Coronary Artery Complicated Lesion Area Is Related to Functional Polymorphism of Matrix Metalloproteinase 9 Gene: An Autopsy Study

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Abstract—Matrix metalloproteinase 9 (MMP9) is expressed in human atherosclerotic plaques, and the protein is localized in human coronary atherosclerotic lesions. The MMP9 gene has a C-to-T promoter polymorphism at position −1562, which affects transcription and leads to promoter low-activity (C/C) and high-activity (C/T, T/T) genotypes. To determine whether these genotypes exert an influence on the atherosclerotic lesion area, we investigated their association with different types of coronary lesions in an autopsy cohort of 276 men aged 33 to 69 years. Areas of the coronary wall covered with fatty streaks and fibrotic, calcified, and complicated lesions were measured, and the percentage of coronary narrowing was determined. MMP9 genotypes were determined by polymerase chain reaction and restriction enzyme digestion. In men aged ≥53 years, the mean area of complicated lesions in 3 coronaries was significantly associated with the MMP9 genotype (P=0.008). Subjects with high promoter activity genotypes had, on average, larger complicated lesion areas than did those with the low-activity genotype. The MMP9 genotype persisted as an independent predictor of complicated lesion area after adjustment for age, body mass index, hypertension, diabetes, and smoking (P=0.012). These data provide evidence that the proposed effect of MMP9 in the process of atherosclerotic lesion development may be modified by the MMP9 genotype. (Arterioscler Thromb Vasc Biol. 2001;21:1446-1450.)

Key Words: matrix metalloproteinase 9 • coronary artery disease • complicated lesions • genetics • polymorphism

The matrix metalloproteinases (MMPs), among them MMP9 (also known as gelatinase B and 92-kDa type IV collagenase), constitute a family of enzymes required for the degradation of extracellular matrix during embryonic development, morphogenesis, and tissue remodeling. MMP3 (stromelysin 1), MMP1 (interstitial collagenase), and MMP9 (92-kDa gelatinase) are present and enzymatically active in atherosclerotic plaques and are known to be associated with the progression and development of atherosclerotic lesions.1,2 The MMP family consists of ≥15 metal-dependent endopeptidases with activity against most extracellular matrix macromolecules.

MMP9 has recently been identified in human atherosclerotic lesions.2,3 It is active against denatured collagens (gelatin) and type IV, V, and XI collagens in addition to the proteoglycans and elastin also found in atherosclerotic lesions.4 MMP9 expression is primarily regulated at the transcription level, with the promoter of the gene responding to different growth factors and cytokines.5 Zhang et al6 have found a functional MMP9 gene promoter C-to-T polymorphism at position −1562, which affects gene transcription and yields promoter low-activity (C/C) and high-activity (C/T, T/T) genotypes.6 They also observed an association between this polymorphism and the severity of angiographically measured atherosclerosis.

No data are as yet available on the impact of the MMP9 genotypes at the vessel-wall level in respect to the coronary complicated lesion area and plaque rupture. Nor has it hitherto been sought to establish whether MMP9 genotypes affect coronary artery lesion development or plaque rupture. Therefore, we investigated in an autopsy series of 276 Finnish men included in the Helsinki Sudden Death Study (HSDS) whether the MMP9 promoter low- and high-activity genotype groups are related to the areas of different types of coronary lesions.7

Methods

Subjects
The HSDS was designed to investigate lifestyle and genetic factors predisposing to sudden death in Finnish middle-aged men living in...
Rubber Casts of Coronary Arteries

Measuring Area of Atherosclerosis Lesions by Silicone Rubber Casts of Coronary Arteries

At autopsy, coronary angiography was performed by using vulcanizing liquid silicone rubber mixed with lead oxide as contrast medium. This procedure does not dislodge an attached thrombus from its site and has been successfully used in the routine diagnostics of thrombotic and other complications after coronary artery bypass surgery. Proximal, medial, and distal narrowing of the main trunks of the LAD, RCA, and LCX were measured on the rubber cast model with a mauser. The percentage of coronary narrowing was obtained by dividing the diameter (in millimeters) of the greatest stenosis by that of the nearest proximal undamaged part of the cast model of the same artery.

Measuring Coronary Narrowing on Silicone Rubber Casts of Coronary Arteries

The vessel wall was stained for fat by the Sudan IV fat-staining method. Any flat or slightly elevated intimal lesion that stained distinctly with Sudan IV and showed no apparent change beneath it was classified as a fatty streak. A raised lesion not exhibiting ulceration or thrombosis was regarded as a fibrous plaque. Any plaque area with ulceration or thrombosis was regarded as a complicated lesion. Areas of coronary artery calcification were assessed from the radiograph taken from the dissected arteries. Radiopaque areas were considered to signify areas of calcification. Coronary artery wall areas covered by fatty streaks or fibrous, calcified, and complicated lesions were measured by a computer-assisted planimetric technique. The percentage area of the various atheromatous changes was obtained by dividing the lesion area by the total surface area of the coronary segment studied. The total atherosclerotic lesion area of the arterial wall was the total area of fatty streak and fibrotic lesions. Because the complicated lesions in the arterial wall were always incorporated in either the fatty streak or in the fibrotic lesion area, or both, the complicated lesion area was analyzed separately. Autopsy and MMP9 genotype data were available for 276 men; this autopsy cohort constituted the final study population.

Characteristics and Phenotypes of MI

The presence of myocardial infarction (MI) in the series was confirmed by a macroscopic and histological examination of the myocardium. The presence/absence of neutrophil granulocytes was considered diagnostic of an acute MI, and the presence/absence of fibrous scar tissue was considered diagnostic of an old MI. The presence of recent or organizing macroscopic coronary thrombosis was recorded while the coronary arteries were being opened.

Risk Factors Underlying Coronary Artery Disease

A spouse, a relative, or a close friend of the deceased was interviewed within 2 weeks after death. Apart from questions pertaining to the risk of sudden death (ie, arterial hypertension, diabetes), additional questions were included to establish the past smoking habits of the deceased. Data on smoking habits was acquired for 153 men, of whom 17 were exsmokers and were included in the class of smokers for statistical analysis. In addition to MMP9 genotype and autopsy data, complete data on all risk factors were available for 123 men, who constituted the adjusted study population.

Determination of the MMP9 Genotype

A region of the MMP9 gene in the promoter region was amplified by using primary and secondary (nested) primers designed from those reported by Zhang et al. Genomic DNA extracted from frozen cardiac muscle samples taken at autopsy was used for polymerase chain reaction. After digestion of the polymerase chain reaction product with BbvI restriction endonuclease, the fragments were separated by use of standard agarose gel electrophoresis.

Statistical Analyses

Data analysis was based on ANCOVA and linear regression. Nonnormally distributed morphometric data were analyzed either in square root or arcsine-square root form, but the results were displayed as crude data. These transformations were the most suitable to normalize the distributions. To achieve a suitable group

<table>
<thead>
<tr>
<th>Genotype</th>
<th>&lt;53 y</th>
<th>≥53 y</th>
<th>All Subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>CT/CT</td>
<td>CC</td>
<td>CT/CT</td>
<td>CC</td>
</tr>
<tr>
<td>Genotype</td>
<td>35</td>
<td>93</td>
<td>39</td>
</tr>
<tr>
<td>Age, y</td>
<td>42.06±5.06</td>
<td>43.32±4.20</td>
<td>60.90±5.04</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>23.96±4.18</td>
<td>25.09±5.11</td>
<td>24.57±5.22</td>
</tr>
<tr>
<td>MI, n (%)</td>
<td>6 (17)</td>
<td>10 (11)</td>
<td>17 (44)</td>
</tr>
<tr>
<td>Acute MI, n (%)</td>
<td>5 (14)</td>
<td>7 (8)</td>
<td>4 (10)</td>
</tr>
<tr>
<td>Coronary thrombus, n (%)</td>
<td>2 (6)</td>
<td>5 (5)</td>
<td>3 (8)</td>
</tr>
<tr>
<td>Cardiac disease, n (%)</td>
<td>9 (26)</td>
<td>27 (29)</td>
<td>22 (56)</td>
</tr>
</tbody>
</table>

Values are mean±SD.

*Excluding causes of death due to other diseases, violence, intoxication, and unknown reasons (n=6).
†Data available for 135 men.
‡Data available for 153 men.
Men aged $\geq 53$ years had, on average, a 153.7% larger area of complicated lesion compared with men aged $<53$ years. Men aged $\geq 53$ years, in whom there was a low promoter activity genotype, had, on average, a 65.8% larger area of calcified lesion in the LAD, a 31.7% larger area in the LCX, and a 67.6% larger area in the RCA. Compared with carriers of the promoter low-activity genotype, carriers of the promoter high-activity genotype had, on average, a 63.3% larger area of complicated lesion compared with those with the promoter low-activity genotype ($P=0.07$, borderline significance; Table 2).

For separate vessels, the results were parallel. Compared with carriers of the promoter low-activity genotype (data not shown), carriers of the promoter high-activity genotype had, on average, a 70.0% larger area of calcified lesion compared with those with the promoter low-activity genotype ($P=0.07$, borderline significance; Table 2).

### Results

#### Descriptive Data

In the present cohort of 276 men aged 33 to 69 years, the mean area of complicated lesions was 2.89% in the most severely affected coronary artery. The proportion of areas of complicated lesion ranged from 0% and 78.7% of the total vessel area; 63.3% evinced no complicated lesion. The mean percent areas of complicated lesion in the most severely affected coronary artery was 1.2% in men aged $<53$ years and 4.3% in men aged $\geq 53$ years. MMP9 genotype frequencies for both age groups are shown in Table 1. The genotype proportions were 73% for C/C (95% CI 68% to 78%), 25% for C/T (95% CI 20% to 30%), and 2% for T/T (95% CI 1% to 5%). The distributions of genotypes followed the Hardy-Weinberg equilibrium. There were no differences in genotype and allele frequencies between the 2 age groups. The cause of death, age, BMI, and prevalence of hypertension, diabetes, and smoking for the respective MMP9 genotypes are shown in Table 1.

#### MMP9 Gene Variation and Complicated Lesion Area

Men aged $\geq 53$ years with a high promoter activity genotype had, on average, a 153.7% larger area of complicated lesion than did those with the low-activity genotype ($P=0.008$, Table 2). With analysis by linear regression with age, MMP9 genotype, BMI, hypertension, diabetes, and smoking used as independent variables, the significant variables were age ($P=0.001$, standardized regression coefficient 3.43) and MMP9 genotype ($P=0.012$, standardized regression coefficient $-2.56$). The entire model explained 20.2% of the variation in lesion area ($P<0.005$). For other types of lesions, there were no significant differences between the genotype groups. Men aged $\geq 53$ years with a promoter high-activity genotype had, on average, a 57.0% larger area of calcified lesion compared with those with the promoter low-activity genotype ($P=0.07$, borderline significance; Table 2).

For separate vessels, the results were parallel. Compared with carriers of the promoter low-activity genotype (data not shown), carriers of the promoter high-activity genotype had, on average, a 70.0% larger area of calcified lesion compared with those with the promoter low-activity genotype ($P=0.07$, borderline significance; Table 2).

#### MMP9 Gene Variation and MI

MMP9 promoter high-activity genotype increased the risk of MI significantly (odds ratio [OR] 1.95, 95% CI 1.03 to 3.67; $P=0.04$), as did the older age group (OR 3.47, 95% CI 1.90 to 6.54; $P<0.001$), but BMI was borderline (OR 1.05, 95% CI 0.99 to 1.12; $P=0.08$). However, the occurrence of acute MI, ie, the presence/absence of neutrophil granulocytes (OR 1.10, 95% CI 0.48 to 2.53; $P=0.82$), or coronary thrombi...
(OR 0.79, 95% CI 0.28 to 2.25; P=0.66) was not significantly associated with the MMP9 genotype.

Discussion

In the present study, the MMP9 gene promoter genotype was found to be related to the area of complicated coronary lesions. At the vessel-wall level, the MMP9 promoter high-activity genotypes represented a significant risk factor for complicated coronary lesions, and this applied to all 3 main coronary trunks (LAD, LCX, and RCA). The MMP9 promoter genotype increased the risk of healed MI significantly (presence/absence of fibrous scar tissue). We divided the study population according to median age (53 years) into 2 subgroups. Younger subjects rarely have the complicated lesion phenotype, and early-stage forms of atherosclerosis predominate. In contrast, elderly subjects have a high incidence of the complicated lesion phenotype. We observed no age–MMP9 genotype interaction, although the effect of the MMP9 promoter high-activity genotypes was most clearly seen in men aged ≥53 years.

No previous studies have investigated the association between MMP9 gene promoter polymorphism and the severity of atherosclerosis as measured directly from arterial wall samples obtained at autopsy with simultaneous analysis of coronary narrowing. The approach that was used circumvents many of the problems encountered in phenotyping clinical patients because measurements were made directly from the coronary arteries, and the presence of MI and coronary thrombosis could be determined reliably. Brown et al3 found an association by using immunostaining between the pattern of MMP9 expression and acute ischemic coronary syndrome (unstable angina). Zhang et al6 observed an association between the MMP9 promoter high-activity genotypes and the severity of atherosclerosis as assessed by coronary angiography, but no association emerged between MI and the polymorphism.

Extensive vascular remodeling is likely to take place in atherogenesis and lesion progression. Lesion growth in the early stage of atherosclerosis may result mainly from lipid deposition, because in the late stages of atherosclerosis in more advanced lesions, connective tissue accumulation and smooth muscle cell proliferation are the prominent contributors to plaque growth.10 MMP9 alters postinjury vascular remodeling and, like MMP3 and MMP1, evinces proteolytic activity against several proteins associated with complicated plaques (eg, type IV collagen, a major component in the basement membrane) and facilitates vascular smooth muscle cell migration and proliferation.11–13 We assume that remodeling of the arterial extracellular matrix and subsequent plaque vulnerability or smooth muscle cell migration associated with the MMP9 T allele contributes to the pathogenesis of atherosclerosis.5,11–14 In a previous study, the major MMP expressed by cultured vascular smooth muscle cells was MMP2 and not MMP9, which would suggest that MMP9 may exert its effect through plaque destabilization rather than by promoting vascular smooth muscle migration to the intima.14

MMP9 is expressed in the shoulder region of the atherosclerotic lesion2–3 and is regulated at 3 levels: transcription, activation of proenzymes, and specific inhibition by endogenous tissue inhibitors of MMPs, which, in turn, like the MMPs themselves, are regulated by various cytokines and growth factors through cis elements in their gene promoters.12,15–17 An increase in MMP9 expression in the plaque shoulder areas, especially in MMP9 promoter T-allele carriers, may weaken the lesion and contribute to plaque vulnerability or plaque rupture, leading to MI.

Although we found no association between the MMP9 genotype and acute MI, an association between the MMP9 genotype and old infarct scarring was observed. The absence of association between the MMP9 genotype and coronary thrombi in the present study may be attributable to the fact that there were only 5 men with thrombotic MI among carriers of the T allele, or it may be due to the fact that only macroscopic thrombi were involved in the classification. However, it may equally be due to a role of the MMP9 genotype in clinically silent plaque ruptures/erosions because the great majority of the old infarcts were originally clinically silent and/or unrecognized.18 In their results, Zhang et al6 could find no difference in the frequencies of the MMP9 genotypes between patients with MI and control subjects. On the other hand, in their results, the percentage of patients with triple-vessel disease was higher in the CT/T and TT genotype class than in the CC class. Furthermore, Brown et al3 found an association between the pattern of MMP9 expression and acute ischemic coronary syndrome (unstable angina). Discrepancies in previous epidemiological studies may arise from the heterogeneity in the pathogenesis of MI, because in many cases of sudden cardiac death, instead of acute MI and acute coronary thrombosis, no thrombosis can be found, and death is caused by arrhythmias arising from old MI scars in the absence of a recent MI.18,19 The association of borderline significance between MMP9 promoter genotype and calcified lesion area seen in men aged ≥53 years in the present study may reflect the hypothesized resemblance between arterial calcification and osteogenesis, inasmuch as MMP9 has been shown to be specifically required for the invasion of osteoclasts and endothelial cells into developing bone.20

Although chance might explain our finding of increased lesion area in relation to the MMP9 promoter genotype, it may be advisable in future studies to consider the effect of this genotype on atherosclerotic lesion phenotype. The MMP9 promoter high-activity genotypes, compared with the promoter low-activity genotypes, were found in the present study to be associated with a larger area of coronary complicated plaque. Our findings suggest that the MMP9 gene functional promoter polymorphism is 1 of the genetic factors that participates in the complex process of atherogenesis and, in particular, in the development of complicated coronary lesions.

Acknowledgments

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