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Original Article

Polymorphism in matrix metalloproteinase-9 1562 C/T contributes to the risk of coronary artery disease

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Abstract: We aimed to investigate the association between *MMP-9* 1562 C/T and susceptibility to coronary artery disease in a Chinese Han population. Between May 2012 and January 2015, samples from 194 patients with coronary artery disease and 252 control subjects were collected from the First Affiliated Hospital of Xinxiang Medical University. Genotyping of *MMP-9* 1562 C/T was carried out by polymerase chain reaction (PCR) coupled with restriction fragment length polymorphism (RFLP). There were significant differences in the genotype distributions of *MMP-9* 1562 C/T between patients with coronary artery disease and control subjects in codominant (χ^2=12.45, P=0.002), dominant (χ^2=7.61, P=0.006) and recessive models (χ^2=9.88, P=0.002). Unconditional logistic analysis revealed that individuals with the TT genotype was associated with increased risk of coronary artery disease in a codominant model, and the OR (95% CI) was 3.68 (1.60-9.02). In dominant model, we found that the CT+TT genotype was correlated with increased risk of coronary artery disease (OR=1.64, 95% CI=1.10-2.44). In recessive model, the TT genotype was also associated with an elevated risk of coronary artery disease when compared to the CC+CT genotype (OR=3.24, 95% CI=1.44-7.82). In conclusion, we suggest that the *MMP-9* 1562 C/T polymorphism is correlated with an increased risk of coronary artery disease in the Chinese population.

Keywords: *MMP-9* 1562 C/T, polymorphism, coronary artery disease, chinese population

Introduction

Coronary artery disease is the most common type of cardiovascular disease and is associated with high morbidity in both developed and developing countries. The etiology of coronary artery disease is not well-understood. Both multifactorial environmental and lifestyle factors are involved in the development of coronary artery disease, such as high intake of high calorie and high fat food, hypertension, hypercholesterolemia, diabetes, obesity, lack of physical activity and tobacco smoking as well as alcohol consumption [1-3]. Currently, many studies indicate that the morbidity of coronary artery disease shows highly discrepancies between different ethnicities, and individuals who had the similar risk factors of coronary artery disease would not develop this disease. Thus, the genetic factors may contribute to the development of this cancer. It is reported that susceptibility to coronary artery disease is accounted for 40% to 60% inherited [4].

Previous experimental studies have reported that inflammation plays a role in the development of atherosclerosis, including processes such as oxidative damage, cell proliferation, plaque evolution, and destabilization [5-8]. Matrix metalloproteinases (MMPs) are well known inflammatory mediators and they are a family of structurally related zinc-binding proteolytic enzymes, and it is widely observed in human tissues. The expression of MMPs is associated with the development of atherosclerosis through the activation of migration and proliferation of smooth muscle cells, and with the destabilization of atherosclerotic plaques [8, 9]. Previous studies have reported that the alteration of MMP expression is associated with cardiovascular and cerebrovascular diseases [10, 11]. The *MMP-9* gene is located on chromosome 20q12.2-13.1, and previous studies have reported that high expression of *MMP-9* plays an important role in the regulation of inflammation in stroke [11, 12]. In our study, we conducted a case-control study to investigate the role

Table 1. The demographic and clinical information of patients with coronary artery disease and control subjects

	Patients N=194	%	Controls N=252	%	χ^2 test or *t* test	*P* value
Mean age, years	55.60 ± 10.42		56.21 ± 9.83		0.63	0.26
Sex						
Male	144	74.23	155	61.51		
Female	50	25.77	97	38.49	8.03	0.005
Body Mass Index, kg/m^2	23.89 ± 3.37		23.39 ± 3.41		1.45	0.07
Systolic blood pressure, mmHg	139.42 ± 22.79		127.15 ± 24.47		5.41	<0.001
Diastolic blood pressure, mmHg	87.15 ± 18.31		84.52 ± 14.63		1.69	0.04
Diabetes mellitus						
No	162	80.41	223	88.49		
Yes	32	19.59	29	11.51	2.31	0.13
Alcohol drinking						
Never	128	65.98	173	68.65		
Current or ever	66	34.02	79	31.35	0.36	0.55
Tobacco smoking						
Never	112	57.73	174	69.05		
Current or ever	82	42.27	78	30.95	6.10	0.01
TC, mmol/L	4.21 ± 1.09		4.54 ± 1.01		3.30	<0.001
LDL-c, mmol/L	2.41 ± 0.88		2.59 ± 0.85		2.18	0.01
HDL-c, mmol/L	1.10 ± 0.29		1.18 ± 0.22		3.31	<0.001
TG, mmol/L	2.28 ± 1.47		1.95 ± 1.19		2.62	0.005

of one single nucleotide polymorphism (SNP) of *MMP-9* 1562 C/T in the development of coronary artery disease.

Material and methods

Patients

Between December 2013 and January 2015, sample from 194 patients with coronary artery disease were collected from the First Affiliated Hospital of Xinxiang Medical University. The diagnosis of coronary artery disease was based on the following criteria as follows: a diameter stenosis of 50% in any of the main coronary arteries through angiography. Patients who had a history of cardiomyopathy, auto-immunologic disease, and severe kidney and liver disease, as well as malignant tumors were excluded from our study.

A total of 252 control subjects were randomly selected from individuals that received the health examination in the same hospital during the same period time. Controls that had a history of coronary artery disease, myocardial bridge, congenital heart disease and peripheral artery disease were excluded from our study.

The demographic and clinical information of patients with coronary artery disease and control subjects were collected from a self-designed questionnaire and their medical records. The demographic information included sex, age, Body Mass Index, and tobacco smoking and alcohol drinking. The clinical information included systolic blood pressure, diastolic blood pressure, diabetes mellitus, total cholesterol (TC), triglyceride (TG), low density lipopolysaccharide cholesterol (LDL-c) and high density lipopolysaccharide (HDL-c). The signed written informed consents were obtained from patients with coronary artery disease and control subjects. Our study was approved by the ethics committee of the First Affiliated Hospital of Xinxiang Medical University.

DNA extraction and genotyping

Five ml of fasting venous blood was drawn from each patient and control subject after participating into this study. The blood samples were

Table 2. Association between *MMP-9* 1562C/T gene polymorphisms and risk of coronary artery disease

Genotypes	Patients	%	Controls	%	χ^2 test	*P* value	*P* value *for HWE*	OR (95% CI)[1]	*P* value
Codominant									
CC	98	50.52	157	62.30				1.0 (Ref.)	-
CT	73	37.63	84	33.33				1.39 (0.91-2.12)	0.11
TT	23	11.86	10	4.37	12.45	0.002	0.77	3.68 (1.60-9.02)	<0.001
Dominant									
TT	98	50.60	157	62.40	7.61	0.006		1.0 (Ref.)	-
CT+TT	96	49.48	94	37.70				1.64 (1.10-2.44)	0.01
Recessive									
CC+CT	171	88.14	241	95.63				1.0 (Ref.)	-
TT	23	11.86	10	4.37	9.88	0.002		3.24 (1.44-7.82)	0.002

[1]Adjusted for sex, systolic blood pressure, diastolic blood pressure, tobacco smoking, TC, TG, ILDL-c and HDL-c.

stored in tubes with ethylene diamine tetraacetic acid (EDTA), and then the blood was centrifuged to separate the plasma content. Genomic DNA was extracted from the peripheral leukocytes using the TIANamp Blood DNA Kit (Tiangen, Beijing, China). The *MMP-9* 1562 C/T was amplified with 435-bp DNA fragment using polymerase chain reaction (PCR) coupled with restriction fragment length polymorphism (RFLP). Primers for *MMP-9* 1562 C/T were designed using the Sequenom Assay Design 3.1 software. The PCR reaction was performed at 95°C for 5 minutes for the initial denaturation, followed by 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 minutes. The restriction enzyme was Sph I. An agarose gel stained with ethidium bromide and ultraviolet light was used to confirm the PCR products of *MMP-9* 1562 C/T.

Statistical analysis

Differences between demographic and clinical characteristics were compared using the chi-square test and t-test. The Fisher's exact test was performed to evaluate whether the genotype distributions of *MMP-9* 1562 C/T had a deviation from the Hardy-Weinberg equilibrium. The association between *MMP-9* 1562 C/T and risk of coronary artery disease was analyzed using conditional logistic regression analysis. Codominant, dominant and recessive models were used to analyze the association between *MMP-9* 1562 C/T and risk of coronary artery disease. The odds ratio (OR) and 95% confidence intervals (CIs) are also used. Statistical analysis was conducted using the SPSS 17.0 package (SPSS Inc., Chicago, IL, USA). A $P<0.05$ was considered to indicate a statistically significant difference.

Results

The demographic and clinical information of patients with coronary artery disease and control subjects were shown in **Table 1**. The mean ages of patients and controls were 55.60 ± 10.42 and 56.21 ± 9.83, respectively, there was no significant difference between them ($\chi^2=0.63$, $P>0.05$). When compared with control subjects, patients with coronary artery disease were more likely to be male ($\chi^2=8.03$, $P<0.005$) and smokers ($\chi^2=6.10$, $P=0.01$), have higher systolic blood pressure ($t=5.41$, $P<0.001$) and diastolic blood pressure ($t=1.69$, $P=0.04$), have lower TC ($t=3.30$, $P<0.001$), LDL-c ($t=2.18$, $P=0.01$) and HDL-c ($t=3.31$, $P<0.001$), and have higher TG ($t=2.62$, $P=0.005$) ($P<0.05$).

By Fisher's exact test, we found that the genotype distributions of *MMP-9* 1562 C/T were in line with the Hardy-Weinberg equilibrium in control subjects ($P=0.13$; **Table 2**). There were significant differences in the genotype distributions of *MMP-9* 1562 C/T between patients with coronary artery disease and control subjects in codominant ($\chi^2=12.45$, $P=0.002$), dominant ($\chi^2=7.61$, $P=0.006$) and recessive models ($\chi^2=9.88$, $P=0.002$). Using conditional logistic analysis, individuals with the TT genotype was associated with increased risk of coronary artery disease in a codominant model, and the OR (95% CI) was 3.68 (1.60-9.02). In a dominant model, we found that the CT+TT genotype was correlated with increased risk of coronary artery disease (OR=1.64, 95% CI=1.10-2.44). In a recessive model, the TT genotype was also

Table 3. Association between *MMP-9* 1562C/T gene polymorphism and risk of coronary artery disease stratified by lifestyle and clinical characteristics

Genotypes	Patients		Controls		OR (95% CI)[1]	*P* value
	CC	CT+TT	CC	CT+TT		
Sex						
Male	73	71	95	60	1.54 (0.95-2.50)	0.07
Female	25	25	62	35	1.77 (0.84-3.75)	0.1
Systolic blood pressure, mmHg						
<135	32	38	87	58	1.78 (0.96-3.30)	0.05
≥135	66	58	70	36	1.71 (0.94-3.03)	0.06
Diastolic blood pressure, mmHg						
<85	40	38	84	57	1.40 (0.77-2.54)	0.24
≥85	58	58	73	37	1.75 (0.96-3.14)	0.06
Tobacco smoking						
Never	65	63	105	68	1.50 (0.92-2.44)	0.09
Current or ever	33	33	52	27	1.93 (0.93-3.98)	0.06
TC, mmol/L						
<4.50	62	61	77	45	1.68 (0.98-2.90)	0.06
≥4.20	36	35	80	49	1.59 (0.85-2.97)	0.12
LDL-c, mmol/L						
<2.6	55	51	76	43	1.64 (0.92-2.90)	0.07
≥2.6	43	45	81	51	1.66 (0.93-2.97)	0.07
HDL-c, mmol/L						
<1.20	59	56	73	44	1.57 (0.90-2.75)	0.03
≥1.20	39	40	84	50	1.72 (0.94-3.14)	0.06
TG, mmol/L						
<2.00	60	56	75	45	1.56 (0.89-2.70)	0.07
≥2.00	38	40	82	49	1.76 (0.96-3.23)	0.05

associated with an elevated risk of coronary artery disease when compared to the CC+CT genotype (OR=3.24, 95% CI=1.44-7.82).

We also performed a gene-environmental association of *MMP-9* 1562 C/T polymorphism with sex, SBP, DBP, tobacco smoking, TC, LDL-c, HDL-c and TG and the risk of coronary artery disease; however, we did not find a significant association between the *MMP-9* 1562 C/T polymorphism and these lifestyle and clinical characteristics in the risk of coronary artery disease (**Table 3**).

Discussion

It is well known that individuals may not develop the same type of disease despite being exposed to similar environmental and lifestyle factors. Therefore, inherit factors may play an important role in the development of diseases. Previous epidemiological studies have reported that SNPs in the candidate genes could contribute to coronary artery diseases, such as IL-6, IL-23, CYP2C19 and TRIB3 [13-15]. In our study, we conducted a case-control study to investigate whether *MMP-9* 1562 C/T could influence the susceptibility to coronary artery disease. We found that the TT genotype of *MMP-9* 1562 C/T was associated with an increased risk of coronary artery disease.

The gene encoding *MMP-9* 1562 C/T is located on chromosome 20q12.2-13.1 at 1562 bp upstream of the transcriptional start site. The presence of either a C or a T influences the transcriptional activity of *MMP-9* 1562 C/T. It was previously reported that the T allele of *MMP-9* 1562 C/T has a higher promoter activity compared to the C allele due to the binding of a transcriptional repressor [16].

Many studies have reported an association between the *MMP-9* 1562 C/T polymorphism

and the development of coronary artery disease [17-21]. Goracy et al. firstly conducted a study in polish population, and they found that T allele of *MMP-9* 1562 C/T was significantly associated with the risk of ischemic heart disease [17]. Zhi et al. conducted a case-control study composed of 762 coronary artery disease patients, and they found that CT and TT genotypes of *MMP-9* 1562 C/T might contribute to the occurrence of coronary artery disease [18]. Xu et al. evaluated the *MMP-9* 1562 C/T polymorphism in 1574 individuals, and they indicated that the genetic variation in *MMP-9* 1562 C/T was associated with the occurrence of coronary artery disease [19]. Opstad et al. conducted two studies with 1000 patients, and they found that the T allele *MMP-9* 1562 C/T increased the risk of coronary artery disease [20, 21]. All these studies suggest that *MMP-9* 1562 C/T is a risk factor for coronary artery disease.

However, two another studies reported inconsistent results that are inconsistent with the previously mentioned studies [22, 23]. Alp et al. conducted a study in a Turkish population, and they found that no association between the *MMP-9* 1562 C/T polymorphism and coronary artery disease [22]. Ghaderian et al. also did not find a statistically significant association between genetic variation of *MMP-9* 1562 C/T and acute myocardial infarction [23]. Finally, a recent meta-analysis with 16 case-control studies suggested that *MMP-9* 1562 C/T polymorphism was only correlated with coronary artery disease in East Asians, but with no association in either West Asians [24]. In our study, we found that the TT genotype of *MMP-9* 1562 C/T was associated with the occurrence of coronary artery disease. The discrepancies of the above results may be caused by differences in ethnicities, selection of subjects and sample size.

Two limitations in our study should be taken into consideration. First, patients with acute pancreatitis and control subjects were selected from only one hospital, which may not represent well the populations in China and other places. However, the genotype distributions did not deviate from the Hardy-Weinberg equilibrium, which could reduce the selection bias. Second, the sample size is small, which could reduce the statistical power of determining the differences between the groups. Therefore, further studies with a larger sample size are required to confirm our findings.

In conclusion, we suggest that the *MMP-9* 1562 C/T polymorphism is correlated with an increased risk of coronary artery disease in codominant, dominant, and recessive models. This finding could be helpful for identifying the genetic characteristics of coronary artery disease and developing more efficient strategies for prevention and treatment.

Disclosure of conflict of interest

None.

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