Variants at the *APOA5* locus, association with carotid atherosclerosis, and modification by obesity: the Framingham Study[S]

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Abstract **Genetic variation at the apolipoprotein A5 gene (*APOA5*) is associated with increased triglyceride concentrations, a risk factor for atherosclerosis. Carotid intimal medial thickness (IMT) is a surrogate measure of atherosclerosis burden. We sought to determine the association of common *APOA5* genetic variants with carotid IMT and stenosis. A total of 2,273 Framingham Offspring Study participants underwent carotid ultrasound and had data on at least one of the five *APOA5* variants (−1131T>C, −3A>G, 56C>G, IVS3+476G>A, and 1259T>C). Although none of the individual variants was significantly associated with carotid measures, the haplotype defined by the presence of the rare allele of the 56C>G variant was associated with a higher common carotid artery (CCA) IMT compared with the wild-type haplotype (0.75 vs. 0.73 mm; $P < 0.05$). The rare allele of each of the −1131T>C, −3A>G, IVS3+476G>A, and 1259T>C variants and the haplotype defined by the presence of the rare alleles in these four variants were each significantly associated with CCA IMT in obese participants. These associations remained significant even after adjustment for triglycerides. *APOA5* variants were associated with CCA IMT, particularly in obese participants. The mechanism of these associations and the effect modification by obesity are independent of fasting triglyceride levels.**—Elosua, R., J. M. Ordovas, L. A. Cupples, C-Q. Lai, S. Demissie, C. S. Fox, J. F. Polak, P. A. Wolf, R. B. D'Agostino, Sr., and C. J. O'Donnell. **Variants at the *APOA5* locus, association with carotid atherosclerosis, and modification by obesity: the Framingham Study. *J. Lipid Res.* 2006.** 47: **990–996.**

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The apolipoprotein A5 gene (*APOA5*) was recently identified by comparison of genomic DNA from human and mouse (1) and by searching for genes involved in liver regeneration (2). This gene is located near the *APOA1/C3/A4* gene cluster in human 11q23 and is involved in triglyceride metabolism (1, 3). More recently, apolipoprotein A-V has been described to reduce plasma triglycerides by inhibiting VLDL-triglyceride production (4) and by stimulating lipoprotein lipase-mediated VLDL-triglyceride hydrolysis (4, 5). Three *APOA5* genetic variants were initially described in humans (−1131T>C, IVS3 + 476G>A, and 1259T>C) (1), and two others were reported subsequently (−3A>G and 56C>G) (6). The less common alleles of these five variants have been consistently associated with higher plasma triglyceride concentrations (1, 6–13). *APOA5* genetic variants have also been associated with larger LDL (12, 14) and VLDL (15) particle sizes.

Although hypertriglyceridemia is an independent risk factor for coronary heart disease (16), there are limited data regarding the association between the *APOA5* locus and a phenotypic manifestation of atherosclerosis in humans, particularly in unselected men and women from the general population. Most of the available studies have reported associations of *APOA5* with atherosclerosis progression in men after coronary bypass (15), with coronary heart disease in a selected population of patients referred for coronary bypass surgery (17) or for angiog-

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[S] The online version of this article (available at http://www.jlr.org) contains an additional two figures.

raphy (18), and with myocardial infarction (19, 20) and cardiovascular disease (12) in the general population.

Carotid intimal medial thickness (IMT) measured by ultrasound is associated with prevalent cardiovascular disease (21), incident myocardial infarction and stroke (22), and premature parental coronary heart disease (23). Therefore, carotid IMT, and carotid stenosis as well, are widely used as surrogate measures of atherosclerosis burden and risk. There is a substantial heritable component to both internal and common carotid IMT (24), but each may represent distinct underlying pathophysiologies (25).

In this report, we sought to determine the association of *APOA5* genetic variants and *APOA5* haplotypes with carotid IMT and stenosis, to assess whether other cardiovascular risk factors modify this association, and to establish whether any observed associations are mediated through plasma triglyceride concentration in a large community-based sample of men and women.

METHODS

Study population

The design of the Framingham Heart Study has been detailed previously (26). Subjects included in this analysis were participants in the offspring cohort of the Framingham Heart Study. There were 3,532 participants in Offspring Study examination cycle 6 (1995–1998). A total of 3,380 (96%) of these participants underwent B-mode carotid ultrasonography. *APOA5* genotype data were available in 2,273–2,367 participants (67–70%) with available carotid IMT data, depending on the genetic variant analyzed. The research protocol and genotype analyses were approved by the Institutional Review Boards at Boston University and Tufts University. All participants provided informed consent.

Carotid ultrasonography

Ultrasound measures were acquired and images analyzed according to a standard protocol (27), as described previously (28). Common carotid artery (CCA) and internal carotid artery (ICA) IMT were defined as the means of the maximal IMT measurements for the right and left sides. A subjective estimate of ICA narrowing, graded as 0%, 1–24%, and 25–49%, was made by the sonographer when Doppler-derived peak systolic velocities in the ICA were <150 cm/s. ICA narrowing of hemodynamic significance (≥50%) was defined as present when peak systolic velocities in the ICA were ≥150 cm/s. We defined the degree of stenosis based upon the maximum stenosis in either ICA, and stenosis was defined as present if it was ≥25%. More details are available in the supplementary data.

APOA5 genotype

DNA was isolated from blood samples using DNA Blood Midi Kits (Qiagen, Hilden, Germany) according to the protocol recommended by the vendor. Five previously reported variants were determined (1, 6): −1131T>C (initially named SNP3-[1]), −3A>G, 56C>G (also known as S19W-[6]), IVS3+476G>A (initially named SNP2-[1]), and 1259T>C (also known as SNP1-[1]).

Genotyping was carried out using the ABI Prism SnaPshot multiplex system (Applied Biosystems, Foster City, CA). The primers and probes used have been reported previously (11).

The haplotype structures and frequencies were established among unrelated participants using Haplo.score (29). Selecting unrelated participants with complete data on all of the analyzed variants (n = 1,535), the four variants other than 56C>G were in almost complete linkage disequilibrium, with correlation coefficients ranging from 0.88 to 0.98, whereas the correlation coefficients between 56C>G and the other four variants ranged from 0.06 to 0.07 (12). There was a limited number of common haplotype variants (frequency>1%) (**Table 1**). When analyzing the whole sample, we could define the haplotype structures without ambiguous linkage phase in 2,047 of the 2,129 participants who had complete data on *APOA5* genotypes. Three haplotype-genotype groups were defined (**Table 2**): haplotype-genotype *APOA5**1/1, which includes homozygotes for haplotype variant *1, the wild-type (n = 1,578); haplotype-genotype *APOA5**1/2 and 2/2, the carriers of haplotype variant *2 (n = 223); and haplotype-genotype *APOA5**1/3 and 3/3, the carriers of haplotype variant *3 (n = 233). Because they were extremely infrequent (n = 13), *APOA5**2/3 heterozygous individuals were excluded from the association analyses, as were those subjects with ambiguous or very rare haplotypes (n = 82).

TABLE 1. Structure and frequency of the three common *APOA5* haplotype variants (frequency > 1%)

APOA5 Haplotype Variants	−1131T>C	−3A>G	56C>G	IVS + 476G>A	1259T>C	Frequency
Variant *1	T	A	C	G	T	86.7
Variant *2	C	G	C	A	C	6.0
Variant *3	T	A	G	G	T	5.6
Others	—	—	—	—	—	1.7

APOA5, apolipoprotein A5 gene.

Other atherosclerosis risk factor variables

Data regarding medical history and physical examination were derived from the sixth examination cycle. The following variables were included in the analyses: diabetes, smoking, hypertension, total cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides, height, weight, body mass index (BMI), waist circumference, obesity, and abdominal obesity. C-reactive protein levels were also determined, as reported previously (28). More details are available in the supplementary data.

Statistical methods

Chi-square tests were used to compare proportions across groups, and ANOVA was used to compare means of continuous variables across groups. Analysis of covariance was used to determine the carotid IMT mean across *APOA5* genotypes, adjusting for covariates. Logistic regression analysis was con-

TABLE 2. Frequency of the three common *APOA5* haplotype-genotype groups defined

APOA5 Haplotype-Genotype Groups	Haplotype-Genotype	N	Frequency
*APOA5**1/1	TACGT–TACGT	1,578	74.11
*APOA5**1/2 or 2/2	TACGT–CGCAC or CGCAC–CGCAC	223	10.47
*APOA5**1/3 or 3/3	TACGT–TAGGT or TAGGT–TAGGT	233	10.95
*APOA5**2/3	CGCAC–TAGGT	13	0.61
Other or ambiguous	—	82	3.85

ducted to determine the association between genetic variants and ICA stenosis. Familial correlations were accounted for using generalized estimating equations with Proc Genmod in SAS (version 8.0). For these analyses, a dominant genetic model was assumed, as the frequency of the rare alleles was low. In these models, we adjusted for the following covariates: age, sex, smoking, diabetes, systolic blood pressure, hypertension treatment and BMI, triglycerides, HDL-cholesterol, and LDL-cholesterol. We ran three models, one adjusting for age and sex, a second adjusting for all covariates except the lipid measures, and a third with all covariates.

Additionally, haplotype analyses were performed in the subset of individuals with unambiguous linkage phase (2,047 of 2,129). According to the individual haplotype structures, we defined three haplotype-genotype groups using a dominant genetic model, as explained above (Table 2). In these analyses, we also used generalized estimating equations and logistic regression with Proc Genmod in SAS, as described above.

According to the prespecified aims of the study, we also tested for the effects on carotid phenotypes of interactions between the genetic variants (individual variants and haplotypes) and hypertension, smoking, diabetes, and obesity. For all analyses, a two-tailed nominal P value of <0.05 was considered statistically significant, and we accounted for familial correlations.

RESULTS

The frequencies of the different *APOA5* rare alleles (analyzed in an unrelated subsample of the study) were 0.06 for the 56C>G and IV53+476G>A variants and 0.07 for the −1131T>C, −3A>G, and 1259T>C variants. These frequencies were consistent with Hardy-Weinberg equilibrium, except for the 56C>G genetic variant. For 56C>G, there was a small but statistically significant difference between the observed genotype frequencies (89.1, 10.1, and 0.8 for the CC, CG, and GG genotypes, respectively) and the expected frequencies (88.7, 11.0, and 0.3, respectively) in this large sample size. Given the very small magnitude of difference, we continued to include this variant in subsequent analyses.

In **Table 3**, we present the characteristics of the participants by each of the *APOA5* genetic variants. With respect to lipids, there was a consistently higher triglyceride level associated with the presence of the rare allele in all of the analyzed *APOA5* variants, with increases ranging from 22% to 27%. Compared with noncarriers, total cholesterol levels were higher in carriers of the rare allele in the −1131T>C, −3A>G, and IVS3+476G>A variants, and HDL-cholesterol levels were lower in those with the rare allele of the 56C>G variant. Additionally, the prevalence of diabetes was lower among carriers of the rare allele of the −1131T>C, −3A>G, and IVS3+476G >A variants. The prevalence of obesity was lower among carriers of the rare allele of the 1259T>C genetic variant.

There were no significant associations of the individual *APOA5* genetic variants with carotid IMT or stenosis (Table 3). Similarly, there were no overall associations in age- and sex-adjusted or multivariable-adjusted models. In analyses of the association of haplotypes with carotid measures, the global test for differences across the three *APOA5*

TABLE 3. Characteristics of the participants, CCA and ICA IMT, and prevalence of carotid stenosis by individual *APOA5* genotypes

Characteristic	−1131T>C		−3A>G		56C>G		IVS + 476G>A		1259T>C	
	TT (N = 1,971)	C Carriers (N = 296)	AA (N = 2,026)	G Carriers (N = 310)	CC (N = 2,084)	G Carriers (N = 277)	GG (N = 2,021)	A Carriers (N = 273)	TT (N = 2,009)	C Carriers (N = 314)
Age (years)	58 (10)	58 (10)	58 (10)	59 (10)	59 (10)	57 (10)	58 (10)	58 (10)	58 (10)	58 (10)
Women (%)	53.0	49.0	53.0	48.7	52.7	50.5	52.9	49.1	53.4	49.4
Smoking (%)	15.0	13.2	14.6	14.2	15.1	11.9	15.0	13.9	14.9	14.7
Diabetes (%)	12.8	8.5 b	12.8	7.7[a]	12.0	12.6	12.7	7.7[a]	12.4	9.6
Hypertension (%)	42.0	37.5	41.2	38.4	40.8	40.4	41.3	38.1	41.5	38.5
Body mass index (kg/m^2)	28.1 (5.3)	28.0 (5.7)	28.1 (5.2)	28.1 (5.7)	28.1 (5.3)	27.9 (4.6)	28.1 (5.2)	28.0 (5.7)	28.1 (5.3)	27.8 (5.6)
Obesity (%)	29.9	25.4	29.9	26.2	29.7	26.4	30.0	26.1	30.1	24.6[a]
Waist (mm)	98 (14)	98 (14)	98 (14)	98 (14)	98 (14)	98 (13)	98 (14)	98 (14)	98(14)	98 (14)
Abdominal obesity (%)	49.5	45.0	49.5	45.9	48.7	52.2	49.7	45.9	49.8	43.7
Total cholesterol (mg/dl)	205 (37)	212 (41)[b]	205 (36)	212 (40)[b]	206 (37)	206 (38)	205 (36)	211 (40)[b]	205 (36)	209 (40)
LDL-cholesterol (mg/dl)	128 (32)	129 (35)	127 (32)	130 (35)	127 (33)	125 (33)	127 (32)	130 (36)	127 (32)	128 (35)
HDL-cholesterol (mg/dl)	51 (16)	50 (16)	51 (16)	50 (16)	51 (16)	48 (15)[b]	51 (16)	50 (16)	51 (16)	50 (16)
Triglycerides (mg/dl)	137 (91)	172 (135)[b]	136 (90)	172 (131)[b]	137 (93)	167 (132)[b]	136 (91)	172 (132)[b]	136 (92)	169 (128)[b]
CCA IMT (mm)	0.73 (0.18)	0.74 (0.16)	0.73 (0.18)	0.74 (0.17)	0.73 (0.17)	0.73 (0.26)	0.73 (0.18)	0.74 (0.17)	0.73 (0.18)	0.74 (0.16)
ICA IMT (mm)	0.79 (0.52)	0.79 (0.52)	0.78 (0.51)	0.80 (0.52)	0.79 (0.51)	0.75 (0.50)	0.78 (0.51)	0.78 (0.51)	0.78 (0.51)	0.79 (0.51)
Stenosis > 25% (%)	18.4	19.6	18.1	21.3	18.8	16.3	18.0	19.4	18.2	20.1

CCA, common carotid artery; ICA, internal carotid artery; IMT, intimal medial thickness. Continuous variables are presented as means and (SD).
[a] $P < 0.05$ (for triglycerides, P value is based on log triglycerides).
[b] $P < 0.01$ (for triglycerides, P value is based on log triglycerides).

haplotypes for CCA IMT was marginal ($P = 0.09$). However, there were significant haplotype-specific differences: the *APOA5**1/3-3/3 haplotype-genotype was associated with significantly higher CCA IMT compared with the *APOA5**1/1 haplotype-genotype ($P = 0.040$) (**Table 4**). These significant differences persisted in multivariable-adjusted models, even after adjustment for triglycerides and other lipid levels. There were no associations between the different haplotype-genotype groups and ICA IMT or stenosis. These haplotype results were similar in analyses conducted separately in men and women (data not shown).

We further analyzed whether other cardiovascular risk factors modulate the association of individual variants with carotid phenotypes. Significant interactions were noted consistently for BMI as well as a number of other obesity-related measures. BMI was directly associated with CCA IMT independently of the *APOA5* genotype, but the association was significantly stronger in carriers of the rare alleles of the −1131T>C, −3A>G, IVS+476G>A, and 1259T>C genetic variants. These interactions remained significant (*P* values ranging from 0.004 to 0.016) even after adjusting for triglycerides and other lipid levels. (Data for the individual *APOA5* genotypes are shown in supplementary Figure I.) There was also a statistically significant interaction when we used waist circumference instead of BMI. In analyses to examine for interaction between the *APOA5* genotypes and the dichotomous measure of obesity (BMI $\geq$ 30 kg/m^2), each of the rare alleles was associated with higher CCA IMT in obese subjects (see supplementary Figure II). Similar significant interactions were also noted with the dichotomous measure of abdominal obesity and were observed in analyses conducted separately in men and women (data not shown).

In analyses for interactions between the *APOA5* haplotype variants and obesity-related variables, the interaction between the *APOA5* haplotype-genotype groups and BMI was marginally significant ($P = 0.059$), and the results were consistent with those noted for the individual genetic variants (**Fig. 1**). The association of BMI with CCA IMT was stronger in carriers of the *APOA5**1/2-2/2 haplotype-genotype. The interaction between *APOA5* haplotype-genotype groups and obesity was statistically significant, even after adjusting for lipids ($P = 0.031$): the *APOA5**1/2-2/2 haplotype-genotype was associated with higher CCA IMT in obese people (**Fig. 2**). These interactions were also observed in analyses conducted separately in men and women (data not shown). Aside from the obesity interac-

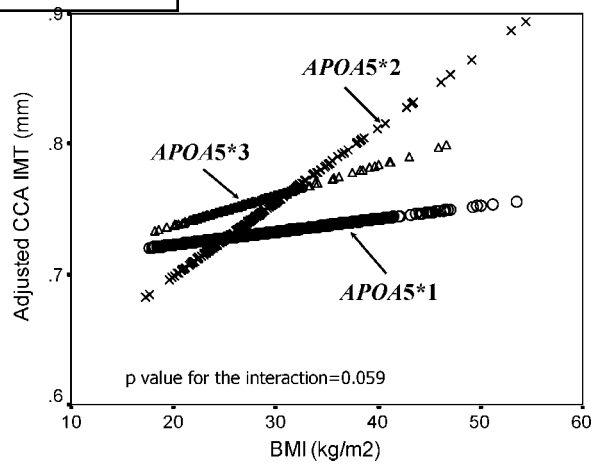

Fig. 1. Predicted values from the regression of the adjusted common carotid artery (CCA) intimal medial thickness (IMT) on body mass index (BMI) within each apolipoprotein A5 gene (APOA5) haplotype-genotype group, and *P* value for the interaction between *APOA5* haplotype-genotype and BMI. The models were adjusted for age, sex, smoking, diabetes, systolic blood pressure, hypertension treatment, triglycerides, HDL-cholesterol, and LDL-cholesterol.

TABLE 4. CCA and ICA IMT, and odds ratios for the prevalence of carotid stenosis across *APOA5* haplotype-genotype groups

Variable	*APOA5**1/1 (TACGT–TACGT) (N = 1,578)	*APOA5**1/2-2/2 (TACGT–CGCAC or CGCAC–CGCAC) (N = 223)	*APOA5**1/3-3/3 (TACGT–TAGGT or TAGGT–TAGGT) (N = 233)	*P*
CCA IMT (mm)				
Unadjusted	0.73 (0.01)	0.74 (0.01)	0.74 (0.01)	0.61
Model 1	0.73 (0.00)	0.74 (0.01)	0.75 (0.01)	0.24
Model 2	0.73 (0.00)	0.74 (0.01)	0.75 (0.01)[a]	0.09
Model 3	0.73 (0.01)	0.74 (0.01)	0.75 (0.01)[b]	0.09
ICA IMT (mm)				
Unadjusted	0.78 (0.01)	0.80 (0.03)	0.76 (0.03)	0.64
Model 1	0.78 (0.00)	0.80 (0.03)	0.77 (0.03)	0.78
Model 2	0.77 (0.00)	0.81 (0.03)	0.78 (0.03)	0.49
Model 3	0.77 (0.01)	0.81 (0.03)	0.76 (0.03)	0.51
ICA stenosis > 25%				
Unadjusted	1	1.00 (0.69–1.44)	0.81 (0.55–1.18)	
Model 1	1	1.04 (0.72–1.50)	0.87 (0.58–1.31)	
Model 2	1	1.19 (0.83–1.71)	0.92 (0.60–1.41)	
Model 3	1	1.18 (0.80–1.74)	0.94 (0.61–1.43)	

IMT values are means and (SEM); stenosis values are odds ratios and (95% confidence intervals). Model 1, adjusted for age and sex; model 2, adjusted for age, sex, smoking, diabetes, systolic blood pressure, hypertension treatment, and body mass index; model 3, model 2 adjustments plus further adjustment for triglycerides, HDL-cholesterol, and LDL-cholesterol.

[a] $P = 0.041$ compared with haplotype-genotype *APOA5**1/1.

[b] $P = 0.040$ compared with haplotype-genotype *APOA5**1/1.

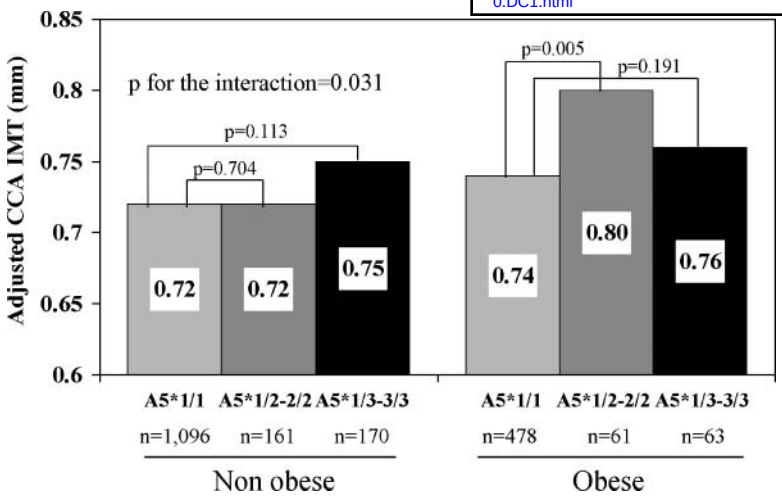

Fig. 2. Multivariable adjusted CCA IMT according to *APOA5* haplotype-genotype groups and obesity, and *P* values for the interactions between these haplotypes and obesity. The models were adjusted for age, sex, smoking, diabetes, systolic blood pressure, hypertension treatment, triglycerides, HDL-cholesterol, and LDL-cholesterol.

tions, there were no other significant interactions of other risk factors with *APOA5* variants.

DISCUSSION

In this large community-based study, we have found that the association between *APOA5* −1131T > C, −3A > G, IVS + 476G > A, and 1259T > C genetic variants and CCA IMT was modified by obesity-related indicators. Three common haplotype variants were formed by the five individual variants examined in this study. The haplotype defined by the substitution of G for C at nucleotide 56 was associated with higher CCA IMT compared with the wild-type haplotype, whereas the haplotypes defined by the presence of the rare allele in the −1131T>C, −3A>G, IVS+476G> A, and 1259T>C genetic variants were associated with higher CCA IMT only in obese subjects. Surprisingly, all of these associations were independent of cardiovascular risk factors, including fasting triglycerides and other lipid levels. No significant associations were noted between *APOA5* genetic variants and ICA IMT or carotid stenosis.

The results of our study extend and add new information to the existing data regarding the association between *APOA5* and atherosclerosis measures. Although the association between *APOA5* genetic variants and hypertriglyceridemia has been studied extensively and replicated consistently in different studies (1, 6–11), there have been few studies, particularly in large population-based cohorts, of the association between these genetic variants and clinically relevant atherosclerotic phenotypes (12, 15, 17, 19, 20). We report an association between *APOA5* genetic variants and CCA IMT, and although the magnitude of differences in IMT is modest, similar magnitudes of difference in other studies have been associated with clinically significant atherosclerotic outcomes (21–23).

Our data further add to prior studies by analyzing the association of haplotypes and individual genetic variants at the *APOA5* locus with atherosclerosis-related phenotypes. Although we did not observe statistically significant associations between the individual *APOA5* variants and carotid measures, the haplotype defined by the presence of the 56C > G variant (*APOA5**1/3-3/3) was associated with higher CCA IMT compared with the wild-type haplotype (*APOA5**1/1). Thus, the discordance of the results for CCA IMT observed between the association of the haplotype defined by the presence of the 56C > G variant and this individual single-nucleotide polymorphism may be explained by a more precise genetic classification of the individuals. The haplotype analysis clearly defines three different haplotype-genotype groups. However, the individual single-nucleotide polymorphism analysis defines only two groups, comparing the carriers of the 56C > G variant and noncarriers. Because the noncarriers include both wild-type individuals (haplotype *APOA5**1/1) and carriers of the other four genetic variants (haplotype *APOA5**1/2-2/2), the association results for CCA IMT may be diluted by the introduction of misclassification. These results support the idea that analyses based upon haplotypes may be more efficient than separate analyses of individual markers when studying complex diseases (30).

Of potential clinical relevance, we report a significant effect modification of obesity on the association of individual *APOA5* variants with CCA IMT. This effect modification was noted consistently in analyses of haplotypes involving the *APOA5**1/2-2/2 haplotype-genotype, which was present in 11% of the population. These results suggest that 56C>G acts as a restrictive variant, whereas −1131T>C, −3A>G, IVS + 476G > A, and 1259T > C act as conditional genetic variants expressing the deleterious phenotype under certain environmental conditions, such as obesity. Interestingly, although all of the individual variants were associated with higher triglyceride levels, only the 56C > G variant was also associated with lower HDL-cholesterol, whereas the −1131T>C, −3A > G, and IVS + 476G > A variants were also associated with higher total cholesterol. These different associations may be related to a different impact of these variants on apolipoprotein A-V function (31): although the *APOA5* 56C>G variant reduces *APOA5* expression, the other four variants, which define the haplotype *APOA5**2, do not influence translation efficiency or gene expression, suggesting that the effect of these variants may reflect a strong linkage disequilibrium with other functional variants. The *APOA5*

gene is a constituent of the well-known *APOA1/C3/A4/A5* gene cluster that has been the subject of intense research to gain understanding about lipid metabolism and cardiovascular disease risk (32). Recent data suggest that the haplotype *APOA5**2 is in linkage disequilibrium with the minor alleles of some *APOC3* variants (Sst1 −3238G> C, −482C>T, and −455T>C) (33, 34), such that the association between this haplotype and carotid IMT in obese men and women could be explained by variations in the *APOC3* gene. The *APOC3* Sst1 variant has been associated with carotid atherosclerosis in different studies (35–37), although we did not observe this association in the Framingham Heart Study (38). Recent studies suggest that haplotypes in *APOC3* but not in *APOA5* increase susceptibility to myocardial infarction (39). Further studies are warranted to explore this cluster, define the linkage disequilibrium pattern, and determine the association with lipid traits and cardiovascular risk susceptibility.

All of the associations of *APOA5* variants with CCA IMT were independent of risk factors, including fasting triglycerides or other lipid levels. This surprising result was also reported by Szalai et al. (17), who found that the association between the *APOA5* −1131T > C variant and coronary heart disease susceptibility was independent of triglyceride levels. These observations suggest additional mechanisms explaining the association between *APOA5* and atherosclerosis independent of the classical lipid risk factors included in these analyses. It is possible that other lipid variables, such as postprandial lipemia or LDL particle size, reported previously to be associated with the *APOA5* genotype (12, 14), may play a role. We explored other possible mechanisms by including C-reactive protein, a marker of inflammation, and the ratio between triglycerides and HDL-cholesterol, an indirect marker of insulin resistance, in the multivariate models; however, the inclusion of these covariates did not alter the reported associations (data not shown).

We observed an association of the *APOA5* variants with CCA IMT but not with ICA IMT or stenosis. These different carotid phenotypes represent different stages in the complex process of atherosclerosis (40). The observation of associations specific to CCA but not ICA, the effect modification by obesity, and the independence of effect from circulating triglyceride and other lipid levels leads to speculation regarding the function of apolipoprotein A-V in atherogenesis. Apolipoprotein A-V has been shown to stimulate lipoprotein lipase hydrolytic capacity (4, 5), and it is possible that it also stimulates other lipoprotein lipase functions, such as lipoprotein bridging and selective cholesteryl ester uptake (41). These functions are important in the delivery of atherogenic particles to the arterial wall (41) and could be especially significant in arterial segments with laminar blood flow, such as the CCA, where the wall shear stress decreases (25).

There are possible study limitations. Although we examined a priori for plausible interactions, it is possible that our observed interaction between *APOA5* variants and obesity is falsely positive, given the number of tests examined during our analyses. Therefore, our observations, although strong, should be considered with caution and confirmed in other studies. Second, we have not conducted extensive single-nucleotide polymorphism genotyping across the *APOA5* gene and have not measured carotid atherosclerosis measures other than IMT and stenosis; thus, it is possible that we have not completely accounted for the contribution of all possible genetic variations at this locus with all possible carotid measures. However, we have focused on the *APOA5* variants that have been defined recently and studied intensively as well as on carotid phenotypes that are both heritable (24) and independently associated with incident cardiovascular disease in prospective studies (22). Third, our findings are restricted to a Caucasian cohort and thus may not be generalizable to other ethnic cohorts.

In conclusion, in this community-based study, we report that common *APOA5* genetic variants and haplotypes are associated with CCA IMT, particularly in obese subjects. If confirmed, these associations may have substantial public health implications, given the recent epidemic increases in obesity. The independence of these associations from fasting triglyceride levels suggests the existence of an alternative mechanism in the association between *APOA5* and atherosclerosis. Further studies are certainly warranted to confirm and further explore these findings.

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