Heritability of Coronary Artery Calcium Quantity Measured by Electron Beam Computed Tomography in Asymptomatic Adults

Patricia A. Peyser, PhD; Lawrence F. Bielak, DDS, MPH; Julia S. Chu, BS; Stephen T. Turner, MD; Darrell L. Ellsworth, PhD; Eric Boerwinkle, PhD; Patrick F. Sheedy, II, MD

Background—Electron beam computed tomography is an accurate, noninvasive method to detect and quantify coronary artery calcification (CAC), a marker of subclinical and clinical coronary artery atherosclerosis. CAC quantity predicts future coronary artery disease end points in asymptomatic adults, but measured risk factors explain less than half the variability in CAC quantity. Although several candidate genes for CAC have been identified, the relative importance of genetic influences on CAC quantity has not been assessed in asymptomatic adults in a community.

Methods and Results—We quantified the relative contributions of measured risk factors and genetic influences on CAC quantity measured by electron beam computed tomography in 698 asymptomatic white adults from 302 families. Before adjusting for any risk factors, 43.5% of the variation in CAC quantity was attributable to genetic factors ($P = 0.0007$). Independent predictors of CAC quantity were identified with stepwise linear regression. After adjusting for these risk factors, including age, sex, fasting glucose level, systolic blood pressure, pack-years of smoking, and LDL cholesterol, 41.8% of the residual variation in CAC quantity was attributable to genetic factors ($P = 0.0003$).

Conclusions—These results demonstrate the importance of genetic factors in subclinical coronary atherosclerosis variation as measured by CAC quantity. The presence of genetic effects suggests that unknown genes that influence CAC quantity are yet to be identified. (Circulation. 2002;106:304-308.)

Key Words: genetics ■ calcium ■ atherosclerosis ■ imaging ■ epidemiology

Atherosclerosis, the major cause of coronary artery disease (CAD), is influenced by a complex interplay among numerous environmental and genetic factors. Familial aggregation of CAD has been recognized for almost 100 years, and many established CAD risk factors have a genetic basis. The increased risk of CAD associated with a family history of disease even after controlling for these risk factors, however, suggests that numerous genetic factors underlying disease susceptibility are yet to be identified.

A major limitation of the use of CAD events as study end points to identify susceptibility genes is substantial disease misclassification, because many individuals with coronary atherosclerosis are asymptomatic. One half of all sudden coronary deaths and first myocardial infarctions occur in persons without previous symptoms.

Coronary artery calcification (CAC), a marker of atherosclerosis, can be quantified noninvasively and accurately by electron beam computed tomography (EBCT). A direct relationship exists between CAC and both histological and in vivo intravascular ultrasound measures of atherosclerotic plaque. CAC quantity is an independent predictor of angiographically defined CAD after controlling for established CAD risk factors, and CAC predicts future CAD end points in asymptomatic and symptomatic adults. Many established CAD risk factors, such as male sex, older age, smoking, abnormal lipid levels, high blood pressure, and ponderosity are related to CAC quantity. Much variation in CAC quantity, however, remains unexplained after accounting for these factors. The purpose of the current study was to assess the overall genetic contribution (ie, heritability) to CAC quantity measured by EBCT in asymptomatic adults from the community.

Methods

Sample

The Epidemiology of Coronary Artery Calcification (ECAC) Study is an ongoing community-based study of the pathogenesis of CAC in Rochester, Minn. Participants in the ECAC Study are not self or

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From the Department of Epidemiology, University of Michigan, Ann Arbor, Mich (P.A.P., L.F.B., J.S.C.); Division of Hypertension, Department of Internal Medicine (S.T.T.), and Department of Diagnostic Radiology (P.F.S.), Mayo Clinic and Foundation, Rochester, Minn; Gene and Drug Discovery Center, Windber Research Institute, Windber, Pa (D.L.E.); and Human Genetics Center and Institute of Molecular Medicine, University of Texas, Houston Health Science Center, Houston (E.B.).

Guest editor for this article was Bruce Brundage, MD, Bend Memorial Clinic, Bend, Ore.

Correspondence to Patricia A. Peyser, University of Michigan, Department of Epidemiology, 109 Observatory, Ann Arbor, MI 48109. E-mail ppeyser@umich.edu

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physician referred and do not have a history of previous bypass surgery, angioplasty, or other coronary surgery. ECAC Study participants were recruited from the community-based Rochester Family Heart Study.15,16

Between December 1990 and May 1998, 787 participants in the ECAC study who were at least 45 years of age were examined for CAC with EBCT. Of these 787 participants, we excluded 76 participants because they were nonwhites (n=4), had a history of myocardial infarction and/or stroke (n=24), or had missing CAC (n=11) or incomplete risk factor (n=37) data. Thirteen participants were also excluded because LDL cholesterol could not be calculated because their triglyceride levels were >400 mg/dL. The final study group consisted of 698 (360 women) asymptomatic, nonreferral participants at least 45 years of age from 302 families. Most individuals were a sibling, spouse, parent, or offspring of another individual in the study. These different relationships allowed comparisons between genetically related and unrelated individuals. All participants gave written informed consent. The Mayo Clinic and University of Michigan Institutional Review Boards approved the study protocols and process for obtaining informed consent.

Measures

Participants reported current medication use, education, history of smoking, and physician-diagnosed hypertension, myocardial infarction, stroke, or diabetes. Standard enzymatic methods were used to measure total cholesterol, HDL cholesterol, and triglycerides after overnight fasting.15 LDL cholesterol was calculated with the Friedewald equation. Plasma glucose was measured by the glucose oxidase method after overnight fasting. Body mass index (kg/m²) and waist-to-hip ratio were calculated.

Resting systolic blood pressure (SBP) and diastolic blood pressure (DBP) levels were measured in the right arm with a random-zero sphygmomanometer (Hawksley and Sons). Three measures at least 2 minutes apart were taken and the average of the second and third measurements was used. Participants were considered hypertensive if the average SBP was ≥140 mm Hg and/or the average DBP was ≥90 mm Hg, or if they reported a prior diagnosis of and treatment for hypertension and were currently using antihypertensive medications. Participants were considered diabetic if they were using insulin or oral hypoglycemic agents or if they reported a physician diagnosis of diabetes but were not currently taking a pharmacological agent to control their high glucose levels.

The Framingham risk equation was used to estimate the 10-year probability of coronary heart disease on the basis of the participant’s sex, age, SBP, total cholesterol/HDL cholesterol ratio, history of cigarette smoking in the past year, and diabetes status.18 Measurements of left ventricular hypertrophy were not included in the risk equations because they were not available.

Imaging

CAC was measured with an Imatron C-100 or C-150 EBCT scanner (Imatron Inc). A scan run consisted of 40 contiguous 3-mm-thick tomographic slices from the root of the aorta to the apex of the heart. Scan time was 100 ms per tomogram. Electrocardiographic gating was used and all images were triggered at end-diastole during 2 to 4 breath-holds.

A radiological technologist scored the tomograms with an automated scoring system.19 CAC was defined as a hypodensitivating focus within 5 mm of the arterial midline and at least 4 adjacent pixels in size (ie, 1.04 mm²), with CT number above 130 HU throughout the focus. An experienced radiologist inspected the technical quality and scoring accuracy of each tomogram and interpreted their findings. Quantity of CAC was defined as the CAC score developed by Agatston and coworkers.20 In the analyses described below, we used the natural logarithmic transformation of CAC score +1 to reduce skewness.

Statistics

A generalized estimating equations approach, assuming an identity link and allowing for correlation within families, was used to investigate associations between risk factors and log (CAC score +1).21 All statistical tests for associations were 2-sided, and P<0.05 was considered statistically significant. Two linear regression models were fit to predict log (CAC score +1). The first model included age and sex as predictors. The most efficient model of independent predictors of log (CAC score +1) was identified with the use of stepwise linear regression. This second model included age, sex, fasting glucose level, SBP, log (pack-years +1), and LDL cholesterol. Intraclass correlations for sibling pairs and interclass correlations for parent-offspring and spouse pairs were estimated for log (CAC score +1) and for the residuals from the 2 regression models to assess familial aggregation.22

To determine the contribution of genes to CAC quantity, the level of a quantitative trait, y, for individual i was modeled as y = μ + j = β jX j + g i + e i, where μ is the trait mean, X j is the j-th covariate, and β j is its regression coefficient. The remaining variables represent the random deviations from μ for individual i that are attributable to additive genetic and residual error effects, respectively. The residual error component includes true random error, measurement error, and any nonadditive genetic components. The effects of g i and e are assumed to be not correlated and normally distributed with mean zero and variances σ_g^2 and σ_e^2, respectively. Maximum likelihood methods were used to simultaneously estimate the mean and variances as well as the covariate and genetic effects.23 Significance of covariate effects was assessed with a Wald test.24 Heritability was defined as the relative proportion of the residual variance in the quantitative trait explained by additive genetic factors divided by the residual variance after adjustment for covariates. This definition of heritability is the same as that used by others in studies of calciumification.13,25 The significance of genetic effects was assessed by comparing twice the difference in natural logarithm likelihoods between a model with genetic effects estimated and a model with these effects constrained to zero.26

Results

The sex-specific characteristics of the participants are shown in Table 1. The 698 participants were distributed among 302 families. There were 98 families of size 1, 156 of size 2 to 3, 39 of size 4 to 6, 8 of size 7 to 9, and 1 of size 14 individuals. Sixty-one families included just siblings or siblings and at least one spouse of a sibling. Twenty-five families included at least two generations of participants.

Table 2 shows associations between risk factors and log (CAC score +1). The association between age and CAC quantity was adjusted for male sex, and the association between male sex and CAC quantity was adjusted for age. All other associations were adjusted for both age and male sex. All of the factors considered, except HDL cholesterol, DBP, and a college education, were positively and significantly associated with CAC quantity. A college education had a negative, significant association with CAC quantity, whereas HDL cholesterol and DBP were not associated with CAC quantity. Age, male sex, fasting glucose level, log (pack-years +1), and LDL cholesterol were the only risk factors shown to have a positive and statistically significant association with CAC quantity in a multivariable model (data not shown). There were no significant interactions among any of these risk factors.

The unadjusted and adjusted estimated correlations for siblings, parents and offspring, and spouses are shown in Table 3. After adjusting for covariates, the estimated correlations between siblings and between parents and offspring were higher than the estimated correlation between spouses. These higher correlations in genetically related individuals compared with unrelated individuals (ie, the spouses) are consistent with a genetic basis for variation in CAC quantity.
Table 4 presents the estimated components of variance for CAC quantity. All estimates of heritability for CAC quantity were statistically significantly different from zero ($P<0.001$). Before adjusting for any covariates, the estimate of heritability was 0.435. After adjusting for age and male sex, the estimate of heritability was 0.449, suggesting that genetic factors account for $\approx 0.292 \times (1.0 - 0.350) \times 0.449$ of the total variance in CAC quantity. After further adjustment for additional covariates, the estimate of heritability was 0.418, suggesting that genetic factors account for $\approx 0.246 \times (1.0 - 0.412) \times 0.418$ of the total variance in CAC quantity. The estimates after adjusting for covariates are similar because the additional covariates only explain an additional 0.062 of the total variance in CAC quantity beyond the variation explained by age and male sex.

**Discussion**

The associations between risk factors and quantity of CAC found here agree with previous reports. Age, sex, and measures of body size, lipid metabolism, and blood pressure, as well as history of diabetes, smoking, and hypertension, have all been found to be related to quantity of CAC. As shown here and elsewhere, these factors explain, at most, $\approx 40\%$ of the variability in CAC quantity. Only a small amount of variability unexplained by CAD risk factors is due to noise or artifact in EBCT measures of CAC quantity. In this study, we report that $>40\%$ of the interindividual variation in quantity of CAC not explained by traditional risk factors is attributable to genetic factors.

Studies of candidate genes for CAC are limited. Pfohl et al. reported an association between the insertion/deletion polymorphism of the angiotensin 1-converting enzyme gene and CAC detected by intravascular ultrasound in patients with angiographically documented CAD. Apolipoprotein E genotype was found to influence the relationship between risk factors and EBCT-measured CAC presence in ECAC Study participants. Ellsworth et al. reported a significant association in ECAC Study participants between the S128R poly-

### Table 1. Characteristics of Study Participants

<table>
<thead>
<tr>
<th>Variable</th>
<th>Women (n=360)</th>
<th>Men (n=338)</th>
<th>$P^*$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>58.0 (9.0) (45.1; 87.6)</td>
<td>57.1 (8.6) (45.0; 80.5)</td>
<td>0.1685</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>27.3 (5.4) (16.7; 50.1)</td>
<td>27.6 (4.1) (17.1; 48.3)</td>
<td>0.3768</td>
</tr>
<tr>
<td>Waist-to-hip ratio</td>
<td>0.8 (0.08) (0.6; 1.0)</td>
<td>0.9 (0.06) (0.8; 1.1)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Triglycerides, mg/dL</td>
<td>135.1 (64.1) (30.0; 372.0)</td>
<td>136.3 (62.6) (43.0; 400.0)</td>
<td>0.8027</td>
</tr>
<tr>
<td>LDL cholesterol, mg/dL</td>
<td>124.8 (32.9) (33.0; 266.9)</td>
<td>130.7 (32.0) (26.4; 236.8)</td>
<td>0.0162</td>
</tr>
<tr>
<td>HDL cholesterol, mg/dL</td>
<td>53.3 (14.4) (22.0; 101.0)</td>
<td>40.9 (10.2) (19.7; 74.0)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>122.2 (19.0) (85.0; 207.0)</td>
<td>123.9 (17.5) (92.5; 184.0)</td>
<td>0.2353</td>
</tr>
<tr>
<td>DBP, mm Hg</td>
<td>74.7 (8.8) (56.0; 104.0)</td>
<td>79.4 (10.0) (54.0; 120.0)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Fasting glucose, mg/dL</td>
<td>90.7 (13.9) (63.7; 165.0)</td>
<td>93.6 (15.5) (44.3; 194.7)</td>
<td>0.0086</td>
</tr>
<tr>
<td>Measurable CAC, %</td>
<td>37.2 (237.2) (0.0; 2488.5)</td>
<td>171.8 (362.1) (0.0; 3036.7)</td>
<td>...</td>
</tr>
<tr>
<td>10-year Framingham risk, %</td>
<td>6.0 (5.0) (0.5; 27.2)</td>
<td>14.0 (8.0) (1.8; 46.0)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Values are presented as mean (SD) (minimum; maximum) unless otherwise noted.

*Sex differences in participant characteristics were tested using t test, $\chi^2$ test, and Fisher’s Exact test.

### Table 2. Association Between Sex- and Age-Adjusted Covariates and Log (CAC Score+1)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Parameter Estimate*</th>
<th>Standard Error</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>1.167</td>
<td>0.068</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Male</td>
<td>1.766</td>
<td>0.150</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>0.195</td>
<td>0.085</td>
<td>0.022</td>
</tr>
<tr>
<td>Waist-to-hip ratio</td>
<td>0.434</td>
<td>0.118</td>
<td>0.002</td>
</tr>
<tr>
<td>Triglycerides, mg/dL</td>
<td>0.120</td>
<td>0.088</td>
<td>0.024</td>
</tr>
<tr>
<td>LDL cholesterol, mg/dL</td>
<td>0.177</td>
<td>0.070</td>
<td>0.011</td>
</tr>
<tr>
<td>HDL cholesterol, mg/dL</td>
<td>-0.103</td>
<td>0.088</td>
<td>0.243</td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>0.363</td>
<td>0.086</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>DBP, mm Hg</td>
<td>0.139</td>
<td>0.086</td>
<td>0.104</td>
</tr>
<tr>
<td>Fasting glucose, mg/dL</td>
<td>0.299</td>
<td>0.070</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Log (pack years+1)</td>
<td>0.455</td>
<td>0.074</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>History of smoking, %</td>
<td>0.784</td>
<td>0.145</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diabetes</td>
<td>1.663</td>
<td>0.445</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hypertension</td>
<td>0.678</td>
<td>0.202</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>College education</td>
<td>-0.469</td>
<td>0.161</td>
<td>0.004</td>
</tr>
</tbody>
</table>

*Parameter estimates are for a 1 standard deviation increase in the continuous variables or change in status of the dichotomous variables. All associations are adjusted for age and male sex except for the variables age and male sex.
morphism of the E-selectin gene and presence and quantity of EBCT-measured CAC in asymptomatic women 50 years of age or younger after adjusting for CAD risk factors. In the Helsinki Sudden Death Study, a polymorphism within the tumor necrosis factor locus was associated with extent of calcified lesions in coronary arteries. In all of these studies, the effects of the genes have been small and no findings have been replicated yet.

Wagenknecht et al observed that CAC quantity clustered in 56 families (135 individuals) with 2 or more siblings concordant for type 2 diabetes. The estimated heritability was 0.495 (SE=0.216) after adjusting for age, sex, age by sex interaction, race, and diabetes status. Despite the differences between this and the present study, the estimates of heritability for CAC quantity are remarkably similar. The estimates for heritability reported in the present study also agree well with estimates from the Framingham Heart Study of abdominal aortic calcific deposits assessed from lateral lumbar radiographs.

**Study Limitations**

The findings from this study are limited to asymptomatic whites age 45 and older living in Rochester, Minn. Our study participants are similar, in many ways, to others. The prevalence of hypertension (25.3% in women and 31.1% in men) is slightly higher than age-adjusted prevalences for non-Hispanic white women (20.5%) and men (25.2%) ≥20 years of age. In our study, 58.2% of those with hypertension were on medication, whereas nationally, 53.6% are on medication. The prevalence of obesity (body mass index ≥30 kg/m²) was 24.7% in women and 23.4% in men in the present study compared with 23.2% and 20.8% among non-Hispanic white women and men ages 20 to 74 nationally. The prevalence of diabetes here (2.5%) was lower than national estimates of 7.3%. The proportion of our participants who report that they currently smoke (9.4% of women and 10.7% of men) is substantially lower than national estimates for non-Hispanic whites ≥18 years of age (23.1% of women and 25.5% of men). The higher proportions nationally may reflect the tremendous increase in smoking initiation among younger individuals. Finally, the 10-year Framingham risk for our participants ranged from 0.5% to 46%. Only 1.4% of women but 22.2% of the men would be considered high risk with a 10-year Framingham risk ≥20%.

In the present study, the estimates for heritability may overestimate the genetic contribution because we have not estimated shared environments. All siblings reported living in separate households from one another and from their parents at the time of the study. It is possible that shared environments early in life contribute to the correlations for CAC quantity seen among adult relatives. For the spouses, we cannot separate how much of their correlation is due to shared environments and how much is due to assortative mating for factors related to CAC quantity. We could assume that all the spouse correlation is due to shared environments, which is unlikely, and that the strength of the shared environments between spouses is similar to that among relatives. Under these assumptions, genetic factors would be stronger than shared environmental factors in our study because the estimated spouse correlation is much lower than the corresponding estimate of heritability, especially after adjustment for risk factors.

**Conclusions**

In conclusion, our findings suggest a substantial genetic component for subclinical coronary atherosclerosis variation as measured by CAC quantity, even after accounting for effects of genes acting through some measured atherosclerosis risk factors. Although the CAC process has a complex pathogenesis that likely is influenced by the interaction of numerous environmental and genetic factors, the evidence for genetic effects suggests it should be possible to localize previously unknown genes that influence CAC quantity. Studies to localize such genes are just beginning. These genes may act through other measurable atherosclerosis risk factors or through novel pathways that have not or cannot be

**TABLE 4. Components of Variance for Log (CAC Score+1)**

<table>
<thead>
<tr>
<th>Covariates</th>
<th>Proportion of Variance Associated With Covariates</th>
<th>Heritability: Proportion of Variance Associated With Additive Polygenes After Adjusting for Covariates (SE, P value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>. . .</td>
<td>0.435 (0.137, 0.0007)</td>
</tr>
<tr>
<td>Age, male sex</td>
<td>0.350</td>
<td>0.449 (0.126, 0.0001)</td>
</tr>
<tr>
<td>Age, male sex, fasting glucose, SBP, log (pack years+1), LDL cholesterol</td>
<td>0.412</td>
<td>0.418 (0.127, 0.0003)</td>
</tr>
</tbody>
</table>
measured in vivo. Identification of such genes will provide a better basis for prevention and treatment of subclinical coronary atherosclerosis.

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