Relations of Plasma Matrix Metalloproteinase-9 to Clinical Cardiovascular Risk Factors and Echocardiographic Left Ventricular Measures

The Framingham Heart Study

Johan Sundström, MD, PhD; Jane C. Evans, DSc; Emelia J. Benjamin, MD, ScM; Daniel Levy, MD; Martin G. Larson, ScD; Douglas B. Sawyer, MD, PhD; Deborah A. Siwik, PhD; Wilson S. Colucci, MD, PhD; Patrice Sutherland, BS; Peter W.F. Wilson, MD; Ramachandran S. Vasan, MD

Background—Plasma levels of matrix metalloproteinase-9 (MMP-9), a key determinant of extracellular matrix degradation, are increased in heart failure and in acute coronary syndromes. We investigated cross-sectional relations of plasma MMP-9 to vascular risk factors and echocardiographic left ventricular (LV) measurements.

Methods and Results—We studied 699 Framingham Study participants (mean age, 57 years; 58% women), free of heart failure and previous myocardial infarction, who underwent routine echocardiography. We examined sex-specific distributions of LV internal dimensions (LVEDD) and wall thickness (LVWT) and sampled persons with both LVEDD and LVWT below the sex-specific median (referent, n = 299), with increased LVEDD (LVEDD ≥90th percentile, n = 204) and increased LVWT (LVWT ≥90th percentile, n = 221) in a 3:2:2 ratio. Plasma MMP-9 was detectable in 138 persons (20%). In multivariable models, increasing heart rate (OR per SD, 1.41; 95% CI, 1.17 to 1.71) and antihypertensive treatment (OR, 1.63; 95% CI, 1.06 to 2.50) were key clinical correlates of detectable plasma MMP-9. In multivariable-adjusted models, detectable plasma MMP-9 was associated with increased LVEDD (OR, 2.84; 95% CI, 1.13 to 7.11), increased LVWT (OR, 2.54; 95% CI, 1.00 to 6.46), and higher LV mass (P = 0.06) in men but not in women (OR for increased LVEDD, 1.37; 95% CI, 0.54 to 3.46; for increased LVWT, 0.99; 95% CI, 0.39 to 2.52; P = 0.59 for LV mass).

Conclusions—In our community-based sample, detectable plasma MMP-9 levels were associated with increased LV diastolic dimensions and increased wall thickness in men. These observations indicate that plasma MMP-9 level may be a marker for cardiac extracellular matrix degradation, a process involved in LV remodeling. (Circulation. 2004;109: 2850-2856.)

Key Words: heart failure ■ hypertrophy ■ metalloproteinases ■ remodeling ■ echocardiography

Left ventricular (LV) dilation and concentric LV hypertrophy (LVH) are known precursors of heart failure (HF) and are associated with increased collagen turnover in the cardiac extracellular matrix. The breakdown and accumulation of myocardial collagen is regulated by various extracellular matrix metalloproteinases (MMPs) and their tissue inhibitors (TIMPs).

Several lines of evidence suggest a fundamental role for myocardial MMP-9 in LV remodeling. In experimental studies, increased myocardial expression of MMP-9 has been observed in parallel with increasing LV mass as the result of pressure overload. In animal HF models, MMP inhibition reduces LV dilation and preserves cardiac systolic function. Furthermore, mice with deletion of the MMP-9 gene have less LV dilation after an ischemic insult. Observations in small case series of patients with HF also support the importance of MMP-9 in LV remodeling. Both myocardial and blood levels of MMP-9 are elevated in patients with HF. After implantation of an assist device, myocardial MMP activity in patients with HF declines, coinciding with functional LV recovery. More recently, plasma MMP-9 levels have been reported to predict fatal events in patients with known coronary artery disease, raising the possibility that circulating levels may be a marker of vascular remodeling.

Given the emerging importance of MMP-9 in ventricular and vascular remodeling, it is noteworthy that the clinical correlates of circulating MMP-9 remain incompletely understood. Previous investigations have been conducted in small samples of highly selected individuals. Some studies have
Sampling schema for study based on sex-specific distribution of LVEDD and LVWT, and derivation of study sample. All individuals with both LVWT and LVEDD at or below the sex-specific 50th percentile (upper left shaded area) are included and constitute the control group. Individuals with LVWT or LVEDD at or above the sex-specific 90th percentile are also sampled and constitute the groups with increased LVWT (bottom shaded area) and increased LVEDD (shaded area on right), respectively. Twenty-five participants had both increased LVWT and LVEDD (lower right-hand shaded area). Percentile areas are not drawn to scale for simplicity.

reported that plasma MMP-9 levels are higher in patients with coronary disease,24,25 diabetes,26 and dyslipidemia25 and in smokers.27 Others have reported lower serum MMP-9 levels in hypertensive individuals.28

We hypothesized that cardiovascular disease risk factors are related to plasma MMP-9 levels; that LV dilation and dysfunction are associated with remodeling of the cardiac extracellular matrix reflected in plasma MMP-9 levels; and that the relation of plasma MMP-9 to LV measures varies according to plasma TIMP-1 levels, the natural inhibitor of MMP-9. Accordingly, we examined the clinical and echocardiographic correlates of plasma MMP-9 in Framingham Study participants.

Methods

Study Sample
The 3532 attendees of the sixth examination of the Framingham Offspring Study,29 a primarily white cohort, were eligible. Also eligible were 506 attendees of the first examination of the Framingham Omni Study cohort,29 sampled from Framingham residents 40 to 75 years old, who identified themselves as members of an ethnic minority (58% women; 36% black, 40% Hispanic). Both groups were examined between 1985 and 1998. Participants with echocardiographic measurements available constituted our sampling frame. Initially, we examined sex-specific distributions of end-diastolic LV internal diameter (LVEDD) and wall thickness (LVWT). We selected a stratified random sample consisting of 3 groups to obtain a 3:2:2 ratio (Figure): a referent group (n=776) consisting of participants with values of LVEDD and LVWT below the sex-specific medians; an increased LVEDD group with LVEDD values greater than or equal to the sex-specific 90th percentile; and an increased LVWT group with LVWT greater than or equal to the sex-specific 90th percentile. We chose this sampling strategy to combine statistical efficiency with cost containment and prudent use of nonrenewable specimens.

Of the selected participants, we excluded 77 individuals for the following reasons: prevalent congestive HF (n=13), prior myocardial infarction (n=27), serum creatinine >2 mg/dL or missing (n=17), or missing covariates (n=20). After exclusions, 699 participants remained eligible (58% women): referent group, n=299; increased LVEDD group, n=204; and increased LVWT group, n=221. 25 individuals had both increased LVEDD and increased LVWT (Figure). For analyses of clinical correlates of MMP-9, we further excluded 51 individuals with clinically apparent cardiovascular disease. However, these individuals were included for the analyses of echocardiographic correlates.

The Institutional Review Board at Boston Medical Center approved the study, and all subjects gave written informed consent.

Clinical Examination
Participants underwent a standardized medical history and physical examination, including measurements of blood pressure, phlebotomy for assessment of cardiovascular risk factors, and a 12-lead ECG. Diabetes and hypertension were defined according to current guidelines.30,31 Prevalence of cardiovascular disease was established by a panel of 3 physicians using published criteria.32

Plasma Extracellular Matrix
Marker Measurements
Blood samples drawn from fasting participants in a supine position were centrifuged, and plasma was frozen at −70°C until assayed. Plasma MMP-9 was measured in duplicate with the use of a 2-site sandwich ELISA assay (Amersham Pharmacia Biotech), which measures MMP-9, ProMMP-9, and the ProMMP-9/TIMP-1 complex, with an assay range of 4 to 128 ng/mL.33 Plasma TIMP-1 was measured similarly with a 2-site sandwich ELISA assay (Amersham Pharmacia Biotech), which measures free TIMP-1 and TIMP-1 complexed with various MMPs. The intra-assay coefficient of variation was <18% for MMP-9 and <5% for TIMP-1 measurements.

Echocardiographic Methods
Participants underwent routine transthoracic echocardiography with Doppler flow imaging. M-mode measurements of LV dimensions were obtained by use of the leading edge-to-leading edge technique.34 The interventricular septum thickness (IVS), posterior LV wall thickness (PW), and LVEDD were measured at end-diastole. LVWT was calculated as IVS+PW. LVM was calculated as 0.8[(IVS+LVEDD+PW)3−(LVEDD)3]+0.6 g.35 Valve disease was defined as greater than a mild degree of stenosis or regurgitation of the aortic or mitral valves on Doppler echocardiography. Reproducibility of echocardiographic measurements was excellent.

Statistical Analyses
Plasma MMP-9 was detectable (lower detection limit of 4 ng/mL) in only 20% of individuals, so plasma MMP-9 was modeled as a binary variable (detectable versus undetectable) in all analyses.

We investigated the relations of select clinical variables (age, sex, ethnicity [white versus nonwhite], body mass index, smoking, alcohol intake [log-transformed for normality], diabetes, total/HDL cholesterol ratio, statin treatment, systolic blood pressure, antihypertensive treatment, heart rate, atrial fibrillation, and plasma TIMP-1) to plasma MMP-9 levels, using logistic regression.36 All models were fitted initially for each clinical variable separately; stepwise selection was then used to obtain a multivariable model.

We used logistic regression36 to examine the associations of detectable plasma MMP-9 with increased LVEDD and with increased LVWT, participants in the referent group being the comparison group. Participants with increased LVEDD and increased LVWT were included in both models. All analyses were sex specific. Two sets of models were examined in a hierarchical fashion adjusting for (1) age and height, and (2) age, height, weight, ethnicity, smoking, alcohol intake, diabetes, total/HDL cholesterol, systolic blood pressure, antihypertensive treatment, valve disease, and heart rate.

We also used sex-specific multiple linear regression to examine the relations of LV mass, LVEDD, and LVWT (analyzed as continuous variables) to detectable MMP-9.
Table 1. Characteristics of Study Sample

<table>
<thead>
<tr>
<th>Clinical characteristics</th>
<th>Men (n=295)</th>
<th>Women (n=404)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Referent Group</td>
<td>Increased LVEDD</td>
</tr>
<tr>
<td></td>
<td>(n=137)</td>
<td>(n=75)</td>
</tr>
<tr>
<td>Age, y</td>
<td>54±8</td>
<td>59±10</td>
</tr>
<tr>
<td>Ethnicity, % nonwhite</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>26.4±3.3</td>
<td>28.3±3.9</td>
</tr>
<tr>
<td>Smoking, %</td>
<td>10</td>
<td>9</td>
</tr>
<tr>
<td>Alcohol drinks/wk</td>
<td>5.5±6.3</td>
<td>6.0±6.6</td>
</tr>
<tr>
<td>Diabetes, %</td>
<td>4</td>
<td>17</td>
</tr>
<tr>
<td>Total/HDL cholesterol ratio</td>
<td>4.8±1.3</td>
<td>4.7±1.2</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>123±16</td>
<td>133±18</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>76±8</td>
<td>78±10</td>
</tr>
<tr>
<td>Pulse pressure, mm Hg</td>
<td>47±13</td>
<td>54±15</td>
</tr>
<tr>
<td>Antihypertensive treatment, %</td>
<td>16</td>
<td>31</td>
</tr>
<tr>
<td>Hypertension, %</td>
<td>29</td>
<td>51</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>62±9</td>
<td>59±11</td>
</tr>
<tr>
<td>Atrial fibrillation, %</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>Valve disease, %</td>
<td>1</td>
<td>12</td>
</tr>
<tr>
<td>Cardiovascular disease, %</td>
<td>3</td>
<td>15</td>
</tr>
<tr>
<td>Statin treatment, %</td>
<td>4</td>
<td>13</td>
</tr>
</tbody>
</table>

Matrix metalloproteinase-9 and tissue inhibitor of metalloproteinase-1 data

| Detectable MMP-9, % | 12 | 27 | 34 | 16 | 19 | 19 |
| Plasma MMP-9, ng/mL* | 33 (26–44) | 43 (25–72) | 28 (24–44) | 42 (26–62) | 31 (25–45) | 35 (27–61) |
| Plasma TIMP-1, ng/mL | 788 (720–856) | 793 (735–898) | 818 (760–912) | 747 (675–821) | 763 (700–871) | 830 (712–935) |

Echocardiographic data

| LV mass, g | 154±18 | 250±42 | 255±47 | 108±13 | 183±28 | 187±33 |
| LWWT, cm   | 1.84±0.10 | 2.00±0.24 | 2.53±0.21 | 1.64±0.10 | 1.85±0.21 | 2.26±0.20 |
| LVEDD, cm  | 4.76±0.25 | 5.93±0.28 | 4.97±0.48 | 4.22±0.23 | 5.24±0.16 | 4.52±0.45 |
| LVFS, %    | 36±5 | 33±7 | 38±6 | 39±5 | 36±6 | 39±6 |

FS indicates fractional shortening.

Plasma MMP-9 and TIMP-9 values are medians and interquartile ranges. Other values are mean ± SD and percentages. Twenty-five individuals had both increased LVEDD and increased LWT.

*Levels in persons with detectable plasma MMP-9.

Additional Analyses

Investigators have underscored the importance of computing the stoichiometric ratio of MMP-9 to TIMP-1 to better characterize the matrix degradation capacity of MMP-9.22 Because MMP-9 was below the detection limit in 80% of individuals, a ratio of MMP-9/TIMP-1 was not estimated. We therefore included plasma TIMP-1 levels as a covariate (less than versus greater than or equal to sex-specific median) in multivariable models.

A 2-sided probability value of ≤0.05 was considered statistically significant. All statistical analyses were performed with the use of SAS statistical software (version 8, SAS Institute).37

Results

Plasma MMP-9 was detectable in 138 of 699 individuals (20%, 73 women). In those with detectable MMP-9, the median plasma MMP-9 was 33 ng/mL (interquartile range, 25 to 52). Clinical characteristics of the sample are shown in Table 1.

Clinical Correlates of Detectable Plasma MMP-9 in Participants Without Clinical Evidence of Cardiovascular Disease

In age- and sex-adjusted logistic regression models, smoking, diabetes, antihypertensive treatment use, and heart rate were statistically significant correlates of detectable MMP-9 (Table 2). In stepwise logistic regression, only antihypertensive treatment and heart rate were selected.

Relations of Detectable Plasma MMP-9 to LV Dilation and Increased LV Wall Thickness

In men, detectable plasma MMP-9 was associated with nearly 3-fold higher odds of increased LVEDD and with 2.5-fold higher odds of increased LV wall thickness (Table 3, multivariable-adjusted models). The results of linear regression models comparing echocardiographic LV mass, LVWT, and LVEDD in people with detectable versus those with...
Clinical Correlates of Detectable Plasma MMP-9: Logistic Regression Analyses

<table>
<thead>
<tr>
<th>Variable (SD Increment)</th>
<th>Detectable MMP Age- and Sex-Adjusted Models</th>
<th>Detectable MMP Multivariable-Adjusted Model (Stepwise Selection)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR (95% CI)</td>
<td>(P)</td>
</tr>
<tr>
<td>Age (9.6 y)</td>
<td>1.13 (0.94–1.38)</td>
<td>0.20</td>
</tr>
<tr>
<td>Women vs men</td>
<td>0.78 (0.53–1.15)</td>
<td>0.21</td>
</tr>
<tr>
<td>White vs nonwhite</td>
<td>0.84 (0.44–1.62)</td>
<td>0.61</td>
</tr>
<tr>
<td>Body mass index (5.2 kg/m²)</td>
<td>1.17 (0.97–1.41)</td>
<td>0.099</td>
</tr>
<tr>
<td>Smokers vs nonsmokers</td>
<td>1.82 (1.08–3.07)</td>
<td>0.026</td>
</tr>
<tr>
<td>Natural log alcoholic drinks/wk (1.0)</td>
<td>0.98 (0.80–1.19)</td>
<td>0.83</td>
</tr>
<tr>
<td>Diabetic vs nondiabetic</td>
<td>1.95 (1.10–3.43)</td>
<td>0.022</td>
</tr>
<tr>
<td>Total/HDL cholesterol (1.38)</td>
<td>1.09 (0.90–1.33)</td>
<td>0.38</td>
</tr>
<tr>
<td>Statin treatment vs none</td>
<td>1.73 (0.92–3.26)</td>
<td>0.089</td>
</tr>
<tr>
<td>Systolic blood pressure (21 mm Hg)</td>
<td>1.13 (0.92–1.40)</td>
<td>0.25</td>
</tr>
<tr>
<td>Diastolic blood pressure (10 mm Hg)</td>
<td>1.11 (0.91–1.35)</td>
<td>0.32</td>
</tr>
<tr>
<td>Pulse pressure (17 mm Hg)</td>
<td>1.09 (0.88–1.36)</td>
<td>0.42</td>
</tr>
<tr>
<td>Antihypertensive treatment vs none</td>
<td>1.57 (1.01–2.46)</td>
<td>0.046</td>
</tr>
<tr>
<td>Heart rate (9.9 bpm)</td>
<td>1.45 (1.20–1.76)</td>
<td>0.0002</td>
</tr>
<tr>
<td>Atrial fibrillation vs none</td>
<td>1.08 (0.21–0.54)</td>
<td>0.92</td>
</tr>
<tr>
<td>TIMP-1 ≥ median vs &lt; median</td>
<td>0.92 (0.61–1.37)</td>
<td>0.67</td>
</tr>
</tbody>
</table>

*Blank cells indicate that the variable was available for entry into the stepwise model but did not enter (at \(P\) = 0.10) or did not remain at \(P\) < 0.05.

Clinical Correlates of Detectable Plasma MMP-9

A striking finding of our investigation was that the majority of participants did not have detectable plasma MMP-9. Although circulating MMP-9 levels may be elevated in patients with HF and in patients with coronary disease, our data suggest that plasma MMP-9 is unlikely to be an informative biomarker in a low-risk sample of the general population.

Heart rate and antihypertensive treatment emerged as key correlates of detectable plasma MMP-9 in multivariable-adjusted analyses. The association of plasma MMP-9 with heart rate is intuitive because a higher heart rate is associated with increased myocardial oxygen consumption and wall stress and is an indicator of increased HF risk. Activation of non-detectable MMP-9 are displayed in Table 4. Detectable plasma MMP-9 was related positively to LV mass and LVWT in men but not in women; men with detectable MMP-9 had adjusted LV mass 13 g higher and adjusted LVWT thickness 2 mm greater relative to those without detectable MMP-9. LVEDD was not related to detectable MMP-9 in either sex. In secondary analyses, the relations of detectable MMP-9 to increased LVEDD and increased LVWT were maintained after additional adjustment for plasma TIMP-1.

In women, there was no significant relation of plasma MMP-9 to the odds of increased LVEDD or increased LVWT in any model (Table 3). Additionally, in models evaluating LV mass, LVWT, and LVEDD as continuous variables, none of the echocardiographic variables evaluated were related to detectable plasma MMP-9 (Table 4).

### Table 3. Odds Increased Left Ventricular Dimensions and Wall Thickness Associated With Detectable Plasma MMP-9

<table>
<thead>
<tr>
<th></th>
<th>OR (95% CI)</th>
<th>(P)</th>
<th>OR (95% CI)</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Men</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age and height adjusted</td>
<td>2.20 (0.99–4.91)</td>
<td>0.054</td>
<td>4.18 (1.97–8.84)</td>
<td>0.0002</td>
</tr>
<tr>
<td>Multivariable adjusted*</td>
<td>2.84 (1.13–7.11)</td>
<td>0.026</td>
<td>2.54 (1.00–6.46)</td>
<td>0.050</td>
</tr>
<tr>
<td><strong>Women</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age and height adjusted</td>
<td>1.59 (0.79–3.19)</td>
<td>0.19</td>
<td>1.41 (0.72–2.77)</td>
<td>0.31</td>
</tr>
<tr>
<td>Multivariable adjusted*</td>
<td>1.37 (0.54–3.46)</td>
<td>0.51</td>
<td>0.99 (0.39–2.52)</td>
<td>0.98</td>
</tr>
</tbody>
</table>

Odds ratios indicate odds of increased LVEDD or LVWT in participants with detectable MMP-9 compared with those without detectable MMP-9 (referent).

*Multivariable models adjusted for age, height, weight, smoking, alcohol intake, diabetes, total/HDL cholesterol ratio, systolic blood pressure, antihypertensive treatment, valve disease, ethnicity and heart rate.

### Discussion

Clinical Correlates of Detectable Plasma MMP-9

A striking finding of our investigation was that the majority of participants did not have detectable plasma MMP-9. Although circulating MMP-9 levels may be elevated in patients with HF and in patients with coronary disease, our data suggest that plasma MMP-9 is unlikely to be an informative biomarker in a low-risk sample of the general population.

Heart rate and antihypertensive treatment emerged as key correlates of detectable plasma MMP-9 in multivariable-adjusted analyses. The association of plasma MMP-9 with heart rate is intuitive because a higher heart rate is associated with increased myocardial oxygen consumption and wall stress and is an indicator of increased HF risk.
myocardial MMP-9 may be noted in LVH and in early HF. The positive association with antihypertensive treatment is in contrast with one previous study in which serum MMP-9 was lower in a small sample of hypertensive patients. In our experience, antihypertensive use is a marker of chronicity and severity of blood pressure elevation. Only 30% of the hypertensive subjects in our sample had adequately controlled blood pressure. Pressure overload has been noted to be a marker of vascular remodeling. The increased LVWT associated with detectable MMP-9 may represent two time points in the same process; for example, persons with detectable MMP-9 and increased LVWT may have increased LVEDD. Second, activation of MMP-9 is well documented in diastolic HF characterized by increased LVWT, perhaps as a counterbalance to the increased levels of TIMPs. Third, it is possible that plasma MMP-9 is also a marker of vascular remodeling. The increased LVWT observed with high MMP-9 levels could be viewed as a marker of adverse vascular remodeling processes such as increased vascular stiffness. The positive relations to antihypertensive treatment may reflect greater chronic vascular load in those with higher MMP-9. Fourth, MMP-9 has been demonstrated to promote growth in some tissues through alterations of growth factor/cell surface receptor processing, a process inhibited by TIMPs. Many growth factors, including TGF-β family members, receptors of inflammatory cytokines including IL-6, and the erbB receptor tyrosine kinases, are proteolytically processed by MMPs, resulting in alterations in signaling associated with growth in some tissues. Furthermore, some growth factors also upregulate MMPs, including MMP-9. Additional research is warranted to clarify if such complicated mechanisms operate in the adult human heart.

In summary, the increased plasma MMP-9 observed with LV remodeling phenotypes in men may indicate increased LVWT associated with detectable MMP-9 may represent two time points in the same process; for example, persons with detectable MMP-9 and increased LVWT may have increased LVEDD. Second, activation of MMP-9 is well documented in diastolic HF characterized by increased LVWT, perhaps as a counterbalance to the increased levels of TIMPs. Third, it is possible that plasma MMP-9 is also a marker of vascular remodeling. The increased LVWT observed with high MMP-9 levels could be viewed as a marker of adverse vascular remodeling processes such as increased vascular stiffness. The positive relations to antihypertensive treatment may reflect greater chronic vascular load in those with higher MMP-9. Fourth, MMP-9 has been demonstrated to promote growth in some tissues through alterations of growth factor/cell surface receptor processing, a process inhibited by TIMPs. Many growth factors, including TGF-β family members, receptors of inflammatory cytokines including IL-6, and the erbB receptor tyrosine kinases, are proteolytically processed by MMPs, resulting in alterations in signaling associated with growth in some tissues. Furthermore, some growth factors also upregulate MMPs, including MMP-9. Additional research is warranted to clarify if such complicated mechanisms operate in the adult human heart.

In summary, the increased plasma MMP-9 observed with LV remodeling phenotypes in men may indicate increased LVWT associated with detectable MMP-9 may represent two time points in the same process; for example, persons with detectable MMP-9 and increased LVWT may have increased LVEDD. Second, activation of MMP-9 is well documented in diastolic HF characterized by increased LVWT, perhaps as a counterbalance to the increased levels of TIMPs. Third, it is possible that plasma MMP-9 is also a marker of vascular remodeling. The increased LVWT observed with high MMP-9 levels could be viewed as a marker of adverse vascular remodeling processes such as increased vascular stiffness. The positive relations to antihypertensive treatment may reflect greater chronic vascular load in those with higher MMP-9. Fourth, MMP-9 has been demonstrated to promote growth in some tissues through alterations of growth factor/cell surface receptor processing, a process inhibited by TIMPs. Many growth factors, including TGF-β family members, receptors of inflammatory cytokines including IL-6, and the erbB receptor tyrosine kinases, are proteolytically processed by MMPs, resulting in alterations in signaling associated with growth in some tissues. Furthermore, some growth factors also upregulate MMPs, including MMP-9. Additional research is warranted to clarify if such complicated mechanisms operate in the adult human heart.

Sex Differences in Relations of Detectable Plasma MMP-9 to LV Measurements

An intriguing finding in our study was that plasma MMP-9 level was associated with LV remodeling phenotypes in men.
but not in women. It is noteworthy that statistical power to detect an odds ratio of 2.50 for increased LVEDD or increased LVWT was only about 50% (at α of 0.05) in both men and women due to modest sample size. We had slightly more women in our sample, with equal proportions of men and women having detectable plasma MMP-9. It is possible that modest associations of plasma MMP-9 with increased LVEDD or LVWT in women may have been missed as the result of limited statistical power. It is equally conceivable that molecular differences exist between sexes in the LV remodeling process. Investigators have reported sex-related differences in remodeling at the cardiomyocyte level, influenced partly by MMP activation. In the early phase of the volume overload, male animals have smaller myocyte lengths as the result of anisotopic division of binucleate cardiomyocytes, whereas female animals have increased length of myocytes. Estrogen may reduce both the plasma levels and the tissue effects of MMP-9. Transcriptional downregulation of MMP-9 promoter by estrogen-activated estrogen receptors has been reported. Additional investigations are required to confirm our findings and to identify plausible mechanisms for sex-related differences.

**Limitations and Strengths**

Interpretation of the plasma MMP-9 associations found in our study is challenging for several reasons. First, it is simplistic to relate plasma levels of a single metalloproteinase (and its endogenous inhibitor) to clinical and echocardiographic variables with a view to understand the processes of vascular and ventricular remodeling. We chose to measure plasma MMP-9 because experimental data support a more important role of this enzyme in LV remodeling relative to other metalloproteinases, although we are well aware that such an approach carries the penalty of possible uncontrolled confounding by unmeasured MMPs and TIMPs. Additional studies measuring other MMPs (eg, MMPs 2, 8, and 14) are warranted, especially plasma MMP-2, given its role in diastolic HF. Second, total plasma MMP-9 levels measured by an ELISA may not reflect true MMP-9 “activity.” MMP-9 activity may be better assessed by zymographic methods and by measurement of serum levels of collagen breakdown products and by measuring levels of “free” MMP-9 and “free” TIMP-1. Third, analyses relating plasma MMP-9 to echocardiographic measures presuppose that plasma levels correlate with myocardial levels and that the heart is an important source of circulating MMP-9. Fourth, plasma MMP-9 was below the detection limit of the standardized assay in 80% of our sample; as a result, statistical analyses were constrained by the use of MMP-9 as a binary variable. As our sample was predominantly white, generalizability to other ethnic groups is limited.

These caveats notwithstanding, the present study is the first investigation to examine, comprehensively, the clinical and echocardiographic correlates of plasma MMP-9 in a moderately sized, community-based sample, free of confounding by prevalent myocardial infarction and HF.

**Conclusions**

In this moderately sized, community-based sample free of prevalent HF and myocardial infarction, detectable plasma MMP-9 level was associated with increased LVEDD and increased LV wall thickness in men. These observations are consistent with the concept that plasma MMP-9 level may be a marker for cardiac extracellular matrix degradation, a process involved in LV remodeling.

**Acknowledgments**

This study was supported by NHLBI/NIH Contract N01-HC-25195, 1RO1HL67288-01, and 1K24HL04334 (Dr Vasan) and the Swedish Heart Lung Foundation (Dr Sundström).

**References**


Relations of Plasma Matrix Metalloproteinase-9 to Clinical Cardiovascular Risk Factors and Echocardiographic Left Ventricular Measures: The Framingham Heart Study

_Circulation_. 2004;109:2850-2856; originally published online June 1, 2004;
doi: 10.1161/01.CIR.0000129318.79570.84
_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2004 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/109/23/2850

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Circulation_ can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to _Circulation_ is online at:
http://circ.ahajournals.org//subscriptions/