Genetic and Environmental Contributions to Atherosclerosis Phenotypes in Men and Women

Heritability of Carotid Intima-Media Thickness in the Framingham Heart Study

Caroline S. Fox, MD, MPH; Joseph F. Polak, MD, MPH; Irmarie Chazaro, MA; Adrienne Cupples, PhD; Philip A. Wolf, MD; Ralph A. D’Agostino, PhD; Christopher J. O’Donnell, MD, MPH

Background and Purpose—Carotid intima-media thickness (IMT) is a quantitative measure of subclinical atherosclerosis that is predictive of subsequent myocardial infarction and stroke. There is controversy regarding the proportion of variability in IMT explained by genetic factors. Thus, it is uncertain whether carotid IMT is a heritable trait that can be used in genetic studies.

Methods—From 1996 to 1998, we measured carotid IMT in 906 men (mean age, 56.7 years) and 980 women (mean age, 57.4 years) from 586 extended families (1630 sib pairs) in the Framingham Offspring cohort. B-mode carotid ultrasonography was used to define mean and maximum IMT of the common carotid artery (CCA) and internal carotid artery (ICA). Correlation coefficients were calculated in pairs of siblings. Variance component methods were used to estimate heritability with crude, age- and sex-adjusted, and multivariable-adjusted normalized deviates.

Results—Multivariable-adjusted correlation coefficients for mean CCA and ICA IMT were 0.16 and 0.16, respectively. Crude, age- and sex-adjusted, and multivariable-adjusted heritabilities were 0.67, 0.44, and 0.38 for the mean CCA IMT (all P<0.001) and 0.43, 0.37, and 0.35 for the mean ICA IMT (all P<0.001). For CCA IMT, 27% of the overall variance was due to measured covariates; 38% was due to heritable factors.

Conclusions—These data suggest that a substantial proportion of the variability in carotid IMT is explained by genetic factors. Further studies of genetic linkage and candidate gene association are warranted to identify specific genetic variants predisposing to subclinical atherosclerosis and stroke.

Key Words: epidemiology ■ genetics ■ intima-media thickness

G enetic and environmental factors have been linked to the cause of atherosclerosis.1 Risk factors for subclinical atherosclerosis have been shown to be similar to traditional risk factors for clinical cardiovascular disease.2 Carotid intima-media thickness (IMT) is a measure of subclinical atherosclerosis that is correlated with traditional coronary heart disease risk factors3–6 and coronary atherosclerotic burden,7,8 and is predictive of subsequent myocardial infarction and stroke.9,10 It has been suggested that thickening of the common carotid artery (CCA) intima might be more representative of total body atherosclerotic burden, whereas thickening of the internal carotid artery (ICA) IMT might represent focal atherosclerotic plaques.8

Carotid IMT has also been shown to be associated with a family history of cardiovascular disease.11 Evidence from a single study of families of Mexican descent suggests that carotid IMT is heritable, with estimates ranging from 0.86 to 0.92.12 However, these very high heritability estimates have not been reproduced in other populations. Determining whether carotid IMT is heritable in a general population of men and women would suggest that this noninvasive quantitative measure of vascular disease might be of great utility for subsequent use in genetic studies. Furthermore, possible differences in the pathophysiology of CCA and ICA IMT might allow the exploration of differential gene regulation in specific vascular beds. Thus, we hypothesized that carotid IMT is a heritable phenotype, and we sought to test this hypothesis in the Framingham Heart Study Offspring cohort.

Methods

Study Population
This investigation comprised subjects from the Framingham Offspring Study who were undergoing B-mode carotid ultrasonography during examination cycle 6 (1996 to 1998). We measured carotid IMT in 906 men and 980 women from 586 extended families with 1630 sib pairs.
The Framingham Heart Study began in 1948 with the enrollment of 5209 men and women 28 to 62 years of age, with subjects undergoing examinations every 2 years.\textsuperscript{13,14} In 1971, 5124 men and women were enrolled in the Framingham Heart Study Offspring cohort, which included the children or spouses of the children of the original cohort. Offspring subjects underwent examinations approximately every 4 years; the design and methodology have been previously described.\textsuperscript{15,16}

For this particular study, of the total 5124 subjects who attended the initial offspring examination, 3532 attended cycle 6. Of these, 154 did not undergo carotid ultrasound, 1066 were excluded because they were not part of a biological family, and 426 were further excluded because they were not part of a biological family whose members had carotid ultrasound data. Thus, a total of 1886 subjects made up the study population.

Assessment of Risk Factors and Cardiovascular Disease

Details regarding the methods of risk factor measurement and laboratory analysis have been given elsewhere.\textsuperscript{17} Each examination included a cardiovascular disease assessment, 12-lead ECG, and blood testing. Measured covariates for the present study were assessed at the time of carotid ultrasonography. Subjects with a fasting glucose level $\geq 140$ mg/dL, with a random nonfasting glucose level $\geq 200$ mg/dL, and/or on treatment for diabetes were defined as diabetic. Subjects with a systolic blood pressure $\geq 140$ mm Hg, with a diastolic blood pressure $\geq 90$ mm Hg (average of 2 readings taken by the examining physician), and/or on antihypertensive medication were defined as hypertensive. Fasting cholesterol measures included total cholesterol, high-density lipoprotein (LDL) cholesterol, and triglycerides. Smoking status was defined as number of cigarettes smoked per day in the year preceding the examination. Body mass index was defined as weight (kilograms) divided by the square of height (meters). Fatal and nonfatal cardiovascular outcomes were monitored by clinic examinations, hospital surveillance, and communication with participants who did not attend a clinic examination. A panel of 3 experienced investigators reviewed and adjudicated the occurrence of all incident cardiovascular events.

Carotid IMT Assessment

Subjects underwent ultrasonography according to standard protocol. Imaging was conducted with a Toshiba SSH-140A imaging unit that used a high-resolution, 7.5-MHz transducer for the CCA and a 5.0-MHz transducer for the ICA. Images were gated to an ECG; end-diastolic images were acquired.

The following images were obtained from the right and left sides: 2 longitudinal images of the distal CCA, 1 at end diastole and 1 at end systole, and 2 longitudinal views of the ICA at end diastole. Measurement of the peak systolic velocity in the ICA was obtained with color Doppler imaging and duplex ultrasound. Measurements were made by a single trained sonographer blinded to all clinical information and overread by 1 of the investigators (J.F.P.). Based on 25 readings by 2 separate readers, correlation coefficients for the mean and maximum ICAs were 0.83 and 0.84, respectively. This is comparable to previously reported results with similar techniques.\textsuperscript{18}

All studies were recorded on optical disk and read according to a standardized protocol. The high-resolution images of the CCA and ICA were analyzed to calculate near- and far-wall IMT, lumen diameter, and vessel width at each arterial site. All measurements of lumen and wall thickness were calculated with a specially designed computer program.\textsuperscript{8,9}

To quantify the degree of thickening of the carotid artery walls, IMT measures were summarized into 2 variables: 1 for CCA and 1 for ICA. Mean and maximum wall thicknesses of the CCA and ICA were defined as the mean of the wall thickness or the mean of the maximum wall thickness for the near and far walls on the left and right sides. The number of available measurements for averaging ranged from 1 to 4 for the CCA and from 1 to 8 for the ICA.

<table>
<thead>
<tr>
<th>TABLE 1. Baseline Characteristics of Study Subjects</th>
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<tbody>
<tr>
<td><strong>Men, n=906</strong></td>
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<tr>
<td><strong>Mean (SD)</strong></td>
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<tr>
<td>Age, y</td>
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<tr>
<td>Systolic blood pressure, mm Hg</td>
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<tr>
<td>Total cholesterol, mmol/L</td>
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<tr>
<td>Triglycerides, mmol/L</td>
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<tr>
<td>HDL cholesterol, mmol/L</td>
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<tr>
<td>Body mass index, kg/m²</td>
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<tr>
<td>Current smoking, %</td>
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<tr>
<td>Postmenopause</td>
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<tr>
<td>Hormone replacement therapy, %</td>
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<td>Prevalent hypertension, %</td>
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<tr>
<td>Hypertension treatment, %</td>
</tr>
<tr>
<td>Diabetes, %</td>
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<td>Cardiovascular disease, %</td>
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Statistical Analysis

Phenotypes of interest included mean and maximum IMT measures of the CCA and ICA. If either side was missing, we used the available measure at 1 side. Statistical analyses were conducted with SAS version 6.12\textsuperscript{19} and SOLAR.\textsuperscript{20} Subjects were contained in sibships (of a total of 657 sibships) with the following distributions: 62\% had 2 members, 24\% had 3 members, 10\% had 4 members, and 4\% had $\geq$ 5 members. There were 1630 sibling pairs: 378 male-male, 825 male-female, and 427 female-female pairs. Descriptive statistics using means, medians, and SD were performed on all variables when appropriate.

We calculated crude, age- and sex-adjusted, and multivariable-adjusted normalized deviates. For these adjustments, we used multiple linear regression separately for men and women. Covariates (continuous measures except when otherwise specified) in the multivariable models included age, systolic blood pressure, cigarettes smoked per day, total cholesterol, HDL cholesterol, log triglycerides, diabetes status (yes/no), body mass index, antihypertensive treatment (yes/no), and menopausal status (yes/no) and hormone replacement therapy in women (yes/no). From these regression models, we used standardized residuals. Because these residuals were skewed and the variance component method is sensitive to the assumption of normality, we obtained normalized deviates from the rank of the residuals.

We calculated heritability in 2 ways. First, we used FCOR in SAGE to calculate the intraclass correlations for sibling pairs.\textsuperscript{21} An estimate of heritability is obtained by doubling the sib pair correlation.\textsuperscript{22} In addition, the variance component model implemented in SOLAR was used to calculate heritabilities from the normalized deviates. Similarly, we obtained estimates of heritability for men and women separately from normalized deviates.

Heritability measurements estimate the proportion of variability in the measure attributable to the additive effect of genes and represent the contribution of both genes and early common environment. The underlying model assumes that variation in the trait can be partitioned into genetic, known covariates, and environmental components. It is assumed that the genetic component is polygenic with no variation attributable to dominance components. To determine the portion of variation resulting from measured covariates, we used a regression model with men and women combined.

Results

Baseline Characteristics

Baseline characteristics of our study sample are shown in Table 1. The mean age was 56.7 years for men and 57.4 years for women. We found that 40\% of men and 36\% of women...
were hypertensive, 6% of men and 4% of women were diabetic, and 13% of men and 8% of women had cardiovascular disease. Mean and maximum values of CCA and ICA IMT are shown in Table 2.

Pearson’s correlation coefficient data between covariates and measures of IMT are shown in Table 3. Age, systolic blood pressure, triglycerides, cigarette smoking, and body mass index were all significantly correlated with measures of IMT; HDL cholesterol was inversely correlated with IMT. Mean IMT measures were significantly higher in diabetics (P<0.001) and in individuals with cardiovascular disease (P<0.001).

Correlation Coefficients and Heritability Estimates
For mean and maximum CCA and ICA IMT, correlation coefficients for sibling:sibling pairs were calculated with SAGE FCOR. The correlation coefficients are shown in Table 4. In crude, age- and sex- adjusted, and multivariable-adjusted analyses giving equal weights to pedigrees, the correlation coefficients for mean CCA IMT were 0.36, 0.20, and 0.16, respectively. For mean ICA IMT, the correlation coefficients were 0.25, 0.18, and 0.16, respectively. The maximum and mean results were similar for CCA and ICA, respectively. The adjusted correlation coefficients can be used to calculate simple estimates of heritability with the equation \( h^2 = 2r \) (\( h^2 \) indicates heritability and \( r \) is sibling pair correlations). When the multivariable-adjusted sibling:sibling correlation coefficients in Table 4 are used, the estimates of \( h^2 \) for mean CCA and mean ICA were 0.32 and 0.32, respectively.

Heritability estimates derived from SOLAR are presented in Table 5. Heritability indicates the proportion of variance in IMT attributed to the additive effect of genes and early common environment. Heritability estimates were derived from normalized deviates of crude, age- plus sex-, and age-, sex-, plus multivariable-adjusted residuals. The age-, sex-

<table>
<thead>
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<th>TABLE 2. CCA and ICA IMT Measures</th>
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<tr>
<td>Mean (SD)</td>
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<tr>
<td>CCA mean, mm</td>
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<tr>
<td>CCA maximum, mm</td>
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<tr>
<td>ICA mean, mm</td>
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<td>ICA maximum, mm</td>
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<tr>
<th>TABLE 3. Correlation of Carotid Artery IMT with Covariates</th>
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<tr>
<td>Covariate</td>
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<tr>
<td>Age</td>
</tr>
<tr>
<td>Systolic blood pressure</td>
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<tr>
<td>Total cholesterol</td>
</tr>
<tr>
<td>HDL cholesterol</td>
</tr>
<tr>
<td>Triglycerides/day</td>
</tr>
<tr>
<td>Cigarettes/day</td>
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<tr>
<td>Body mass index</td>
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‡P value<0.001.

TABLE 4. Sibling-Sibling Correlation Coefficients for Carotid IMT Using SAGE FCOR

<table>
<thead>
<tr>
<th></th>
<th>CCA Mean</th>
<th>CCA Maximum</th>
<th>ICA Mean</th>
<th>ICA Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude</td>
<td>0.36</td>
<td>0.36</td>
<td>0.25</td>
<td>0.21</td>
</tr>
<tr>
<td>Age-, sex-adjusted*</td>
<td>0.20</td>
<td>0.21</td>
<td>0.18</td>
<td>0.16</td>
</tr>
<tr>
<td>Multivariable-adjusted*</td>
<td>0.16</td>
<td>0.17</td>
<td>0.16</td>
<td>0.14</td>
</tr>
</tbody>
</table>

*Includes adjustment for age, sex, systolic blood pressure, number of cigarettes/day, total cholesterol, HDL cholesterol, triglycerides, diabetes status, body mass index, antihypertensive treatment, menopausal status, and hormone replacement therapy.

Components of Variance Analysis
The overall contribution of genetic factors and measured covariates to carotid IMT was examined. The contribution of genetic factors to overall variation in mean CCA was 38% (heritability), and the contribution of measured covariates to overall variation was 27%, leaving a residual of 35%. The contribution of genetic factors to the overall variation in mean ICA was 35% (heritability), and the contribution of measured covariates was 18%, leaving a residual of 47%. Results for the maximum CCA and maximum ICA were similar.

Discussion
We have found that both mean and maximum measurements of CCA and ICA IMT are heritable. Heritability estimates using correlation coefficients and variance components methods are similar, and the magnitude of heritabilities for carotid IMT is similar to those reported for other quantitative cardiovascular traits such as systolic blood pressure and serum cholesterol. These data suggest that a substantial
proportion of the variability in carotid IMT is explained by genetic factors.

Carotid IMT is a marker of subclinical cardiovascular disease that has been shown to be associated with traditional coronary heart disease risk factors \(^1\)–\(^6\) and coronary atherosclerotic burden \(^7\), \(^8\) and to be predictive of subsequent cardiovascular events \(^9\), \(^10\). Multiple prior studies have demonstrated the contribution of genetic factors to individual coronary heart disease risk factors, including systolic blood pressure \(^23\), HDL cholesterol \(^24\), and diabetes mellitus \(^25\). A large study of 21,000 twins demonstrated an increased risk of premature coronary heart disease among monozygotic and dizygotic twins \(^26\), but there is a paucity of population-based genetic epidemiologic studies linking myocardial infarction or the underlying condition of atherosclerosis to genetic factors. Thus, our findings represent the first report of a large, population-based heritability analysis of carotid IMT.

Although prior studies have examined the association of specific genetic variants with carotid IMT, it has not been definitively demonstrated that carotid IMT is heritable. In a sample drawn from 1742 residents of Mexico City, CCA IMT and ICA IMT were highly heritable, with estimates of 0.92 for CCA and 0.86 for ICA after adjustment for covariates; however, the number of sibships studied (n = 46) was small \(^12\). Our finding of consistent evidence of heritability of the CCA and ICA IMT in our large, well-characterized population reinforces the validity of these prior findings and extends them to a large white cohort.

It has been suggested that CCA IMT might represent diffuse wall thickening resulting from smooth muscle accumulation and matrix deposition, whereas ICA IMT might be more prone to focal atherosclerotic plaques \(^8\), possibly related to endothelial dysfunction and hemodynamic flow in the ICA \(^2\), \(^27\). Indeed, thickening of the ICA intima has been shown to be somewhat more strongly associated with an increased risk of incident disease than in the CCA \(^2\)–\(^8\). Arterial bifurcations are lesion-prone areas that have increased activity of thrombosis, lipid deposition, and atherosclerosis \(^29\), thought to be a direct result of hemodynamic factors \(^30\), \(^31\). In vitro experimental models have demonstrated that atherosclerotic plaques are most often formed along bifurcations and along the inner wall of curvatures \(^32\), \(^33\).

In contrast, the CCA is exposed primarily to laminar blood flow, and in vivo experiments, CCA intimal thickness has been shown to be inversely related to wall shear stress, independent of age, blood pressure, body mass index, and diabetes mellitus \(^24\)–\(^37\). Low wall shear stress is hypothesized to increase the duration of time that blood comes into contact with the endothelial wall, enhancing atherogenic particle delivery and vessel wall adherence \(^27\), \(^35\). Our finding of heritability in the CCA and ICA IMT suggests that both processes might be examined through carotid artery imaging and that the hypothesized pathophysiological differences in CCA and ICA IMT might be attributable to different sets of genes.

Because carotid IMT is a quantitative, intermediate phenotype for clinical atherosclerosis, it may be a useful intermediate phenotype for genetic studies. There is a growing body of evidence for associations between carotid IMT or related carotid phenotypes, but the genes implicated in carotid IMT variability remain undefined. Genes in several pathways implicated in the pathogenesis of atherosclerosis have been considered. Some studies have yielded suggestive results for the T/T genotype of the b-fibrinogen gene \(^38\), factor V Leiden \(^39\), the D/D genotype of the angiotensin-converting enzyme insertion/deletion polymorphism \(^40\), and the paraxone gene \(^38\), whereas others have not found a relationship \(^31\), \(^41\)–\(^44\). In our analysis, heritability was only modestly attenuated by adjustment for known cardiac risk factors, suggesting that genes implicated in the variability of these phenotypes may not be major contributors to carotid IMT.

Certain limitations of our study deserve attention. The predominantly white population that makes up most of the Framingham Offspring cohort may limit the generalizability of our findings. Significant differences in the prevalence of genes important to heritability and different magnitudes of genes by environment effects may lead to estimates of heritability that differ among ethnic groups. However, coronary heart disease risk factor relationships from Framingham have been validated in 6 ethnically and geographically diverse cohorts and were found to be applicable in other populations, reinforcing the representativeness of our data \(^45\).

Second, in ≈25% of our ICA measurements, we were able to assess only the right or the left ICA but not both. In a subanalysis restricted to those with both right and left ICA measurements present, heritability measurements were actually higher, reflecting lower measurement error in the combined ICA measure. Last, we used CCA and ICA IMT as a marker of subclinical atherosclerosis. However, CCA and ICA IMT may not reflect similar atherosclerotic processes, and these hypothesized pathophysiological differences may lead to the eventual discovery of specific genes that are responsible for causing region-specific atherosclerosis.

In conclusion, a substantial proportion of the variability in carotid IMT is explained by genetic factors. Further studies of genetic linkage and candidate gene association are warranted to identify the specific genetic variants associated with this marker of increased risk for atherosclerosis and stroke.

Acknowledgments

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