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Matrix metalloproteinase 9 gene polymorphism 1562C>T is significantly associated with acute coronary syndrome susceptibility in the Vietnamese population

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ABSTRACT: The role of the 1562C>T single nucleotide polymorphism of the matrix metalloproteinase 9 (MMP-9) gene promoter in acute coronary syndrome (ACS) development has been reported in various populations. In this work, we investigated the association between MMP-9 1562C>T polymorphism and ACS patients in Vietnam. This study was conducted on 138 ACS patients and 68 control subjects recruited from the Vietnam National Heart Institute. The genotype of 1562C>T polymorphism was determined by RFLP-polymerase chain reaction and the serum MMP-9 level was measured by the ELISA method. We found that the frequencies of CT and TT genotypes in the ACS patients (30% and 4%) were higher than those in the control (18% and 1%). The 1562T allele in the MMP-9 promoter was found to have a significantly higher frequency in ACS patients than in control subjects (ACS versus control: 19% versus 10%, $p = 0.001$). Multiple logistic regression analysis indicated that the MMP-9 1562T allele carriers had an increased risk of developing ACS (odds ratio $= 2.37$; 95% confidence intervals: 1.04–5.75, $p = 0.045$). The serum MMP-9 level in the polymorphism-carrying group was considerably higher than in the group without polymorphism in both ACS patients (229 ± 83 versus 194 ± 108, $p = 0.037$) and controls (220 ± 41 versus 171 ± 80, $p = 0.006$). Our results showed that the MMP-9 1562C>T polymorphism is significantly associated with the ACS susceptibility in the Vietnamese population.

KEYWORDS: acute coronary syndrome patient, single nucleotide polymorphism (SPN), serum MMP-9 level, logistic regression analysis, Vietnam

INTRODUCTION

Acute coronary syndrome (ACS) has become a major cause of mortality in developing countries, as well as in Vietnam. ACS is evoked by the rupture or erosion of coronary atherosclerotic plaque and subsequent thrombus formation[1]. Matrix metalloproteinases (MMPs) belong to a large family of Zn^{2+} dependent endoproteinases which degrade extracellular matrix proteins such as collagen and elastin. MMPs are found abundantly in human coronary atherosclerotic plaque[2,3] and implicated in the pathogenesis of several atherosclerotic cardiovascular diseases[4].

Among MMPs, MMP-9, also known as 92 kDa type IV collagenase or 92 kDa gelatinase B, is involved in the breakdown of extracellular matrix. MMP-9 is overexpressed in atherosclerotic plaque and plays a role in the rupture of plaque[2,3,5,6]. Increasing circulating MMP-9 level has been observed in patients with acute coronary syndrome and cardiovascular disease[7–12]. Potentially functional single nucleotide polymorphism (SNP) in the MMP-9 gene promoter has been found in which a cytosine (C) to thymidine (T) transition at position 1562 may strongly contribute to the susceptibility of ACS. Variation in the MMP-9 genotypes may modulate the circulating phenotype of the protein and consequently increase the risk of a coronary event. Although association of MMP-9 variants and its concentrations in the development of ACS has been reported, the results are controversial[13–18]. The aim of our study is therefore to investigate the role of MMP-9 1562C>T polymorphism in the ACS development by the case-control study in the Vietnamese population.

MATERIALS AND METHODS

Subjects

We enrolled 138 patients with ACS and 68 healthy subjects, all of Vietnamese origin, and blood samples were collected from the Vietnam National Heart Institute–Bach Mai Hospital, Hanoi, Vietnam during periods of 2014–2015. Peripheral blood was drawn from ACS patients within 24 h after the onset of chest pain. The patients and control subjects in this study had no history of neoplastic, hepatic, infectious or autoimmune disease, or cancer. The healthy volunteers were randomly selected from individuals who have no history or evidence of cardiovascular disease, hypertension, or diabetes mellitus. All ACS patients were diagnosed with their medical history, clinical symptoms, ultrasonic echocardiogram, 12-lead electrocardiogram, laboratory examinations, and coronary angiography according to criteria issued by the Vietnam National Heart Association and World Health Organization. The Ethics Committee of the Bach Mai Hospital approved the project and all participants gave written informed consent to take part in the study. The study conformed to the declaration of Helsinki 1964 and its later amendments.

Genotyping of MMP-9 gene

The genomic DNA was extracted from peripheral venous blood using the Qiagen Blood and Tissue DNA kit (Qiagen NV, Hilden, Germany) according to the manufacture's instruction. To determine the genotypes of MMP-9 promoter at position 1562, polymerase chain reaction (PCR) - restriction fragment length polymorphism analysis (RFLP) was performed using a primer pair consisting of the forward primer, 5′-GCCTGGCACATAGTAGGCCC-3′ and the reverse primer, 5′-CTTCCTAGCCAGCCGGCATC-3′. The PCR standard reaction conditions were 30 cycles of denaturation at 94 °C for 30 s, annealing at 58 °C for 30 s, elongation at 72 °C for 60 s, and a final step at 72 °C for 3 min after a pre-denaturation step at 94 °C for 3 min. The PCR product was digested with *Sph* I restriction endonuclease before subjecting to electrophoresis on a 1.5% agarose gel. Three potential DNA bands (435, 247, and 188 bp) were visualized by UV exposure using ethidium bromide stain. Producing fragment was a single undigested 435 bp band in the case of the CC homozygotes (*Sph* I enzyme is not effective on C allele) while those with T allele (CT/TT genotypes) produce two fragments of 188 bp and 247 bp (Fig. 1).

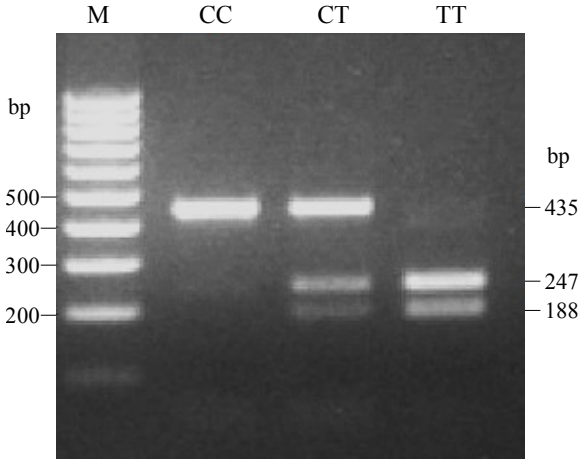

Fig. 1 1.5% agarose gel electrophoresis of MMP-9 promoter PCR products (three different fragments). M is 100 bp ladder.

Serum MMP-9 assays

To measure serum MMP-9 level, 5 ml of blood was drawn and allowed to clot for 30 min. These samples were centrifuged at 1000 rpm for 15 min and the supernatants were extracted and stored at −80 °C before assay. MMP-9 levels were measured by sandwich enzyme immunoassay method using human MMP-9 Elisa Pair Set (Sino Biological Inc., Beijing, China) according to the manufacturer's instructions.

Statistical analysis

The clinical characteristics are shown as mean ± SD and were analysed using Welch's t-test for continuous variables. The categorical variables were performed using chi-squared test (χ^2 test). The distribution of genotype and allele frequencies and the serum level of MMP-9 between patient and control groups were compared by using χ^2 test. The association among ACS with SNP in the MMP-9 promoter and the traditional risks for ACS were tested by odds ratio (OR) and 95% confidence intervals (CIs) obtained using univariate and/or multivariate logistic regression analysis. Statistical significance was set at the $p < 0.05$.

RESULTS

Characteristic of the subjects

This case-control study enrolled 138 patients with ACS (mean age 59 ± 9 years, 94 men) and 68 healthy volunteers (mean age 49 ± 12 years, 27 men). The comparison of clinical characteristics

Table 1 Baseline characteristics of participants.

Variables†	Control ($n = 68$)	ACS ($n = 138$)	p value
Gender(M/F)	27/41	94/44	
Age (years)	49 ± 12	59 ± 9	< 0.001
Glucose (mM)	5.7 ± 1.0	7.6 ± 5.5	< 0.001
Cholesterol (mM)	4.52 ± 0.74	5.0 ± 1.3	0.002
Triglyceride (mM)	2.1 ± 3.2	2.7 ± 1.8	0.150
HDL-C (mM)	1.29 ± 0.63	1.06 ± 0.32	0.006
LDL-C (mM)	2.97 ± 0.94	3.0 ± 1.1	0.854
Creatinine (μM)	78 ± 23	96 ± 44	< 0.001
Troponin (ng/l)	0.01 ± 0.02	1.8 ± 3.1	< 0.001
CK-MB (U/l)	14.5 ± 5.2	66 ± 118	< 0.001
BNP (pM)	19 ± 59	253 ± 452	< 0.001

† Continuous variables are presented as mean ± SD; categorical variables were compared using a χ^2 test. ACS: acute coronary syndrome; HDL-C: high density lipoprotein-cholesterol; LDL-C: low density lipoprotein-cholesterol; CK-MB: creatinine kinase-MB; BNP: B-type natriuretic peptide.

between the ACS and control groups is presented in Table 1. The results showed that age, glucose, total cholesterol, creatinine, troponin, CK-MB, and BNP were significantly higher in the ACS group than in the control group ($p < 0.05$). Triglyceride and LDL-cholesterol levels were slightly higher in ACS group, however, the differences is not statistically significant ($p > 0.05$). In contrast, HDL-cholesterol was significantly lower in the ACS group ($p < 0.05$).

MMP-9 gene polymorphism and ACS

Genotype and allele frequencies of MMP-9 1562C>T in controls and ACS patients are shown in Table 2. 47 out of 138 patients with ACS (34%) had MMP-9 (1562C>T) polymorphism which notably was found in 13 out of 68 control subjects (19%). Homozygote TT genotype was found in one subject in the control (1%) and five in the ACS (4%) groups. According to the Hardy-Weinberg equilibrium expectation, we did not observe statistically significant distribution of genotypes in all subjects (all $p > 0.05$). The frequency of T allele and CT and TT genotypes in the ACS (19% and 34%, respectively) were significantly higher than that in the control (10% and 19%, respectively) ($p < 0.001$ and $p < 0.05$) (Table 2). A logistic regression analysis showed that individuals with variant genotypes (CT/TT) and T allele have higher crude risk of ACS compared to MMP-9 CC genotype and T allele (OR = 2.185, 95% CI: 1.085–4.395, $p = 0.028$ and OR = 2.023, 95% CI: 1.077–3.798, $p = 0.029$, respectively) (Table 2). The multiple logistic regression analysis showed three independent risk factors for ACS including age (adjusted OR = 1.097, 95% CI: 1.058–1.143, $p < 0.001$), gender (adjusted OR = 4.33, 95% CI: 2.083–9.433, $p < 0.001$), and glucose concentration (adjusted OR = 1.333, 95% CI: 1.101–1.697, $p = 0.01$) (Table 3). The increased risk of developing ACS in the 1562T allele carriers is 2.37 fold (95% CI: 1.042–5.75, $p = 0.045$) in comparison with the CC genotype when interacting with age, gender, and glucose concentration.

Table 3 Multiple logistic regression analysis of interaction among the MMP-9 polymorphism and the risks of ACS.

Factors	B	SE	p value	OR	95% CI
Age	0.092	0.019	< 0.001	1.097	1.058–1.143
Gender	1.465	0.383	< 0.001	4.330	2.083–9.433
MMP-9	0.865	0.432	0.045	2.375	1.042–5.750
Glucose	0.287	0.111	0.01	1.332	1.101–1.697

Table 2 Distribution of MMP-9 genotypes, odds ratio of MMP-9 1562C>T genotypes and alleles in ACS versus control individuals.

MMP-9 polymorphism	Control n (%)	ACS n (%)	Crude OR (95% CI)	p value
Genotypes				
CC	55 (81%)	91 (66%)	1 (reference)	
CT	12 (18%)	42 (30%)	2.115 (1.026–4.363)	0.042
TT	1 (1%)	5 (4%)	3.021 (0.344–26.541)	0.318
CT+TT	13 (19%)	47 (34%)*	2.185 (1.085–4.395)	0.028
Alleles				
C	122 (90%)	224 (81%)	1 (reference)	
T	14 (10%)	52 (19%)**	2.023 (1.077–3.798)	0.028

* $p < 0.05$; ** $p < 0.001$ (ACS group according to control group)

Table 4 Serum MMP-9 concentrations by MMP-9 1562C>T genotypes and alleles.

Subjects	Serum MMP-9 concentrations (ng/ml)			p value
	Total	CC	CT+TT	
Control	179 ± 77	171 ± 80	220 ± 41	0.006
ACS	206 ± 101*	194 ± 108	229 ± 83	0.037
		Allele C	Allele T	
Control		180 ± 77	220 ± 41	0.017
ACS		207 ± 102	229 ± 83	0.065

* $p = 0.05$: comparison according to control

MMP-9 polymorphism and the serum level of MMP-9

The distribution of serum MMP-9 concentrations according to genotype in ACS and control groups is illustrated in Table 4. The level of serum MMP-9 in ACS was significantly higher than that in the control (206 ± 101 ng/ml versus 179 ± 77 ng/ml, $p = 0.05$). The MMP-9 level from the individuals with the 1562 CT/TT genotypes was significantly higher than in the group without the CC genotype in both ACS patients (229 ± 83 versus 194 ± 108, $p = 0.037$) and controls (220 ± 41 versus 171 ± 80, $p = 0.006$) (Table 4). However, according to the C allele, the significantly higher level of MMP-9 in the T allele carriers was observed only in the control group ($p = 0.017$), not in the ACS patient group ($p = 0.065$).

DISCUSSION

We introduced the association of MMP-9 polymorphism and the risk of ACS in Vietnamese population. Our data, as mentioned above, imply that the 1562 MMP-9 genotypes were significantly associated with the ACS in Vietnamese population.

The rupture or erosion of coronary vulnerable atherosclerotic plaque and subsequent thrombus formation is currently considered as the main occurrence in the pathophysiology and progression of ACS. Among MMPs, MMP-9 is abundantly expressed in the rupture prone region of coronary plaque[2,6], and its expression is regulated primarily at the transcription level. Zhang et al reported that the 1562T allele in the promoter region of MMP-9 leads to a higher promoter activity compared to the C allele, and that T allele carriers have a higher risk for coronary artery diseases (CAD)[19]. Over the last two decades, many studies have examined the relationship between this polymorphism and cardiovascular diseases; however, the impact of ethnicity is still in question. In our study on Vietnamese subjects, the frequency of the 1562T allele in the MMP-9 promoter as well as the serum expression level of MMP-9 were significantly higher in the ACS patients than in the control subjects. In the ACS group, the serum level of MMP-9 is higher than at the SNP group when compared to a group without. These results suggest that individuals carrying the T allele have a high risk of developing ACS. It was explained that there is binding in the promotor between T allele and the transcriptional repressor factor as a result increasing the MMP-9 expression[19]. In addition, the combination of MMP-9 1562 CT/TT genotypes and several other factors such as age, gender, and glucose concentration were significantly associated with increasing of the ACS risk in Vietnamese population. Our result thus is similar to many other studies in terms of ACS or CAD published previously. Koh et al found the 1562C>T polymorphism in the MMP-9 promoter has a significant and independent role in the development of acute myocardial infarction (AMI) in Korean population and the age was associated independently for development of AMI[14]. Later, three reports investigated on the different ethnicity of Chinese populations and showed the same results. In a study composed of 762 Chinese CAD patients, Zhi et al reported that MMP-9 1562C>T polymorphism may contribute to the occurrence of CAD when combining with other polymorphisms in MMP-9 gene[16]. They also detected the 1562 CT/TT genotypes as significantly associated with CAD risk in diabetic subjects[16]. This result shares similarity with ours showing that high glucose level together with MMP-9 1562C>T polymorphism contributes to the risk of ACS. Another study focusing on Uyghur population in China indicated that the 1562T allele carriers might confer a high risk of developing ACS[17]. Recently, Yin et al revealed the correlation between the MMP-9 1562C>T polymorphism and the elevated risk of CAD when studying on Chinese Han population as well as age, gender, and smoking factors have significantly correlated with the progression of AMI[18].

In contrast to our results and the results from several studies as mentioned above, Alp et al showed that 1562C>T polymorphism was not correlated with CAD when conducting a study in Turkish population[20]. Similarly, Wang et al suggested that this polymorphism had no association with CAD (including ACS patients) in Caucasian patients[13]. In another study on Iranian population, Ghaderian et al also failed to indicate that MMP-9 T allele had

an association with increasing risk of developing AMI[15]. Although these studies showed inconsistent results, a plausible explanation may be drawn from the study of Wang and Shi[21]. Using meta-analysis of 16 case-control studies, the authors suggested that MMP-9 1562C>T polymorphism was an occurrence of coronary artery diseases in the East Asian but not in the West Asian[21]. This difference could be explained by the genetic specificity of population ethnicity[18,21].

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