Genetic Basis of Variation in Carotid Artery Plaque in the San Antonio Family Heart Study

Kelly J. Hunt, PhD; Ravindranath Duggirala, PhD; Harald H.H. Göring, PhD; Jeff T. Williams, PhD; Laura Almasy, PhD; John Blangero, PhD; Daniel H. O’Leary, MD; Michael P. Stern, MD

Background and Purpose—In contrast to the commonly used quantitative marker of subclinical atherosclerosis, namely intima-media thickness, we investigated the extent to which the presence or absence of carotid artery plaque (CAP) was under genetic control.

Methods—The study population consisted of 750 individuals distributed across 29 randomly ascertained extended Mexican American pedigrees who participated in the second examination cycle of the San Antonio Family Heart Study. Extracranial focal CAP was identified by B-mode ultrasound bilaterally in the internal carotid artery or the carotid bulb. Using a variance decomposition approach implemented in the SOLAR computer program, we performed genetic analysis on the discrete trait CAP (ie, liability to disease) using a threshold model. Covariates considered in the analysis included age, sex, diabetes, current smoking status, lipid levels, and markers of hypertension and obesity.

Results—Fifty-one of 461 women and fifty-seven of 289 men with a mean age of 42.1 years had evidence of a plaque in the right and/or left carotid artery. The age- and sex-adjusted heritability \( h^2 \) for CAP was significant \( (h^2=0.28\pm0.15, P=0.01) \). Furthermore, after adjustment for additional covariates that contributed significantly to the model \( (P<0.05; \text{diabetes, hypertension, body mass index, waist circumference, and smoking status}) \), heritability remained significant \( (h^2=0.23\pm0.15, P=0.03) \).

Conclusions—Our data indicate that after established cardiovascular risk factors are controlled for, the variation of the discrete trait CAP is under appreciable additive genetic influences. (Stroke. 2002;33:2775-2780.)

Key Words: epidemiology ■ genetics ■ risk factors

Atherosclerosis is a complex cardiovascular disorder that can be detected at a subclinical stage. It is almost always the pathology underlying coronary heart disease; in many cases, it is also the pathology underlying cerebrovascular events. Although plaques in the carotid arteries are directly responsible for only a fraction of clinical cardiovascular events, carotid atherosclerosis is associated with atherosclerosis in other arterial beds (coronary, femoral, and aorta) and can serve as a marker of generalized atherosclerosis.1–3 Furthermore, B-mode ultrasound is a noninvasive procedure that can be used to systematically identify and quantify subclinical atherosclerosis in the carotid arteries.

The genetic basis of B-mode ultrasound–measured carotid artery intima-media thickness (IMT), a quantitative marker of subclinical atherosclerosis, remains unclear despite several previous studies.4–9 In both the present study population and the National Heart, Lung, and Blood Institute (NHLBI) Family Heart Study, the published estimates of heritability for common carotid artery IMT are low, 0.13 and 0.23, respectively.5,7 In genetic studies enriched with diabetic individuals or individuals with cardiovascular disease, on the other hand, estimates of heritability for common carotid artery IMT tend to be higher, ranging from 0.49 to 0.92.4,8,9 In contrast to IMT, the extent of the additive genetic effects on carotid artery plaque (CAP) is yet to be explored; consequently, we investigated the extent to which the presence or absence of CAP was under genetic control in the San Antonio Family Heart Study (SAFHS).

Materials and Methods

SAFHS Design and Population

The SAFHS population consisted of 1431 individuals distributed across >40 extended Mexican American families recruited and first examined between 1992 and 1995. The study population for the present analyses was limited to 750 individuals distributed across 29 families who participated in the second examination cycle of the SAFHS between 1996 and 1999 and had complete B-mode ultrasound information available.10 Probands in the SAFHS were 40- to 60-year-old men and women chosen at random, independently of disease status, from a low-income Mexican American barrio in San Antonio (Texas). Apart from age, there were 2 additional eligibility
criteria: that the proband have a living spouse who was willing to participate in the study and that the proband have at least 6 first-degree relatives, excluding parents, who were at least 16 years of age and living in the San Antonio area. The latter requirement was imposed to ensure the availability of a reasonably large number of family members for recruitment into the study. All first-, second-, and third-degree relatives of both the proband and spouse were invited to participate. The study was approved by the Institutional Review Board of the University of Texas Health Science Center at San Antonio, and all subjects gave informed consent.

SAFHS Follow-Up Examination

The SAFHS follow-up examination consisted of a standardized medical examination that included interviews, measurement of blood pressure, anthropometry, a fasting venipuncture, and an oral glucose tolerance test. Trained interviewers obtained information on medical history, medication use, and current smoking status. Examinations occurred in the morning after participants had fasted for 12 hours. Measurement of blood pressure, body mass index (BMI), waist circumference, fasting total and high-density lipoprotein (HDL) cholesterol, fasting triglycerides, fasting plasma glucose, and plasma glucose 2 hours after a standardized oral glucose load has been previously described in detail.10

Hypertension was defined as systolic blood pressure ≥140 mm Hg, diastolic blood pressure ≥90 mm Hg, or current treatment with antihypertensive medication. Diabetes was defined according to the 2002 criterion of the American Diabetes Association11: plasma glucose ≥7.0 mmol/L (126 mg/dL). Participants who did not meet these criteria but who self-reported physician-diagnosed diabetes and who reported current therapy with either oral antidiabetic agents or insulin were also considered to have diabetes. Finally, prevalent cardiovascular disease was defined as self-reported physician-diagnosed stroke, heart attack, or heart surgery.

B-Mode Ultrasound Examination

B-mode ultrasound evaluations of atherosclerosis were completed on bilateral segments of the extracranial carotid arteries. The scanning and reading protocols were identical to those used in the Cardiovascular Health Study.12 Ultrasound images were recorded on super-VHS tapes and sent monthly to the central carotid ultrasound reading center, where 1 of 2 readers read each participant’s images. Sonographers completed central training at the reading center. Readers, trained and working at the reading center, were continuously monitored.

The ultrasound scanning protocol required sonographers to obtain on the right and left sides 1 lateral view of the common carotid artery and 3 views of the internal carotid artery in different fixed planes. The common carotid artery was defined as the 10-mm segment of the carotid artery immediately proximal to the origin of the bulb, where the near and far walls of the artery were parallel. The 3 views of the internal carotid artery were centered on the site of maximum wall thickness within the carotid bulb or the initial 10 mm of the internal carotid artery or when the sonographer considered the artery to be normal (without evidence of plaque) on the initial 10 mm of the internal carotid artery. Additionally, pulsed-wave Doppler was recorded at the point of maximum velocity in each carotid artery.

The ultrasound readers were asked to measure the near- and far-wall IMT of the single common carotid artery view and of the 3 different views of the internal carotid artery centered on the site of maximum wall thickness. In addition, using the images centered on the site of maximum wall thickness in the internal carotid artery, readers reported the absence or presence of a focal CAP. Focal CAP was defined as a focal widening of the IMT relative to the adjacent wall segment, measuring at least 1.5 mm in thickness. When focal plaque was present, the readers were asked to make a subjective assessment of the artery or plaque surface, morphology, stenosis, location, and density. Because plaques are less frequent in the common carotid artery and the focus of the ultrasound image in the common carotid artery was on systematically measuring IMT, plaque characterization was based on the recorded images of the site of maximum wall thickness in the left and right internal carotid arteries and hence was limited to the largest plaque identified in either the carotid bulb or internal carotid artery. A participant was considered to have a CAP if the morphology of either the left or right internal carotid artery was centered on the site of maximum wall thickness was considered a homogeneous or heterogeneous plaque, whereas a participant was considered to be without a plaque if the morphology of the right and left internal carotid images was reported to be normal. In addition, in cases of 100% stenosis, judging from pulsed-wave Doppler, of either the right or left internal carotid artery, a participant was considered to have a CAP.

Statistical Analyses

Using a variance decomposition approach implemented in the SOLAR computer program, we performed genetic analysis on the discrete trait carotid artery plaque (ie, liability to disease) using a threshold model.13 This approach assumes that an individual belongs to a specific disease class if an underlying genetically determined risk or liability exceeds a certain threshold, T = 0, on a normally distributed liability curve. The liability is assumed to have an underlying multivariate normal distribution. The correlation in liability between individuals i and j is given by

\[ \rho_{ij} = \rho_0 + \rho_1 \rho_{ij} + \rho_2 \text{E}_{ij} \]

where \( \rho_0 \) is the correlation in liability to disease between individuals i and j; \( \rho_2 \) is the kinship coefficient for individuals i and j; \( \rho_1 \) is the heritability attributed to additive polygenic effects; \( \rho_2 \) is the coefficient for the random environmental component for individuals i and j, which equals 1 if i equals j and 0 if i does not equal j; and \( e^2 \) is equal to 1 - \( h^2 \). The null hypothesis of no genetic effect \( (h^2 = 0) \) on CAP was tested with a likelihood ratio test by comparing the likelihood of a restricted model in which the parameter \( h^2 \) was constrained to a value of 0 with that for a general model in which the same parameter was estimated. Twice the difference between the In-likelihood values of these models yields a test statistic that is asymptotically distributed, as a \( \chi^2 \) distribution and a point mass at zero.14,15 For each covariate, the null hypothesis of no influence of a given covariate \( (\beta_{\text{covariate}} = 0) \) on liability to CAP was tested with a likelihood ratio test. A covariate test involves a likelihood ratio comparison for a given parameter and has 1 df. A statistically significant test \( (P < 0.05) \) was considered evidence of a nonzero estimate for a given parameter.

By extending the univariate variance component model to the multivariate situation, bivariate genetic analysis was used to partition the phenotypic correlation \( (\rho_{12}) \) between a given pair of quantitative traits into their additive genetic \( (\rho_{a12}) \) and random environmental \( (\rho_{e12}) \) components by means of information on genetic relationships between relatives and the maximum likelihood technique.14,16–18 An extension of this approach was used to conduct the bivariate analysis of a quantitative trait and a dichotomous trait or 2 dichotomous traits.19,20 The nature of the phenotypic correlation \( (\rho_{12}) \) between a pair of traits is given by the following:

\[ \rho_{12} = \sqrt{(h_1^2 + h_2^2)\rho_0 + (e_1^2 + e_2^2)\rho_0} \]

where \( \rho_{12} \) is the phenotypic correlation, \( \rho_{0} \) is the additive genetic correlation, \( \rho_{12} \) is the random environmental correlation, \( h_1^2 \) is the heritability of trait 1, \( h_2^2 \) is the heritability of trait 2, \( e_1^2 \) is equal to 1 - \( h_1^2 \), and \( e_2^2 \) is equal to 1 - \( h_2^2 \).

By using likelihood ratio tests, the significances of the phenotypic, additive genetic, and random environmental correlations were determined. For example, whether \( \rho_{12} \) between 2 traits is significantly different from 0 was tested by comparing the In-likelihood of a model in which the parameter \( \rho_{12} \) was constrained to equal zero with a model in which the same parameter was estimated. Twice the difference in In-likelihoods of these models yields a test statistic that is asymptotically distributed as a \( \chi^2 \) statistic. The degrees of freedom are equal to the difference in the number of parameters estimated in the 2 competing models.

In all of the analyses, age and sex were included as covariates. Additionally, because of the association between BMI and the technical difficulty of identifying CAP on B-mode ultrasound scans, models both excluding and including BMI as a covariate were considered in all analyses involving CAP.21 Additional covariates...
considered included ultrasound reader, waist circumference, lipid levels (total cholesterol, HDL cholesterol, and triglycerides), hypertensive status, diabetes status, diabetes medication, and current smoking status. Because the triglyceride distribution was skewed, this variable was ln-transformed. All covariate outliers, defined as >4 SD from the mean, were excluded from the analyses. A value of \( P = 0.05 \) was used as a nominal value for retention in the model. For each cardiovascular risk factor found to be statistically significantly associated with CAP in our final model, (1) age- and sex-adjusted estimates of heritability were calculated, and (2) phenotypic, additive genetic, and environmental correlations with the discrete trait CAP (liability to disease) were estimated with bivariate analysis. The procedures of the analysis used here are incorporated into the SOLAR computer program.\(^\text{22}\)

### Results

A total of 750 individuals 18 to 89 years of age from 29 families were examined; the distribution of pedigree size is presented in Table 1. Extracranial focal CAP was identified by B-mode ultrasound in the internal carotid arteries or the carotid bulb on the right and/or left side in 51 of 461 women and 57 of 289 men. Characteristics of the study participants

### Table 1. Structure of Pedigrees Used for Genetic Analysis of Plaque

<table>
<thead>
<tr>
<th>Family Members Examined</th>
<th>No. of Pedigrees</th>
<th>Total No. of Individuals</th>
</tr>
</thead>
<tbody>
<tr>
<td>1–10</td>
<td>8</td>
<td>37</td>
</tr>
<tr>
<td>11–20</td>
<td>3</td>
<td>55</td>
</tr>
<tr>
<td>21–30</td>
<td>8</td>
<td>208</td>
</tr>
<tr>
<td>31–40</td>
<td>5</td>
<td>185</td>
</tr>
<tr>
<td>41–50</td>
<td>3</td>
<td>135</td>
</tr>
<tr>
<td>51–60</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>61–70</td>
<td>2</td>
<td>130</td>
</tr>
<tr>
<td>Total</td>
<td>29</td>
<td>750</td>
</tr>
</tbody>
</table>

### Table 2. Characteristics (mean ±SD or %) of the San Antonio Family Heart Study Participants in Individuals With and Without Carotid Artery Plaque

<table>
<thead>
<tr>
<th></th>
<th>Without CAP (n=642(^\ast))</th>
<th>With CAP (n=108(^\ast))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>39.3 ± 14.3</td>
<td>59.2 ± 13.9</td>
</tr>
<tr>
<td>BMI, kg/m(^2)</td>
<td>30.9 ± 7.0</td>
<td>30.5 ± 6.0</td>
</tr>
<tr>
<td>Waist circumference, mm</td>
<td>1000 ± 167</td>
<td>1049 ± 152</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>4.58 ± 0.93</td>
<td>4.77 ± 0.93</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L</td>
<td>1.25 ± 0.32</td>
<td>1.26 ± 0.33</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>1.54 ± 1.11</td>
<td>1.91 ± 1.76</td>
</tr>
<tr>
<td>Female, %</td>
<td>63.9</td>
<td>47.2</td>
</tr>
<tr>
<td>Type 2 diabetes, %</td>
<td>13.7</td>
<td>42.1</td>
</tr>
<tr>
<td>Diabetes medication, %</td>
<td>9.5</td>
<td>26.2</td>
</tr>
<tr>
<td>Hypertension, %</td>
<td>22.7</td>
<td>65.7</td>
</tr>
<tr>
<td>Current smokers, %</td>
<td>19.7</td>
<td>26.2</td>
</tr>
<tr>
<td>Clinical CVD, %</td>
<td>4.4</td>
<td>27.1</td>
</tr>
</tbody>
</table>

CAP indicates carotid artery plaque; BMI, body mass index; BP, blood pressure; CVD, cardiovascular disease.

\(^\ast\)No more than 1% of participants are missing information on any individual trait.

### Table 3. Age- and Sex-Adjusted Estimates of Heritability for Selected Cardiovascular Risk Factors

<table>
<thead>
<tr>
<th></th>
<th>( h^2 ) ± SE</th>
<th>( P ) Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body mass index</td>
<td>0.517 ± 0.073</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Waist circumference</td>
<td>0.481 ± 0.076</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Hypertension</td>
<td>0.101 ± 0.099</td>
<td>&lt;0.1178</td>
</tr>
<tr>
<td>Diabetes (ADA criteria)</td>
<td>0.634 ± 0.126</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Current smoking status</td>
<td>0.261 ± 0.098</td>
<td>0.0007</td>
</tr>
</tbody>
</table>

with and without CAP are presented in Table 2. Overall, point estimates indicate that participants with CAP were older, more likely to be male, more likely to be smokers, and more likely to have diabetes, hypertension and clinical cardiovascular disease than participants without CAP (Table 2). However, participants with and without CAP had similar lipid levels, BMIs, and waist circumferences. Finally, each ultrasound reader reported a similar percentage of individuals with CAP (14.3% versus 14.6%). The age- and sex-adjusted heritability (\( h^2 \) ± SE) for CAP was significant (\( h^2 = 0.28 ± 0.15, P = 0.01 \)); furthermore, after additional adjustment for BMI, the heritability increased slightly and remained significant (\( h^2 = 0.29 ± 0.15, P = 0.01 \)). After accounting for additional covariates that contributed significantly to the model (\( P < 0.05 \); BMI, waist circumference, diabetes, hypertension, and smoking status), the heritability was attenuated slightly (\( h^2 = 0.23 ± 0.15, P = 0.03 \)) but remained statistically significant. Furthermore, in our final model including significant covariates, the prevalence of CAP increased with age and waist circumference; decreased with increased BMI; and was higher in men, individuals with hypertension, individuals with diabetes, and smokers. However, lipid levels (total cholesterol, HDL cholesterol, or triglycerides), being on diabetes medication, and ultrasound reader were not significantly associated with the presence of CAP.

Age- and sex-adjusted estimates of heritability for the cardiovascular risk factors included in our final model ranged from 0.10 for hypertension to 0.63 for diabetes (Table 3). Furthermore, the phenotypic, additive genetic, and random environmental correlations between CAP and each of the cardiovascular risk factors included in our final model, as well as clinical cardiovascular disease, are presented in Table 4. For each trait, phenotypic, additive genetic, and random environmental correlations are shown adjusted for age and sex, as well as for age, sex, and BMI. After adjustment for age and sex, the phenotypic correlations with CAP were statistically significant for diabetes, hypertension, smoking, and clinical cardiovascular disease, ranging from 0.24 (\( P = 0.0076 \)) for smoking to 0.30 (\( P = 0.0029 \)) for clinical cardiovascular disease. Furthermore, additive genetic correlations with CAP were moderately significant for type 2 diabetes (\( h^2 = 0.48, P = 0.0305 \)) and strongly significant for clinical cardiovascular disease (\( h^2 = 0.75, P = 0.0107 \)), whereas no environmental correlations with CAP were significant. After adjustment for age, sex, and BMI, results remained similar with the exception of the bivariate analysis.
between CAP and waist circumference. Adjusted for age, sex, and BMI, the phenotypic correlation between waist circumference and CAP was significant ($\rho_p = 0.19$, $P = 0.0050$), and although the additive genetic correlation was weak and did not approach statistical significance ($\rho_h = -0.04$, $P = 0.8845$), the environmental correlation was statistically significant ($\rho_e = 0.29$, $P = 0.0327$).

### Discussion

Identifying factors associated with the presence of atherosclerosis could help to quantify an individual’s risk of developing subclinical and clinical cardiovascular disease. Atherosclerosis itself is a complex cardiovascular disorder, is almost always the pathology underlying coronary heart disease, and is often the pathology underlying cerebrovascular events. We report here a statistically significant heritability for focal CAP after accounting for age and sex effects. Adjusting for the established cardiovascular risk factors attenuates the focal CAP heritability by <20%. Furthermore, bivariate analysis in our study population indicates that the phenotypic correlations observed between focal CAP and diabetes ($\rho_p = 0.30$, $P = 0.0002$) and between focal CAP and self-reported clinical cardiovascular disease ($\rho_p = 0.30$, $P = 0.0029$) are largely explained by the genetic correlation between these traits ($\rho_h = 0.48$, $P = 0.0350$; and $\rho_e = 0.75$, $P = 0.0107$, respectively).

B-mode ultrasound of the carotid arteries is a noninvasive procedure used to identify and measure subclinical atherosclerosis for research purposes. B-mode ultrasound–derived mean common carotid artery IMT has been established as an early quantitative marker of generalized atherosclerosis because of its association with atherosclerosis in other arterial beds1–3 and its association with cardiovascular diseases4,5,23–26 and its risk factors.12,27–29 Results from familial studies of the heritability of mean carotid artery thickness are inconsistent. Published estimates of heritability for common carotid artery IMT are low in the present study population and the NHLBI Family Heart Study, 0.13 and 0.23, respectively.5,12 However, in genetic studies enriched with diabetics, estimates of heritability for carotid artery IMT tend to be higher.4,8,29 In a study of sibships located in Mexico City with a 33% prevalence of diabetes, Duggirala et al14 reported high heritabilities for common ($h^2 = 0.92$) and internal ($h^2 = 0.86$) carotid artery IMT. Furthermore, the Diabetes Heart Study, a study enriched with type 2 diabetic individuals, reported a heritability of 0.49 for common carotid artery IMT, and in sibling pairs with type 2 diabetes, Rich et al reported a heritability of 0.84 for common carotid artery IMT.15

Mean carotid artery IMT can reflect a combination of arterial characteristics, including (1) an early diffuse thickening of the carotid arteries that is preatherosclerotic, (2) a single focal thickening of the carotid arteries that contributes disproportionately to the overall mean IMT measured across multiple sites, and (3) at lower levels a nonatherosclerotic thickening that is an adaptive response to altered flow and shear stress on the arterial wall.30,31 In addition, variation in methodology across studies, with some studies including and other studies excluding sites of focal CAP in their measurement, may alter the interpretation of mean carotid artery IMT. Finally, B-mode ultrasound cannot distinguish between the intimal and medial arterial layers, which may differentially contribute to the pathophysiological significance of IMT.

In contrast to previous studies that have focused on carotid artery IMT, our study focused on the genetic basis of the discrete marker of subclinical atherosclerosis focal CAP. Focal CAP is believed to represent a later stage of atherosclerosis than diffuse carotid artery intima-media thickening;
hence, focal CAP is closer to the disease end point of interest, clinical cardiovascular disease. Interestingly, a study that examined the relationship between a parental history of premature death from coronary heart disease and common carotid IMT and CAP reported a positive association between a parental history and CAP but no association between parental history and IMT.\textsuperscript{32} One limitation associated with focal CAP compared with mean carotid artery IMT is the possible lower statistical power inherent in the use of discrete traits compared with their quantitative counterparts.

Factors associated with the presence of CAP in our study, except for the absence of a statistically significant association with lipid levels, are consistent with earlier population based studies.\textsuperscript{21,33,34} Increased age, male sex, smoking, hypertension, diabetes, and increased waist circumference were associated with the presence of focal CAP. In addition, as in earlier population-based studies, an inverse association with BMI was observed.\textsuperscript{21}

Strengths of our study include the randomly ascertained study population, eliminating the need to correct for ascertainment bias with respect to a disease, and the nonissue of temporality in genetic studies. One limitation is that our heritability estimates may have been influenced by shared environmental factors because we did not account for these among family members in our model.

In summary, we report that liability to focal CAP is heritable even after accounting for the established cardiovascular risk factors. The results also support the concept that focal CAP, diabetes, and self-reported clinical cardiovascular disease have a common genetic background (ie, pleiotropy). Despite the wide array of approaches currently being used to elucidate the genetic determinants of complex disorders such as cardiovascular disease, our understanding remains limited.\textsuperscript{35} However, as our knowledge of the human genome increases and our ability to noninvasively image, characterize, and quantify subclinical cardiovascular disorders improves, studies such as this will increase our ability to identify the genetic determinants of the complex disorder cardiovascular disease.

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