Heritability of Carotid Artery Intima-Medial Thickness in Type 2 Diabetes

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Background and Purpose—Carotid artery intima-medial thickness (IMT), a marker of subclinical atherosclerosis, is a strong predictor of subsequent cardiovascular morbidity. The role of genetic factors in thickening of the carotid wall remains largely unknown. We hypothesize that in families with multiple members having diabetes, carotid IMT is likely to be associated with both inherited and environmental factors.

Methods—To determine the extent of the familial aggregation of carotid IMT in the presence of type 2 diabetes, we studied 252 individuals with type 2 diabetes (mean age 60.6 years) from 122 families. Common carotid artery IMT was measured by high-resolution B-mode ultrasonography. Other measured factors included lipid levels, body mass index, fasting glucose, hemoglobin A1c, albumin/creatinine ratio, and self-reported medical history. Heritability estimates were obtained by using variance component methodology, as implemented in the SOLAR software package. Tests for association between carotid IMT and variables were performed by using mixed model analysis while accounting for the correlation due to family structure.

Results—The age-, sex-, and race-adjusted heritability estimate for carotid IMT was 0.32 (SE 0.17, P = 0.02). Further adjustment for total cholesterol, hypertension status, and current smoking status resulted in a heritability estimate of 0.41 (SE 0.16, P = 0.004). The strongest predictors of carotid IMT, after adjusting for age and sex, were ethnicity (African American versus white), total cholesterol, and smoking status.

Conclusions—These data provide empirical evidence that subclinical cardiovascular disease has a significant genetic component and merits a search for the genes involved in susceptibility to the atherosclerotic complications of diabetes. (Stroke. 2002;33:1876-1881.)

Key Words: atherosclerosis • carotid arteries • diabetes mellitus • epidemiology • genetics • hereditary disease • ultrasonics

Carotid artery intima-medial thickness (IMT), measured by high-resolution B-mode ultrasonography, has proven to be an excellent marker of subclinical atherosclerotic cardiovascular disease (CVD). Increasing carotid IMT has been associated with an enhanced risk of myocardial infarction and stroke in older adults lacking a prior history of CVD.1,2

Several studies (Cardiovascular Health Study [CHS],3 Atherosclerosis Risk in Communities [ARIC],4 and Insulin Resistance Atherosclerosis Study [IRAS]5) have shown that hypertension, hyperlipidemia, male sex, age, smoking, and postmenopausal status are consistent and independent factors that contribute to increasing carotid IMT. Additionally, certain forms of antihypertensive6 and lipid-lowering7 therapies have been associated with regression or reduced progression of IMT. However, the role of genetic factors in determining carotid IMT is still largely unknown. Two family studies have attempted to determine the contribution of inherited and environmental factors to variability in carotid IMT. These reports, in nondiabetic families, estimated heritability to be ≈0.908 and ≈0.30,9 supporting the presence of strong independent genetic contributions to carotid IMT.

Atherosclerotic CVD is known to develop at an accelerated rate in individuals with impaired glucose tolerance and type 2 diabetes mellitus.10,11 This is particularly true in individuals with diabetic nephropathy12 or microalbuminuria.13 It is likely that a combination of environmental and inherited factors (pleiotropic genes mediating susceptibility to both diabetes and atherosclerosis) are involved in this relationship, although their relative contributions are unknown. Therefore, we measured common carotid artery IMT in affected siblings from multiplex diabetic families to examine its heritability in this population.

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Subjects and Methods

The present study was organized and conducted in Forsyth County, NC, to study CVD in families affected with type 2 diabetes mellitus. Siblings concordant for diabetes were recruited from internal medicine clinics, endocrinology clinics, and community advertising. Type 2 diabetes was defined as a clinical diagnosis of diabetes after the age of 34 years in the absence of historical evidence of diabetic ketoacidosis. Unaffected siblings, similar in age to the siblings with diabetes, were also invited to participate. For the purposes of this analysis, only diabetic family members were included. The sample includes white and African American participants. Individuals with serious health conditions, eg, renal replacement therapy, were not eligible to participate. Individuals reporting previous carotid endarterectomy (n = 2) were excluded from all analyses. The present study was approved by the Institutional Review Board at Wake Forest University School of Medicine. All participants gave informed consent.

The participant examinations were conducted in the General Clinical Research Center of the Wake Forest University Baptist Medical Center and included interviews for medical history and health behaviors, anthropometric measures, resting blood pressure, a 12-lead ECG, a fasting blood draw, and a spot urine collection. Laboratory assays included urine albumin and creatinine, total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides, hemoglobin A1c, fasting glucose, and blood chemistries. DNA was isolated from a blood sample and stored for later studies.

For measurement of IMT, the participant was examined in the supine position. A plastic arc marked with an angle scale and positioned near the shoulders of the participant was used as a reference to measure the ultrasound transducer interrogation angle during the study. The sonographer scanned from the head of the examination table.

High-resolution B-mode carotid ultrasonography was performed by using a 7.5-MHz transducer and a Biosound Esaote (AUS) ultrasound machine, and B-mode images were recorded on an S-VHS videocassette. Scans were performed on both the right and left extracranial carotid arteries by trained and certified ultrason technicians. A preliminary exploratory transverse scan was performed to assess the participant’s anatomy and to detect the presence of significant atherosclerotic disease. A second exploratory longitudinal scan was then performed on the common carotid, the bulb, and the internal carotid to assist in interpreting the presence of significant disease. Standardized longitudinal images were then acquired from the neck to above the clavicle. The mean value of up to 20 mean common carotid IMT values is the outcome variable reported in the present study.

Statistical Methods

To determine the contribution of genetic factors to carotid IMT, we analyzed the carotid IMT data obtained on diabetes-affected family members by using the SOLAR software package (Version 1.4.1). SOLAR performs a variance components analysis of family data that decomposes the total variance of the phenotype (carotid IMT) into components that are due to genetic (polygenic) effects (additive genetic variance), measured covariates, and random environmental effects. The relative contribution of genetic factors to carotid IMT variation is then estimated by the heritability ($h^2$), defined by the ratio of the genetic variance component to the residual (after removal of covariates) phenotypic variance. A series of models was developed that incorporates an increasing number of covariates known to be associated with IMT to determine the extent of genetic factors contributing to the variation in carotid IMT independent of the measured risk factors. Model 1 was adjusted for age, sex, and ethnicity. Model 2 was additionally adjusted for fasting glucose and total cholesterol. Model 3 was additionally adjusted for hypertension status and current smoking status. Significance of the $h^2$ values was determined by likelihood ratio tests, in which the likelihood of the models with the additive genetic variance component and covariates was compared with the model with the likelihood in which the additive genetic variance component was constrained to be 0.

To investigate the potential relationships between carotid IMT and the above-mentioned demographic characteristics and anthropometric, clinical, and laboratory measures, we used a mixed model ANOVA approach, implemented by the SAS software package, assuming exchangeable correlation among pedigree members and the sandwich estimator of the variance-covariance matrix. This approach allows for the correlation due to the family structures present in our data.

Transformations were performed on body mass index (square root), HDL, triglycerides, albumin/creatinine ratio, and pack years (logarithm) to reduce the influence of extreme values. All continuous covariates were standardized to a mean of 0 and a variance of 1 to facilitate the interpretation of the results. All tests for covariate effects were based on models that included age and sex. A multivariable model containing age, sex, ethnicity, smoking status (current and former), education level (high-school graduate versus not and college graduate versus not), diabetes treatment (insulin, oral hypoglycemic, neither), hypertension status, total cholesterol, and fasting glucose was then examined to test each covariate effect after adjusting for all other covariates. The covariates for this model were selected a priori and not as a result of exploratory analysis.

Results

The analysis sample consisted of 252 diabetic individuals from 122 families. Pedigree size ranged from 2 to 7, with 99 pedigrees consisting of sibling pairs, 9 consisting of 3 members, and 1 each of 4, 5, and 7 members. Participants were all informed by their physician that they were diabetic, and 241 (95.6%) of 252 are currently receiving, or at some point received, oral hypoglycemic/insulin therapy. Of the remaining 11 patients, 6 had a fasting blood sugar $>126$ mg/dL, and 1 patient had hemoglobin A1c $>7.5$% at the screening visit. Four diet-controlled patients (4 [1.5%] of 252) had normal fasting glucose and hemoglobin A1c levels. Historical fasting glucose levels were not available for these 4 participants. Eleven singletons were also included in the sample descriptives and the association analysis but were excluded in the $h^2$ analysis. The average age of participants was 60.6 years, and the average duration of diagnosed diabetes was 11.2 years. Fifty-nine percent (n = 149) of the sample were female, and 11% (n = 28) were African American. Thirty-three percent (n = 82) were being treated with insulin, 76% (n = 192) were taking oral hypoglycemics, and 36% (n = 91) were taking lipid-lowering medications. The mean carotid IMT for this sample was 0.674 mm (SD 0.134), with a median value of 0.644 mm and a range from 0.460 to 1.316 mm. Additional sample descriptives are shown in Table 1.

Results of the heritability analysis are listed in Table 2. The $h^2$ value of carotid IMT, adjusted for age, sex, and race was 0.32 ($P = 0.02$). After additional adjustment was made for total cholesterol, $h^2$ was determined to be 0.38 ($P = 0.008$). Further adjustment for hypertension status and current smoking status resulted in an even larger estimate ($h^2$ 0.41, $P = 0.004$). These increases occur because adjustment for measured covariates reduces the overall remaining unexplained variance, allowing the genetic contribution to become more apparent.

Age and male sex were positively and significantly associated with IMT ($P = 0.0001$ and $P = 0.0002$, respectively). Ethnicity ($P = 0.04$) was also a significant predictor of carotid IMT, with African American ethnicity being associated with
higher IMT. Because of this observation, all subsequent models were also adjusted for ethnicity. The results from the sex-, age-, and ethnicity-adjusted bivariate analysis are presented in Table 3. The strongest predictors of carotid IMT were total cholesterol ($P=0.009$), smoking status (never, former, and current); (former, $P=0.002$; current, $P=0.0007$), and LDL cholesterol ($P=0.049$). Log-triglycerides were marginally positively associated with carotid IMT ($P=0.095$). No other covariates met statistical significance at the 5% level.

A multivariable model containing age, sex, ethnicity, smoking status (current smoker, former smoker), education level (high-school graduate versus not), hypertension status, fasting glucose, and total cholesterol was created, and smoking status, total cholesterol, and hypertension status were determined to be statistically significant, with adjustment made for the other covariates. The direction and magnitude of the coefficients for each measure remained similar to the bivariate analyses. The results of this analysis are shown in Table 4.

**Discussion**

To our knowledge, this is the first analysis of the heritability of carotid IMT in families with multiple members having type 2 diabetes mellitus. The observed $h^2$ value (0.41) for carotid IMT is similar to that observed for coronary artery calcium (CAC) content (0.50) in the same study.16
adjusted estimate of CAC heritability was measured in a subsample of 56 of these families whose members had a measure of CAC estimated via fast-gated helical CT. Taken together, these 2 reports provide empirical evidence that subclinical CVD has a significant genetic component and merits a search for the genes involved in susceptibility to the atherosclerotic complications of diabetes.

The heritability of carotid IMT has been determined in 2 other family studies. Duggirala et al^8 detected an extremely high heritability of carotid IMT among siblings from Mexico City (from 0.86 to 0.92), suggesting a strong genetic component to atherosclerosis. This estimate, adjusted for a number of established CVD risk factors, in 46 mixed-ethnicity sibships of various sizes was based on a sample from an epidemiological survey in Mexico City. A potential explanation for this extremely high heritability estimate value may be that the participants in that study had a higher degree of similarity among environmental exposures than is usually seen in other, more general, populations, or it may be that the estimate was dominated by a single large family. In another family study, genetic factors were reported to contribute to 30% of the variability for carotid IMT. Although these 2 reports detected a genetic component to carotid IMT, they were in nondiabetic families with different ethnicity than the families in the present study. Additionally, the methodology used by Zannad et al^9 to estimate heritability was different from that used by us. In Zannad et al^9 nuclear families were ascertained with relatively young children, in whom the carotid IMT measures are not expected to be variable in the absence of disease. Thus, the power for estimating the contribution of genetic factors in that setting would not be substantial.

Several studies have examined candidate genes for association with IMT, although the findings have been inconsistent. Candidate genes that have been examined include apoE,^17–20 paraoxonase,21–24 ACE,22,25–27 cholesterol ester transfer protein,28 β2-adrenergic receptor,29 and methylenetetrahydrofolate-late.22,30,31 The inconsistency among the results from these

### TABLE 2. Heritability Analysis of Carotid IMT in Participants With Type 2 Diabetes

<table>
<thead>
<tr>
<th>Adjustments</th>
<th>Proportion of Variance Accounted for by Demographic, Metabolic, and Behavioral Factors</th>
<th>h²</th>
<th>SE of h²</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model 1 (age, sex, race)</td>
<td>0.187</td>
<td>0.306</td>
<td>0.166</td>
<td>0.030</td>
</tr>
<tr>
<td>Model 2 (age, sex, race, total cholesterol)</td>
<td>0.206</td>
<td>0.359</td>
<td>0.163</td>
<td>0.012</td>
</tr>
<tr>
<td>Model 3 (age, sex, race, total cholesterol, hypertension status, current smoking status)</td>
<td>0.249</td>
<td>0.408</td>
<td>0.160</td>
<td>0.005</td>
</tr>
</tbody>
</table>

The β coefficients represent the change in IMT for a 1-SD increase in the predictor. All models are age-, sex-, and ethnicity-adjusted, except for ethnicity, which is age- and sex-adjusted.
studies provides additional support for the necessity of a genome-wide linkage scan for this trait.

The associations we observed between carotid IMT and total serum cholesterol, LDL cholesterol, and smoking are consistent with other studies of individuals with type 2 diabetes and with studies of nondiabetic individuals. These findings are congruent with the results of a study by Stamler et al., in which the risk factors for CVD mortality were similar in diabetic and nondiabetic men. We did not observe a significant association between carotid IMT and hypertension status until we adjusted for a number of other CVD risk factors. Hypertension was extremely prevalent (76%) in this sample. This result may indicate that the effects of hypertension on CVD development may not be as strong in such a high-risk population, as has been detected in a number of studies in nondiabetic subjects. In another study of 271 patients with type 2 diabetes, hypertension status was not a significant predictor of IMT after adjustment was made for other CVD risk factors. The nonsignificant positive trend for IMT with myocardial infarction and stroke are of smaller magnitude, as reported previously by others in nondiabetic subjects.

There was no statistical evidence of an association between the disease duration of diabetes and IMT. However, there was suggestive evidence of a trend for fasting glucose levels and carotid IMT, especially after multivariable adjustment. These results are consistent with those reported by Wagenknecht et al., who examined these factors in 489 unrelated individuals with type 2 diabetes. They observed that common carotid IMT was increased by 26 μm per 1 SD of fasting glucose, but they did not observe an association between the duration of diabetes in participants with established diabetes and IMT. Our estimate of a 12-μm (0.012-mm) increase in carotid IMT per 1 SD of fasting glucose is of somewhat smaller magnitude. We also examined diabetes treatment (insulin, oral hypoglycemic, none) as a surrogate measure of disease severity. Treatment was not associated with carotid IMT in this sample. However, the observed null findings may be unique to this sample and should not be interpreted as proof that these associations do not exist.

Our observation of significantly greater carotid IMT in African Americans versus whites is consistent with findings reported by D’Agostino et al. in a study of 1020 nondiabetic subjects from IRAS. In that study, the authors reported that even after adjustment was made for major CVD risk factors and insulin sensitivity, African Americans had a significantly greater IMT than did non-Hispanic whites (0.864 versus 0.823 mm, respectively). Our finding is similar, with African Americans having an average carotid IMT 0.060 mm greater than that of whites, an effect equivalent in the present study to 10 years of increased age. A number of other large studies of carotid IMT did not report an association between carotid IMT and race and did not adjust for its effects in other analyses. Our finding is in accordance with the increased rate of stroke in African Americans versus whites. It is interesting to note that the opposite trend (ie, whites with a higher rate than African Americans) was apparent in CAC measured in this same study group. In contrast, IMT and CAC are both positively associated with age, male sex, smoking, and (to a lesser degree) triglycerides.

A limitation of the present study is that in using a traditional sibling-pair design, we can only estimate heritability by using sibling correlations. Thus, the estimated heritability includes both dominance and epistatic effects (potentially inflating the “true” estimate). Furthermore, the power of the variance components approach is due, in part, to the inclusion of additional relative pairs. In restricting the sample to sibling pairs, some loss of power and accuracy occurs. On the other hand, inclusion of covariates in the model reduces the phenotypic variance, thereby increasing the ability to estimate the genetic contribution to variation in IMT. Thus, our estimates of heritability of IMT were found to be statistically significant and of moderate magnitude, providing further support for the genetic basis of IMT in families affected with type 2 diabetes.

In conclusion, the established CVD risk factors (older age, male sex, African American race, total cholesterol, and LDL cholesterol) are important correlates of IMT in persons with type 2 diabetes. In addition, our findings of IMT heritability in the range of 40% warrants further investigation for specific genes contributing to variation in this trait. Although candidate gene studies can be informative, they may have limited utility in identifying the quantitative trait loci involved in complex diseases and in determining whether genome-wide linkage analysis scans are necessary. The moderate degree of heritability that we observed for IMT and the heritabilities observed for other traits associated with CVD in the present study indicate that this sample may be useful in efforts to map genes contributing to atherosclerotic CVD.

Acknowledgments

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