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Genomewide Linkage and Peakwide Association Analyses of Carotid Plaque in Caribbean Hispanics

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Abstract

Background and Purpose—Atherosclerosis is a complex subclinical cardiovascular disorder with a substantial genetic component. This study sought to identify genetic loci influencing carotid plaque in 2 independent samples.

Methods—B-mode ultrasound was performed to determine the presence and area of carotid plaque. Variance components analysis was used to test for linkage using 383 autosomal microsatellite markers in 1308 subjects from 100 Dominican families. Multiple linear and logistic regression models were used to investigate the association between plaque traits and 18 904 single nucleotide polymorphisms under the 1-logarithm of odds unit down regions of linkage peaks in an independent community-based data set (N=941, 41% Dominicans) from the Northern Manhattan Study.

Results—After adjustment for age, hypertension, diabetes mellitus, cigarette pack-years, body mass index, and waist-to-hip ratio, significant heritability was detected for plaque presence (h^2=0.50±0.14, P<0.0001) and plaque area (h^2=0.17±0.04, P<0.0001). Quantitative and dichotomous trait linkage analyses obtained similar results and identified 4 regions with multipoint logarithm of odds scores ≥2.00 on 7q36, 11p15, 14q32, and 15q23. In the association analysis of the 4 linkage peaks, several single nucleotide polymorphisms in or near *SOX6*, *FSD2*, *AP3S2*, *EFTUD1*, and *MYOD1* were associated with carotid plaque traits with a nominal P≤0.0005 in the Northern Manhattan Study data set and with a P≤0.01 in Northern Manhattan Study Dominican subset.

Conclusions—Carotid plaque has considerable heritability and may be influenced by loci on chromosomes 11p15, 14q32, and 15q23. The *SOX6* gene within the bone morphogenic protein pathway could be a candidate for carotid plaque. Larger independent studies are needed to validate these findings.

Keywords

association; Caribbean Hispanics; carotid plaque; heritability; linkage

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Disclosures
None.

Atherosclerosis, a complex subclinical cardiovascular disorder, is the pathology underlying most ischemic strokes and heart attacks, currently the leading causes of disability and death in the United States and, soon, worldwide.[1–3] Dissecting the genetic underpinnings of atherosclerosis, therefore, is of great value in assessing an individual's future risk for stroke and cardiovascular disease. Although carotid plaque and intima-media thickness (IMT) are well-documented and widely used subclinical surrogate markers of vascular events, they may reflect different biological aspects of atherogenesis having distinctive relationships with clinical vascular end points and likely have distinct genetic determinants.[4,5] Thus, evaluation of these individual subclinical phenotypes may reduce heterogeneity and facilitate discovery of the genetic variants that influence susceptibility to atherosclerosis.

Several lines of evidence suggest that atherosclerotic disease is hereditary. First, population studies have shown that a positive family history of cardiovascular disease confers an independent risk.[6,7] Second, family-based studies have demonstrated that a substantial portion of the phenotypic variance of subclinical markers is genetic in origin with the heritabilities ranging from 30% to 60% for IMT[8,9] and from 23% to 28% for plaque.[10] Lastly, twin studies have also indicated that genetic factors have a substantial influence on the risk of stroke and variation of IMT.[11–15] To date, the vast majority of genetic studies on subclinical atherosclerosis have investigated carotid IMT, whereas studies on the genetic basis of atherosclerotic plaque have been lacking.

The primary goal of the Family Study of Stroke Risk and Carotid Atherosclerosis is to identify the genetic determinants of the specific precursors to stroke in multigeneration Dominican families. Previously we reported quantitative trait loci for IMT on chromosomes 7p and 14q.[9] In the present study, we extend these findings for carotid plaque phenotypes[16] followed by a validation study using a peakwide association in an independent prospective community-based subcohort from the Northern Manhattan Study (NOMAS).[17]

Subjects and Methods

Subjects

Two independent data sets were included in the present study. The first consisted of 1308 participants with carotid plaque measures from 100 Dominican families for linkage analysis and the second included a stroke-free subcohort of 941 individuals from NOMAS for association analysis (Table 1). Informed consent was obtained from all participants and the study was approved by the Institutional Review Boards of Columbia University, the University of Miami, the National Bioethics Committee, and the Independent Ethics Committee of Instituto Oncologico Regional del Cibao in the Dominican Republic.

The research design and detailed ascertainment scheme for the family study and NOMAS were described previously.[9,16,17] For the family study, probands were selected from the Caribbean Hispanic participants in NOMAS using the following criteria: (1) reporting a sibling with a history of myocardial infarction or stroke; or (2) having 2 of 3 quantitative risk phenotypes (maximal carotid plaque thickness, left ventricular mass, or homocystine level above the 75th percentiles in the NOMAS cohort). For NOMAS, a total of 3497 community subjects were enrolled between 1993 and 2008. In the association analysis, however, NOMAS subjects were excluded for the following reasons: no genotype data for those who were not enrolled for MRI examination (n=2207), no or low DNA (n=153), failure in passing genotyping or postgenotyping sample quality control (n=87), and no carotid plaque assessment or missing values in covariates (n=57). To ensure independence of samples, 52 probands who overlapped between the family sample and NOMAS data set were also excluded in the association analysis. This yielded a sample size of 941 subjects in the final peakwide association analysis.

Carotid Plaque Phenotypes

High-resolution B-mode 2-dimensional carotid ultrasound imaging was performed according to a standardized scanning and reading protocol by a certified sonologist as previously described.[18] Both internal and common carotid arteries as well as the bifurcations were examined for atherosclerotic plaque. Presence of plaque was defined as an area of focal wall thickening >50% greater than surrounding wall thickness in millimeters. If plaques were identified in any of carotid segments, in-depth imaging of plaques was performed in long axes and multiple angles. The optimized and normalized images were stored and analyzed offline by M'Ath (Intelligence in Medical Technologies, Inc, Paris, France). Plaque boundaries were traced using automated computerized edge detection algorithm and each plaque area was measured using the automated system in M'Ath. The sum of all plaque areas within the carotid segments (mm^2) was calculated and expressed as a total carotid plaque area, our final phenotype of interest.

Genotyping and Quality Control

For the linkage study, a total of 383 autosomal microsatellite markers were genotyped by the Center for Inherited Disease Research at an average interval of 10 centimorgans. To verify and adjust family structure, the putative relationship between pairs of individuals was compared with those constructed based on the autosomal genotypes by performing a maximized log-likelihood ratio test using PREST.[19] Relationships with a probability value $<1.0\times10^{-6}$ in a consistent manner across the family were considered an error. Mendelian errors were also checked on the final family structure using PEDCHECK.[20]

For the NOMAS association analysis, DNA samples were genotyped using the Genome-Wide Human SNP Array 6.0 chip at the Genotyping Core of the Hussman Institute for Human Genomics at the University of Miami. Vigorous quality control was applied to both samples and single nucleotide polymorphisms (SNPs). Post-genotyping, samples were removed from further analysis if they had call rates <95%, relatedness or sex discrepancies, or were outliers beyond 6 SDs from the mean based on EIGENSTRAT analysis.[21] SNPs were removed if they were not in Hardy-Weinberg equilibrium ($P<1.0\times10^{-6}$), had a genotyping call rate <95%, or minor allele frequency <5% as identified by PLINK.[22]

Statistical Analysis

Power transformation on total plaque area was first conducted to reduce the skewness and kurtosis in both linkage and association analyses. For the linkage analysis, polygenic modeling was performed using SOLAR to screen for a set of covariates: age, sex, age by sex, age^2, cigarette pack-years, body mass index (BMI), waist-to-hip ratio, hypertension, diabetes mellitus, and dyslipidemia with a cutoff of $P<0.1$ for inclusion of any potentially significant covariates. The residual skewness and kurtosis of total plaque area in the polygenic model were 0.74 and 0.65, respectively. A maximum likelihood approach was then implemented in SOLAR to calculate heritability, proportion of alleles shared identical-by-descent, and multipoint logarithm of odds (LOD) scores.[23] For the presence of plaque, the heritability was estimated using a pedigree-based maximum likelihood method that models disease status by a liability threshold model.[24,25] To evaluate the robustness of the results, simulation analysis was also conducted to compute empirical probability values for LOD scores based on 10 000 replicates in which a fully informative marker, unlinked to the specific trait, was simulated and used to compute possible LOD scores.

For the association analysis of linkage peaks, population stratification was controlled for with a principal component approach using EIGENSTRAT. In addition, the analysis was performed in Dominicans only to check if the observed associations in the whole NOMAS subcohort remained similar and significant. The same covariates used in the family study

analysis were screened using a stepwise selection procedure with SAS 9.2 (SAS Institute Inc, Cary, NC) for the whole subcohort and Dominicans only analysis separately. Any covariates with $P<0.10$ were kept in the model. Additionally, the top 2 principal components from EIGENSTRAT (PCA1 and PCA2) were included as covariates in the whole NOMAS subcohort analysis and the top principal component (PCA1) for the Dominican only analysis. Assuming an additive genetic model, multiple linear regression analysis was performed with PLINK to investigate the association between plaque area and 18 904 SNPs located within the 1-LOD down regions of the 4 linkage peaks on 7p36 (1424), 11p15 (5644), 14q32 (5606), and 15q23 (6230) after adjusting for the selected covariates. Similarly, multiple logistic regression analysis was performed to examine the association between plaque presence and the selected SNPs. Because many SNPs tested within the 4 regions are highly correlated due to linkage disequilibrium, we computed the effective number of tests based on the sum of singleton SNPs plus linkage disequilibrium blocks identified using Gabriel's method and then applied the Bonferroni procedure to adjust for multiple testing in each region as suggested by Nicodemus et al.[26]

Results

Overall, the Dominican family sample and the NOMAS subcohort had a similar sex distribution ($P=0.31$) but significant difference in age (45.6±17.0 versus 69.8±8.9, $P<0.0001$). Due to an older age distribution, the NOMAS subcohort had a greater proportion of hypertension (64%), diabetes (19%), dyslipidemia (47%), and carotid artery plaque (60%) compared with the family sample. However, both samples had a comparable average BMI (28.7±5.8 versus 28.4±5.0, $P=0.39$; Table 1).

Covariate Effects and Trait Heritabilities

Table 2 shows the effects of vascular risk factors on carotid plaque traits and the adjusted heritability estimates for plaque traits in Dominican families. Age, diabetes, smoking, BMI, and hypertension were significant covariates for both presence and area of carotid plaque, whereas waist-to-hip ratio and age^2 were only significantly associated with plaque area. No significant sex effect or sex-by-age interaction was found for either plaque phenotype. The selected covariates explained approximately 33% of the variance for both plaque traits. After adjustment for the significant covariates, SOLAR polygenic analyses detected a highly significant genetic component for both plaque traits with heritabilities of 0.17 ($P<0.0001$) for plaque area and 0.50 ($P<0.0001$) for plaque presence.

Autosomal Genomewide Linkage Analysis

Table 3 lists the chromosomal regions with a maximum multipoint LOD score ≥2.00 for plaque area or presence. The Figure displays the multipoint LOD scores across 22 autosomal chromosomes. The linkage analysis results were similar for both phenotypes. One region (14q32.13) had a LOD score >2.00 for both plaque area (2.66 at 115 centimorgans, empirical $P=0.0008$) and presence (2.07 at 117 centimorgans, empirical $P=0.0002$). Linkage signals of LOD ≥2.00 were detected in 2 additional regions for plaque area (2.00 on 7q36.2, empirical $P=0.003$; 2.58 on 15q23, empirical $P=0.001$) and in 1 region for plaque presence (2.09 on 11p15.1, empirical $P=0.0002$).

Association Analysis of Linkage Peaks

Within the 4 regions of the linkage peaks, linkage disequilibrium block analysis yielded an effective number of tests of 691 for 7q36, 2243 for 11p15, 2254 for 14 q32, and 2806 for 15q23. Table 4 reports the associated SNPs with a nominal $P\leq0.0005$ in the NOMAS data set and with a nominal $P\leq0.01$ in Dominican subset. Among them, the most significant associations were found for rs14067314 ($P=0.00002$, multiple testing adjusted $P=0.043$) and

rs2665109 (*P*=0.00002, multiple testing adjusted *P*=0.056) located in the flanking region of elongation factor Tu GTP binding domain containing 1 gene (*EFTUD1*). Additionally, 4 SNPs are located within 3 known genes: rs16933090 in the sex-determining region Y-box 6 gene (*SOX6*), rs7163402 in fibronectin type III and additive allelic effect on a plaque trait (*P*<0.001) in the NOMAS data set. SPRY domain containing 2 gene (*FSD2*), and rs2174292 and rs7174330 in adaptor-related protein complex 3, sigma 2 subunit gene (*AP3S2*); rs3935159 near myogenic differentiation 1 gene (*MYOD1*), 1 additional SNP (rs2654209) in the flank of *EFTUD1*; and 3 SNPs on chromosome 14 are located in or near hypothetical gene *LOC7320217* (rs7144551, rs17095330, and rs12433290). Supplemental Table I (available at http://stroke.ahajournals.org) lists all 37 SNPs with an

Discussion

The present study represents detailed genetic analyses of both qualitative and quantitative plaque phenotypes and is complemented by the use of 2 independent samples: well-characterized Caribbean Hispanic multigeneration families and a community-based cohort that is mainly composed of Hispanics. Thus, it provides a unique opportunity to systematically investigate the link between genetic loci and carotid plaque in a moderately sized and well-characterized minority population.

Our polygenic analysis provides further evidence that carotid artery plaque is under genetic control by showing a modest but significant heritability of 0.17 (*P*<0.0001) for plaque area and 0.50 (*P*<0.0001) for plaque presence after adjustment for significant covariates. These estimates are similar to those from 3 large family heart studies and support previous findings that heritability of carotid plaque is lower than that of IMT.[27] The National Heart, Lung, and Blood Institute Family Heart Study reported a heritability of 0.52 for carotid plaque without covariate adjustment and 0.13 after adjusted for age, sex, field center, and major vascular risk factors.[28] The San Antonio Family Heart Study showed a heritability of 0.23 after adjustment for age, sex, waist circumference, BMI, diabetes, hypertension, and smoking status.[10,27] The Diabetes Heart Study observed an adjusted heritability of 0.40 for carotid artery calcified plaque in European Americans (*P*<0.0001). Although there were also 2 studies that did not detect a significant heritability for carotid plaque after adjustment for traditional vascular risk factors, both studies reported significant heritabilities for IMT and stiffness/stenosis, which are highly correlated with plaque traits.[27,29] It should also be noted that inclusion of the covariates diabetes, hypertension, and body weight may affect these estimates if some genes have pleiotropic effects on both plaque traits and the covariates known to be clustered in families.[10]

Our autosomal genomewide linkage analysis of carotid plaque traits revealed suggestive linkage in 4 regions (7p36, 11p15, 14q32, and 15q23). Among these regions, 14q32 has been previously reported to be linked with coronary artery calcification in the National Heart, Lung, and Blood Institute's Framingham Heart Study (LOD, 2.33) and the Genetic Epidemiology Network of Arteriopathy study in sibships at high risk for hypertension (LOD, 1.16).[30,31] To date, relatively few linkage studies have been conducted for carotid plaque phenotypes and the findings are inconsistent. The National Heart, Lung, and Blood Institute Family Heart Study did not detect suggestive linkage in any region in the whole sample, although a region on chromosome 2p11 had a suggestive LOD score (2.43) for carotid plaque presence in 26 affected sibling pairs aged ≤55 years,[28] whereas the Diabetes Heart Study identified a region of significant linkage for carotid calcified plaque (LOD, 4.39; *P*=0.00001) on chromosome 16p13 in diabetes-enriched European American families.[32] The lack of replication may be explained by multiple factors, including varying sample size and population and/or genetic heterogeneity.

Our association analysis found several polymorphisms with an additive allelic effect on a plaque trait with a nominal $P \geq 0.0005$ in the NOMAS subcohort and $P \geq 0.01$ in the Dominican sample within *SOX6*, *EFTUD1*, *FSD2*, and *AP3S2* genes. Of these 4 genes, *SOX6* is of particular interest. Animal models have suggested that *SOX6* is associated with cardioskeletal myopathy and heart block,[33] plays an important role in obesity-related insulin resistance,[34] and is within the bone morphogenic protein pathway in cardiac differentiation.[35] Bone morphogenic proteins have been implicated in the pathophysiology of type 2 diabetes and cardiovascular calcification.[36,37] Very recently, a bivariate human genomewide association study supported the hypothesis that *SOX6* plays pleiotropic roles in both obesity and osteoporosis.[38] *FSD2* might be another candidate of interest given the role of the fibronectin family in endothelial adhesion and atherosclerosis.[39] The function of other associated genes observed in these regions is largely unknown; however, they may also be candidates for further investigation.

Our results, however, need to be taken with caution because there are several weaknesses, including the follow-up association analysis in a relatively small convenience sample with an older population, a small sample size in the Dominican subcohort, and the lack of similar findings reported in the literature due to very few genomewide association studies on these traits.

In summary, in the present study of multigeneration Caribbean Hispanic families and an independent community-based cohort, our findings indicate that carotid artery plaque has considerable heritability and may be influenced by multiple loci on chromosomes 11p15, 14q32, and 15q23 in Caribbean Hispanics. The *SOX6* gene may be of particular interest. Further studies in larger populations are needed to validate the observed association as well as an in-depth investigation of the genes in these regions to provide fundamental insight to the understanding of atherosclerotic disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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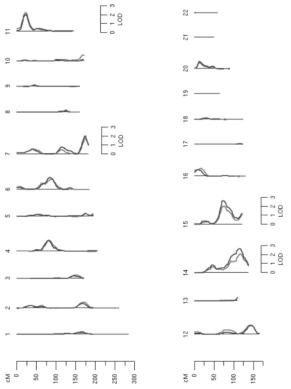

Figure.
Results of autosomal genome scan for carotid plaque phenotypes in Dominican families. Multipoint LOD scores are shown in gray and black lines, respectively, for presence and area of carotid plaque.

Table 1

Characteristics of Study Samples

		NOMAS Subcohort			
		All		Dominicans	
Characteristics	**Dominican Family Sample (n=1308)**	**(n=941)**	***P*‡**	**(n=384)**	***P*‡**
Demographics					
Age, years, mean ± SD	45.6±17.0	69.8±8.9	<0.0001	67.7±8.3	<0.0001
Female, %	61.9	59.8	0.31	64.8	0.30
Hispanic, %	100.0	64.6	<0.0001	100.0	1.00
Vascular risk factors					
Ever smoke, %	33.0	52.1	<0.0001	46.9	<0.0001
Cigarette pack-years,* mean±SD	12.3±14.6	23.7±26.9	<0.0001	19.4±20.9	<0.0001
BMI, kg/m^2, mean±SD	28.7±5.8	28.4±5.0	0.39	28.8±4.8	0.41
Waist-to-hip ratio, mean±SD	0.90±0.09	0.92±0.08	<0.0001	0.92±0.08	<0.0001
Hypertension, %	38.8	64.1	<0.0001	68.5	<0.0001
Diabetes mellitus, %	13.8	19.5	0.0003	22.7	<0.0001
Dyslipidemia, %	32.2	47.0	<0.0001	45.7	<0.0001
Phenotypes					
Carotid plaque, %	21.6	60.5	<0.0001	53.1	<0.0001
Plaque area, mm^2,† mean±SD	16.2±20.5	22.3±21.8	<0.0001	19.4±20.9	0.004

*Among smokers.

†Among those with carotid plaque.

‡Compared with the Dominican family sample.

Table 2

Covariates and Adjusted Heritability Estimates for Carotid Plaque

	Carotid Plaque Trait*	
Polygenic Model	**Presence**	**Area**
Covariates screened, *P* value		
Age	8.92×10^{-14}	4.24×10^{-19}
Sex	0.48	0.51
Age* sex	0.50	0.33
Age^2	0.74	0.00004
Dyslipidemia	0.17	0.13
Hypertension	0.08	0.02
Diabetes mellitus	0.003	0.00001
Cigarette pack-years	0.02	0.0001
BMI	0.001	0.0004
Waist-to-hip ratio	0.08	0.09
Proportion of variance due to covariates	0.33*	0.33
Trait residual skewness		0.74
Trait residual kurtosis		0.65
Adjusted heritability		
h^2±SE	0.50±0.14	0.17±0.04
P value	1.63×10^{-5}	2.20×10^{-6}

*Kullback-Leibler R^2.

Table 3

Chromosomal Regions With SOLAR Multipoint LOD Scores >1.5 by Carotid Plaque Trait

Carotid Artery Plaque	Location	Position, Centimorgans	Nearest Marker	LOD Score	Empirical *P*[*]	h2q[†]	LOD-1 Interval, Mb
Presence	7q36.2	174	D7S3058	1.79	0.001	0.53	
	11p15.1	22	D11S1981	2.09	0.0002	0.53	
	14q32.13	117	D14S1434	2.07	0.0002	0.54	
	15q23	75	D15S131	1.98	0.0004	0.53	
Area	7q36.2	174	D7S3058	2.00	0.003	0.19	152–156
	11p15.1	22	D11S1981	1.85	0.003	0.18	8–25
	14q32.13	115	D14S1434	2.66	0.0008	0.19	82–101
	15q23	74	D15S131	2.58	0.001	0.19	68–93

[*] Based on 10000 replicates.

[†] Locus-specific heritability.

Table 4

Plaque-Associated SNPs With a $P<0.0005$ in the NOMAS Subcohort and With a $P<0.01$ in Dominicans

				All NOMAS Subcohort				NOMAS Dominicans					
Chromosome	**Carotid Plaque**	**SNP**	**Position, bp**	**Minor Allele**	**MAF***	**OR/β (95% CI)†**	***P***	**Minor Allele**	**MAF***	**OR/β (95% CI)†**	***P***	**Nearby Gene**	**SNP Location**
11	Presence	rs16933090	16412370	T	0.13	0.52 (0.38 to 0.72)	0.00005	T	0.14	0.48 (0.30 to 0.78)	0.003	*SOX6*	Intron
	Area	rs16933090	16412370	T	0.13	−0.36 (−0.55 to −0.17)	0.0002	T	0.14	−0.39 (−0.65 to −0.12)	0.005	*SOX6*	Intron
	Presence	rs3935159	17630806	A	0.49	1.51 (1.21 to 1.88)	0.0003	G	0.47	0.63 (0.45 to 0.88)	0.007	*MYOD1*	Flanking‡
	Area	rs1406314	23902665	G	0.16	0.37 (0.20 to 0.53)	0.00002§	G	0.17	0.37 (0.12 to 0.62)	0.004		
14	Area	rs7144551	96913863	T	0.30	0.26 (0.13 to 0.40)	0.0002	T	0.30	0.29 (0.09 to 0.50)	0.006	*LOC730217*	Flanking‡
	Area	rs17095330	96919653	A	0.30	0.25 (0.11 to 0.38)	0.0003	A	0.31	0.27 (0.07 to 0.46)	0.008	*LOC730217*	Flanking‡
	Area	rs12433290	97029784	G	0.13	−0.36 (−0.56 to −0.16)	0.0003	G	0.11	−0.42 (−0.73 to −0.10)	0.01	*LOC730217*	Intron
	Presence	rs12433290	97029784	G	0.13	0.56 (0.40 to 0.77)	0.0004	G	0.11	0.45 (0.26 to 0.78)	0.004	*LOC730217*	Intron
15	Area	rs2654209	80196782	A	0.30	0.24 (0.11 to 0.38)	0.0004	A	0.33	0.27 (0.08 to 0.47)	0.007	*EFTUD1*	Flanking‡
	Presence	rs2665109	80202412	T	0.15	2.01 (1.46 to 2.78)	0.00002	T	0.14	2.05 (1.27 to 3.31)	0.003	*EFTUD1*	Flanking‡
	Area	rs2665109	80202412	T	0.15	0.34 (0.16 to 0.52)	0.0002	T	0.14	0.38 (0.11 to 0.65)	0.006	*EFTUD1*	Flanking‡
	Area	rs7163402	81250549	A	0.12	0.37 (0.18 to 0.56)	0.0002	A	0.11	0.46 (0.16 to 0.77)	0.003	*FSD2*	Intron
	Presence	rs2174292	88216585	C	0.45	0.68 (0.55 to 0.84)	0.0004	C	0.40	0.59 (0.42 to 0.83)	0.003	*AP3S2*	Intron
	Presence	rs7174330	88235583	C	0.49	0.68 (0.55 to 0.84)	0.0003	C	0.46	0.59 (0.42 to 0.82)	0.002	*AP3S2*	Intron

*MAF indicates minor allele frequency.

†OR indicates odds ratio for presence of carotid plaque; β, regression coefficient for carotid plaque area; CI, confidence interval.

‡The distance from the gene <100 kb.

§Bonferroni-corrected $P<0.05$ based on the sum of singleton SNPs and linkage disequilibrium blocks.