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Apolipoprotein E Gene Polymorphism and Subclinical Carotid Atherosclerosis: The Northern Manhattan Study

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Abstract

Background and Aims—APOE polymorphism has previously been associated with carotid intima-media thickness (cIMT) in predominantly Caucasian populations. We sought to test the strength of the relationship between APOE-ε4 carrier status and subclinical atherosclerosis in a tri-ethnic population with a large Hispanic representation.

Methods—We assessed the association between APOE polymorphism and cIMT and plaque burden among 1,243 stroke-free individuals (mean age 69 years, 65% Hispanic, 18% Black, 17% White) using a sequence of multivariable regression models.

Results—After adjusting for demographics, vascular risk factors and plasma LDL levels, APOE-ε4 carrier status was positively associated with cIMT (mean difference, 0.013 mm; 95% CI, 0.003 to 0.023 mm). The APOE-ε4 association with cIMT appeared to be segment-specific with greater differences in IMT between APOE-ε4 carriers and non-carriers in the common carotid artery, (CCA, 0.014 mm) and bifurcation (0.017 mm) than in the internal carotid artery (ICA) IMT (0.007 mm). This relationship was not modified by race-ethnicity. Presence of diabetes modified the ε4-cIMT relationship in CCA (p=0.045) and ICA (p=0.046). APOE-ε4 carrier status was not associated with plaque presence or plaque area.

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Conclusion—APOE-ε4 carriers had elevated cIMT independent of demographics and vascular risk factors including LDL levels. Diabetes was an effect modifier of the relationship between APOE-ε4 and IMT, such that ε4 carriers with diabetes had greater IMT in the CCA and ICA than those without diabetes. The APOE-IMT relationship was not modified by race-ethnicity.

Keywords

apolipoproteins E; carotid arteries; populations; genetics

Introduction

Carotid atherosclerosis is a major risk factor for ischemic cardiovascular disease (CVD).¹ Carotid intima-media thickness (cIMT) has frequently been utilized as a surrogate marker of atherosclerosis^{2,3} as cIMT has been associated with future vascular events including stroke and myocardial infarction (MI) in some studies.⁴ There does not appear to be a relationship between cIMT progression and cardiovascular risk.^{5,6} cIMT may be a precursor of carotid plaque, but their association was not present in prospective analysis,⁶ indicating that the two cardiovascular markers may be related but biologically distinct entities.² A significant proportion of variability in cIMT is unaccounted for by traditional cardiovascular risk factors, which implies that genetic contributions may play an important role in the development of this disease phenotype.⁷⁻⁹

Apolipoprotein E is one of the most studied proteins in relationship to CVD. ApoE is crucial to the structural integrity of lipoproteins, plays a significant role in lipid metabolism, and is responsible for altering circulating levels of cholesterol. The APOE gene, located at chromosome 19q13.2, is polymorphic, and the three most common protein isoforms are E2, E3, and E4.¹⁰ Generally, the ε2 allele corresponds with lower mean plasma cholesterol levels and the ε4 allele with higher levels.¹¹ ApoE polymorphisms do not appear to be an important risk factor for development of carotid plaque¹² but previous literature has demonstrated a significant relationship between carrier status of the ε4 allele and cIMT.^{13,14} However, the investigations of APOE genotype in relation to cIMT have included predominantly Caucasian individuals. Only the Atherosclerosis Risk in Communities (ARIC) study included an African-American cohort, but lacked a significant inclusion of Hispanics.¹⁴ APOE allele frequencies differ significantly between race-ethnicities,^{13,15,16} and relationships between APOE and other health outcomes including Alzheimer's disease¹⁶ and cognitive decline¹⁷ vary by race, which underscores the importance of analyses in multi-ethnic populations.

In the present study, we sought to investigate the relationship between APOE genotype and cIMT in a tri-ethnic cohort of the Northern Manhattan Study (NOMAS), with a large, self-identified Hispanic representation. Given previous literature and the observation that Hispanics and blacks in NOMAS have a greater risk of stroke than whites,¹⁸ we expected APOE-ε4 carrier status to be associated with increased cIMT and plaque burden in these race-ethnic groups.

Methods

Study Participants

A subsample of participants in the Northern Manhattan Study (NOMAS), an ongoing, prospective, population-based study of stroke incidence and vascular risk factors, were enrolled in the Oral Infections and Vascular Disease Epidemiology Study (INVEST) and had cIMT assessed for the purpose of both studies. A total of 1,243 participants had available data for APOE genotype and cIMT/plaque measurements using high-resolution two-dimensional (2D) carotid ultrasound imaging. Details on subject ascertainment, extensive assessments, and methods used in NOMAS and INVEST are described elsewhere.^{19,20} NOMAS and INVEST are approved by the IRBs of the Columbia University Medical Center and the University of Miami. All subjects signed written informed consent for participation.

Baseline Evaluation

Data were collected through interviews with trained bilingual research assistants in English or Spanish. Physical and neurological examinations were conducted by study neurologists. Race-ethnicity was based upon self-identification through a series of questions modeled after the US census and conforming to standard definitions outlined by Directive 15. The ethnic label “Hispanic” was defined as “A person of Mexican, Puerto Rican, Cuban, Central or South American or other Spanish culture or origin, regardless of race.”²¹ Standardized questions were adapted from the Behavioral Risk Factor Surveillance System by the Centers for Disease Control regarding hypertension, diabetes, smoking, and cardiac conditions.²² Blood pressure (BP) was measured with mercury sphygmomanometers and appropriately-sized cuffs. Hypertension was defined as a BP >140/90 mmHg (based on the average of two measurements during one sitting), the patient’s self-reported hypertension, or use of antihypertensive medications. Diabetes mellitus was defined by the patient’s self-reported diabetes, use of insulin or oral anti-diabetic medications, or fasting glucose ≥ 126 mg/dl. The fasting lipid profile was measured at enrollment. Body mass index (BMI) was calculated as weight in kg/(height in meters)².

Carotid Ultrasound

High-resolution B-mode ultrasound imaging (GE LogIQ 700, 9-to 13-MHz linear-array transducer) was performed according to the standardized scanning and reading protocols by trained and certified sonographers as previously described.²⁴ Presence of carotid plaque was defined as a focal wall thickening or protrusion in the lumen more than 50% greater than the surrounding thickness. Carotid plaque areas were measured with the automated computerized edge tracking software program M’Ath (M’Ath Inc, Paris, France).⁷ cIMT was measured in areas without plaque. cIMT was calculated as a composite of 12 separate mean IMT measures from the near and the far walls of the common carotid artery (CCA), bifurcation (BIF), and internal carotid artery (ICA) bilaterally. The mean of the mean measurements from each carotid site in mm is used as the outcome. We also examined cIMT in the bifurcation and ICA exclusively and cIMT in the CCA exclusively. The high reliability of cIMT measurements in our laboratory was reported previously.²³

APOE Genotyping

APOE $\epsilon 2$, $\epsilon 3$, and $\epsilon 4$ alleles were determined by two single-nucleotide polymorphisms (SNPs), i.e. *rs429358* and *rs7412*. The two SNPs were genotyped using TaqMan® allelic discrimination assays (Applied Biosystems, Foster City, CA) following manufacturer's instructions.

Statistical Evaluation

Allele frequencies were estimated by gene counting and the Hardy-Weinberg equilibrium was tested by a Chi-square goodness-of-fit test. The participants were classified into APOE- $\epsilon 4$ carriers and non-carriers. First we examined the relation of APOE- $\epsilon 4$ to demographics, vascular risk factors, carotid IMT, plaque presence and plaque area among all participants using a t-test for continuous variables and a Chi-square test for categorical variables. For total and segment-specific cIMT analysis, two multivariable linear regression models were constructed as follows: model 1) adjusted for age, sex, smoking, alcohol drinking, BMI, hypertension, and diabetes (and race-ethnicity if applicable); and model 2) model 1 + LDL-C level and lipid-lowering medication use. After exploring the global relationship between APOE and IMT, we investigated the possible differences between the groups for effect modification. APOE- $\epsilon 4$ carrier status was assessed in relation to plaque presence (yes or no) as well as total plaque area (mm^3) among those with plaque, utilizing the same regression models 1 and 2. cIMT and plaque analyses were stratified by race-ethnic groups.

Results

Figure 1 depicts APOE allele distributions, stratified by race-ethnic group. APOE- $\epsilon 4$ allele frequencies were highest among blacks, followed by Hispanics, and whites. The characteristics of the study population are represented in Table 1. Among the 1,243 participants (mean age 69 ± 9 , 38% male, 65% Hispanic, 18% non-Hispanic black, 17% non-Hispanic white) the mean total IMT was 0.71 ± 0.08 mm and 55% had plaque. Individuals with the $\epsilon 4$ allele had significantly higher LDL levels than those without the $\epsilon 4$ allele ($p=0.0003$). The total cIMT for those with the $\epsilon 4$ allele was 0.72 ± 0.09 mm (0.74 ± 0.10 mm for CCA, 0.65 ± 0.10 mm for ICA, and 0.76 ± 0.11 mm for BIF) and for those without the $\epsilon 4$ allele was 0.71 ± 0.08 mm (0.73 ± 0.09 mm for CCA, 0.65 ± 0.08 mm for ICA, and 0.74 ± 0.10 mm). The difference in cIMT was significant between APOE- $\epsilon 4$ carriers and non-carriers for total cIMT ($p=0.02$), CCA ($p=0.009$) and BIF ($p=0.03$), but not for ICA ($p=0.24$). The presence of carotid plaque was not significantly different between $\epsilon 4$ carriers and non-carriers ($p=0.26$); neither was plaque area ($p=0.90$) among those with plaque. Independent cardiovascular risk factors including smoking, alcohol consumption, hypertension, diabetes, age, and BMI were not significantly different between the two allele groups.

Associations between APOE- $\epsilon 4$ carrier status and segment-specific IMT measurements, broken down by total study population and individual race-ethnic groups, are represented in Table 2. In Model 1 analyses APOE- $\epsilon 4$ carrier status was significantly associated with total IMT ($p=0.009$), CCA-IMT ($p=0.01$), and BIF-IMT ($p=0.01$) but not ICA-IMT ($p=0.24$). Associations between $\epsilon 4$ carrier status and cIMT measurements by individual race-ethnic

group were only significant among blacks, specifically for total IMT ($p=0.03$) and CCA-IMT ($p=0.02$). In model 2 analysis, all associations between $\epsilon 4$ carrier status and segment-specific IMT measurements remained significant among the total study population, but were no longer significant among the black group. No significant associations were found among the Hispanic subgroup.

Table 3 represents analyses between APOE- $\epsilon 4$ carrier status and plaque assessments, employing the same regression models 1 and 2. The $\epsilon 4$ allele was not associated with plaque presence or plaque area among the total study population or individually among race-ethnic groups.

Diabetes was found to be an effect modifier for the association between APOE- $\epsilon 4$ and CCA IMT, as well as ICA IMT. CCA and ICA IMT values across APOE- $\epsilon 4$ carrier status, stratified by diabetes are listed in Figure 2. Multivariate analysis adjusted for covariates listed in Model 1. The p -value for the overall diabetes-APOE4 interaction in CCA was $p=0.045$ and in ICA was $p=0.046$. This interaction was not significant in BIF or Total IMT ($p=0.411$ and $p=0.067$, respectively). Age had a significant interaction with APOE- $\epsilon 4$ only in ICA IMT ($p=0.017$).

Discussion

The present investigation found an association between APOE- $\epsilon 4$ and cIMT in the total study population independent of demographics and vascular risk factors, including plasma LDL levels. The cIMT association was segment-specific: APOE- $\epsilon 4$ carriers had greater wall thickness in the common carotid artery and carotid bifurcation. Diabetes modified the relationship between APOE- $\epsilon 4$ and IMT. Many studies have corroborated the association between APOE- $\epsilon 4$ and total cIMT,^{13–14, 25} while others did not.^{26–28} The discrepancy between these studies may be influenced by race-ethnic composition of study populations, sample sizes, and inclusion of lipid parameters in regression analysis.¹³ However, a meta-analysis of genetic contributions to cIMT found that, among many candidate genes, only APOE- $\epsilon 4$ had a convincing association with cIMT in larger study populations.²⁹

The present study was of modest sample size but benefited from significant inclusion of Hispanics and blacks which raised the population of $\epsilon 4$ carriers (Fig. 1). It should be cautioned that self-reported ethnic and race labels in our study were based on sociologic definitions which were not designed for genetics research and therefore any genetic conclusions to be drawn from our subpopulations are fundamentally imperfect. A large proportion of the Hispanic population in our study were Caribbean Hispanics, mostly Dominicans, who, genetically, have greater African ancestry than other Hispanic subgroups. However, The Race, Ethnicity, and Genetics Working Group still supports a selective use of racial and ethnic labels in genetic association studies, whereby researchers believe that the differing experience of their self-selected subpopulations reveal interactions between “particular alleles and environmental factors that may influence health.”³⁰ Given that APOE allele frequencies differed significantly among our study subpopulations and that genetic researchers have found extraordinary correlation between self-identified race-ethnicity and

genetic cluster categories,³¹ we deemed it appropriate to proceed, albeit with caution, in genetic subgroup analysis.

A significant interaction between diabetes and APOE-ε4 was found in CCA IMT and ICA IMT, but not in BIF or total IMT. The Framingham Offspring Study found a significant relationship between APOE-ε4 and ICA IMT only among diabetic men.²⁸ APOE-ε4 has been established as a risk factor for higher cIMT values in individuals with early stage diabetes.³² Other studies have found an interaction between APOE-ε4 and diabetes in the prevalence of peripheral arterial disease,³³ and Alzheimer's Disease,³⁴ and between ε4 and obesity in fasting insulin and glucose levels.³⁵ Animal models suggest that APOE may play a role in energy metabolism and adipose tissue regulation,³⁶ and ε4 carriers are at higher risk of metabolic syndrome.³⁷ Taken together, these results suggest that APOE-ε4 and diabetes in combination may produce an advanced risk for an atherogenic state. Future studies should investigate the APOE-ε4 effect modification on the role of cIMT as a predictor of vascular events in diabetics.

A previous study of Hispanics in NOMAS found that segmental cIMTs were under different genetic controls, based on variation in anatomy, hemodynamics, and pathogenesis of atherosclerosis between the carotid artery segments.³⁸ However, in our study, race-ethnicity did not significantly modify the ε4-IMT relationship (p=0.47 for total IMT, p=0.30 for CCA, p=0.62 for ICA, and p=0.72 for BIF).

Our data demonstrate a segment-specific nature of the APOE-cIMT relationship. A greater influence of APOE-ε4 on CCA-IMT values versus ICA-IMT has been reported elsewhere,³⁹ although no mechanistic explanation has been provided. ApoE is pleiotropic in function, including immunomodulatory capacities⁴⁰ as well as functioning as an inhibitor of smooth muscle cell proliferation in the presence of platelet derived growth factor through binding of the LDL-receptor.⁴¹ CCA-IMT mainly represents changes in medial smooth muscle cells and matrix deposition while ICA-IMT is more influenced by focal plaques.⁴² ApoE's role in regulating smooth muscle proliferation could represent a pathway underlying the segment-specific nature of the ApoE-cIMT relationship as well as the broader impact of ApoE on carotid arterial wall thickness outside of lipid metabolism. Further, higher expression of hydroperoxidases in diabetics could increase oxidization of APOE,⁴³⁻⁴⁴ which presumably weakens APOE binding to the LDL-receptor.⁴³ However, at this time, mechanistic details regarding the segment-specific nature of APOE-ε4's effect on IMT remain speculative.

We did not find an association between APOE-ε4 and presence of carotid plaque or plaque area. This finding is consistent with other studies.^{12, 45} Carotid IMT and carotid plaque represent two distinct phenotypes of atherosclerosis. Plaque forms as a result of chronic inflammatory events and immune cell infiltration into arterial walls,⁴⁶ while cIMT mainly represents pathological hypertrophy of the medial layer.² cIMT may serve as a surrogate marker of early atherosclerosis⁶ rather than a bonafide manifestation of atherosclerosis.⁴⁷ Carotid plaque and cIMT both have significant heritability,^{7,8} but genetic influences appear to be stronger in cIMT, whereas environmental factors play a greater role in plaque.⁶

Strengths of this study include its multi-ethnic population-based design, a systematic collection of vascular risk factors, adjustment for plasma LDL levels, and segment-specific cIMT analysis. Study participants lived in the same limited geographic area within northern Manhattan, which minimizes some of the environmental heterogeneity that would be incurred in multiregional cohort studies. In terms of limitations, the majority of our study population is older with a high burden of vascular risk factors. As such, our results may not be applicable to younger and healthier individuals. Finally, sample size may have limited statistical evaluations among individual race-ethnic groups.

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Figure 1.
APOE Allele Distributions.

Sample	APOE		
	ε2	ε3	ε4
White	6.2%	84.7%	9.1%
Black	11.1%	67.5%	21.4%
Hispanic	5.8%	81.1%	13.1%
All	6.8%	79.2%	14.0%

Figure 2.
Interaction between diabetes and APOE- ϵ 4 in Common Carotid and Internal Carotid Arteries.

Table 1

Population study characteristics

Characteristics	Total (n=1243)	Non-carrier (n=934)	e4 carrier (n=309)	p
Sex, n (%)				0.49
Female	765 (62)	580 (62)	185 (60)	
Male	478 (38)	354 (38)	124 (40)	
Race-ethnicity, n (%)				<0.0001
NH-White	209 (17)	174 (18)	35 (11)	
NH-Black	229 (18)	146 (16)	83 (27)	
Hispanic	805 (65)	614 (66)	191 (62)	
Moderate alcohol drinking, n (%)				0.28
No	744 (60)	551 (59)	193 (62)	
Yes	499 (40)	383 (41)	116 (38)	
Smoking, n (%)				0.38
Never	607 (49)	463 (50)	144 (47)	
Former	442 (35)	322 (34)	120 (39)	
Current	194 (16)	149 (16)	45 (14)	
Hypertension, n (%)				0.62
No	404 (33)	300 (32)	104 (34)	
Yes	839 (67)	634 (68)	205 (66)	
Diabetes, n (%)				0.63
No	1006 (81)	753 (81)	253 (82)	
Yes	237 (19)	181 (19)	56 (18)	
Blood Pressure Rx, n (%)				
No	751 (60)	569 (61)	182 (59)	0.53
Yes	492 (40)	365 (39)	127 (41)	
Diabetes Rx, n (%)				0.99
No	1090 (88)	819 (88)	271 (88)	
Yes	153 (12)	115 (12)	38 (12)	
Lipids Rx, n (%)				
No	1047 (84)	790 (85)	257 (83)	0.56
Yes	196 (16)	144 (15)	52 (17)	
Age (y), Mean $\pm$ SD	69 $\pm$ 9	69 $\pm$ 9	69 $\pm$ 9	0.90
BMI (kg/m ²), Mean $\pm$ SD	28 $\pm$ 5	28 $\pm$ 5	28 $\pm$ 5	0.72
SBP (mmHg), Mean $\pm$ SD	140 $\pm$ 20	140 $\pm$ 19	141 $\pm$ 22	0.65
DBP (mmHg), Mean $\pm$ SD	83 $\pm$ 11	83 $\pm$ 10	83 $\pm$ 11	0.55
BS (mg/dL), Mean $\pm$ SD	102 $\pm$ 41	100 $\pm$ 37	105 $\pm$ 51	0.11
LDL-C (mg/dL), Mean $\pm$ SD	128 $\pm$ 35	126 $\pm$ 35	135 $\pm$ 37	0.0003
HDL-C (mg/dL), Mean $\pm$ SD	47 $\pm$ 15	47 $\pm$ 15	46 $\pm$ 15	0.13
Total IMT(mm), Mean $\pm$ SD	0.71 $\pm$ 0.08	0.71 $\pm$ 0.08	0.72 $\pm$ 0.09	0.02
CCA-IMT(mm), Mean $\pm$ SD	0.73 $\pm$ 0.10	0.73 $\pm$ 0.09	0.74 $\pm$ 0.10	0.009
ICA-IMT(mm), Mean $\pm$ SD	0.65 $\pm$ 0.09	0.65 $\pm$ 0.08	0.65 $\pm$ 0.10	0.24

Characteristics	Total (n=1243)	Non-carrier (n=934)	e4 carrier (n=309)	p
BIF-IMT(mm), Mean $\pm$ SD	0.75 $\pm$ 0.10	0.74 $\pm$ 0.10	0.76 $\pm$ 0.11	0.03
Carotid plaque, n (%)				0.26
No	561 (45)	430 (46)	131 (42)	
Yes	682 (55)	504 (54)	178 (58)	
Carotid plaque area [#] (mm ³), Median (IQR)	13.55 (6.96-27.64)	13.39 (6.94-28.83)	13.82 (6.96-25.50)	0.90

[#] Among those (n=682) with carotid plaque.

Table 2

Association of APOE-ε4 carrier status with carotid IMT assessments

Carotid IMT assessment	Sample	Model 1		Model 2	
		Difference (95% CI) for ε4 carrier vs. non-carrier	P	Difference (95% CI) for ε4-carrier vs. non-carrier	P
Total IMT	All	0.013 (0.003, 0.023)	0.009	0.013 (0.003, 0.023)	0.01
	White	0.013 (−0.017, 0.042)	0.41	0.019 (−0.011, 0.049)	0.21
	Black	0.023 (0.002, 0.044)	0.03	0.020 (−0.002, 0.041)	0.07
	Hispanic	0.010 (−0.002, 0.022)	0.11	0.009 (−0.003, 0.022)	0.15
P for interaction			0.54		0.47
CCA-IMT	All	0.015 (0.003, 0.026)	0.01	0.014 (0.003, 0.026)	0.02
	White	0.016 (−0.017, 0.048)	0.35	0.021 (−0.012, 0.054)	0.22
	Black	0.029 (0.004, 0.054)	0.02	0.023 (−0.002, 0.048)	0.07
	Hispanic	0.009 (−0.005, 0.023)	0.21	0.009 (−0.005, 0.023)	0.20
P for interaction			0.38		0.30
ICA-IMT	All	0.007 (−0.005, 0.018)	0.24	0.007 (−0.005, 0.018)	0.25
	White	0.014 (−0.022, 0.050)	0.44	0.022 (−0.014, 0.058)	0.23
	Black	0.010 (−0.012, 0.031)	0.39	0.009 (−0.013, 0.032)	0.42
	Hispanic	0.004 (−0.010, 0.018)	0.55	0.003 (−0.010, 0.017)	0.68
P for interaction			0.80		0.62
BIF-IMT	All	0.017 (0.004, 0.030)	0.01	0.017 (0.004, 0.030)	0.01
	White	0.014 (−0.026, 0.053)	0.49	0.018 (−0.021, 0.057)	0.36
	Black	0.026 (−0.001, 0.053)	0.06	0.024 (−0.004, 0.051)	0.10
	Hispanic	0.015 (−0.001, 0.030)	0.07	0.014 (−0.002, 0.030)	0.08
P for interaction			0.78		0.72

Model 1: adjusted for age, sex, smoking, alcohol drinking, BMI, hypertension, diabetes, and race-ethnicity if applicable.

Model 2: adjusted for the covariates in model 1 and additionally adjusted for LDL-C level and lipid-lowering medication use.

Table 3

Association of APOE-ε4 carrier status with carotid plaque assessments

Carotid plaque	Sample	Model 1			Model 2		
		OR (95% CI)	ε4 carrier vs. non-carrier	p	OR (95% CI)	ε4 carrier vs. non-carrier	p
Plaque presence	All	1.23 (0.93, 1.62)		0.15	1.16 (0.87, 1.54)		0.31
	NH-White	1.35 (0.57, 3.25)		0.50	1.50 (0.60, 3.76)		0.39
	NH-Black	1.38 (0.76, 2.53)		0.29	1.33 (0.72, 2.49)		0.37
	Hispanic	1.16 (0.82, 1.63)		0.42	1.06 (0.74, 1.51)		0.75
Plaque area [#]		Beta (95% CI) ε4 carrier vs. non-carrier			Beta (95% CI) for APOE-ε4 vs. Non-ε4		
	All	0.00 (−0.13, 0.13)		0.99	−0.01 (−0.14, 0.12)		0.89
	NH-White	−0.10 (−0.43, 0.23)		0.55	−0.13 (−0.46, 0.21)		0.46
	NH-Black	0.04 (−0.23, 0.32)		0.76	0.02 (−0.27, 0.31)		0.89
	Hispanic	−0.01 (−0.16, 0.15)		0.87	−0.01 (−0.17, 0.15)		0.90

Model 1: adjusted for age, sex, smoking, alcohol drinking, BMI, hypertension, diabetes, and race-ethnicity if applicable.

Model 2: adjusted for the covariates in model 1 and additionally adjusted for LDL-C level and lipid-lowering medication use.

[#] cubic root transformed only among those with plaque (n=696).