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Association between matrix metalloproteinase-9 rs3918242 polymorphism and development of coronary artery disease in a Chinese population

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ABSTRACT. We conducted a case-control study to investigate the role of one single nucleotide polymorphism of *MMP-9* rs3918242 in the development of coronary artery disease. The rs3918242 was amplified with 435-bp DNA fragments using polymerase chain reaction coupled with restriction fragment length polymorphism. When compared with control subjects, patients with coronary artery disease had higher systolic and diastolic blood pressure, as well as higher triglycerides ($P < 0.05$), were more likely to suffer from diabetes mellitus, and had lower total cholesterol and high-density lipopolysaccharides. Using unconditional logistic analysis, we found that individuals with CT and TT genotypes were associated with increased risk of coronary artery disease in a co-dominant model, and the ORs (95%CI) were 1.50 (1.02-2.20) and 6.89 (2.51-23.41) for CT and TT, respectively. We observed that the T allele of rs3918242 was correlated with increased

risk of coronary artery disease (OR = 1.88, 95%CI = 1.39-2.55). In conclusion, we suggest that the TT and CT genotypes and T allele of *MMP-9* rs3918242 polymorphism is correlated with an increased risk of coronary artery disease in a Chinese population

Key words: Matrix metalloproteinase-9; Polymorphism; Coronary artery disease

INTRODUCTION

Cardiovascular disease is one of the major causes of morbidity worldwide. Coronary artery disease is the most common type of cardiovascular disease and it is caused by atherosclerosis. The development of coronary artery disease results from multiple complex factors, including many environmental factors and their interactions, such as hypertension, hypercholesterolemia, diabetes, obesity, tobacco, and alcohol (Lindahl et al., 2000). However, not all individuals develop coronary artery disease, even when they are exposed to the risk factors of coronary artery disease, which suggests that hereditary factors also play an important role in the development of coronary artery disease. Recent studies have reported that the genetic factors account for about 40 to 60% in the development of coronary artery disease (Roberts and Stewart, 2012).

Matrix metalloproteinases (MMPs) are well-known gelatinase B or 92-kDa type IV collagenase and they form a large family of structurally related zinc-binding proteolytic enzymes that can degrade the extracellular matrix and cause both normal and pathological tissue remodeling. It is reported that the high plasma of MMP expression could influence the development of cardiovascular and cerebrovascular diseases (Siefert and Sarkar, 2012; Chen et al., 2013). Three main polymorphisms were observed in the promoter, coding and untranslated region of *MMP-9* (Zhang et al., 1999). The *MMP-9* rs3918242 polymorphism (C®T) is located at the promoter region, and previous studies have reported that the *MMP-9* rs3918242 gene polymorphism plays an important role in the development of coronary artery diseases, but the results are inconclusive (Mishra et al., 2012; Opstad et al., 2013; Xu et al., 2013; Opstad et al., 2014; Wang and Shi, 2014; Zhang et al., 2014). Therefore, we carried out a case-control study to assess the role of one single nucleotide polymorphism (SNP) of *MMP-9* rs3918242 in the development of coronary artery disease.

MATERIAL AND METHODS

Patients

A hospital-based case-control study was carried out. Samples from 261 patients with coronary artery disease were consecutively recruited from the Xianyang Central Hospital of Shaanxi between October 2012 and December 2014. Clinical diagnosis of coronary artery disease was given by angiography, and coronary artery disease was defined as a diameter stenosis of 50% in any of the main coronary arteries. The exclusion criteria of this study were patients that had a history of primary or secondary cardiomyopathy, chronic and acute serious infection disease, and cancer.

Control subjects (261) were selected from the Xianyang Central Hospital of Shaanxi

during the period of October 2012 and December 2014. Control subjects that had a history of coronary artery disease, cardiomyopathy, chronic and acute serious infection disease, myocardial bridge, congenital heart disease, and peripheral artery disease, as well as malignant tumors, were excluded from our study.

The lifestyle and baseline information of coronary artery disease and control subjects was collected from a self-designed questionnaire or their medical records. The lifestyle and baseline information of coronary artery disease and control subjects included gender, age, body mass index, and tobacco and alcohol consumption habits. The clinical data were collected from medical records, including hypertension, diabetes mellitus, total cholesterol (TC), triglycerides (TG), and low-density lipopolysaccharide cholesterol (LDL-c), as well as high-density lipopolysaccharide (HDL-c). Written informed consents were signed by both patients with coronary artery disease and control subjects. Our study was approved by the Ethics Committee of the Xianyang Central Hospital of Shaanxi.

DNA extraction and genotyping

Fasting venous blood (5 mL) was drawn from each patient and control subject after enrolling in this study. Isolation of genomic DNA from the peripheral leukocytes was done using the QIAamp DNA Blood Mini kit (Qiagen, Valencia, CA, USA), according to the manufacturer's instructions. Genotyping of the rs3918242 was carried out using polymerase chain reaction (PCR) coupled with restriction fragment length polymorphism (RFLP). The forward and reverse primers for rs3918242 were 5'-GGTGGTGAGGATGAAACGAGAG-3', respectively. The PCR thermal cycling conditions started at 95°C for 5 min in the initial denaturation, followed by 35 cycles of denaturation at 95°C for 30 s, annealing at 61°C for 30s, extension at 72°C for 60 s, and final extension at 72°C for 20 min. An agarose gel (2%) stained with ethidium bromide and ultraviolet light was used to confirm the PCR products of rs3918242.

One fragment was observed for the CC genotype, and the length was 662 bp. Two fragments were observed for the TT genotype, and the lengths were 469 and 193 bp. Three fragments were observed for the CT genotype, and the lengths were 662, 469 and 193 bp.

Statistical analysis

The differences within the demographic variables of the two study groups were done using independent sample *t*-test or chi-square (χ^2) test. Whether the genotype distributions of rs3918242 had a deviation from the Hardy-Weinberg equilibrium was calculated using the Fisher exact test. Unconditional logistic regression analysis was carried out to assess the association between rs3918242 and risk of coronary artery disease. The odds ratio (OR) and 95% confidence intervals (CIs) are also calculated. Statistical analysis was carried out using the SPSS 20.0 package (SPSS Inc., Chicago, IL, USA). A $P < 0.05$ was considered to indicate a statistically significant difference.

RESULTS

Since the gender and age were matched between patients and controls, there were no significant differences between them (Table 1). When compared with control subjects, patients with coronary artery disease had higher systolic and diastolic blood pressure, as well as higher

TG ($P < 0.05$), were more likely to suffer from diabetes mellitus, and had a lower TC and HDL-c.

The genotype distributions of rs3918242 are presented in Table 2. We observed that the genotype frequencies of rs3918242 had no deviation from the Hardy-Weinberg equilibrium in control subjects ($P = 0.13$). There were significant differences in the genotype ($\chi^2 = 20.83$, $P < 0.001$) and allele ($\chi^2 = 18.36$, $P < 0.001$) distributions of rs3918242 between patients with coronary artery disease and control subjects. Using unconditional logistic analysis, we found that individuals with CT and TT genotypes were associated with increased risk of coronary artery disease, and the ORs (95%CI) were 1.50 (1.02-2.20) and 6.89 (2.51-23.41) for CT and TT, respectively. We observed that the T allele of rs3918242 was correlated with increased risk of coronary artery disease (OR = 1.88, 95%CI = 1.39-2.55).

Table 1. Demographic and clinical information of enrolled subjects.

	CAD cases (N = 261)	%	Controls (N = 261)	%	χ^2 test or *t*-test	P value
Mean age (years)	58.75 ± 9.36		59.21 ± 10.10		0.54	0.29
Gender						
Male	186	71.26	186	71.26		
Female	75	28.74	75	28.74	0.00	1.00
Body mass index (kg/m^2)	25.42 ± 3.16		23.15 ± 3.26		0.96	0.17
SBP	138.54 ± 25.85		134.48 ± 23.66		1.87	0.03
DBP	86.32 ± 17.45		83.62 ± 15.72		1.86	0.03
Diabetes mellitus						
No	208	79.69	242	92.72		
Yes	53	20.31	19	7.28	18.62	<0.001
Alcohol consumption						
Never	179	68.58	190	72.80		
Current or ever	82	31.42	71	27.20	1.12	0.29
Tobacco consumption						
Never	163	62.45	176	67.43		
Current or ever	98	37.55	85	32.57	1.42	0.23
TC (mM)	4.22 ± 1.15		4.45 ± 1.02		2.42	0.01
LDL-c (mM)	2.47 ± 0.95		2.56 ± 0.87		1.13	0.13
HDL-c (mM)	1.08 ± 0.32		1.16 ± 0.28		3.04	0.001
TG (mM)	2.27 ± 1.53		2.04 ± 1.26		1.87	0.03

SBP = systolic blood pressure; DBP = diastolic blood pressure; TC = total cholesterol; LDL-c = low-density lipopolysaccharide cholesterol; HDL-c = high-density lipopolysaccharide cholesterol; TG = triglycerides.

Table 2. Association between *MMP-9* rs3918242 gene polymorphisms and risk of CAD.

Genotypes	Patients	%	Controls	%	χ^2 test	P value	P value for HWE	OR (95%CI)[1]	P value
CC	134	51.34	171	65.52				1.0 (Ref.)	-
CT	100	38.31	85	32.57				1.50 (1.02-2.20)	0.03
TT	27	10.34	5	1.92	20.83	<0.001	0.13	6.89 (2.51-23.41)	<0.001
Allele									
C	368	70.5	427	81.8	18.36	<0.001		1.0 (Ref.)	-
T	154	29.5	95	18.2				1.88 (1.39-2.55)	<0.001

[1]Adjusted for age, gender, body mass index, systolic and diastolic blood pressure, diabetes mellitus, TC, TG, LDL-c, and HDL-c.

We also performed a gene-environmental association of rs3918242 polymorphism with systolic and diastolic blood pressure, diabetes mellitus, TC, HDL-c and TG, and the risk of coronary artery disease. However, no significant gene-environmental interaction was found between groups (Table 3).

Table 3. Association between *MMP-9* rs3918242 gene polymorphisms and risk of coronary artery disease stratified by SBP, DBP, diabetes mellitus, TC, HDL-c, and TG.

Genotypes	Patients		Controls		OR (95%CI)[1]	P value
	TT	CT+TT	TT	CT+TT		
SBP						
<135	78	69	82	41	1.77 (1.05-3.00)	0.02
≥135	56	58	89	49	1.88 (1.10-3.22)	0.01
DBP						
<85	61	56	93	48	1.78 (1.04-3.03)	0.02
≥85	73	71	78	42	1.81 (1.07-3.07)	0.02
Diabetes mellitus						
No	115	93	159	83	1.65 (1.11-2.45)	0.01
Yes	19	34	12	7	3.07 (0.91-10.73)	0.04
TC (mM)						
<4.20	56	73	51	39	1.70 (0.96-3.04)	0.06
≥4.20	78	54	120	51	1.63 (0.98-2.70)	0.06
HDL-c (mM)						
<1.10	74	68	72	38	1.74 (1.01-3.01)	0.03
≥1.10	60	59	99	52	1.87 (1.11-3.15)	0.01
TG (mM)						
<2.00	52	49	85	43	1.86 (1.05-3.30)	0.02
≥2.00	82	78	86	47	1.74 (1.06-2.87)	0.02

[1]Adjusted for age and gender.

DISCUSSION

In the present study, we conducted a study to assess the correlation between *MMP-9* rs3918242 and the susceptibility to coronary artery disease in a Chinese population. We revealed that the TT and CT genotypes and T allele of *MMP-9* rs3918242 significantly influence the risk of coronary artery disease in the Chinese population.

Increasing experimental studies have reported the association between the *MMP-9* rs3918242 expression and the risk of coronary artery disease (Yongxin et al., 2013; Li et al., 2013; Li et al., 2015; Ma et al., 2015). Li et al. (2013) reported that the decreasing expression of MMP-9 protein could influence the pathogenesis of coronary atherosclerotic heart disease in rats. Li et al. (2015) suggested that inhibited expression of MMP-9 protein could promote the permeability of coronary artery in Wistar rats, and thus influence the susceptibility to coronary artery disease. Ma et al. (2015) revealed that human coronary artery endothelial cells significantly inhibited the MMP-9 expression and activity. All these studies indicate that MMP-9 expression and activity may affect the pathogenesis of coronary artery disease.

Currently, several studies investigated the role of MMP-9 genetic variation and the susceptibility to coronary artery disease, but the results are inconclusive (Goracy et al., 2003; Alp et al., 2009; Ghaderian et al., 2010; Zhi et al., 2010; Mishra et al., 2012; Opstad et al., 2013; Xu et al., 2013; Opstad et al., 2014). Four studies reported carried out case-control studies in different ethnicities, and they suggested that T allele of *MMP-9* rs3918242 elevated the susceptibility to coronary artery disease (Goracy et al., 2003; Zhi et al., 2010; Opstad et al., 2013; Xu et al., 2013). Mishra et al. (2012) reported that MMP9 R668Q plays an important role in conferring susceptibility of left ventricular dysfunction in coronary artery disease patients. However, some studies reported different results (Alp et al., 2009; Ghaderian et al., 2010). Alp et al. (2009) and Ghaderian et al. (2010) did not find a significant association between MMP-9 rs3918242 genetic variation and development of coronary artery disease and

myocardial infarction in Turkish and Chinese population. Two recent meta-analysis studies reported that the MMP-9 rs3918242 genetic factors may have association with the risk of coronary artery disease in Asian populations (Wang and Shi, 2014; Zhang et al., 2014). In our study, we found that the TT and CT genotypes and T allele of *MMP-9* rs3918242 could influence the development of coronary artery disease in a Chinese population. Further large scale studies are greatly to verify the results of our findings.

In conclusion, we suggest that the TT and CT genotypes and T allele of *MMP-9* rs3918242 polymorphism is correlated with an increased risk of coronary artery disease in a Chinese population.

Conflicts of interest

The authors declare no conflict of interest.

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