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 endoproteases 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...
Helsinki and its environs. The autopsy series was collected during the period 1991 to 1992 at the Department of Forensic Medicine, University of Helsinki. The indications for autopsy were out-of-hospital death of a previously healthy person, accidental death, suspected intoxication, suicide, and death in connection with medical treatment. The original study population was composed of a prospectively collected series of 276 white men aged 33 to 69 years. This autopsy series covered 42% of all deaths among men aged <65 years in the area of Helsinki during the years in question. The cause of death was cardiac disease in 39% (n = 109), other diseases in 20% (n = 56), and violent death (suicides and accidents) in 40% (n = 111), as shown in Table 1. The body mass index (BMI) was calculated by dividing the weight (in kilograms) of the cadaver by the height (in meters) squared. The proximal parts of the left anterior descending coronary artery (LAD), right coronary artery (RCA), and left circumflex coronary artery (LCX) were collected for analysis.

**Measuring Coronary Narrowing on 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 Area of Atherosclerosis Lesions by Computer-Assisted Morphometry**

The definition of atherosclerosis was based on the protocols of 2 international studies: the International Atherosclerosis Project, Standard Operating Protocol 1962, and the World Health Organization Study Group in Europe. Coronary arteries were dissected free, opened, attached to a card, and then fixed in 10% buffered formalin. 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.6 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 Bsal 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

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**TABLE 1. Subjects by MMP9 Genotype and Age Subgroup**

<table>
<thead>
<tr>
<th>MMP9 genotype group</th>
<th>&lt;53 y</th>
<th>≥53 y</th>
<th>All Subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>CT/TT (n = 35)</td>
<td>CC (n = 93)</td>
<td>CT/TT (n = 39)</td>
<td>CC (n = 109)</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>
<tr>
<td>Coronary risk factor</td>
<td>†Diabetes (yes/no), n</td>
<td>3/18</td>
<td>2/38</td>
</tr>
<tr>
<td>†Hypertension (yes/no), n</td>
<td>7/14</td>
<td>12/28</td>
<td>4/16</td>
</tr>
<tr>
<td>‡Smoking (yes/no), n</td>
<td>5/16</td>
<td>12/33</td>
<td>11/14</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.
size, MMP9 genotype was categorized into promoter low-activity (C/C) and high-activity (C/T, T/T) genotype groups. Furthermore, we divided the series according to median age (53 years) into 2 subgroups to study the genotype association: elderly subjects (aged ≥53 years), in whom the complicated lesion phenotype dominates, and young subjects (aged <53 years), in whom there was a low incidence of the complicated lesion phenotype. MMP9 genotype status was used as a factor in the 1-way ANCOVA, in which BMI was taken into the model as a covariate. We also used linear regression analysis to find independent risk factors for the complicated lesion, taking the mean area of complicated lesion as an independent variable and age, BMI, hypertension, diabetes, and smoking used as independent variables, the significant variables were age (P=0.012, 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 ≥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 74.0% larger area of complicated lesion in the LAD, a 419.0% larger area in the LCX, and a 51.4% larger area in the RCA. Compared with carriers of the low-activity genotype, carriers of the high-activity genotype had, on average, a 65.8% larger area of complicated lesion in the LAD, a 31.7% larger area in the LCX, and a 67.6% larger area in the RCA (data not shown). There were no significant differences between the genotype groups with respect to other types of lesion. The percentage of coronary narrowing was not significantly associated with MMP9 genotype in any of the 3 coronary arteries. There was no significant association between the MMP9 genotype and severity of atherosclerosis as assessed by numbers of coronary arteries that had a stenosis >50%.

### 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 ≥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 ≥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 ≥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 74.0% larger area of complicated lesion in the LAD, a 419.0% larger area in the LCX, and a 51.4% larger area in the RCA. Compared with carriers of the low-activity genotype, carriers of the high-activity genotype had, on average, a 65.8% larger area of complicated lesion in the LAD, a 31.7% larger area in the LCX, and a 67.6% larger area in the RCA (data not shown). There were no significant differences between the genotype groups with respect to other types of lesion. The percentage of coronary narrowing was not significantly associated with MMP9 genotype in any of the 3 coronary arteries. There was no significant association between the MMP9 genotype and severity of atherosclerosis as assessed by numbers of coronary arteries that had a stenosis >50%.

### 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 1.84 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...
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 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 participate in the complex process of atherogenesis and, in particular, in the development of complicated coronary lesions.

Acknowledgments

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