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Research Paper

Relationship between matrix metalloproteinase-9 polymorphism and acute coronary syndrome

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Abstract

Objective: To investigate the relationship of matrix metalloproteinase-9 polymorphism to acute coronary syndrome and its affect on the severity of coronary artery disease. **Methods:** By means of polymerase chain reaction (PCR) and restriction fragment length polymorphism, genotypes of 245 patients with acute coronary syndrome(ACS) and 205 healthy subjects were tested. Genotypes displaying C-1562T functional promoter polymorphism (of the MMP-9 gene) were determined. The relationship between the polymorphism of the MMP-9 gene and ACS and the severity of coronary vessels diseased was analyzed. **Results:** The frequency of C/T plus T/T genotypes and T allele in patients with ACS was significantly higher than that in healthy subjects (22.1% vs 12.7% and 11.4% vs 6.6% respectively). But they were not associated with the number of coronary arteries diseased. **Conclusion:** The MMP-9 polymorphism may be susceptible to ACS. But there was not significant difference between the AMI and UAP subgroups.

Keywords: matrix metalloproteinases(MMP); polymorphism; acute coronary syndrome

INTRODUCTION

It was known that acute coronary syndrome is the result of plaque disruption superimposed thrombosis formation. In this process, the degradation of extracellular matrix(ECM) may play an important role[1]. The degradation of ECM usually happened, following plaque instability at the shoulder region of plaque and caused a degradation of the fabric cap to accelerate plaque disruption[2]. MMP-9 is an important member of the MMP family and a large amount of evidence has revealed that MMP-9 has a greater function than does its counterparts [3]. Reports show an increase of MMP-9 expression in the site of rupture plaque[4-6]. However there are also some contradictory conclusions that have been made and there are few supporting studies [7]. We believe evidence has been significant enough for the further investigation of the relationship between the polymorphism of the MMP-9 gene and coronary vessel diseases[8-10].

MATERIALS AND METHODS

Study cohort

The case-control study was carried out at Cardiology Department, the First Affiliated Hospital of Nanjing Medical University. The study cohort comprised 245 patients with ACS including 114 patients displaying myocardial infarction and 131 patients with unstable angina (the experimental group), and the 204 normal subjects (130 men and 74 women) for the control. This was carried out from November, 2003 to September, 2005. Coronary angiography was operated on patients with no stenosis of the coronary artery. ACS was based on 2003 ACC/AHA guidelines of diagnosis and treatment. Stenosis of the coronary artery was confirmed by coronary angiography.

Extraction and amplification of genomic DNA

Genomic DNA was extracted from frozen periph-

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eral-blood leukocytes by standard methods. Briefly, venous blood(7 ml) was collected from each subject into tubes containing 50 mmol/L of EDTA, and genomic DNA was isolated by the phenol-chloroform method. Polymorphic regions of each MMP-9 were amplified by the polymerase chain reaction (PCR) with upstream primer 5'-GCCTGGCACATAGTAG-GCCC-3' and downstream primer 5'-CTTCC-TAGCCAGCCGGCATC-3'. The reaction mixture (20 μl) contained 0.1 μg of DNA, 1.25 μmol of each primer, 0.2 mmol/L of each deoxynucleoside triphosphate, 2 mmol/L of magnesium chloride, and 3 U of DNA polymerase in corresponding DNA polymerase buffer. The amplification protocol comprised an initial period of denaturation at 95℃ for 5 minutes, 30 cycles of denaturation at 94℃ for 30 seconds, annealing at 64℃ for 30 seconds, extension at 72℃ for 1 minute, and a final period of extension at 72℃ for 10 minutes. The PCR products were digested by restriction enzyme *Nsp*I at 37° for 5 hours. The reaction mixture (20 μl) contained the PCR products 1 μg and *Nsp*I 2U in corresponding restriction enzyme buffer. The extreme products (1 μg/μl) were denatured and separated by agarose-gel electrophoresis. Allele frequency equaled twice homozygote number plus the heterozygote number divided twice(subjects).

Statistical Analysis

All calculations were performed with SPSS statistical software. Measured variables were compared between patients with acute coronary syndrome and controls by the unpaired Student´s *t*-test. Categorical variables were compared with use of the chi-square test. Allele frequencies were estimated by the gene-counting method.

RESULTS

Comparison of clinical characteristics

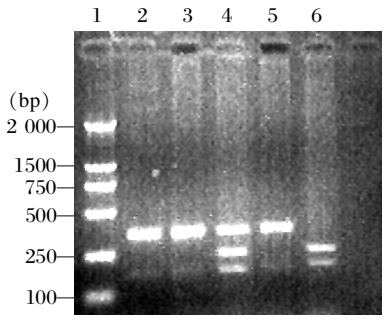
The clinical characteristics of the 449 subjects in study for the mmp-9 polymorphism are shown in ***Tab 1***. Among the two groups, there were no significant differences in age, body-mass index, or the prevalence of some risk factors for coronary artery disease, including hypertension and hypercholesterolemia. But the prevalence of both smoking and diabetes mellitus was higher in the patients with ACS than that in controls.

Tab 1 **Comparison of clinical characteristics between ACS and control group**

Groups	Sample size (M/F)	age (year)	HBP (%)	Smoking (%)	DM (%)	TG (mmol/L)	HDL-C (mmol/L)	LDL-C (mmol/L)	SB (mmHg)	DBP (mmHg)
ACS	176/69	63.4 ± 10.5	42.0	53.61	8.40	1.83 ± 0.83	0.89 ± 0.32	2.65 ± 0.71	134.05 ± 17.90	78.52 ± 9.48
Control	134/70	62.9 ± 11.8	35.9	41.01	2.93	1.68 ± 1.12	1.15 ± 0.32	2.76 ± 0.76	133.98 ± 15.60	78.84 ± 9.40

Electrophoresis result of different genetypes

The extreme products (1 μg/μl) were denatured and separated by agarose-gel electrophores. Allele frequency equaled twice homozygote number plus heterozygote number divided by two subjects. The result was shown in ***Fig 1***.



1:Marker; 4: CT genotype; 6: TT genotype; 2,3,5: CC genotype.

Fig 1 Electrophoresis result of different genetypes

The distributions of genotype frequencies

The distributions of gen otype frequencies were significantly different between the entire population of patients with acute coronary syndrome and the patients without coronary artery disease. Because of the small number of patients with the very rare TT genetype, we analyzed them together with the CT genetypes. The frequencies of CT+TT were 22.1% and 12.7%, respectively ($x^2 = 6.554$, $P = 0.01$). (***Tab 2***). The frequency of T allele was 11.4% and 6.6% in patients with acute coronary syndrome and control patients, respectively($x^2 = 6.136$, $P = 0.013$). As in subgroups, there was no significant difference between patients with acute myocardial infarction (AMI) and unstable angina pectoris(UAP). The frequencies of CT+TT were 22.8%, 21.2%, respectively($x^2 = 0.073$, $P = 0.788$).(***Tab 3***). We also studied the relationship between genetypes and the severity of coronary stenosis. The polymorphism of MMP-9 had no significant effect on the severity of coronary stenosis.(***Tab 4***).

Tab 2 **The distributions of genotype frequencies between ACS and control group**

Group		Genotypic frequency (%)				Allele frequency(%)			
		CC	CT+TT	x^2	*P* value	C	T	x^2	*P* value
ACS	245	191(201.3)	52+2(43.7)	6.554	0.01	434(88.6)	56(11.4)	6.136	0.013
Control	204	178(167.6)	25+1(36.3)			381(93.4)	27(6.6)		

Tab 3 **The distributions of genotype frequencies between AMI and UMP group**

Group		Genotypic frequency(%)			
		CC	CT+TT	x^2	*P* value
AMI	114	88	24+2	0.073	0.788
UAP	131	103	28		

Tab 4 **The relationship between genetypes and the severity of coronary stenosis**

Genotype		Severity of stenosis				x^2	*P* value
		0	1	2	≥3		
CT+TT	54	2	21	24	7	2.787	0.426
CC	191	9	94	62	26		

DISCUSSION

It is already known that the interaction between polygenic character and environmental agents is the main pathopoiesis. In recent years, some researches have presumed some genovariation to predict coronary artery disease and acute coronary syndrome [11], but the results of most of these studies however, remain controversial.

The degradation of ECM was a prerequisite for histoleucocyte and VSCMs to migrate through coronary atherosclerosis[12]. When plaque ruptured, acute coronary syndrome happened. As the main protease to degradate ECM, MMPs had been a hot spot in recent years. MMP-9 belonged to Gelatinases B, and it was famous for the ability of degrading collagenase and interstitial collagen [13]. It could degraded denatured collagens, type IV, V, VII, X, and XII collagens, which was concerned with the degradation of ECM of atherosclerotic plaque.

It was showed that MMP-9 level was significantly high in AMI and UAP patients[14,15]. Inokubo reported compared with control group, MMP-9 level was significantly high in AMI and UAP patients' coronary circulation. The activity of MMP-9 was tightly controlled at several different cellular levels. There were three main points: ① modulation of gene expression by cytokines, growth factors and hormones; ② synthesis and secretion of proMMPs, activation of proenzymes; ③ inhibition of the active enzymes tissue inhibitors of matrix metalloproteinases (TIMPs). The polymorphism of MMP-9 may be one of these regulators.

This study showed the frequencies of CT+TT were 22.1%, 12.7% respectively in patients with ACS and control subjects ($x^2 = 6.554, P = 0.01$). The frequency of T allele was 11.4% and 6.6% respectively ($x^2 = 6.136, P = 0.013$). Our result revealed the C1562T polymorphism in the MMP-9. 37 gene was associated with a significant risk of acute coronary syndrome. The interaction between gene mutation and environmental agents may facilitate plaque rupture by up-regulating the expression of MMP-9. As in subgroups, there was not significant difference between patients with acute myocardial infarction and unstable angina pectoris ($x^2 = 0.073$, $P = 0.788$), which was coincidental with the pathogenesy of AMI and UAP.

We also found that the polymorphism of MMP-9 had no significant effect on the severity of coronary stenosis ($P > 0.05$).This was because acute coronary syndrome had no direct correlation with the severity of coronary stenosis. Pollanen PJ *et al*[16] reported that T allele was associated with a high risk of stenosis of three arteries. Because of the limited size of the study population, we were unable to analyze this aspect.

To summarize, studies revealed that the distribution of the C1562T polymorphism in the MMP-9 gene was fairly different in distinct localities and racial groups. This article indicated C1562T polymorphism in the MMP-9 gene was related to a high risk of ACS in Han people from Eastern China. The T allele may be a predisposing gene for ACS.

Some studies have examined the relations between the polymorphism of MMP-9 gene and ischemic heart disease[17]. The results of these studies, however, remain controversial mainly because of the ethnic diversity of polymorphisms, and complicating environmental factors [18]. Furthermore, acute coronary syndrome was rarely analyzed separately. Wang Meifang's [19] studies in China found that the polymorphism of MMP-9 was significantly different between the UAP and control group. However the sample size was relatively small. Blankenberg S *et al*[20] reported that the T allele in CHD patients was codominant with an increased peripheral level of

MMP-9, and would influence prognosis. The internal relationship between the C1562T polymorphism in the MMP-9 gene and ACS requires more large scale studies in differing localities.

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