 10 µL, SphI 2 U	CC 435/435 CT 435/188, 247 TT 188, 247/188, 247
R279Q (rs17576)	SmaI	37 °C for 16 h: PCR product 8 µL, 10×buffer 2 µL, H ₂ O ₂ 10 µL, SmaI 2 U	GG 96, 181/96, 181 GA 96, 181bp/277 AA 277/277

Table 3. Clinical characteristics of the ACS group and the control group

Variables	ACS group (n=361)	Control group (n=432)	P value
Age (years)	61.53±10.47	59.56±9.52	0.204
TC (mmol/L)	4.41±1.16	4.16±0.99	0.010
TG (mmol/L)	1.97±1.08	1.94±0.65	0.753
LDL-C (mmol/L)	2.46±0.84	2.34±0.75	0.170
HDL-C (mmol/L)	0.97±0.25	1.31±0.67	<0.001
BMI (kg/m ²)	27.10±3.59	24.99±3.17	<0.001
Smoking (no. %)	169 (46.8)	140 (32.4)	<0.001
Drinking (no. %)	135 (37.4)	173 (40.0)	0.446
Diabetes mellitus (no. %)	75 (20.8)	43 (10.0)	<0.001
Hypertension (no. %)	171 (47.4)	141 (32.6)	<0.001

Company Limited. The reaction was performed in a 25 µL final volume and contained 1 µL each primer (10 pmol/µL), 12.5 µL 2× Power TaqPCR Master Mix (Boiteke Corporation, Beijing, China) and 1 µL genomic DNA. The PCR products of -1562C>T and R279Q polymorphism sites were digested with the restriction enzyme SphI and SmaI (Fermentas, Lithuania) at 37 °C for 16 hours, separated by electrophoresis on a 2% agarose gel, and visualized by ethidium bromide.

The MMP-9-1562C allele was not cut; it produced a 436-base pair (bp) fragment, and the MMP-9 -1562T allele was cut into fragments of 194 and 242 bp. The MMP-9 279R allele was not cut, but it produced a 277-bp fragment, and the MMP-9 279Q allele was cut into fragments of 181 and 96 bp. Detailed descriptions of the methods are summarized in Tables 1 and 2.

Statistical analysis

All data were analyzed using SPSS for Windows 13.0 (SPSS Inc, Chicago, Illinois, USA). The polymorphisms

were tested for confirmation with Hardy-Weinberg expectations in the ACS group and control group. Differences in demographic characteristics, selected variables and frequencies of the genotypes, alleles of the two MMP-9 polymorphisms between the two groups were evaluated using the chi-square test (for categorical variables) and Student's *t* test (for continuous variables). The association between MMP-9 variant genotypes and ACS risk was estimated by odds ratios (ORs) and their 95% confidence intervals (CIs) using univariate analysis. The factors associated with ACS were evaluated using multifactor logistic regression. A *P* value less than 0.05 was considered statistically significant.

RESULTS

Clinical characteristics

The comparison of clinical characteristics between the ACS group and the control group is shown in Table 3. There were no significant differences in age, triglyceride, LDL-cholesterol and drinking between the two groups (*P*>0.05). However, total cholesterol and body mass index were significantly higher in the ACS group than in the control group (*P*=0.01 and *P*<0.001, respectively), whereas HDL-cholesterol was significantly lower in the ACS group than in the control group (*P*<0.001). There were significantly higher percentages of smokers and patients with diabetes mellitus, hypertension in the ACS group (*P*<0.001).

MMP-9-1562C>T and R279Q allele frequency and genotype distribution

Table 4 shows the distribution of genotypes and the prevalence of alleles in the ACS group and the control

group. The distribution of genotypes was not significantly different from the Hardy-Weinberg equilibrium in both groups ($P>0.05$). The frequencies of C/C, C/T and T/T of MMP-9 (-1562C>T) polymorphism were 74.5%, 22.4% and 3.1% in the ACS group, and 84.2%, 14.7% and 1.1% in the control group ($P=0.002$). The homozygote TT and the heterozygote CT were more frequent in the ACS group, and the -1562T allele frequency was significantly higher in the ACS group than in the control group (25.5% vs. 15.8%, $P=0.001$ and 14.3% vs. 8.5%, $P<0.001$, respectively). There were no significant differences in genotypes and allele distribution of the MMP-9 gene R279Q polymorphism between the two groups ($P>0.05$). We found that the patients with CT or TT genotype had a higher risk of ACS (vs. CC genotype; CT: $OR=1.73$, $P=0.003$; TT: $OR=3.01$, $P=0.034$). Patients with T allele

had an increased risk of ACS ($OR=1.82$, $P=0.001$).

Association of MMP-9 (-1562C>T) polymorphism with the severity of coronary atherosclerosis

As shown in Table 5, the percentages of patients with two-vessel and triple-vessel disease were higher in the C/T and T/T genotype class (44.6%, 15.2%) than in the C/C class (38.6%, 14.9%). However, the frequencies of CC, CT and TT genotypes were not significantly different in ACS patients with one, two and three or more diseased vessels ($\chi^2=1.97$, $P=0.55$). In the 361 ACS patients with coronary angiographic data, there was no significant association between MMP-9 (-1562C>T) polymorphism and the severity of coronary atherosclerosis.

Association of MMP-9 genotypes with ACS risk

Multifactor logistic regression analysis showed seven independent risk factors for ACS, including hypertension ($OR=2.983$, 95% CI 1.879-4.734; $P<0.01$), smoking ($OR=2.682$, 95% CI 1.602-4.488; $P<0.001$), diabetes mellitus ($OR=1.915$, 95% CI 1.523-2.451; $P<0.001$), total cholesterol ($OR=1.59$, 95% CI 1.137-2.265; $P=0.006$), body mass index ($OR=1.313$, 95% CI 1.356-2.266; $P<0.001$) and high density lipoprotein cholesterol ($OR=0.229$, 95% CI 0.111-0.472; $P=0.001$). After adjustment for age, body mass index, smoking, diabetes mellitus, hypertension and hypercholesterolemia, the odds ratio for ACS in patients with -1562TT or CT genotype was 1.737 (95% CI 1.337-2.257, $P=0.018$) as compared with those with -1562CC genotype (Table 6).

Table 4. Genotype distribution and allele frequencies of MMP-9 polymorphism between the two groups

MMP-9 polymorphisms	ACS group [n=361(%)]	Control group [n=437(%)]	OR (95% CI)	P value
-1562C>T				
CC	269 (74.5)	368 (84.2)	1	
CT	81 (22.4)	64 (14.7)	1.73 (1.204-2.491)	0.003
TT	11 (3.1)	5 (1.1)	3.01 (1.034-8.763)	0.034
CT+TT	92 (25.5)	69 (15.8)	1.82 (1.286-2.587)	0.001
C allele	619 (85.7)	800 (91.5)	1	
T allele	103 (14.3)	74 (8.5)	1.80 (1.311-2.469)	<0.001
R279Q				
RR	123 (34.1)	162 (37.1)	1	
RQ	152 (42.1)	191 (43.7)	1.05 (0.764-1.439)	0.771
QQ	86 (23.8)	84 (19.2)	1.35 (0.921-1.974)	0.124
RQ+QQ	238 (65.9)	275 (62.9)	1.14 (0.852-1.526)	0.379
R allele	398 (55.1)	515 (58.9)	1	
Q allele	324 (44.9)	359 (41.1)	1.17 (0.957-1.425)	0.127

Table 5. Number of patients with 1, 2, and 3 stenotic coronary arteries according to MMP-9 genotype (no. %)

Genotype	With 1 stenotic vessel	With 2 stenotic vessel	With 3 stenotic vessel	Total number
CC	125 (46.5)	104 (38.6)	40 (14.9)	269
CT+TT	37 (40.2)	41 (44.6)	14 (15.2)	92
Total	162 (44.9)	145 (40.2)	54 (14.9)	361

Table 6. Logistic regression analysis of association between the MMP-9 genotypes and risk of ACS

Risk factors	B	SE	Wald χ^2	P	OR	95% CI
Hypertension	1.093	0.236	9.829	0.007	2.983	1.879-4.734
Smoking	0.986	0.263	14.092	0.000	2.682	1.602-4.488
DM	0.591	0.117	21.876	0.000	1.915	1.523-2.451
-1562T allele	0.552	0.133	5.312	0.018	1.737	1.337-2.257
TC	0.459	0.162	7.447	0.006	1.590	1.137-2.265
BMI	0.257	0.064	18.344	0.000	1.313	1.356-2.266
HDL-C	-1.475	0.369	11.415	0.001	0.229	0.111-0.472

DISCUSSION

This is the first study on the two potentially functional polymorphisms of the MMP-9 gene in relation to ACS susceptibility in the Uyghur population of China. The data of this study demonstrated that the -1562 T allele of the MMP-9 gene is significantly associated with an increased risk of ACS. However, no association was found between R279Q polymorphism and ACS risk in this cohort.

MMP-9 possesses proteolytic activity on type IV collagen, a major constitute of the basement membrane that surrounds every vascular smooth muscle cell and underlies the endothelium in the blood vessel wall.^[13] The human MMP-9 gene is located on chromosome 20q12.2-13.1, and Zhang et al^[14] found a number of single nucleotide polymorphisms (SNPs) in the promoter, coding and untranslated regions. Of these, two polymorphisms, namely promoter -1562C>T

polymorphism and codon 279 polymorphism (R279Q), are of special significance. Functional studies indicate that the -1562C>T polymorphism has an allele-specific effect on MMP-9 transcription. DNA-protein interaction assays have revealed that the sequence between nucleotide position -1567 and -1559 relative to the transcription start site of the MMP9 gene, which encompasses the -1562 polymorphic site, can interact with a nuclear protein, but its mechanism is still unknown.^[15] And codon 279 polymorphism is located in the gelatinase-specific fibronectin type II domain, and can cause an amino acid exchange (arginine [R] to glutamine [Q]) in the catalytic domain, which presumably enhances substrate binding.^[16,17] It is possible that the amino acid conversion is associated with this polymorphism, and affect the activity of this enzyme.

Functional studies indicate that the -1562C>T polymorphism has an allele-specific effect on MMP-9 transcription. In this study we found that -1562CT/TT genotypes were associated with a significantly increased risk of ACS. A genetic epidemiological study^[18] indicated that T-1562 allele carriers are predisposed to the development of coronary atherosclerosis, which causes coronary stenosis. Zhang et al^[15] reported a functional 1562C>T polymorphism in the promoter region of MMP-9. Transfection experiments and DNA-protein interaction assays indicated that the T allele had a higher activity. In addition, Blankenberg et al^[19] reported that plasma MMP-9 levels were also higher in -1562T allele carriers than in non-carriers. A study^[20] on aortic tissues showed that MMP-9 mRNA levels, MMP-9 protein levels and MMP-9 activity were higher in -1562 T allele carriers than in non-carriers. MMP-9 knockout studies^[21,22] in mice also demonstrated a role of MMP-9 in the development of atherosclerosis. Compared with MMP-9 wild-type mice, MMP-9 deficient mice had fewer and smaller atherosclerotic lesions.^[23] Thus, increased vascular smooth muscle migration and macrophage infiltration are likely to explain increased coronary atherosclerosis in carriers of the MMP-9 high expression -1562T allele in humans. These findings suggest that the -1562 C>T polymorphism not only affect the MMP-9 promoter activity *in vitro* experiments, but also influence the MMP-9 transcription *in vivo*, and this effect is translated into differences in MMP-9 protein level and activity between individuals with different MMP-9 genotypes.

There are many polymorphisms in the MMP-9 gene for CAD. Zhang et al^[15] reported that -1562T allele was associated with the severity of coronary atherosclerosis measured by the number of coronary arteries showing

a stenosis more than 50%. Mizon-Gerard et al^[24] found that patients carrying the MMP-9 gene -1562T allele had a higher cardiac mortality rate than non-carriers. Recently, Zhi et al^[25] reported that -1562 CT/TT genotypes may contribute to CAD in diabetics and MI in CAD patients in a Chinese population. Fallah et al^[26] found that the -1562 C>T polymorphism in the MMP-9 gene potentially plays a role in the manifestation of coronary atherosclerosis but does not affect the number of diseased vessels. The results of their study were not consistent. Three published studies on 788 Caucasians, 248 Koreans and 2731 German men with angiographically documented CAD failed to confirm an association with the T allele.^[27-29]

Genetic polymorphisms may vary in different ethnic groups. In our study population, the genotype frequencies of -1562C>T polymorphism for CC, CT and TT were 84.2%, 14.7% and 1.1% respectively in the control subjects, and 74.5%, 22.4% and 3.1% in the ACS patients respectively. The genotype frequencies for CT+TT genotypes and the -1562T allele were significantly higher in the ACS group than in the control group (25.5% vs. 15.8% and 14.3% vs. 8.5%, $P=0.001$ and $P<0.001$, respectively). After adjustment for age, gender, smoking, hypertension, diabetes mellitus and hypercholesterolemia, the T allele carriers had an approximately 1.74-fold higher risk of developing ACS than those with the CC homozygote. It has also been found that MMP-9 is highly expressed in the shoulder regions of advanced atherosclerotic lesions and therefore it is suggested that this potent matrix-degrading enzyme also contributes to plaque instability.^[30-32] Similar to the findings of Fallah et al, we did not find that -1562 C>T polymorphism has any significant effect on the number of diseased vessels.

The 279Q allele was found to be associated with increased MMP-9 levels and the combined end point of cardiovascular death and non-fatal myocardial infarction.^[19] Whereas in the other studies,^[18,27,33] R279Q polymorphism was not found to be associated with risk of CAD and stable angina. As well, we did not find any significant differences in both the genotype and allele distribution between the ACS group and the control group in this population.

In conclusion, this study suggests that MMP-9 -1562C>T polymorphism could be associated with the susceptibility to ACS in the Uyghur population of China, and the -1562T allele carriers might be at high risk of the development of ACS. The results are consistent with the notion that MMP-9 plays an important role in the

development of atherosclerotic lesion and arterial plaque rupture.^[9,10] However, this mutation apparently is not related to the severity of coronary arterial stenosis.

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Ethical approval: The study was approved by the Ethics Committee of the First Hospital Affiliated to Xinjiang Medical University.

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