Functional Polymorphism in the Promoter Region of Matrix Metalloproteinase-9 is Strongly Associated with Acute Myocardial Infarction

Pum Joon Kim, M.D.1, Kiyuk Chang, M.D.2, Yoon Seok Koh, M.D.1, Ki Bae Seung, M.D.1, Sang Hong Baek, M.D.1, Woo Seung Shin, M.D.2, Sang Hyun Lim, M.D.1, Jae Hyung Kim, M.D.1, Soon Jo Hong, M.D.1 and Kyu Bo Choi, M.D.1

1Department of Internal Medicine, College of Medicine, The Catholic University of Korea, Seoul, 2Uijeongbu St. Mary’s Hospital, Uijeongbu, Korea

ABSTRACT

Background and Objectives: Matrix metalloproteinase-9 (MMP-9) plays an important role in the genesis of atherosclerotic plaque rupture and acute coronary syndrome (ACS), including an acute myocardial infarction (AMI). A single nucleotide polymorphism (SNP) in the MMP-9 promoter (−1562C>T) has recently been identified. This study investigated whether the SNP of the MMP-9 promoter is a significant risk factor for an AMI due to plaque rupture and if SNPs affect the transcription of the gene that elevates the MMP-9 expression level.

Subjects and Methods: A polymerase chain reaction, followed by a restriction fragment length polymorphism analysis, was performed in 173 control participants and 206 AMI patients. The serum levels of MMP-9 in the groups with or without the SNP were measured, using ELISA, and compared.

Results: There was a significantly higher incidence of the −1562C>T MMP-9 polymorphism in the AMI compared to the control group (27.6% vs. 17.9%, \( \text{p}=0.04 \)). A multiple logistic regression analysis of the risk factors for coronary artery disease and the MMP-9 polymorphism showed the MMP-9 polymorphism to be an important factor in the prediction of an AMI (odds ratio 1.67, 95% confidence interval 1.02–2.67, \( \text{p}=0.04 \)).

Conclusion: The −1562C>T polymorphism in the MMP-9 promoter is a definite risk factor for an AMI, and is associated with elevated MMP-9 expression. These results suggest that a SNP in the MMP-9 promoter is strongly associated with an Acute Myocardial Infarction.

KEY WORDS: Matrix metalloproteinase-9; Polymorphism; Acute myocardial infarction.

Introduction

The rupture of an atherosclerotic plaque is a major event in the pathogenesis of an acute myocardial infarction (AMI).1,2 Plaques that are vulnerable to rupture tend to have a large lipid-rich atheromatous core, a thin fibrous cap covering the core, active inflammatory cellular infiltration, particularly in the plaque shoulders effected by strong shear stress, and a reduced smooth muscle cell density collagen content in the fibrous cap.3-6 Matrix metalloproteinases (MMPs), mainly produced by infiltrating inflammatory cells, degrade the extracellular matrix, thereby contributing to the weakening of the cap and its subsequent rupture.7 Of the many MMPs, MMP-9 (gelatinase B) might play an important role in matrix degradation and the subsequent rupture of the atherosclerotic plaques, owing to its broad substrate specificity and distal position in the matrix proteolytic cascade.8 MMP-9 is involved in the degradation of a broad spectrum of substrates, including collagen types IV, V, VII and X as well as gelatin,9,10 and also degrades the proteoglycans and elastins, which are resistant to degradation by other MMPs, such as stromelysin and interstitial collagenase.9,10 In contrast to the constitutive expression of MMP-2 in the non-atherosclerotic arteries, an analysis of human coronary atherectomy specimens revealed the uniformly active synthesis of MMP-9 in lesions from patients with unstable angina, but not in those with stable angina.9,11 Recently, a functional single nucleotide polymorphism in the MMP-9 promoter, with a cytosine to thymidine transition at position −1562, was found.12 According to in vitro assays of the promoter activity, the T allele
had a higher promoter activity than the C allele, and was associated with an elevation in both the levels of MMP-9 expression level and serum MMP-9 in humans.\(^{12,13}\) However, an association of a SNP in the MMP-9 promoter with AMI has not been reported in Korea, even though an association of a SNP in the MMP-9 promoter with coronary artery disease and coronary artery complicated lesions have been reported.\(^{12,14}\) Therefore, the aim of this study was to determine if the MMP-9 \(-1562C>T\) polymorphism is a significant risk factor in patients with AMI.

**Subjects and Methods**

**Study population**

This study included 206 AMI patients (mean age 61 years, male 60.7%) and 173 control participants, with a mean age 58 years, but all were less than 70 years old: 65.3% were males. The cases consisted of first-event AMI patients admitted to the 3 participating hospitals (Kangnam St Mary’s Hospital, Uijongbu St. Mary’s Hospital, and Bucheon Holy Family Hospital, all in South Korea) between January 2003 and August 2004. The diagnoses of AMI were established according to the World Health Organization criteria and Consensus Document of the Joint European Society of Cardiology/American College of Cardiology Committee for the Redefinition of a Myocardial infarction.\(^{15}\) This study focused on the atherothrombotic coronary plaques resulting from a plaque rupture. Accordingly, all cases had elevated cardiac troponin levels and complex lesion morphology on coronary angiography, which included ulcerating plaques and intraluminal filling defects or occlusive thrombi. This study excluded those patients with normal looking coronary angiograms or previous coronary revascularization. The healthy control subjects were enrolled from those visiting one of the Health Checkup Centers of the above three participating hospitals for their regular checkup.

Assays of serum MMP-9

The peripheral blood was drawn from all AMI patients within 24hrs of admission, and stored at \(-80^\circ\text{C}\). A standard sandwich enzyme immunoassay was performed to measure the serum MMP-9 concentrations, using commercially available kits with a monoclonal antibody against MMP-9 according to the manufacturer’s instructions (R & D systems, MN, USA), but only on those patients with an AMI to determine the serum MMP-9 levels, according to the genotypes, at the time of plaque rupture.

**Genotyping of the MMP-9 gene**

The genomic DNA was extracted from the mononuclear cells of each participant, and the specific genotypes of the MMP-9 promoter identified using a polymerase chain reaction followed by restriction fragment length polymorphism analysis (PCR-RFLP). For an analysis of the \(-1562C>T\) polymorphism in the MMP-9 promoter, PCR was performed using primer pairs designed to amplify the sequence from 1809 to 1374 in the promoter region. The sequences of the sense and antisense oligonucleotide primers were 5’-GCCT-GGCACATAGTAGGGCC-3’ and 5’-CTTCCTAGCCAGCGGCATC-3’, respectively. The resulting 435 bp amplification product was incubated with the restriction enzyme, SphI. This resulted in three potential fragments (435, 247 and 188 bp in size), which were separated on a 1.5% agarose gel and visualized using ethidium bromide. The CC homozygotes produced a single undigested band at 435 bp, whereas the CT heterozygotes showed the three bands: 435, 247 and 188 bp (Fig. 1).

![Fig. 1. The three different fragments of the MMP-9 promoter. M: methionine, CC: cytocine cytocine, CT: cytocine thymin, TT: thymin thymin](image-url)
**Statistical analysis**

All statistical analyses were conducted between the control group and patients with AMI using the PC-SAS system (SAS Institute Inc., Cary, North Carolina, USA). The statistical differences in the frequency of each genotype between the groups were examined using a univariate analysis. The serum levels of MMP-9 in the AMI patients, both with and without the SNP in the MMP-9 promoter (that is, TT+CT) were compared. All the continuous variables were analyzed using paired t-tests and all the categorical variables using $\chi^2$-tests. In order to identify the independent risk factors for an AMI, a multiple logistic regression analysis, with a forward stepwise selection (Wald), was also performed. The independent variables included in the model were a SNP in the MMP-9 promoter, age, gender, body mass index, hypercholesterolemia, and the presence of hypertension, diabetes mellitus and smoking. $P<0.05$ were considered significant.

**Results**

**Characteristics of the study participants**

Table 1 shows the characteristics of the AMI patients and control subjects. Among the coronary artery risk factors, age and LDL cholesterol level were the only statistically different factors between the AMI patients and control subjects.

**MMP-9 1562C>T polymorphism and AMI**

The genotype frequency of the $-1562C>T$ polymorphism in the 206 AMI patients and 173 controls was compared (Table 2). The prevalence of the genotype in each group agreed with those predicted by the Hardy-Weinberg equilibrium. While there were no TT homozygotes in the control group, four appeared in the AMI group. The frequencies of the T allele in the MMP-9 promoter were 14.3 and 8.9% in the AMI and control groups, respectively ($p=0.0001$). The frequencies of the TT and CT genotype in the MMP-9 promoter were 26.7 and 17.9% in the AMI and control groups, respectively ($p=0.0001$).

**Association between the MMP-9 polymorphisms and the serum level of MMP-9 in AMI patients**

Figure 2 shows a higher plasma MMP-9 level in the group with the $-1562C>T$ polymorphism in the MMP-9 promoter (494.8 ng/mL) than in the group without (309.5 ng/mL, $p=0.04$).

A multiple logistic regression analysis, with the known coronary artery disease risk factors and MMP-9 polymorphism, was performed. In order to determine the predictive risk factors for an AMI, a multiple logistic regression analysis, with the forward stepwise selection, using all the clinical risk factors and the MMP-9 genotypes, was performed. The most predictive risk factor for an AMI was age (odds ratio 1.80, 95% confidence interval 1.20−2.72, $p=0.004$), followed by...

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**Table 1. Characteristics of the study participants**

<table>
<thead>
<tr>
<th>Variables</th>
<th>Control group (n=173)</th>
<th>AMI group (n=206)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)*</td>
<td>58.3±11.8</td>
<td>61.1±11.8</td>
</tr>
<tr>
<td>Male (%)</td>
<td>65.3</td>
<td>60.7</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>28.3±2.9</td>
<td>23.9±3.4</td>
</tr>
<tr>
<td>LDL-C (mg/dL)†</td>
<td>120.4±37.3</td>
<td>129.1±35.1</td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>39.9</td>
<td>44.7</td>
</tr>
<tr>
<td>DM (%)</td>
<td>15.6</td>
<td>18.0</td>
</tr>
<tr>
<td>Smoking (%)</td>
<td>39.9</td>
<td>40.8</td>
</tr>
</tbody>
</table>

Values are expressed as the mean±standard deviation. AMI indicates acute myocardial infarction. BMI: body mass index, LDL-C: low density lipoprotein-cholesterol, DM: diabetes mellitus, *: $p=0.01$, †: $p=0.04$.

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**Table 2. Distribution of MMP-9 genotypes**

<table>
<thead>
<tr>
<th>Allele</th>
<th>Control group</th>
<th>AMI group</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>T</td>
<td>31 (8.9%)</td>
<td>59 (14.3%)</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>315 (91.1%)</td>
<td>353 (85.7%)</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

**Table 3. Multiple logistic regression analysis with known coronary artery risk factors and MMP-9 polymorphism (forward stepwise selection)**

<table>
<thead>
<tr>
<th>Variables</th>
<th>OR</th>
<th>95% CI*</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>1.80</td>
<td>1.20−2.72</td>
<td>0.004</td>
</tr>
<tr>
<td>MMP-9 polymorphism</td>
<td>1.67</td>
<td>1.02−2.67</td>
<td>0.04</td>
</tr>
</tbody>
</table>

*: 95% confidence interval. OR: odds ratio

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**Fig. 2. Serum MMP-9 level according to the MMP-9 genotypes.**

Serum MMP-9 level of the CC genotype: 304.5±21.9 ng/mL.
Serum MMP-9 level of the CT & TT genotypes: 494.8±3.66 ng/mL.
CC: cytocine cytocine, CT: cytocine thymin, TT: thymin thymin.
the SNP in the MMP-9 promoter (odds ratio 1.67, 95% confidence interval 1.02–2.67, p=0.04) (Table 3).

Discussion

This study showed that the −1562C>T polymorphism in the promoter region of the MMP-9 gene has a significant and independent role in the development of an acute myocardial infarction in association with increased serum MMP-9 levels in the Korean population. We found that the T allele in the MMP-9 promoter was significantly more frequent in AMI patients than in control subjects, suggesting that patients with the T allele have rupture-prone atherosclerotic plaques in their coronary arteries. Studies of human atherosclerotic specimens have demonstrated that MMP-9 can contribute to remodeling of the coronary artery plaques due to degradation of the extracellular matrix, which contributes to the progression of plaques via a series of events: namely plaque rupture, microthrombus formation and then healing, which eventually results in severe coronary artery disease. Several polymorphisms have been reported to be associated with an AMI in Koreans, including the eNOS gene polymorphism. However, the present study provides the first evidence of the association between a polymorphism in the MMPs and an AMI in the Korean population.

MMP-9 could contribute to the development of an acute myocardial infarction by degrading a broad range of matrices and also some substrates resistant to degradation by other MMPs. An analysis of human coronary atherectomy specimens showed an increased MMP-9 expression in the atheromas of patients with acute coronary syndrome, but weak or absent expressions in the lesions of those with stable angina. Clinical studies have also shown that the serum level of MMP-9 was acutely elevated at the time of acute coronary syndrome compared with those in normal controls and patients with stable angina.

Recently, Zhang et al reported a novel polymorphism (−1562C>T) in the promoter region of the MMP-9. The promoter region contains the 9-bp sequence (GGCGAC/TGGCC), an important regulatory element of gene expression, which appears to be a binding site for a transcription repressor pro-tein. The DNA-protein interaction is lost due to a C to T substitution at the polymorphism site, resulting in a T-allelic promoter, with higher promoter activity, and an increased level of MMP-9 synthesis. An autopsy study of the coronary arteries showed that subjects carrying this T allele had larger complicated lesion areas than those with the CC genotype, suggesting the MMP-9 −1562C>T polymorphism contributes to the development of a plaque rupture by influencing the endogenous MMP-9 production. Indeed, in this study the serum level of MMP-9 was higher in the AMI patients with the MMP-9 polymorphism than in those without. In contrast with our results, Wang et al failed to show any relationship between a MMP-9 polymorphism and acute coronary syndrome in Caucasians. This discrepancy might have arisen from differences between the patients included in the two studies. Their study included patients with a myocardial infarction as well as those with unstable angina, which is a heterogeneous disorder from angina due to plaque rupture to that with a secondary precipitating cause. This might lead to an inappropriate selection of patients. Whereas, our study tried to include only those patients with AMI due to a plaque rupture. Furthermore, Wang’s study included those with atypical chest pain as the control group, which leads to the inclusion of those with other inflammatory conditions. It can also be explained with respect to the population specificity, which suggests that a certain genetic variant may be relevant to a defined population only.

In conclusion, this study showed that a functional polymorphism in the promoter region of the MMP-9 gene is strongly associated with an acute myocardial infarction in the Korean population, which was independent of known coronary risk factors. Further study on a larger population will be required to confirm this polymorphism as a potential novel genetic marker for a plaque rupture in Koreans.

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