Heritability and Correlates of Intercellular Adhesion Molecule-1 in the Framingham Offspring Study

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The notion that inflammation plays a role in the development of clinical manifestations of atherosclerosis now is firmly established (1). The recruitment of inflammatory cells into the arterial wall is dependent upon cellular adhesion molecules and begins with leukocyte rolling on the endothelium that is facilitated by endothelial expression of P-selectin and its interaction with leukocyte P-selectin glycoprotein ligand-1 (2). Firm leukocyte adhesion requires an interaction between leukocyte beta1 and beta2 integrins and endothelial adhesion molecules such as vascular cell adhesion molecule-1 and intracellular adhesion molecule-1, respectively (3). With respect to the latter, several prospective studies have demonstrated that circulating soluble levels of intracellular adhesion molecule-1 (sICAM-1) are predictive of future cardiovascular events (4–6) and the development of peripheral arterial disease (7). The independent predictive value of sICAM-1 with respect to incident cardiovascular disease (CVD) is controversial because it is lost after multivariable adjustment for traditional cardiovascular risk factors (4,5,7).

We sought to determine the clinical factors and heritability associated with inflammation measured as circulating levels of soluble-intercellular adhesion molecule-1 (sICAM-1) in a community-based cohort.

Several prospective studies indicate that circulating sICAM-1 is predictive of future cardiovascular events. However, in some studies this predictive value is lost after multivariable adjustment for traditional cardiovascular disease (CVD) risk factors. We addressed the heritability of sICAM-1 and its relation to CVD risk factors in a community-based cohort.

We examined 3,295 subjects from the Framingham Heart Study and measured sICAM-1 levels. We then used linear and stepwise multivariable regression to determine predictors or sICAM-1 levels.

In age- and gender-adjusted regression models, increased sICAM-1 levels were positively associated with age, total/high-density lipoprotein cholesterol, systolic blood pressure, body mass index (BMI), blood glucose, diabetes, smoking, and prevalent CVD. In stepwise multivariable regression models, sICAM-1 levels remained associated with age, female gender, total/high-density lipoprotein cholesterol ratio, BMI, blood glucose, smoking, and prevalent CVD. The residual heritability of sICAM-1 was 24%.

In addition to prevalent CVD, established CVD risk factors and non-traditional ones such as BMI were associated with systemic inflammation as determined by sICAM-1 levels. There also is significant heritability of sICAM-1, which suggests a genetic component to systemic inflammation. (J Am Coll Cardiol 2004;44:168–73) © 2004 by the American College of Cardiology Foundation

OBJECTIVES

Several prospective studies indicate that circulating sICAM-1 is predictive of future cardiovascular events. However, in some studies this predictive value is lost after multivariable adjustment for traditional cardiovascular disease (CVD) risk factors. We addressed the heritability of sICAM-1 and its relation to CVD risk factors in a community-based cohort.

METHODS

We examined 3,295 subjects from the Framingham Heart Study and measured sICAM-1 levels. We then used linear and stepwise multivariable regression to determine predictors or sICAM-1 levels.

RESULTS

In age- and gender-adjusted regression models, increased sICAM-1 levels were positively associated with age, total/high-density lipoprotein cholesterol, systolic blood pressure, body mass index (BMI), blood glucose, diabetes, smoking, and prevalent CVD. In stepwise multivariable regression models, sICAM-1 levels remained associated with age, female gender, total/high-density lipoprotein cholesterol ratio, BMI, blood glucose, smoking, and prevalent CVD. The residual heritability of sICAM-1 was 24%.

CONCLUSIONS

In addition to prevalent CVD, established CVD risk factors and non-traditional ones such as BMI were associated with systemic inflammation as determined by sICAM-1 levels. There also is significant heritability of sICAM-1, which suggests a genetic component to systemic inflammation. (J Am Coll Cardiol 2004;44:168–73) © 2004 by the American College of Cardiology Foundation

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Abbreviations and Acronyms

BMI = body mass index
BP = blood pressure
CHD = coronary heart disease
CHF = congestive heart failure
CVD = cardiovascular disease
MI = myocardial infarction
sICAM-1 = soluble intracellular adhesion molecule-1

records. The latter were reviewed by a panel of three experienced investigators using previously described criteria (9). Subjects were excluded if they underwent physical examination off-site (n = 205), if serum was unavailable (n = 30), or if covariate data were missing (n = 9), which left 3,295 subjects for the current investigation.

**Determination of sICAM-1**. Serum samples were collected and stored at −70°C. For analysis, samples were thawed at room temperature, vortexed vigorously, and the sICAM-1 determined using a commercially available enzyme-linked immunosorbent assay (R & D Systems, Minneapolis, Minnesota) according to the manufacturer’s instructions and expressed as ng/ml. Samples were analyzed in duplicate with an average intra-assay coefficient of variation greater than two standard deviations from this mean (8.8%) were repeated in duplicate, and the mean of the repeat duplicate values was used in this analysis.

**Statistical analysis**. Ninety-nine percent of the sICAM-1 values were within the range of 29 to 550 ng/ml, and the distribution was approximately normal in this range. Approximately 1% of sICAM-1 values were between 543 ng/ml and the maximum of 1,332 ng/ml. The distribution of sICAM-1 was asymmetric, but analyses performed on raw (untransformed) and log-transformed data were virtually identical. We present untransformed data because they are more easily understood clinically.

Age- and gender-adjusted linear regression models were constructed to assess relations of sICAM-1 to the following individual variables: total/high-density lipoprotein (HDL) cholesterol, systolic blood pressure (BP), diastolic BP, body mass index (BMI), waist-to-hip ratio, fasting glucose level, history of diabetes, smoking, hypertension treatment, and history of myocardial infarction (MI), CHF, or CVD. Gender and age interactions also were tested for each variable listed above. Stepwise linear regression multivariable models (10) were constructed with sICAM-1 against the above variables with a p < 0.10 threshold for inclusion. The Framingham CHD Risk Score was calculated as described previously (11) except age was not included, because we age-adjusted sICAM-1 levels in analyses using the risk score. In addition, adjusted logistic regression models were constructed with prevalent CVD as the outcome to assess its relation with sICAM-1 (12). Analyses were run using SAS version 8.1 (Cary, North Carolina) (13). Any two-sided p value less than 0.05 was considered to be statistically significant.

We used Sequential Oligogenic Linkage Analysis Routines (14) to estimate residual heritability of sICAM-1, that is, the proportion of variability attributable to genetic effects after accounting for measured covariates. The underlying model assumed that trait variation could be partitioned into genetic and environmental (measured covariate) factors. The genetic component was assumed to be polygenic without dominance components. We estimated residual heritability after accounting for gender and age and then after accounting for multiple additional covariates. The portion of variation resulting from measured covariates was determined using a regression model with men and women combined.

**RESULTS**

**Subject characteristics and individual predictors of sICAM-1**. Characteristics of the study sample are displayed in **Table 1**. As expected, CVD was more prevalent in men (18%) than women (9%). Because men and women exhibited similar serum levels of sICAM-1 and because each covariate effect on sICAM-1 was similar for men and women, gender-pooled analyses were performed. Age- and gender-adjusted regression was conducted using variables from **Table 1** as correlates of sICAM-1 (Table 2). Advancing age positively correlated with sICAM-1, as was total/HDL cholesterol ratio, systolic BP, diabetes, history of hypertension treatment, smoking, MI, and CVD. Serum glucose also was positively associated with sICAM-1. Obesity, measured as overall adiposity (BMI) or its pattern (waist-to-hip ratio), also emerged as a strong positive

### Table 1. Baseline Characteristics of the Study Sample

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Men (n = 1,535)</th>
<th>Women (n = 1,760)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yrs (range)</td>
<td>61 ± 10 (37–89)</td>
<td>61 ± 9 (33–87)</td>
</tr>
<tr>
<td>Total cholesterol, mg/dl</td>
<td>192 ± 35</td>
<td>207 ± 37</td>
</tr>
<tr>
<td>Total/HDL cholesterol</td>
<td>4.5 ± 1.4</td>
<td>3.7 ± 1.2</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>128 ± 17</td>
<td>126 ± 20</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>76 ± 10</td>
<td>72 ± 10</td>
</tr>
<tr>
<td>Fasting glucose, mg/dl</td>
<td>109 ± 29</td>
<td>101 ± 25</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>28.8 ± 4.6</td>
<td>27.6 ± 5.8</td>
</tr>
<tr>
<td>Waist-to-hip ratio</td>
<td>1.0 ± 0.1</td>
<td>0.9 ± 0.1</td>
</tr>
<tr>
<td>Diabetes</td>
<td>17%</td>
<td>11%</td>
</tr>
<tr>
<td>Smoking</td>
<td>13%</td>
<td>13%</td>
</tr>
<tr>
<td>History of CVD</td>
<td>18%</td>
<td>9%</td>
</tr>
<tr>
<td>History of MI</td>
<td>8%</td>
<td>2%</td>
</tr>
<tr>
<td>History of CHF</td>
<td>2%</td>
<td>1%</td>
</tr>
<tr>
<td>Hypertension treatment</td>
<td>37%</td>
<td>31%</td>
</tr>
<tr>
<td>Lipid-lowering treatment</td>
<td>25%</td>
<td>17%</td>
</tr>
<tr>
<td>sICAM-1 (ng/ml)</td>
<td>259 ± 81</td>
<td>258 ± 85</td>
</tr>
</tbody>
</table>

All numbers are mean ± SD (continuous), or percent (categorical).

CHF = congestive heart failure; CVD = cardiovascular disease; HDL = high-density lipoprotein; MI = myocardial infarction; sICAM-1 = soluble intracellular adhesion molecule-1.
Table 2. Age- and Gender-Adjusted Regression Models for Serum sICAM-1 Levels

<table>
<thead>
<tr>
<th>Variable, U</th>
<th>Regression Coefficient (95% CI)</th>
<th>p Value</th>
<th>Partial R² (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, 10 yrs</td>
<td>8.02 (6.35, 12.35)</td>
<td>&lt;0.0001</td>
<td>1.0</td>
</tr>
<tr>
<td>Gender, female vs. male</td>
<td>-0.0073 (0.99)</td>
<td>0.99</td>
<td>0.02</td>
</tr>
<tr>
<td>Total/HDL cholesterol, 2 U</td>
<td>16.27 (9.99, 22.54)</td>
<td>&lt;0.0001</td>
<td>0.54</td>
</tr>
<tr>
<td>Systolic blood pressure, 20 mm Hg</td>
<td>4.09 (0.14, 7.96)</td>
<td>0.014</td>
<td>0.02</td>
</tr>
<tr>
<td>Diastolic blood pressure, 10 mm Hg</td>
<td>-0.67 (0.66)</td>
<td>0.66</td>
<td>0.02</td>
</tr>
<tr>
<td>Body mass index, 5 kg/m²</td>
<td>7.40 (5.01, 9.79)</td>
<td>&lt;0.0001</td>
<td>0.54</td>
</tr>
<tr>
<td>Waist-to-hip ratio, 0.075</td>
<td>10.08 (7.79, 12.38)</td>
<td>&lt;0.0001</td>
<td>0.54</td>
</tr>
<tr>
<td>Blood glucose, 25 mg/dl</td>
<td>9.82 (6.89, 12.76)</td>
<td>&lt;0.0001</td>
<td>0.54</td>
</tr>
<tr>
<td>Diabetes, present</td>
<td>26.01 (18.72, 33.30)</td>
<td>&lt;0.0001</td>
<td>0.54</td>
</tr>
<tr>
<td>Smoking, present</td>
<td>63.38 (47.39, 79.36)</td>
<td>&lt;0.0001</td>
<td>0.54</td>
</tr>
<tr>
<td>Hypertension treatment, present</td>
<td>8.30 (5.92, 10.68)</td>
<td>0.010</td>
<td>0.02</td>
</tr>
<tr>
<td>History of CHF, present</td>
<td>16.36 (13.07, 19.65)</td>
<td>0.20</td>
<td>0.02</td>
</tr>
<tr>
<td>History of MI, present</td>
<td>18.02 (14.93, 21.12)</td>
<td>0.009</td>
<td>0.02</td>
</tr>
<tr>
<td>History of CVD, present</td>
<td>22.89 (19.60, 26.18)</td>
<td>&lt;0.0001</td>
<td>0.54</td>
</tr>
</tbody>
</table>

All linear regression coefficients represent the estimated change in sICAM-1 (ng/ml) per specified number of units (continuous, approximately 1 SD) or presence (dichotomous) of the variable. Each covariate was analyzed separately, adjusting for specified number of units (continuous, approximately 1 SD) or presence of the variable. Each covariate was analyzed separately, adjusting for age and gender. Two-tailed p values were obtained for t = coefficient/standard error of coefficient) using the t distribution. Abbreviations as in Table 1.

The results of age- and gender-adjusted stepwise multivariable linear regression models are shown in Table 3. Age remained a highly significant correlate of sICAM-1, and gender emerged as a significant correlate in the multivariable model, with women averaging sICAM-1 levels that were 9 ng/ml higher than men. The emergence of gender as a predictor of sICAM-1 was driven by the entrance of total/HDL cholesterol ratio in the model because women had higher average sICAM-1 levels than men at any given level of total/HDL cholesterol ratio. Other strong positive predictors of sICAM-1 were smoking and total/HDL cholesterol ratio. Smokers demonstrated an adjusted mean sICAM-1 that was 25% higher than non-smokers (309 ± 100 ng/ml vs. 251 ± 78 ng/ml, respectively). Fasting glucose also was associated with increased sICAM-1 levels because each 25-mg/dl increase in fasting glucose was associated with a 6-ng/ml adjusted mean increase in sICAM-1. Although some previous studies have suggested that sICAM-1 is merely a reflection of CVD risk factors, we found that prevalent CVD was highly associated with sICAM-1 levels (Table 3) and that this relation was not related to time from CVD diagnosis (p = 0.70 for time effect).

To examine the relation between established CHD risk and sICAM-1, we plotted age-adjusted mean sICAM-1 (± one standard error) by gender-specific Framingham CHD risk score (11) quintile for men and women without prevalent CVD (Fig. 1). We found that increasing quintiles of risk scores were associated with increasing levels of sICAM-1. To determine whether sICAM-1 adds significantly to the risk factor profile of CVD beyond traditional risk factors, we performed multivariable logistic regression with prevalent CVD as the outcome variable and found that...
The heritability of sICAM-1 was estimated as 25.7% with age and as 24.3% among siblings in the multivariable model had no material impact on the analyses listed in Table 3. Similarity, excluding patients on hypertension treatment or on lipid-lowering medication had no material impact on the results.

**DISCUSSION**

Considerable evidence now links both atherosclerosis and coronary artery disease events to a state of vascular inflammation (15). Consistent with this notion, we found that elevated levels of sICAM-1 were related to CVD risk factors and prevalent CVD. We also found that sICAM-1 was related to CVD risk factors after excluding subjects with prevalent CVD, suggesting sICAM-1 is linked to early atherosclerosis. The notion that sICAM-1 may reflect preclinical early atherosclerosis is not without precedent. Ligands for ICAM-1 include the beta2 integrins CD11a/CD18 (LFA-1) and CD11b/CD18 (Mac-1), which are present on inflammatory cells such as neutrophils, monocytes, and lymphocytes. Atherosclerotic lesions of all stages demonstrate expression of ICAM-1, and mice deficient in either CD18 or ICAM-1 are resistant to diet-induced atherosclerosis (17). Moreover, in vivo studies in apolipoprotein E-deficient mice (18) and humans (19,20) suggest that sICAM-1 levels parallel the extent of atherosclerosis. Therefore, available data indicate that the development of atherosclerosis is, in part, dependent upon ICAM-1.

In addition to the morphologic development of atherosclerosis, clinical studies suggest a relation between ICAM-1 and CVD events. For example, prospective case-control studies have linked elevated sICAM-1 levels to incident CHD (4–6,21) and peripheral vascular disease (7).

We performed several secondary analyses to ensure that our results were not confounded by the presence of CVD or medications. We found that excluding patients with CVD had no material effect on the results contained in Table 3. Similarly, excluding patients on hypertension treatment or on lipid-lowering medication had no material impact on the results.
Correlates of sICAM in the Community

To our knowledge, this is the first report of the heritability of sICAM-1, which we found to be 24%. This level of heritability is comparable with the heritability of left ventricular mass (41). There is increasing interest in the relationship between genetic variation in inflammatory markers and the risk of clinical and subclinical CVD (42). Polymorphisms in sICAM-1 have been related to diabetes (43), CHD, and MI (44). However, the genetic basis of vascular inflammation and the relation between genetic polymorphisms in inflammatory genes and CVD are incompletely understood. Our finding that vascular inflammation is modestly heritable would suggest that its genetic basis merits further investigation.

There are several limitations of the present study that warrant consideration. First and foremost, we used sICAM-1 as an index of inflammation. Although plasma levels of soluble adhesion molecules are thought to originate from vascular cells through proteolytic cleavage and shedding (32), the precise source of sICAM-1 and factors that influence its clearance from plasma remain unknown. Therefore, our results could merely represent some change in the clearance of sICAM-1 rather than a reflection of its production per se. Additionally, because the sample was derived from a single measurement in ambulatory volunteers, sICAM-1 levels may have been confounded by medication status. We investigated such potential confounding by separately analyzing people not receiving lipid-lowering or hypertension medications and noted consistency in findings with those observed in the entire sample. Finally, the Framingham cohort is not ethnically diverse, and the extent to which our findings are applicable to other ethnic groups is not known at this time.

In summary, the data presented here support an association between circulating sICAM-1 levels and prevalent CVD as well as with established CVD risk factors such as age, smoking, increasing glucose levels, and total/HDL cholesterol ratio. Circulating levels of sICAM-1 were also modestly related to obesity, suggesting one mechanism whereby obesity may contribute to the clinical manifestations of CVD. Finally, we found modest heritability of sICAM-1 levels, suggesting that investigation into the genetic determinants of inflammation is likely to be fruitful.

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