A Genome-Wide Scan for Carotid Artery Intima-Media Thickness

The Mexican-American Coronary Artery Disease Family Study

Dai Wang, PhD; Huiying Yang, MD, PhD; Manuel J. Quiñones, MD; Isabel Bulnes-Enríquez, MD; Xochitl Jiménez, MD; Roxana De La Rosa, BS; Tamara Modilevsky, MD; Katherine Yu, MD; Yanjie Li, MD; Kent D. Taylor, PhD; Willa A. Hsueh, MD; Howard N. Hodis, MD; Jerome I. Rotter, MD

Background and Purpose—Carotid artery intima-media thickness (CIMT), a subclinical measure of atherosclerosis, is associated with coronary artery disease (CAD), and stroke. CIMT is also an important predictor of clinical cardiovascular events. To systematically identify the genetic determinants of CIMT, we performed a genome-wide scan using data from 91 2-generation Mexican American families ascertained via a parent with CAD diagnosed.

Methods—CIMT was measured in 274 adult offspring (mean age, 34.6 years) using high-resolution B-mode ultrasound; 413 subjects, including adult offspring and their parents, were genotyped using Marshfield screen set 12 (380 microsatellite markers at ~10-cM interval). Heritability was estimated using the variance component approach implemented in SOLAR. Linkage analyses were performed using both the sib-pair regression approach and the variance component approach.

Results—The estimated heritability was 0.68, 0.45, and 0.40 for unadjusted, gender- and age-adjusted, and multivariate-adjusted CIMT, respectively. The strongest evidence of linkage was found on chromosome 2 at D2S2944 (logarithm of the odds [LOD]=3.08). Other suggestive linkages were also found on chromosome 6 at D6S1022 to D6S2410 (LOD=2.21) and chromosome 13 at D13S796 to D13S895 (LOD=1.34).

Conclusions—These results show that there is a strong genetic effect on CIMT in these Mexican American CAD families. The linkage peak on chromosome 2 suggests that there is a gene (or genes) at this chromosome location influencing CIMT. (Stroke. 2005;36:540-545.)

Key Words: coronary artery disease ■ genetics ■ intima-media thickness ■ linkage mapping ■ stroke

Carotid artery intima-media thickness (CIMT) is a widely used subclinical measure of atherosclerosis in clinical research. Its advantages include that it is a marker of early arterial wall atherosclerotic change and that it can be quantitatively measured with precision and reproducibility by use of B-mode ultrasound. Epidemiological studies have demonstrated an association between CIMT and all of the major cardiovascular risk factors such as gender, age, adiposity, blood pressure, diabetes mellitus, insulin resistance, and smoking. CIMT is also an important predictor of clinical cardiovascular outcomes.

CIMT is associated with a family history of coronary artery disease (CAD), stroke, and type II diabetes, suggesting a genetic basis for CIMT variation. CIMT heritability has been studied in several different populations. These studies suggest that approximately one-fifth to one-half (and in some cases more) of CIMT variation is caused by genetic influences.

Candidate gene association studies with CIMT are emerging. The results to date are inconclusive. An alternative to the candidate gene approach is the systematic genome scan, which has the advantage of not requiring a prior specific candidate gene or pathophysiological hypothesis. The genome scan linkage approach uses a relative small set of markers along the entire set of human chromosomes. Evidence for cosegregation of a marker or markers with the trait variation in families, the phenomenon known as linkage, serves to identify the chromosomal location of genes responsible for the trait of interest. To systematically identify the genetic determinants of CIMT, we performed such a genome-wide scan using data from 91 2-generation Mexican American families.
UCLA and Cedars-Sinai Medical Centers. Procedures were approved by the institutional review boards of the genotyped. All subjects provided written informed consent and the phenotyped and 413 subjects (adult offspring and their parents) were included. In total, 274 subjects (adult offspring) were extensively for CIMT measurements in the adult offspring only. These adult provided information on gender, age, and a fasting blood sample for 2 to 7 (with an average of 3) adult offspring (age older than 18) were patients affected with CAD, determined by evidence of myocardial infarction on electrocardiogram or hospital record, evidence of atherosclerosis on coronary angiography, or history of coronary artery bypass graft or angioplasty, were recruited as probands from southern California. For each proband, the spouse (if available) and 91 2-generation Mexican American families were can families ascertained via a parent with CAD diagnosed. The results provide evidence for 1 or more genetic loci as determinants of CIMT.

Materials and Methods

Subjects
The subjects were participants in the Mexican-American Coronary Artery Disease (MACAD) study, an ongoing study of CAD and insulin resistance in Mexican Americans. Mexican American patients affected with CAD, determined by evidence of myocardial infarction on electrocardiogram or hospital record, evidence of atherosclerosis on coronary angiography, or history of coronary artery bypass graft or angioplasty, were recruited as probands from southern California. For each proband, the spouse (if available) and 7 to 2 (with an average of 3) adult offspring (age older than 18) were enrolled in the study. At the initial clinic visit, all the subjects provided information on gender, age, and a fasting blood sample for setting up cell lines and measurement of standard biochemical variables such as lipids, fasting glucose, and fasting insulin. At the second clinic visit, carotid artery ultrasound images were acquired for CIMT measurements in the adult offspring only. These adult offspring were also measured for body mass index (BMI) and blood pressure. All the offspring were free from clinically evident CAD. In the present report, 91 2-generation Mexican American families were included. In total, 274 subjects (adult offspring) were extensively phenotyped and 413 subjects (adult offspring and their parents) were genotyped. All subjects provided written informed consent and the procedures were approved by the institutional review boards of the UCLA and Cedars-Sinai Medical Centers.

Measurement of IMT
Common CIMT of 274 adult offspring was measured. High-resolution B-mode carotid artery images were obtained with a Toshiba SSH-140A ultrasound system with a 7.5-MHz probe at the University of Southern California Atherosclerosis Research Unit.

Statistical Analysis
Covariates were screened using stepwise regression implemented in SAS software (version 8.2). Only terms that were significant at the 0.05 level or less were retained. Covariates considered included gender, age, BMI, systolic blood pressure (SBP), diastolic blood pressure, lipids, fasting glucose, fasting insulin, and smoking. In addition to gender, age, and SBP, well-known significant predictors of CIMT, BMI, and fasting insulin were also significant when evaluated individually. However, when all 3 phenotypes (SBP, BMI, and fasting insulin) were evaluated simultaneously, BMI failed to enter the final model. Covariates that entered the final model included gender, age, age2, SBP, fasting insulin, and smoking. Three sets of covariate adjustments of CIMT were considered for the heritability estimations and linkage analyses: (1) no adjustment; (2) adjustment for gender, age, and age2 (gender- and age-adjusted); (3) adjustment for gender, age, age2, SBP, fasting insulin, and smoking (multivariate-adjusted).

Heritability was estimated using the variance component model implemented in the SOLAR software. This method allows the total phenotypic variance to be partitioned into a proportion caused by polygenic effects (heritability, h²) and a proportion caused by random environmental effects. The calculated heritability, therefore, estimates the proportion of total variance of a trait caused by genetic effects.

Linkage analyses were performed with 2 different approaches. The first approach was the recently augmented Haseman–Elston regression method implemented in SAGE 4.5 (Statistical Solutions Ltd; 2002). The principle herein is comparison of the quantitative phenotypic difference between relative pairs (ie, siblings) as a function of the number of marker alleles they share in common at each genetic locus. Instead of using the squared sib-pair trait difference in the original method or the mean-corrected cross product of the sib-pair trait values in the second version, this newest method uses a weighted average of the squared sib-pair trait difference and the squared sib-pair mean-corrected trait sum as the dependent variable. The dependent variable is regressed on the estimated proportion of alleles at a locus shared identical by descent (IBD) by the sib-pairs. Single-point and multipoint IBD values were calculated with the GENIBD program and linkage was evaluated with the SIBPAL program, both of the SAGE package. The second approach was the variance component method implemented in the QTDT software. By this approach, the phenotypic covariance among family members is modeled as the sum of an additive genetic variance of the major gene (σ²g), a residual sibling resemblance (σ²r), and a residual environmental variance component (σ²e). Evidence of

Table 1: Descriptive Statistics in Adult Offspring: CIMT and Related Phenotypes

<table>
<thead>
<tr>
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<th>Male (n=111)</th>
<th>Female (n=163)</th>
<th>P Value</th>
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<tr>
<td>CIMT, mm</td>
<td>0.70±0.13</td>
<td>0.64±0.11</td>
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<td>Age, y</td>
<td>34.2±9.5</td>
<td>34.9±9.0</td>
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<td>BMI, kg/m²</td>
<td>28.3±4.4</td>
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<td>SBP, mm Hg</td>
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<td>Smoking, %</td>
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Data (except for smoking) presented are mean±SD. Data for smoking are percentages of smokers. *P value is from χ² test.

BMI indicates body mass index; CIMT, carotid artery intima-media thickness; SBP, systolic blood pressure.

Table 2: Heritability of CIMT Adjusted for Different Sets of Covariates

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*P value represents the significance of heritability.
†Multivariate-adjusted CIMT: adjusted for gender, age, age2, SBP, fasting insulin, and smoking.

Longitudinal views of the far wall of the right distal common carotid artery were recorded with minimum gain necessary for clear visualization of structure. A standardized videotape was used to calibrate the grayscale of the ultrasound monitor and the same intensity setting was maintained throughout the study. Common CIMT was measured with a software program using an automated computerized edge detection algorithm (Prowin; patent pending). The distance between the blood–intima and the media–adventitia echoes was taken as the IMT measure. The measurement of CIMT was performed through the Mammalian Genotyping Services at the Marshall Medical Research foundation (Marshfield, Wis). The average distance between adjacent markers was 10 cM. To ensure the quality of genotype data, we performed the following data cleaning steps. First, family relationships were verified with all available markers by using the RELCHECK program. Second, Mendelian inconsistencies were detected and eliminated using the PEDCHECK program.

Genotyping
DNA extracted from cell lines was used for genotyping; 413 subjects with cell lines available, including 86 probands, 53 spouses, and 274 adult offspring, were genotyped at 380 microsatellites, using Marshfield screen set 12 (http://research.marshfieldclinic.org/genetics). The genotyping was performed through the Mammalian Genotyping Services at the Marshall Medical Research foundation (Marshfield, Wis). The average distance between adjacent markers was 10 cM. To ensure the quality of genotype data, we performed the following data cleaning steps. First, family relationships were verified with all available markers by using the RELCHECK program. Second, Mendelian inconsistencies were detected and eliminated using the PEDCHECK program.

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linkage is evaluated using a likelihood ratio test, which compares the likelihood under null hypothesis of no linkage ($\sigma_r^2=0$) and the alternative hypothesis ($\sigma_r^2\neq 0$). The multipoint IBD values used in QTDT were calculated using GeneHunter 2.1r4.20 The results from quantitative transmission disequilibrium test were transformed to logarithm of the odds (LOD) scores by $\chi^2/2\ln 10$, which is equivalent to the classic LOD score of linkage analysis. Linkage evidence was considered highly suggestive (LOD >1.75 and $P<0.0023$) or significant (LOD >3.0 and $P<0.0001$).21

We performed simulation to assess the possibility of false-positive results. Given the family structure and phenotype availability of our study, we simulated an unlinked quantitative trait by use of the “simqtl” function in SOLAR.15 We conducted 100 of our genome-wide scans by use of the real genotype data and the unlinked phenotype. Only 3% of

![Figure 1. Plot of CIMT linkage results from QTDT for all autosomal chromosomes. CIMT phenotypes are (1) unadjusted, (2) gender- and age-adjusted, and (3) multivariate-adjusted (adjusted for gender, age, age$^2$, SBP, fasting insulin, and smoking).](image-url)
the genome-wide maximum LOD scores were >3. This indicates that the chance of our finding being a false-positive is <3% after controlling for genome-wide multiple testing.

Results

Distribution of Variables

The descriptive statistics for CIMT and related phenotypes are listed in Table 1. Among the 274 extensively phenotyped adult offspring, 111 were males and 163 were females. CIMT was significantly greater in male than in female offspring \((P=0.0004)\). The average age of the male and female offspring was similar. Besides gender and age, for the 3 significant biological predictors of CIMT, SBP was significantly greater in male than in female offspring \((P<0.0001)\), whereas BMI and fasting insulin were not significantly different between male and female offspring. The percentage of smokers was significantly greater for male than female offspring \((P=0.0012)\).

Heritability

Results of the heritability calculation are listed in Table 2. The heritability estimate for CIMT in this sample ranged from 0.68 to 0.45, and to 0.40 for unadjusted, gender- and age-adjusted, and multivariate-adjusted \((gender, age, age^2, SBP, fasting insulin, and smoking)\) CIMT, respectively. These results suggested that there was a strong genetic effect on CIMT in our study population independent of known demographic and physiological risk factors.

A limitation of our and most such studies is caused by only a single measurement being used in the subsequent regression. For covariates such as blood pressure, insulin, and smoking, the within-person variability may cause the actual variance contribution of these variables being underestimated and therefore heritability being overestimated. We would anticipate that repeated measurements, if available, should further improve the accuracy of the heritability estimate.

Linkage Analysis

Results from the multipoint sib-pair regression and the variance component linkage approaches are given in Figure I (available online only at http://www.strokeaha.org) and Figure 1, respectively. Loci with highly suggestive \((LOD >1.75\) and \(P<0.0023)\) or significant \((LOD >3.0\) and \(P<0.0001)\) evidence for linkage are summarized in Table 3. The strongest evidence of linkage that was detected by both linkage approaches in this study was found on chromosome 2 at D2S2944. The LOD score from the variance component method at this location for unadjusted CIMT was 0.92. It increased to 3.08 after adjustment for gender and age, and decreased to 1.94 after further adjustment for other significant physiological predictors of CIMT. Using the sib-pair regression method, we obtained significant single-point and multi-point \(P\) values for gender- and age-adjusted CIMT, \(6\times10^{-6}\) and \(9\times10^{-7}\), respectively. Two other regions showed evidence of highly suggestive linkages with LOD scores >1.75 and \(P<0.0023\). One region was on chromosome 6 at D6S2439 to D6S2410. The greatest LOD scores in this region were 1.98 for unadjusted CIMT, 2.21 for gender- and age-adjusted CIMT, and 2.03 for multivariate-adjusted CIMT.

The other highly suggestive location for linkage was on chromosome 13 at D13S796 to D13S895. The LOD scores were 2.23, 1.34, and 1.62 for unadjusted, gender- and age-adjusted, and multivariate-adjusted CIMT, respectively, and the multipoint probability values from the sib-pair regression method were \(7\times10^{-5}\), \(9\times10^{-5}\), and \(2\times10^{-5}\), respectively. Detailed results from the variance component linkage approach for chromosomes 2, 6, and 13 are given in Figure 2.

Discussion

The objective of this study was to identify the genetic determinants of CIMT, a subclinical measure of atherosclerosis. Although it has been shown that CIMT is associated with CAD, is a powerful predictor of clinical cardiovascular events, and is significantly heritable, little is known about the genes influencing CIMT. Using a 10-cM density marker set, we performed a genome-wide scan of CIMT in 91 Mexican American families ascertained through a CAD proband. To maximize our chances of identifying genes predisposing CIMT, we used a family cohort from one homogeneous population that was ascertained through a disease (namely CAD) that is enriched for the atherosclerotic process that leads to increased CIMT.

Using both sib-pair regression and variance component methods, we found 3 autosomal chromosome loci yielding either significant or highly suggestive evidence of linkage
with CIMT. The locations of the linkage peaks were consistent between the 2 linkage methods and the linkages were maintained with CIMT adjusted for different sets of covariates. The consistency of the linkage results at these locations is reassuring because true linkages should be able to be detected by different approaches. The only locus that attained full criteria for significant evidence of linkage (LOD score $\geq 3.0$ and $P < 0.0001$) was on chromosome 2 at D2S2944 after CIMT was adjusted for gender and age. The LOD score from the variance component method was 3.08 at this locus. The single and multipoint $P$ values from the sib-pair regression were $6 \times 10^{-6}$ and $9 \times 10^{-7}$, respectively. The 2 markers adjacent to D2S2944 also showed highly suggestive evidence of linkage (Table 3). These markers are located in the 2q33–q35 region. A search of available databases revealed several genes in this region, such as nitric oxide synthase trafficker (NOSTRIN; 2q31.1), insulin-like growth factor-binding protein-2 (IGFBP2; 2q33–q34), and insulin-like growth factor-binding protein 5 (IGFBP5; 2q33–q36) (Figure 2A). To our knowledge, none of the candidate genes in this region has been tested for association with CIMT. It is also possible that the genes underlying this locus that influences CIMT have not been identified.

In addition to the chromosome 2 region, 2 other regions showed evidence of highly suggestive linkage by both linkage approaches used in this study. One of them was the 6p12–p22 region on chromosome 6, flanked by markers D6S2439 and D6S1051. The shape of the plot suggested the possibility that there may be multiple genes underlying this region affecting CIMT. The 6p21 region contains many important genes related to inflammation, such as human leukocyte antigens and tumor necrosis factor. Endothelial and inflammatory mechanisms are now believed to play a central role in the origins and complications of atherosclerosis. Vascular endothelial growth factor located at 6p12 may be an interesting candidate gene. In vivo, it has been observed that lower vitreous levels of pigment epithelium-derived factor and higher levels of vascular endothelial growth factor may be related to the angiogenesis in proliferative diabetic retinopathy (Figure 2B). The second region was 13q32–q33 on chromosome 13, at locus D13S796. Candidate genes underlying this region include endothelin receptor type B (EDNRB; 13q22), intimal thickness-related receptor (ITR; 13q31–q32), ATP-binding cassette subfamily C member 4 (ABCC4; 13q32), and insulin receptor substrate 2 (IRS2; 13q34) (Figure 2C). It has been shown that the product of the intimal thickness-related receptor may play an important role in the regulation of vascular remodeling.

Our findings are different from the recent findings of the Framingham Heart Study. They reported evidence for significant linkage to internal carotid artery IMT (2-point LOD score 4.1; multipoint LOD score 3.4) on chromosome 12. However, no LOD scores $\geq 2.0$ were observed for common carotid artery IMT. Several differences between the Framingham Heart Study and our study could contribute to the difference in findings between 2 studies. First, there are major differences in the IMT measurement methodologies used in the Framingham study and ours, which can be an important determinant of the difference in the findings. While we used an average common carotid artery IMT (over $\approx 80$ to 100 points) over 1 cm (a true average IMT) on the far wall of the right distal common carotid artery, the Framingham study used a mean maximum “average” in which maximum single points in the near and far walls of the right and left common (or internal) carotid artery were averaged. Second, the 2 study cohorts are also completely different, except that both are family cohorts. The Framingham Heart Study cohort is from a general population and the predominant majority of subjects in Framingham study are whites. Our study cohort was ascertained from a homogeneous Mexican American population via a disease (namely CAD) that is enriched for the atherosclerotic process that leads to increased CIMT. Differ-
ent genetic characteristics, interacting with different social and environmental factors, may result in different kinds of mechanisms leading to the same phenotype in these 2 populations.

In conclusion, our results suggest 3 loci, on chromosomes 2, 6, and 13, respectively, influencing common carotid artery IMT. Among them, the evidence for linkage on chromosome 2 is most significant. There are several potential candidate genes underlying these linkage peaks. Further fine-mapping and candidate gene association studies will be required to further narrow the linkage region and to identify genes influencing CIMT.

Acknowledgments
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