

# Genomewide Linkage Analysis for Internal Carotid Artery Intimal Medial Thickness: Evidence for Linkage to Chromosome 12

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Carotid intimal medial thickness (IMT) is a heritable quantitative measure of atherosclerosis. A genomewide linkage analysis was conducted to localize a quantitative-trait locus (QTL) influencing carotid IMT. Carotid IMT was measured in 596 men and 629 women from 311 extended families (1,242 sib pairs) in the Framingham Heart Study Offspring cohort. B-mode carotid ultrasonography was used to define mean IMT of the carotid artery segments. Multipoint variance-component linkage analysis was performed. Evidence for significant linkage to internal carotid artery (ICA) IMT (two-point log odds [LOD] score 4.1, multipoint LOD score 3.4) was found 161 cM from the tip of the short arm of chromosome 12; these results were confirmed using the GENEHUNTER package (multipoint LOD score 4.3). No LOD scores >2.0 were observed for common carotid artery (CCA) IMT. Association analysis of a single-nucleotide-polymorphism variant of *SCARB1* (minor allele frequency 0.13), a gene in close proximity to the region of peak linkage, revealed a protective association of the missense variant allele in exon 1 of *SCARB1*, with decreased ICA IMT compared with subjects homozygous for the common allele. Although the exon 1 variant contributed 2% to overall variation in ICA IMT, there was no significant change in the peak LOD score after adjustment in the linkage analyses. These data provide substantial evidence for a QTL on chromosome 12 influencing ICA IMT and for association of a rare variant of *SCARB1*, or a nearby locus, with ICA IMT. Because this rare *SCARB1* variant does not account for our observed linkage, further investigations are warranted to identify additional candidate-gene variants on chromosome 12 predisposing to atherosclerosis phenotypes and clinical vascular disease.

## Introduction

Atherosclerotic cardiovascular disease (CVD) is the leading cause of morbidity and mortality in the United States (American Heart Association 2002). Both genetic and environmental factors have been implicated in the pathogenesis of atherosclerosis (Luis 2000). Carotid artery intimal medial thickness (IMT) is a measure of atherosclerosis that is independently associated with traditional CVD risk factors (Crouse et al. 1996; Espeland et al. 1999) and coronary atherosclerotic burden (O'Leary et al. 1996; Davis et al. 1999) and that is predictive of subsequent myocardial infarction and stroke (Bots et al. 1997; O'Leary et al. 1999). Carotid IMT is associated with a family history of cardiovascular disease, and we

have shown that 35%–45% of the variability in multivariable-adjusted carotid IMT is explained by genetic factors (Fox et al. 2003). However, few large population-based analyses of genomewide linkage exist for vascular phenotypes, and there are few significant genomewide linkage data available in the general population for myocardial infarction or the underlying condition of atherosclerosis.

Thus, we conducted a genomewide linkage analysis in a population-based cohort of families, to identify chromosomal regions linked to interindividual variation in carotid IMT. We followed up with association analyses of polymorphisms in a plausible candidate gene in the region of peak linkage.

## Material and Methods

### Study Population

In the current investigation, subjects from the Framingham Heart Study Offspring cohort underwent B-mode carotid ultrasonography during examination cycle

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6 (1996–1998). Carotid IMT was measured in 596 men and 629 women, included in 311 extended families and 1,242 sib pairs.

The Framingham Heart Study began in 1948 with the enrollment of 5,209 men and women, 28–62 years of age at study entry, with subjects undergoing repeat exams every 2 years (Dawber et al. 1951, 1963). In 1971, 5,124 men and women were enrolled in the offspring cohort of the Framingham Heart Study, 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 of the offspring cohort has been described elsewhere (Feinleib et al. 1975; Kannel et al. 1979).

For the linkage analysis, 3,532 subjects attended cycle 6 of the total 5,124 subjects that attended the initial offspring exam. Of these, 152 did not undergo carotid ultrasound, 2,137 were excluded because they were not a biologic member of a family that was genotyped in the marker set for the linkage analysis, a further 17 were excluded because they were not part of a biologic family whose members had carotid ultrasound data, and 1 was excluded because of inadequate carotid IMT data. Thus, a total of 1,225 subjects in 311 families comprised the study sample. Of these, all had an available common carotid artery (CCA) measurement, and 1,113 had an available internal carotid artery (ICA) measurement.

For the genotyping of the gene encoding scavenger receptor, class B, type I (*SCARB1* [MIM 601040]), previously known as *SR-BI*, in the association portion of this study, DNA samples from 2,342 individuals who attended cycle 6 were used. Of these, 96 subjects were excluded because they did not undergo carotid ultrasound, and 1 was excluded because of inadequate carotid IMT data. In all, 2,245 subjects had at least one carotid IMT measure and were typed for at least one of the *SCARB1* polymorphisms; therefore, they were included in these analyses. A total of 736 subjects appear in both the linkage and association analyses.

#### *Assessment of Risk Factors and Cardiovascular Disease*

Details regarding the methods of risk factor measurement and laboratory analysis have been described (Cupples and D'Agostino 1987). Each examination included an extensive cardiovascular disease assessment, 12-lead electrocardiogram (ECG), and blood testing. Measured covariates for the current study were assessed at the time of carotid ultrasonography.

#### *Carotid Intimal Medial Thickness Assessment*

Subjects underwent ultrasonography according to a modification of a standard protocol (O'Leary et al. 1999). Imaging was conducted by a single trained sonographer, using a Toshiba SSH-140A imaging unit and

7.0-MHz transducer for the CCA and a 5.0-MHz transducer for the ICA.

The following views were obtained from the right and left sides: two longitudinal views of the distal common carotid, one at end diastole and one at end systole, and two longitudinal views of the ICA at end diastole. Measurements were made by a single trained sonographer blinded to all clinical information, and they were over-read by one of the investigators (J.F.P.).

All studies were read according to a standardized protocol. The high-resolution images of the ICA and CCA 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 (O'Leary et al. 1996, 1999). On the basis of 25 replicate readings by two separate readers, intraclass correlation coefficients for the mean and maximum ICA and CCA IMT were 0.74, 0.74, 0.86, and 0.90, respectively.

A summary measure of the mean wall thickness of the ICA was defined as the mean of the wall thickness for the near and far wall on both the left and right sides. Similar measurements were obtained for the CCA.

#### *Genotyping*

Leukocyte DNA was extracted from 5–10 mL of whole-blood or buffy-coat specimens by use of a standard protocol (Gross-Bellard et al. 1973; Miller et al. 1988). Aliquots of DNA from members of the largest Framingham Heart Study families were sent in four batches to the Mammalian Genotyping Service Laboratory at the Marshfield Clinic (Marshfield, WI). A 10-cM density genomewide scan was performed (marker set 8A, heterozygosity 0.77). Genotype-data cleaning, including verification of family relationships and Mendelian inconsistencies, has been described elsewhere (Levy et al. 2000). In brief, familial relationships were checked using the SIBKIN program in ASPEX, which uses maximum-likelihood estimation to test whether the genotyping data are consistent with the specified relationships, and adjustments were made accordingly. In addition, random genotyping errors were checked using Gentest, a precursor to the INFER procedure, in PEDSYS. When we discovered a genotyping error and could not attribute it to a specific person in a nuclear family, all genotypes for that nuclear family were assigned as missing.

Known mutations in *SCARB1* (Acton et al. 1999; Osgood-McWeeney et al. 2000) were identified by direct sequencing of PCR products. Three polymorphisms were typed: one in exon 1, one in exon 8, and one in intron 5. *SCARB1* genotyping was carried out by allelic discrimination by use of the 5' nuclease assay with fluorogenic probes on a 7700 Sequence Detection System

(PE Applied Biosystems), as described elsewhere (Osgood-McWeeney et al. 2000).

### Linkage Analysis

Our statistical analysis focused on mean IMT measures of the ICA and CCA. The heritabilities for CCA and ICA IMT have been published elsewhere (Fox et al. 2003). Before multivariable adjustment, the heritabilities were 0.67 for CCA IMT and 0.43 for ICA IMT. After multivariable adjustment, the heritabilities were 0.43 and 0.35, respectively.

Because the distributions of carotid measures were skewed, normalized deviates were used. Multivariable-adjusted normalized deviates were calculated using the SAS/STAT user's guide, version 8 (SAS Institute Inc. 2000); all linkage data presented is multivariable adjusted. For these adjustments, we used multiple linear regression separately for men and women, to obtain standardized residuals. These standardized residuals were then ranked, and normalized deviates were calculated. Covariates in the multivariable models included age, systolic blood pressure, number of cigarettes per day, total and HDL cholesterol, log-triglycerides, diabetes status (yes/no), body mass index, antihypertensive treatment (yes/no), menopausal status (yes/no), and hormone-replacement therapy in women (yes/no). All covariates were continuous except where otherwise specified.

For the linkage analysis, there were a total of 311 extended families, with 2–24 individuals each. There were 1,242 sib pairs (sibships range from two to seven individuals), 100 avuncular pairs, 39 half siblings, and 716 first cousins. Subjects were contained in sibships in the following distribution: 47% had two members, 32% had three members, 14% had four members, and 7% had five or more members.

Two-point and multipoint quantitative-trait linkage analyses were conducted with the normalized deviates for the carotid IMT measures by use of the SOLAR package (Almasy and Blangero 1998). This approach uses information on available marker data and the family structure to impute genotypes for untyped individuals. Estimates of the proportion of alleles with shared identity by descent (IBD) are then obtained for all relative pairs. By imputing genotypes for untyped individuals, SOLAR makes use of all individuals with carotid IMT measures in the linkage analysis. To assess linkage, a fitted model with no genetic marker information (polygenic model) is used and compared with a fitted model that incorporates genetic marker information at a specific marker (two-point analysis) or across a chromosome (multipoint analysis). To obtain the log odds (LOD) score, the log (base 10) of the ratio of the likelihoods of the marker-specific and polygenic models is computed. We have verified our results by use of GENE-

HUNTER (Kruglyak et al. 1996), an alternative statistical linkage program, conducted to address the suggestion by some critics of SOLAR that the multipoint IBD estimates calculated by GENEHUNTER are superior to those calculated by SOLAR (Ekstrom 2001).

We used SOLAR to estimate power and type 1 error. We used the function "simqtl" in SOLAR (Almasy and Blangero 1998) to simulate both a linked and an unlinked quantitative trait for power and type 1 error analysis, respectively. Given the family structure and the approximate number of individuals with trait values used in these analyses, we have 63%–95% power to detect a QTL that accounts for 25%–35% of the variation in the carotid IMT traits if we use a LOD score  $>2$  as our critical value, and we have 37%–84% power for a LOD score  $>3$ . In addition to power, we conducted 100 simulations of our genomewide scan by use of unlinked phenotypes, to assess the risk of false-positive results. The genomewide chance is only 4% of a LOD score in our data being  $>3$  when there is no genomewide linkage. This indicates that the chance of our results being a false positive is  $<4\%$ , controlling for genomewide multiple testing.

### Association Analysis

To evaluate the association between the *SCARB1* gene and carotid IMT, a total of 2,245 individuals were included in the analysis. These individuals had at least one carotid IMT measure and were genotyped for at least one of the three *SCARB1* polymorphisms considered. The majority of individuals (1,435) included in these analyses were members of families, with 2–14 individuals each. Association analyses for polymorphisms in the *SCARB1* gene were analyzed using the SAS procedure PROC MIXED (SAS Institute Inc. 2000). This methodology uses a maximum-likelihood procedure, assuming a normal distribution of the phenotypes, to estimate the regression coefficients in a mixed-effect model that incorporates fixed effects for known covariates and specific genotypes and random effects to account for the correlation among members in the same families. Normalized deviates of crude IMT measures were used in these analyses to meet the normality assumption.

To evaluate individual effects of the *SCARB1* polymorphisms on carotid IMT, we created two dichotomous variables. The significance of covariates and genotype effects was assessed by comparing a fitted model in which fixed effects for known covariates, genotypes, and random effects were included, with a model in which the two dummy variable-regression coefficients were set to zero. We calculated crude and multivariable-adjusted models, including the following covariates: age, sex, systolic blood pressure, number of cigarettes per day, total and HDL cholesterol, log-triglycerides, diabetes status

(yes/no), body mass index, antihypertensive treatment (yes/no), menopausal status (yes/no), and hormone-replacement therapy in women (yes/no). To parallel the linkage analysis, the association analysis was repeated using sex-specific multivariable-adjusted residuals.

## Results

### Study Sample

The characteristics of the study samples used in the linkage and association analyses are shown in table 1. The mean age was 56 years in the linkage sample and 59 years in the association sample. Mean cholesterol, triglycerides, and HDL cholesterol were similar in both groups; the prevalence of hypertension and hypertension treatment was higher in the association sample.

Mean CCA and ICA IMT values are presented in table 1. The CCA values ranged from 0.33–3.25 mm; the ICA values ranged from 0.20–3.94 mm. The correlation between ICA and CCA IMT is 0.48 ( $P < .001$ ).

### Multivariable-Adjusted Two-Point and Multipoint Linkage

For the ICA, we observed a maximum two-point LOD score of 4.1 at 161 cM on chromosome 12, with a small secondary peak on chromosome 1 (LOD score 2.4 at 76 cM) and a maximum multipoint LOD score of 3.4 at 160 cM on chromosome 12 (fig. 1). The marker at the peak LOD score on chromosome 12 was D12S1045. The high-

est multipoint LOD scores for ICA and CCA IMT for each of the 22 autosomes are presented in figure 2. For CCA IMT, we observed a peak multipoint LOD score of 1.99 on chromosome 2 at 105 cM. The marker nearest to the peak LOD score on chromosome 2 was D2S1790. Using GENEHUNTER, we confirmed our results on chromosome 12, with a multipoint LOD score of 4.3 in the same region identified by SOLAR. Table 2 presents all LOD scores  $>1.0$  for ICA and CCA IMT. The two-point and multipoint ICA IMT linkage results for all chromosomes appear as an online-only supplement.

### Association Analysis of Common Variants in SCARB1

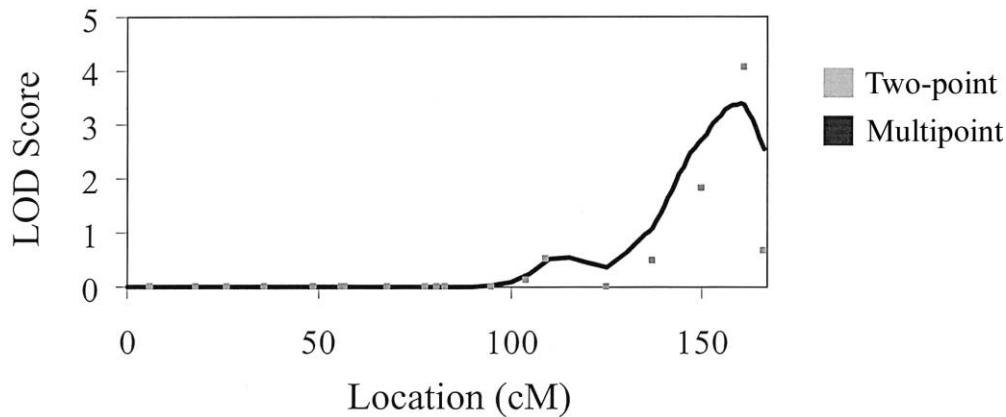
SCARB1 is an atherosclerosis candidate gene that resides at 124.9 Mb, ~5 million base pairs away from the microsatellite marker D12S1045. Allele frequencies for the rare alleles in exons 1 and 8 and intron 5 were 0.13, 0.49, and 0.09, respectively. All polymorphisms were in Hardy-Weinberg equilibrium. In crude analyses, the exon 1 variant was significantly associated with ICA IMT ( $P < .007$ ); crude (i.e., prior to the calculation of normalized deviates) ICA IMT values, arranged by exon 1 variant genotype, are presented in figure 3. In multivariable-adjusted analyses, subjects who are homozygous for the rare allele had a mean ICA IMT that was 0.5 normalized SDs lower than the rest of the population ( $P < .02$  for differences among all three groups). The use of sex-specific multivariable-adjusted residuals resulted in similar differences in the magnitude of mean ICA IMT,

**Table 1**  
Baseline Characteristics of Study Subjects

CHARACTERISTIC	FINDING IN SAMPLE	
	Linkage ( $n = 1,225$ )	Association ( $n = 2,245$ )
Age (years) <sup>a</sup>	56 ± 10	59 ± 10
Sex (% male)	49	48
Systolic blood pressure (mm Hg) <sup>a</sup>	125 ± 18	129 ± 19
Total cholesterol (mg/dL) <sup>a</sup>	206 ± 43	205 ± 40
Triglycerides (mg/dL) <sup>a</sup>	144 ± 178	142 ± 151
High-density lipoprotein cholesterol (mg/dL) <sup>a</sup>	51 ± 15	51 ± 16
Body mass index (kg/m <sup>2</sup> ) <sup>a</sup>	28.2 ± 5.5	28.0 ± 5.1
Current smoking (%)	17	15
Postmenopause (%)	70	79
Hormone replacement therapy (%)	22	28
Prevalent hypertension (%)	35	43
Hypertension treatment (%)	24	30
Diabetes (%)	5	6
Cardiovascular disease (%)	9	11
Carotid artery IMT (mm) <sup>a</sup> :		
Common	.59 ± .15	.61 ± .16
Internal	.56 ± .36	.59 ± .39

NOTE.—To convert total and high-density lipoprotein from mg/dL to mmol/L, multiply by .0259. To convert triglycerides from mg/dL to mmol/L, multiply by .0113.

<sup>a</sup> Mean ± SD.



**Figure 1** Two-point and multipoint LOD scores for ICA IMT, chromosome 12

as compared with the rest of the population, but the  $P$  value was attenuated ( $P = .12$ ). Including the exon 1 variant as a covariate in the variance-component analysis in SOLAR revealed that it accounted for 2% of the overall variability in ICA IMT, whereas the additional covariates accounted for 36% of the total variation in CCA IMT, and 21% of the total variation for ICA IMT. In further multivariable analyses with ICA IMT, there were no significant associations with variants in either exon 8 or intron 5.

In a subanalysis conducted on subjects included in the genomewide linkage analysis and typed for *SCARB1* ( $n = 736$ ), multipoint LOD scores were calculated, after adjustment for the exon 1 genotype of *SCARB1*. The multivariable-adjusted LOD score was 2.33 prior to including the *SCARB1* exon variant and 2.27 after including the *SCARB1* exon 1 variant. The lower peak LOD score is due to the loss of sample size incurred when limiting the analysis to those subjects included in both the linkage and association samples.

## Discussion

We found evidence for genomewide linkage to ICA IMT 161 cM from the tip of the short arm of chromosome 12, with a maximum two-point LOD score of 4.1 and multipoint LOD score of 3.4. To our knowledge, this is the first population-based study that has provided strong evidence for linkage of a QTL to a measure of subclinical atherosclerosis on a genomewide scale. We followed up our finding of genomewide linkage with an association study of variants in a highly plausible candidate gene, *SCARB1*, in the region of linkage. Evidence for association of the exon 1 variant of *SCARB1* with ICA IMT was found. However, although this variant is associated with ICA IMT, the homozygotes are quite rare, and it does not account for the linkage observed, suggesting

that other variants in this gene or in other genes in the region are responsible for the linkage with carotid IMT.

To date, the available evidence from genomewide linkage analyses of atherosclerosis phenotypes derives from selected populations. A recent report in families with two or more affected relatives identified a region of linkage to premature myocardial infarction on chromosome 14 (LOD score 3.9) (Broeckel et al. 2002). An additional genomewide linkage analysis performed in a genetically isolated population in Finland that was enriched for subjects with premature coronary artery disease demonstrated evidence for linkage on chromosome 2 (LOD score 3.0), ~45 million base pairs from our peak LOD score for CCA IMT (Pajukanta et al. 2000). Linkage to chromosome 16 (LOD score 3.1) was found in another population with premature coronary artery disease in Mauritius (Francke et al. 2001). A recent study performed in 29 families enriched for hypertension showed evidence for linkage to coronary artery calcification, assessed by electron-beam computed tomography (LOD score 3.2 on chromosome 10) (Lange et al. 2002). Although these studies provide suggestive results, each was drawn from a sample selected for probands with a high burden of cardiac risk factors or premature atherosclerosis, or from a genetically isolated population. Whereas the findings of these prior studies may represent valid evidence for linkage of genetic loci to subclinical and clinical vascular disease phenotypes in rare families, the selected nature of the cohorts in these studies may limit the generalizability of the results.

It is interesting that QTLs for ICA IMT and CCA IMT map to different chromosomes and that our linkage peak for ICA IMT is stronger than that for CCA IMT. It has been suggested that thickening of the CCA intima may be a better marker for total body atherosclerosis, whereas thickening of the ICA intima might represent focal atherosclerotic plaques (O'Leary et al.

1996). Indeed, thickening of the ICA has been shown to be more strongly associated with incident CVD, compared with the CCA (O'Leary et al. 1996). Differential blood flow in regions of arterial bifurcation, such as the ICA, may increase thrombosis, lipid deposition, and atherosclerosis (Grabowski and Lam 1995), whereas laminar blood flow, as seen in the CCA, is inversely related to wall shear stress (Gnasso et al. 1996). Low wall shear stress, via an increase in the duration of time that blood comes into contact with the endothelial wall, may enhance the delivery of atherogenic particles (Gnasso et al. 1996). Thus, different pathophysiologic mechanisms may underlie observed differences in ICA IMT and CCA IMT, suggesting the role of unique sets of genes in their variation.

The region on chromosome 12 near our peak LOD score for ICA IMT contains a number of atherosclerosis candidate genes located very close to the region of linkage. Among them, *SCARB1* is implicated in the atherogenic process by a considerable body of prior research. The identification of *SCARB1* (Acton et al. 1996) represented a significant advance in understanding the manner in which high-density lipoproteins (HDL) are believed to exert their anti-atherogenic role in cholesterol metabolism through the process of delivering cholesterol from peripheral tissues back to the liver for removal from the body, called "reverse cholesterol transport" (Stein and Stein 1999; Silver et al. 2000). *SCARB1*, a cell-surface glycoprotein expressed in the liver, adrenal glands, and ovaries, was the first HDL receptor to be well defined and characterized in vitro and in animal studies. In murine atherosclerosis models, the absence of *SCARB1* dramatically acceler-

**Table 2**

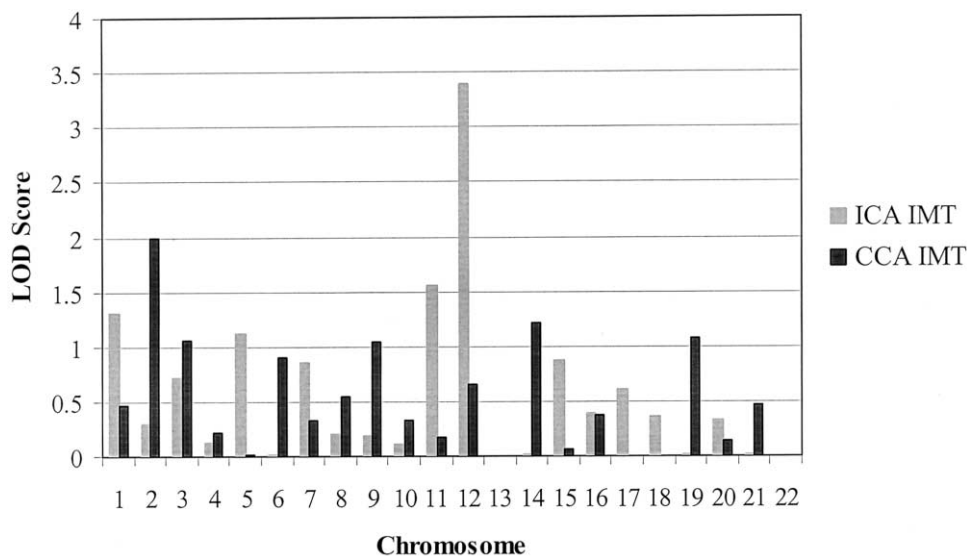
**All Markers and Locations with Two-Point LOD Scores >1.0 for ICA and CCA IMT**

Type and Marker	Chromosome	Location (cM)	LOD Score
ICA:			
D1S2134	1	76	2.37
D1S1653	1	164	1.22
D1S1678	1	218	1.86
D5S817	5	23	1.06
D5S1725	5	98	1.12
D5S1462	5	105	1.14
D7S817	7	50	1.58
D11S199	11	17	1.28
D12S2078	12	150	1.82
D12S1045	12	161	4.06
CCA:			
D1S3721	1	74	1.08
D2S1790	2	103	1.57
D2S2972	2	114	1.03
D3S1768	3	62	1.16
D3S2459	3	119	1.44
D9S319	9	54	1.53
D12S1301	12	56	1.70
D12S1294	12	78	1.18
D14S606	14	92	1.75
D19S586	19	33	1.47

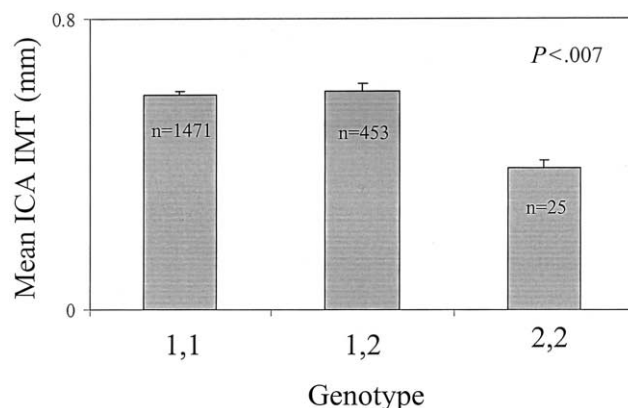
NOTE.—Genetic distance provided by Marshfield map location.

ates the onset of atherosclerosis (Braun et al. 2002), whereas atherosclerosis is suppressed by hepatic over-expression of this gene (Arai et al. 1999; Kozarsky et al. 2000).

The human *SCARB1* gene was characterized and in-



**Figure 2** Peak multipoint LOD scores for ICA and CCA IMT by chromosome



**Figure 3** Mean (SE) ICA IMT by genotype of *SCARB1*; data presented is unadjusted

investigated in a southern European population by Acton et al. (1999). Three common SNP variants were described (with allele frequency  $>0.1$ ) at exon 1 (G→A), exon 8 (C→T), and intron 5 (C→T). The polymorphisms at intron 5 and exon 8 are in linkage disequilibrium with each other and are both probably nonfunctional, whereas the SNP in exon 1 is not in linkage disequilibrium with either of the other two SNPs and encodes an amino acid change from glycine to serine that modifies one of the potential acylation sites in the *SCARB1* molecule. In this study by Acton et al. (1999), associations were found between *SCARB1* and both HDL and LDL (low-density lipoproteins), suggesting that *SCARB1* may play a role in the metabolism of both lipoprotein classes in humans. In addition, associations were detected with triglycerides and body mass index (Acton et al. 1999). In a study of the Framingham Heart Study Offspring cohort, it has been shown that statistically significant interactions exist between the *SCARB1* exon 1 variant and type 2 diabetes. Diabetic subjects with the less-common allele have lower lipid concentrations, including lower LDL cholesterol and HDL cholesterol (Osgood et al. 2003). Taken together, these data allow us to hypothesize that the *SCARB1* gene may also be involved in several features of the metabolic syndrome.

Although our findings support a role for *SCARB1* as an atherosclerosis candidate gene, we acknowledge the limitation that we have not demonstrated that associations of *SCARB1* variants with carotid IMT explain the linkage of chromosome 12 to carotid IMT. Our findings illustrate the challenges inherent in attempts to implicate a specific gene and mutation in the etiology of complex diseases by utilizing the results of genome-wide linkage analyses (Altshuler et al. 2000). In this regard, we note that several other candidate genes of interest reside near the region of linkage. For example, the neuronal *nitric oxide synthase 1* (*NOS1* [MIM

163731]) gene lies near the region of peak linkage that was found in the current study, and upregulation of juxtaglomerular *NOS1* has been shown to precede glomerulosclerosis in fawn-hooded hypertensive rats (Weichert et al. 2001). In human studies of asthma, polymorphisms in the *NOS1* gene were associated with eosinophil cell counts and IgE levels (Immervoll et al. 2001). Thus, *NOS1* may play a role in inflammation and human atherosclerosis. Mutations in the *TCF1* (MIM 142410) gene, mapped to chromosome 12q24, have been shown to cause biliary-acid-transport defects, with impaired liver-cholesterol synthesis and HDL metabolism (Shih et al. 2001). Maturity-onset diabetes of the young (MODY3 [MIM 600496]) is a rare, dominantly inherited form of early-onset diabetes that is caused by mutations in the *hepatocyte nuclear factor-1 alpha* gene (*HNF-1 alpha*, encoded by the *TCF1* gene) (Yamagata et al. 1996). The *ATP-binding cassette, subfamily B, member 9* (*ABCB9*) (Zhang et al. 2000) [MIM 605453], a member of the ABC family of genes, also resides near the region of peak linkage that was found in the current study. Several genes in the ABC gene family have been shown to impact lipid metabolism (Borst and Elferink 2002). The *insulin-like growth factor 1* gene (*IGF1* [MIM 147440]) also resides near the peak linkage region and has been shown to be associated with type 2 diabetes and glucose tolerance (Frayling et al. 2002).

Some additional limitations of our study deserve mention. First, because the phenotype information for the study was not collected until 1996, we may have incurred a survival bias, since subjects with a greater genetic predisposition inducing more severe disease may have died prematurely or been lost to follow-up. However, this loss would likely attenuate our results. Second, most of the subjects in our study were white, potentially limiting the generalizability of the findings to other eth-

nicities. Last, the sample size and the marker spacing of 10 cM in the genomewide linkage analysis may have limited the power of our study to localize a disease-influencing QTL that is in linkage with carotid IMT. However, we do not believe the low power affects the validity of our primary findings, since we obtained a LOD score of 3.4, which is typically regarded as of sufficient magnitude to be of nearly genomewide significance. Regardless, there are likely to be QTLs that were not detected in our study but that contribute significantly to variability in carotid IMT, and there may be additional genes in other regions of the genome that we have missed, given the low power of our study.

These data suggest substantial evidence for a QTL on chromosome 12 that influences the IMT of the ICA. *SCARB1* represents an attractive candidate gene, and a common missense variant in exon 1 of *SCARB1* or a nearby locus is associated with lower IMT. However, we have not demonstrated that allelic variants in this candidate gene explain our observed linkage. Although our findings suggest that further investigation is warranted to determine the role of *SCARB1* in atherosclerosis, additional variants on chromosome 12, including those in nearby candidate genes, should be examined for evidence of association to atherosclerosis traits. These findings support the continued use of genomewide approaches to discover genetic variants predisposing to quantitative measures of subclinical atherosclerosis.

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## Electronic-Database Information

The URL for data presented herein is as follows:

Online Mendelian Inheritance in Man (OMIM), <http://www.ncbi.nlm.nih.gov/Omim/> (for *SCARB1*, *NOS1*, *TCF1*, *MODY3*, *ABCB9*, and *IGF1*)

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## Erratum

Because of a clerical error, incorrect “received” dates were printed for manuscripts published in the December 2003 and February 2004 issues of the *Journal*. The corrected dates are as follows:

The article entitled “*MDR1* Ala893 Polymorphism Is Associated with Inflammatory Bowel Disease,” by Brant et al. (73:1282–1292), was received May 30, 2003.

The article entitled “Genomewide Linkage Analysis for Internal Carotid Artery Intimal Medial Thickness: Evidence for Linkage to Chromosome 12,” by Fox et al. (74:253–261), was received April 16, 2003.

The article entitled “Premature Myocardial Infarc-

tion Novel Susceptibility Locus on Chromosome 1P34-36 Identified by Genomewide Linkage Analysis,” by Wang et al. (74:262–271), was received July 31, 2003.

The article entitled “Evidence of a Novel Quantitative-Trait Locus for Obesity on Chromosome 4p in Mexican Americans,” by Arya et al. (74:272–282), was received September 12, 2003.

The report entitled “Mutations in the *VLGR1* Gene Implicate G-Protein Signaling in the Pathogenesis of Usher Syndrome Type II,” by Weston et al. (74:357–366), was received October 16, 2003.

The *Journal* regrets these errors.