Evidence for Heritability of Abdominal Aortic Calcific Deposits in the Framingham Heart Study

Christopher J. O’Donnell, MD, MPH; Irmarie Chazaro, MA; Peter W.F. Wilson, MD; Caroline Fox, MD; Marian T. Hannan, ScD; Douglas P. Kiel, MD, MPH; L. Adrienne Cupples, PhD

Background—Atherosclerosis is a systemic disease that underlies clinical cardiovascular disease. The radiographic finding of abdominal aortic calcific deposits is an indicator of the presence of aortic atherosclerosis and an independent predictor of cardiovascular disease events. Little is known about the heritability of aortic calcification.

Methods and Results—Original Framingham Heart Study cohort participants (2151) in 1109 extended pedigrees had a lateral lumbar radiograph. The presence and severity of abdominal aortic calcific (AAC) deposits at the levels of the first through fourth lumbar vertebrae was graded by a previously validated rating scale. Correlation coefficients were calculated in pairs of siblings, parent-offspring, and spouses. Age-, sex-, and multivariable-adjusted correlation coefficients for AAC were 0.52 for parent-offspring pairs and 0.20 for sibling pairs. In contrast, the multivariable-adjusted correlation for AAC in spouse pairs was −0.02. Using variance component methods implemented in SOLAR, the estimated heritability for age-, sex-, and multivariable-adjusted AAC was 0.49 (P<0.001). Thirty-one percent of the overall variance in AAC deposits was due to measured covariates, and 49% to heritable factors.

Conclusions—In our large, population-based sample, heritable factors play a role in the presence and extent of abdominal aortic calcification. Thus, a substantial proportion of the variation in AAC is due to additive effects of genes, which have yet to be characterized. Measures of aortic atherosclerosis may provide heritable quantitative phenotypes for the genetic dissection of the complex condition of atherosclerosis in human populations. (Circulation. 2002;106:337-341.)

Key Words: atherosclerosis ■ calcification ■ abdominal aorta ■ genetics ■ heritability

Calcification is an integral process in the developing atherosclerotic plaque.1,2 Vascular calcific deposits of the abdomen and chest can be reliably detected by plain lateral radiography as well as high-resolution computed tomography. Calcific deposits of the abdominal aorta,3,4 thoracic aortic arch,5,6 and the coronary arteries7 predispose to increased risks of morbidity and mortality from coronary heart disease and cardiovascular disease. Exogenous environmental factors such as cigarette smoking, as well as endogenous factors, such as elevated blood pressure and increased levels of serum cholesterol, have all been associated with prevalent vascular calcification.4 However, it remains uncertain whether genetic factors (ie, heritability) determine any significant proportion of the interindividual variability in vascular calcification, and to what extent this variability is determined by genetic versus environmental factors.

Two important considerations in the evaluation of associations between arterial calcification and environmental or genetic factors are the assessment methods and the age of the cohort. The natural sequence for atherosclerotic lesions includes migration of white blood cells through endothelial surfaces, development of fatty streaks, and later accretion of matrix in the artery wall. Calcification of the endothelial and medial regions of arteries typically occurs during the course of atherosclerosis development. Large radiographic studies that include families and sibling pairs with middle-aged and elderly persons allow estimation of heritability in this setting. Although research has demonstrated the capability of computerized tomography to detect small amounts of arterial calcification, the investigation of older conventional radiographs offers the advantage of utilizing previously obtained films, low cost, and assessment of a large number of persons who are relatively unaffected by the process. Ongoing and future investigations in large population studies will rigorously examine atherosclerotic calcification with computerized tomography techniques.

The Framingham Heart Study is a large, prospective population-based study containing multiple extended pedigrees, so it is possible to test hypotheses regarding the heritability of vascular calcification. We sought to assess the

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From the National Heart, Lung and Blood Institute’s Framingham Heart Study (C.J.O., P.W.F.W., C.F.), Framingham, Mass; the Cardiology Division (C.J.O.), Department of Medicine, Massachusetts General Hospital, the Hebrew Rehabilitation Center for Aged Research and Training Institute and the Division on Aging (M.H., D.K.), Harvard Medical School, Boston, Mass; the Boston University School of Public Health Department of Epidemiology and Biostatistics (I.C., L.A.C.), Boston, Mass; and the National Heart, Lung and Blood Institute, National Institutes of Health (C.J.O.), Bethesda, Md.

Correspondence to Christopher J. O’Donnell, MD, MPH, Framingham Heart Study, 73 Mount Wayte Ave, Framingham, MA 01702. E-mail chris@fram.nhlbi.nih.gov

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contribution of measured and unmeasured genetic and environmental influences on interindividual differences in abdominal aortic calcific deposits. The current project evaluates the heritability of arterial calcification with methods that are less sensitive than current techniques and provides conservative estimates of disease severity, but our study cohort is well past middle age and a considerable amount of abdominal aortic disease is present. As most of the participants in this study have some degree of arterial calcification, the heritability estimates and investigation of the contribution of genetic and environmental effects should provide information regarding the potential usefulness of such measures in genetic studies.

Methods

Study Sample and Risk Factor Measurements

The study participants consisted of men and women participants in the original cohort of the Framingham Heart Study. In 1948, 5209 men and women from the town of Framingham, Mass. agreed to participate. Subjects who attended routine examinations in 1965 to 1969 (examination cycles 9 through 11) were considered for analysis of abdominal aortic calcific (AAC) deposits. Men and women (2151) had lateral lumbar radiographs as part of a special osteoporosis examination. Of these, 2151 were members of 1109 extended pedigrees who could be used for heritability analyses. Characteristics of the subset of subjects undergoing lumbar aortic radiography have been described in detail. The clinical history included information on recognized risk factors for vascular morbidity and mortality, and subjects reported on cigarette smoking during the year before the examination. Blood pressure was measured with the subject in the sitting position for at least 5 minutes. Height and weight were measured and the body mass index was calculated as the height in kilograms divided by the weight expressed in meters squared. Left ventricular hypertrophy on the ECG was determined by standard criteria. Blood tests at the time of the examination (or the examination immediately before or after the index examination) included measurement of blood cholesterol, HDL cholesterol, and blood glucose. Persons taking oral hypoglycemic agents, insulin, with fasting glucose >140 mg/dL, or a history of casual glucose >200 mg/dL were considered diabetic. The methods for anthropomorphic measurements, physician history, physical examination, and blood assays for cardiovascular risk factor information have been described.

Determination of Aortic Calcifications

The lateral lumbar spine radiographs were acquired in the standing position, as previously described. The methods for scoring of lumbar aortic calcific deposits and its validation have been previously described. Briefly, an index of AAC deposits index was used to grade the severity of calcification in the aorta at the level of the first through fourth lumbar vertebrae. The radiodensity of the aortic wall was assessed systematically at each vertebral segment, and calcific deposits were regarded as present if densities were visible in an area parallel to and anterior to the lumbar spine. Calcific densities were graded on a 0 to 3 scale at each lumbar vertebral segment. A score of 0 denoted no aortic calcific deposits; 1, small scattered calcific deposits filling less than one third of the longitudinal wall of the aorta; 2, one third or more, but less than two thirds of the longitudinal wall of the aorta calcified; 3, two thirds or more of the longitudinal wall of the aortal calcified. A separate score was determined for the anterior and posterior aorta, and the values were summed across the 4 vertebrae, resulting in an AAC index that could range from 0 to 24 points. As reported previously, the interrater intraclass correlation was 0.93 and the intrarater intraclass correlation was 0.98.

Statistical Methods

To analyze genetic contributions to abdominal aortic calcific deposits, separate analyses were conducted on siblings and spouse pairs, and on extended pedigrees. A total of 2151 subjects were analyzed in 1109 extended pedigrees. The majority of the subjects were contained in sibships with the following distributions: 46% had one member, 38% had 2 members, 7% had 3 members, 4% had 5 to 6 members, and 5% had a least 7 members. There were 597 spouse pairs, 51 parent-offspring pairs, 392 sibling pairs (78 male-male, 187 male-female, and 127 female-female pairs), 15 avuncular pairs, and 5 cousin pairs. Spouse pairs, rather than randomly selected unrelated individuals, were included as a comparison group because, due to assortative mating, spouses may be similar in age, weight, and other variables, including household environment, that tend to be common among siblings.

In separate linear regression analyses for men and women, the AAC score was adjusted for the effects of the following covariates (continuous measures unless otherwise indicated): age, body mass index, number of cigarettes smoked per day, systolic blood pressure, antihypertensive therapy (yes or no), total cholesterol, high density lipoprotein (HDL) cholesterol, presence of diabetes mellitus (yes or no) and left ventricular hypertrophy (yes or no). These adjustments yielded standardized residuals, which were then ranked to produce normalized deviates because AAC and the non-normalized residuals are highly skewed. These ranked normalized deviates were then used to estimate heritability of AAC. Different regression models were used to produce age- and sex-adjusted heritability and multivariable-adjusted heritability (adjusted for age-, sex-, and other covariates above). For estimates of the heritability of crude AAC, we ranked the crude values of the AAC score and obtained normalized deviates.

We calculated estimates of heritability in 2 ways. First, we used familial correlation (FCOR) in SAGE (Statistical Analysis for Genetic Epidemiology) to calculate the correlations (Pearson and intraclass) for different types of relative pairs. For our data, the primary relationships were sibling pairs and spouse pairs. An estimate of heritability is obtained by doubling the sibling-pair intrainclass correlation. In addition, we used variance component methods as implemented in SOLAR with extended pedigrees to obtain heritability estimates. This methodology applies maximum likelihood estimation to a mixed effects model that incorporates fixed effects for known covariates and variance components for genetic effects. The model partitions the total variation in the trait, AAC, into additive genetic and individual environmental components. We applied these methods to the normalized deviates, crude and adjusted, without additional adjustment for covariates. In addition, we also used this approach to the crude normalized deviate and included the covariates in the model for fixed effects to obtain estimates of the relative proportion of variance explained by known covariates and the proportion explained by additive genetic effects. Thus, we used 2 methods for adjustment: the first used separate regression models for men and women before analyses of the normalized deviates from the residuals; the second method adjusted for covariates directly in SOLAR. We used the former for our primary results, because the associations between known covariates and AAC may differ for men and women. We used the latter method only to obtain estimates of the proportion of variation explained by known covariates and the proportion explained by additive genetic effects. Therefore, heritability estimates reflect the residual variation in the trait due to a genetic contribution to the trait after adjusting for known covariates.

Results

Participant Characteristics

The eligible study sample consisted of 926 men and 1225 women. Characteristics of the sample are shown in Table 1. The mean age was 60.8 years for men and 60.5 years for women. The correlations of AAC with age and established risk factors are shown in Table 2. As expected, significant associations were seen with age and with several cardiovascular risk factors. A total of 364 attended the examinations...
TABLE 1. Characteristics of Study Subjects

<table>
<thead>
<tr>
<th>Variable</th>
<th>Men (n=926)</th>
<th>Women (n=1225)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>60.8 (7.8)</td>
<td>60.5 (7.9)</td>
</tr>
<tr>
<td>Systolic BP, mm Hg</td>
<td>138.7 (21.4)</td>
<td>140.5 (24.9)</td>
</tr>
<tr>
<td>Hypertension treatment, %*</td>
<td>11.9</td>
<td>17.5</td>
</tr>
<tr>
<td>Total cholesterol, mg/dL†</td>
<td>220.0 (40.6)</td>
<td>243.8 (42.4)</td>
</tr>
<tr>
<td>HDL cholesterol, mg/dL†</td>
<td>45.0 (12.7)</td>
<td>57.2 (16.0)</td>
</tr>
<tr>
<td>Diabetes, %</td>
<td>9.8</td>
<td>7.3</td>
</tr>
<tr>
<td>Cigarettes, per day†</td>
<td>8.6 (13.9)</td>
<td>5.8 (9.9)</td>
</tr>
<tr>
<td>Smoking status, %</td>
<td>36.4</td>
<td>33.3</td>
</tr>
<tr>
<td>LVH by ECG, %</td>
<td>2.7</td>
<td>2.3</td>
</tr>
<tr>
<td>Body mass index*</td>
<td>26.2 (3.4)</td>
<td>25.4 (4.1)</td>
</tr>
<tr>
<td>AAC scores</td>
<td>3.8 (4.4)</td>
<td>3.6 (4.8)</td>
</tr>
</tbody>
</table>

Values are means (standard deviation) or proportions. AAC indicates abdominal aortic calcification; BP, blood pressure; HTN, hypertension; HDL, high-density lipoprotein; and LVH by ECG, left ventricular hypertrophy.

Only n=690 and n=904 have HDL measures among men and women, respectively. *P-values for differences between men and women: *P<0.001; †P<0.001.

and underwent lateral lumbar radiography but were not included in the analysis (n=123 men and n=241 women) because each was not a member of an extended family. The baseline characteristics of the excluded group were similar to the included subjects.

Correlation Coefficients and Heritability of Aortic Calcific Deposits

For AAC deposits, correlation coefficients for parent-offspring, sibling-sibling and spousal pairs were calculated using SAGE FCOR. The correlation coefficients are shown in Table 3. In age- and sex-adjusted analyses giving equal weight to pedigrees, the correlation coefficient was 0.30 for parent-offspring pairs and 0.26 for sibling pairs. In the multivariable-adjusted analysis (adjusted for age-, sex-, and all other covariates), the correlation coefficient was 0.52 for parent-offspring pairs and 0.20 for sibling pairs. The

TABLE 2. Correlation of AAC Scores With Age and Other Covariates

<table>
<thead>
<tr>
<th>Variable</th>
<th>n=2151</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y*</td>
<td>0.47</td>
</tr>
<tr>
<td>Systolic BP, mm Hg*</td>
<td>0.27</td>
</tr>
<tr>
<td>HTN treatment, %*</td>
<td>0.12</td>
</tr>
<tr>
<td>Total cholesterol, mg/dL*</td>
<td>0.13</td>
</tr>
<tr>
<td>HDL cholesterol, mg/dL†</td>
<td>-0.07</td>
</tr>
<tr>
<td>Diabetes, %*</td>
<td>0.10</td>
</tr>
<tr>
<td>Cigarettes, per day†</td>
<td>0.02</td>
</tr>
<tr>
<td>LVH by ECG, %*</td>
<td>0.10</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>-0.03</td>
</tr>
</tbody>
</table>

AAC indicates abdominal aortic calcification; BP, blood pressure; HTN, hypertension; HDL, high-density lipoprotein; and LVH by ECG, left ventricular hypertrophy.

Only n=1594 have HDL measured. *P<0.0001; †P<0.01.

Multivariable-adjusted correlation coefficient for spousal pairs was −0.02. Similar correlation coefficients for familial pairs and spousal pairs were noted in separate analyses giving equal weights to sibling pairs and to nuclear families (data not shown). In subanalyses of same sex and opposite sex pairs, the multivariable-adjusted correlation coefficients ranged from 0.11 for sister-sister pairs (n=127 pairs) to 0.42 for brother-brother pairs (n=78 pairs).

The variance components estimates for heritability of AAC deposits are shown in Table 4. The unadjusted heritability estimate was 0.40, the age-adjusted estimate was 0.47, and the multivariable-adjusted estimate was 0.49. The heritability estimate of 0.49 means that 49% of the variance of the AAC deposits scores is attributed to the additive effect of genes. Heritability estimates were also calculated using normalized deviates of the calcification scores.

Components of Variance Analysis

The overall contribution of genetic factors and measured covariates to the abdominal aortic calcification scores was examined. In the fully adjusted model, the contribution of genetic factors to overall variation in abdominal aortic calcific deposits responses was 49%. In the same models, the contribution of measured covariates to overall variation was 31%, leaving a residual of 20%.

Discussion

In this study of sibships drawn from a population-based sample, we found significant heritability for abdominal aortic calcific deposits. Indeed, the magnitude of the correlation coefficients for abdominal aortic calcific deposits were comparable to or exceeded the correlations for interindividual differences in many heritable cardiovascular risk factors, such as systolic blood pressure and HDL cholesterol.

Calcific plaques occur during the development of atherosclerosis and precede the onset of clinical events by decades.

TABLE 3. Correlation Coefficients for Abdominal Aortic Calcific Deposits Using SAGE FCOR

<table>
<thead>
<tr>
<th>Level of Adjustment</th>
<th>Spousal (n=597)</th>
<th>Parent-Offspring (n=51)</th>
<th>Sibling-Sibling (n=392)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unadjusted</td>
<td>0.15</td>
<td>0.35</td>
<td>0.40</td>
</tr>
<tr>
<td>Age- and sex-adjusted</td>
<td>-0.02</td>
<td>0.30</td>
<td>0.26</td>
</tr>
<tr>
<td>Multivariable-adjusted</td>
<td>-0.02</td>
<td>0.52</td>
<td>0.20</td>
</tr>
</tbody>
</table>

TABLE 4. Estimated Variance Component Heritabilities for Abdominal Aortic Calcific Deposits

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Heritability</th>
<th>Measured Covariates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unadjusted</td>
<td>2151</td>
<td>0.40 (0.09)*</td>
<td>N/A</td>
</tr>
<tr>
<td>Age- and sex-adjusted</td>
<td>2151</td>
<td>0.47 (0.10)*</td>
<td>0.25</td>
</tr>
<tr>
<td>Multivariable-adjusted</td>
<td>1594</td>
<td>0.49 (0.14)*</td>
<td>0.31</td>
</tr>
</tbody>
</table>

Values in parentheses are standard errors. Due to missing covariates, 557 fewer subjects were available for the multivariable-adjusted estimates compared with the age-adjusted estimates.

*P<0.0001; †P<0.001.
Calcification is noted in atherosclerotic plaques of the aorta, predominantly the abdominal aorta, as well as the coronary arteries early in life. Hypertension, hypercholesterolemia, diabetes mellitus, and cigarette smoking are important “traditional” risk factors associated with coronary heart disease and other atherosclerotic cardiovascular diseases.\textsuperscript{17,18} We have shown that these risk factors are associated with the burden of atherosclerotic plaque detected by AAC, as expected. In addition, we show that the variability in AAC is heritable, even after adjustment for traditional risk factors.

Wagenknecht et al\textsuperscript{19} recently reported the presence of familial aggregation of coronary artery calcium in 135 subjects from 56 families with a predominance of type 2 diabetes. Their multivariable heritability estimate of 0.40 is consistent with our findings for AAC. However, there are no published reports of heritability of vascular calcification, particularly aortic calcification, in unsellected population-based cohorts. Recent advances allow the imaging of calcific deposits in the coronary arteries as well as the aortoiliac system using electron beam computed tomography or multi-detector computed tomography. In a companion article,\textsuperscript{20} evidence is provided for multivariable heritability estimates very similar to our estimates for AAC for coronary arterial calcific deposits detected by electron-beam computed tomography. Together, our articles provide strong, complementary evidence that genetic factors underlie the variability in vascular calcific deposits in the aorta and in the coronary arteries.

Several specific gene pathways may contain plausible candidate genes in the genetic etiology of aortic atherosclerosis, although there has been little study to date of genetic variants for aortic calcification. Genetic variants that control some of the variability in traditional risk factors such as systolic blood pressure and lipoproteins may play a role in atherosclerosis and, specifically, vascular calcification. In small studies, associations have been reported between coronary calcification and the angiotensin-converting enzyme deletion-insertion polymorphism,\textsuperscript{21} the apolipoprotein E genotype,\textsuperscript{22} and the E-selectin S128R polymorphism.\textsuperscript{23} However, the substantial heritability of vascular calcification that persists even after multivariable adjustment for heritable atherosclerosis risk factors suggests that other genetic mechanisms beyond those in the causal pathway of traditional risk factors may play a role. Rare families have been reported with disorders including calcification of arteries. Matrix \textit{\gamma}-carboxyglutamic acid (Gla) protein is an important inhibitor of cartilage calcification that is expressed in human calcified, atherosclerotic plaques and could modify atherosclerotic calcification.\textsuperscript{24} Mice lacking matrix Gla protein show spontaneous calcification of the arteries and cartilage,\textsuperscript{25} and osteoprotegerin-deficient mice are noted to have diffuse calcific deposits in the aorta an renal arteries.\textsuperscript{26} One study provides weak evidence that variants in matrix Gla protein, may be related to vascular calcification.\textsuperscript{27} To date there are no reports of genome-wide scans for aortic atherosclerosis, although a recent genome-wide scan in hypertensive sibships provided evidence in hypertensive sibships for coronary artery calcification loci on chromosomes 6 and 10.\textsuperscript{28} Our evidence for heritability suggests that the presence and extent of aortic calcific deposits may provide useful quantitative traits for dissection of the genetic component of vascular calcification and atherosclerosis in general.

There are several study limitations. First, the method for detection and quantification of AAC deposits is semiquantitative. Errors in classifying AAC scores on abdominal radiographs would be expected to diminish findings in heritability. However, we have reported that this method has high inter-rater and intrarater reliability.\textsuperscript{8} Currently available higher-resolution computed axial tomography tests would be expected to provide more reproducible and quantitative estimates of aortic calcific deposits. Another potential limitation is that our study cohort is Caucasian, so our findings may not be generalizable to other ethnicities.

The contribution of genetics and the environment to arterial calcification will continue to be an area of active research. Conventional radiographs have been superseded by scanning and ultrasound methods, as each of the latter modalities is more sensitive in detection. The greater cost of newer methods does limit use in population and family studies and does affect interpretation of the results. It is likely that genetic factors will be of greatest interest in persons with a “premature” onset of disease at an age, between 30 and 50 years, when subclinical atherosclerosis develops and is detectable. Inclusion of much younger or much older persons in such investigations may have limitations. In the case of younger persons, the disease may not be detected with the newest techniques; in the elderly the disease process may affect too great a proportion of the population. Only increased experience with the evaluation of arterial calcification in populations across a wide age spectrum and in families will allow us to make firmer statements concerning the genetic and environmental influences.

In conclusion, we have found that AAC deposits are heritable atherosclerosis traits. Our findings support the further investigation of genetic determinants for these traits using candidate gene and genome-wide screening methods.

Acknowledgments

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O’Donnell et al. Heritability of Abdominal Aortic Atherosclerosis


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