



## Original Contribution

# Apolipoprotein E Polymorphisms Predict Low Density Lipoprotein Cholesterol Levels and Carotid Artery Wall Thickness but Not Incident Coronary Heart Disease in 12,491 ARIC Study Participants

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Elevated levels of low density lipoprotein (LDL) cholesterol is a well-established risk factor for cardiovascular disease, and recent advancements have provided evidence that carotid artery intima-media thickness (IMT) is associated with increased occurrence of cardiovascular events. Apolipoprotein E (ApoE) has been widely studied in regard to its role in lipid transport and metabolism, but the role that ApoE genetic variation plays in relation to carotid artery IMT and risk of incident coronary heart disease remains a subject of debate. In 1987–2001, the authors examined the effect of each *ApoE* allele ( $\epsilon$ 2,  $\epsilon$ 3,  $\epsilon$ 4) on LDL cholesterol and carotid IMT, as well as the association with coronary heart disease risk, in 12,491 participants of the US Atherosclerosis Risk in Communities Study. *ApoE*  $\epsilon$ 2,  $\epsilon$ 3, and  $\epsilon$ 4 allele frequencies were determined, respectively, in Whites (0.08, 0.77, 0.15) and African Americans (0.11, 0.67, 0.22). These alleles did not predict incident coronary heart disease in either racial group. The *ApoE*  $\epsilon$ 2 allele was associated with lower LDL cholesterol and the  $\epsilon$ 4 allele with higher LDL cholesterol in both Whites and African Americans. The *ApoE*  $\epsilon$ 2 and  $\epsilon$ 4 alleles were associated with carotid IMT measures in both racial groups, but, after adjusting for lipid parameters, only the  $\epsilon$ 4 allele was associated with carotid IMT measures in African Americans.

apolipoproteins E; carotid arteries; coronary disease; lipoproteins, LDL cholesterol; polymorphism, genetic

Abbreviations: ApoE, apolipoprotein E; ARIC, Atherosclerosis Risk in Communities; CHD, coronary heart disease; HDL, high density lipoprotein; IMT, intima-media thickness; LDL, low density lipoprotein.

Among the numerous genetic and environmental risk factors for atherosclerosis and coronary heart disease (CHD), including smoking, hypertension, and diabetes, the association between elevated low density lipoprotein (LDL) cholesterol levels and cardiovascular risk has been well established over the past several decades. LDL particles are taken up by the vessel wall, and the cholesterol from the LDL becomes

an integral component of the atherosclerotic lesion (1, 2). There are increasing interest and advances in noninvasive assessment of atherosclerosis, including B-mode ultrasound assessment of carotid artery intima-media thickness (IMT) (3). Previous studies have shown that increased carotid IMT is a marker of atherosclerotic disease, with associations detected between carotid IMT and traditional cardiovascular

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risk factors (4–7), as well as evidence that higher carotid IMT measures are associated with increased occurrence of cardiovascular events (8–11).

Apolipoprotein E (ApoE) plays an important role in lipid transport and metabolism. Three common alleles ( $\epsilon 2$ ,  $\epsilon 3$ , and  $\epsilon 4$ ) of the *ApoE* gene code for the three major ApoE isoforms (E2, E3, and E4), which differ in sequence at amino acid positions 112 and 158. Allelic variation within the *ApoE* gene has been shown to account for as much as 7 percent of the interindividual variability in LDL and total cholesterol, with multiple studies in human populations demonstrating associations of the  $\epsilon 4$  allele with increases in LDL cholesterol and of the  $\epsilon 2$  allele with decreased LDL cholesterol levels (12–16). Beyond effects on lipid metabolism, the role that coding variation within the *ApoE* gene plays with regard to carotid artery IMT and risk of incident CHD remains a subject of debate across genetic epidemiologic studies (17–21). A recent meta-analysis compiling results from 48 studies evaluating both incident and prevalent CHD concluded that, compared with carriers of the  $\epsilon 3/\epsilon 3$  genotype, carriers of the  $\epsilon 4$  allele had a significantly higher risk of CHD whereas the  $\epsilon 2$  allele had no significant association with CHD risk (22). Song et al. (22) revealed that results from independent studies often were not consistent because of differences in geographic settings, ethnic backgrounds, CHD endpoints, overall study design, and inadequate statistical power or consideration of potential gene-environment interactions. Additionally, the majority of previous studies were cross-sectional in design and were small, involving populations of less than 1,000 subjects.

In the present study, we investigated the effect of each *ApoE* allele on LDL cholesterol levels and carotid artery IMT, as well as the association with CHD risk in the prospective Atherosclerosis Risk in Communities (ARIC) Study cohort. The large sample size of the ARIC Study cohort allows a valuable opportunity for in-depth analysis of *ApoE* allele effects in both African Americans and Whites.

## MATERIALS AND METHODS

Study participants were selected from the ARIC Study, a prospective investigation of atherosclerosis and its clinical sequelae involving 15,792 persons aged 45–64 years at recruitment (1987–1989). Subjects were selected by probability sampling from four US communities: Forsyth County, North Carolina; Jackson, Mississippi; the northwestern suburbs of Minneapolis, Minnesota; and Washington County, Maryland. A detailed description of the ARIC Study design, methods, and sampling procedures is published elsewhere (23, 24).

Briefly, approximately 75 percent of age-eligible persons in each community completed a home interview. In three of the communities, 86–88 percent of those who took part in the home interview also completed the clinic examination, whereas only 65 percent did so in Jackson, Mississippi. Among White participants, response rates were similar for men and women and between communities. Each field center's institutional review board approved the study, and each participant provided their written informed consent at each examination.

Participants underwent a baseline clinic examination in 1987–1989, were examined every 3 years thereafter, and were interviewed annually by telephone. Details of classification of CHD events have been published previously (25). Incident CHD cases were defined by a definite or probable myocardial infarction, a silent myocardial infarction between examinations detected by electrocardiogram, a definite CHD death, or a coronary revascularization procedure. Exclusions from analyses ( $n = 2,868$ ) were made for missing phenotype (LDL cholesterol, carotid IMT, and/or incident CHD) information, history of stroke or CHD at study baseline, participant-requested restriction of DNA usage, and race other than African American or White. Additional exclusions were made for missing genotype information ( $n = 433$ ). Following these exclusions, data on 3,187 African Americans and 9,304 Whites were included in the present analyses.

Seated blood pressure measurements were taken three times with a random-zero sphygmomanometer, and the last two measurements were averaged. Hypertension was defined as systolic blood pressure of  $\geq 140$  mmHg or diastolic blood pressure of  $\geq 90$  mmHg, or current use of antihypertensive medications. Diabetes was defined by a fasting glucose level of  $\geq 126$  mg/dl, a nonfasting glucose level of  $\geq 200$  mg/dl, and/or a history of or treatment for diabetes. Cigarette smoking status was analyzed by comparing current smokers with participants who had formerly or never smoked. Body mass index ( $\text{kg}/\text{m}^2$ ) was calculated from height and weight measurements. Plasma total cholesterol was measured by an enzymatic method (26), and LDL cholesterol was calculated (for participants with triglyceride levels of  $>400$  mg/dl, LDL cholesterol measurements were set to missing) (27). High density lipoprotein (HDL) cholesterol was measured after dextran-magnesium precipitation of non-HDL lipoproteins (28). The percentage of persons using cholesterol-lowering medication at baseline was 2 percent. Carotid artery IMT was determined by high-resolution B-mode ultrasound, as described previously (29, 30). The reliability coefficient for mean carotid IMT was 0.67, estimated from repeat measurements at three visits, 7–14 days apart in 36 volunteers from each of the four ARIC field centers (31). The estimated site-specific reliability coefficients appropriate to the ARIC study population were 0.77, 0.73, and 0.70 for mean carotid far-wall IMT at the carotid bifurcation and the internal and common carotid arteries, respectively (31).

Genotyping of the *ApoE* polymorphisms at codons 112 and 158 in exon 3 was performed by using the TaqMan assay (Applied Biosystems, Foster City, California). For each polymorphism, forward primers included 5'-GGGCACGGCTGTCCAA-3' for codon 112 and 5'-TCCCACCTGCGCAAGCT-3' for codon 158, and reverse primers included 5'-CCTCGCCGCG-GTACTG-3' for codon 112 and 5'-GCACGCGGCC-CTGTT-3' for codon 158. Sequence-specific labeled probes used in the allele discrimination assay were as follows: 5'-6FAM-CGGCCGCAC-ACGTCCTCC-TAMRA-3' and 5'-VIC-CGGCC-GCGCACGTCCT-TAMRA-3' for the codon 112 polymorphism, and 5'-6FAM-CCTGCAGA-AGCGCC-TGGCA-TAMRA-3' and 5'-VIC-GACCTGC-AGAAGTGCCTGGCAGT-TAMRA-3' for the codon 158 polymorphism. Allele detection and genotype calling were

**TABLE 1. Baseline characteristics† of the ARIC‡ Study population by race and ApoE‡ genotype, United States, 1987–1989**

ApoE genotype	No.	Model 1§					Model 2¶: carotid IMT‡ (mm)	Model 3#: carotid IMT (mm)
		Body mass index (kg/m <sup>2</sup> )	HDL cholesterol (mg/dl)	LDL cholesterol (mg/dl)	Triglycerides (mmol/liter)	Carotid IMT (mm)		
<b>Whites</b>								
ε2/ε2, ε2/ε3, ε2/ε4	1,459	26.9 (0.1)	52.6 (0.3)*	125.6 (0.8)**	1.46 (0.01)	0.723 (0.003)*	0.722 (0.003)*	0.729 (0.003)
ε3/ε3	5,534	26.8 (0.05)	51.6 (0.2)	135.7 (0.4)	1.44 (0.008)	0.733 (0.002)	0.733 (0.002)	0.734 (0.002)
ε3/ε4, ε4/ε4	2,311	26.6 (0.08)	50.7 (0.3)*	145.7 (0.7)**	1.43 (0.01)	0.743 (0.003)*	0.744 (0.003)*	0.739 (0.003)
<b>African Americans</b>								
ε2/ε2, ε2/ε3, ε2/ε4	634	29.6 (0.2)*	57.6 (0.6)*	124.0 (1.4)**	1.20 (0.02)	0.722 (0.005)*	0.720 (0.005)*	0.725 (0.005)
ε3/ε3	1,427	29.2 (0.1)	56.0 (0.3)	135.0 (0.7)	1.20 (0.01)	0.734 (0.003)	0.734 (0.003)	0.734 (0.003)
ε3/ε4, ε4/ε4	1,126	28.8 (0.1)*	54.5 (0.5)*	146.0 (1.1)**	1.21 (0.02)	0.746 (0.004)*	0.74 (0.004)*	0.744 (0.004)*

\*  $p < 0.05$ , ε3/ε3 as the referent; \*\* $p < 0.00001$ , ε3/ε3 as the referent.

† All values are presented as mean (standard error of the mean).

‡ ARIC, Atherosclerosis Risk in Communities; ApoE, apolipoprotein E gene; IMT, intima-media thickness.

§ Adjusted for age and gender.

¶ Adjusted for age, gender, body mass index, and smoking, diabetes, and hypertension status.

# Adjusted for age; gender; body mass index; smoking, diabetes, and hypertension status; total triglycerides; high density lipoprotein (HDL) cholesterol; and low density lipoprotein (LDL) cholesterol.

performed by using ABI 7900 and Sequence Detection System software (Applied Biosystems). The ARIC Study has extensive quality control measures for all genotyping assays, including but not limited to robotic liquid handling, separate pre- and post-polymerase chain reaction areas, standard protocols and quality control analyses, a blind duplicate program, positive and negative controls, computerized sample tracking, and data validity checks.

All statistical analyses were conducted by using Stata version 8.0 software (Stata Corporation, College Station, Texas), and data for African Americans and Whites were evaluated separately. Allele frequencies were estimated by gene counting. Hardy-Weinberg equilibrium expectations were tested by using a  $\chi^2$  goodness-of-fit test. Proportions, means, and standard errors of the mean for established cardiovascular risk factors were calculated. Multiple linear regression models were used to assess the relation between ApoE genotype and baseline LDL cholesterol level, as well as baseline carotid IMT. ApoE genotypes were coded into three groups for analysis: ε2 (ε2/ε2, ε2/ε3, ε2/ε4), ε3 (ε3/ε3), and ε4 (ε3/ε4, ε4/ε4). In each model, the homozygous ε3/ε3 genotype served as the referent group. The ε2/ε4 genotype was included with the ε2 group because, in preliminary analyses, LDL cholesterol levels for this genotype were more similar to the ε2/ε2 and ε2/ε3 genotypes as opposed to the ε3/ε4 and ε4/ε4 genotypes (data not shown). Cox proportional hazards models were used to estimate the hazard rate ratios for incident CHD. For analyses of incident CHD cases, follow-up time intervals were defined as the time between the initial clinic visit and the date of the first CHD event. For noncases, follow-up continued until December 31, 2001, the date of death, or the date of last contact if lost to follow-up, whichever occurred first. Covariates evaluated in the Cox proportional hazards models included age, gender, HDL cholesterol, LDL cholesterol, total triglycerides,

and body mass index, as well as diabetes, smoking, and hypertension status. Covariates were assessed for statistical significance in the models by using the Wald  $\chi^2$  statistic.

## RESULTS

In accordance with previous studies, ε3/ε3 was the most prevalent ApoE genotype in both Whites and African Americans (0.59 and 0.45, respectively), followed by ε3/ε4 (0.23 and 0.30), ε2/ε3 (0.13 and 0.14), ε2/ε4 (0.02 and 0.05), ε4/ε4 (0.02 and 0.04), and ε2/ε2 (0.01 and 0.01). As noted from these frequencies, the ε2 and ε4 alleles are both more commonly observed in African Americans (0.11 and 0.22, respectively) than in Whites (0.08 and 0.15, respectively), and the ε3 allele is more frequent in Whites (0.77) compared with African Americans (0.67); each of these individual allele frequency differences between racial groups was statistically significant ( $p < 0.00001$ ).

Study population characteristics are presented in table 1, subdivided by race and ApoE genotype group. For model 1 analyses in which adjustments were made for age and gender, both Whites and African Americans with the ε2 allele had, respectively, higher HDL cholesterol levels ( $p = 0.006$ ,  $p = 0.02$ ), lower LDL cholesterol levels ( $p < 0.0001$ ,  $p < 0.0001$ ), and lower carotid IMT measures ( $p = 0.009$ ,  $p = 0.04$ ) than carriers of the ApoE ε3/ε3 genotype. Also in model 1 analyses, both Whites and African Americans with the ε4 allele had, respectively, lower HDL cholesterol levels ( $p = 0.002$ ,  $p = 0.006$ ), higher LDL cholesterol levels ( $p < 0.0001$ ,  $p < 0.0001$ ), and higher carotid IMT measures ( $p = 0.004$ ,  $p = 0.01$ ).

For model 2 analyses, in which adjustments were made for age, gender, body mass index, and smoking, diabetes, and hypertension status, both the ε2 and the ε4 allele groups

**TABLE 2. ApoE\* genotype and allele frequencies for incident CHD\* cases and noncases in the ARIC\* Study population, by race, United States, 1987–2001**

	Whites					African Americans				
	Incident CHD cases		Noncases		<i>p</i> value†	Incident CHD cases		Noncases		<i>p</i> value†
	No.	%	No.	%		No.	%	No.	%	
<i>ApoE</i> genotype										
ε2/ε2	11	1	58	1	0.3	2	1	36	1	0.4
ε2/ε3	119	12	1,054	13		31	12	403	14	
ε2/ε4	18	2	199	2		19	7	143	5	
ε3/3	602	59	4,932	59		112	42	1,315	45	
ε3/4	241	24	1,880	23		89	33	898	31	
ε4/4	26	2	164	2		14	5	125	4	
<i>ApoE</i> allele										
ε2	159	8	1,369	8	0.5	54	10	618	11	0.2
ε3	1,564	77	12,798	77		344	64	3,931	67	
ε4	311	15	2,407	15		136	26	1,291	22	

\* *ApoE*, apolipoprotein E gene; CHD, coronary heart disease; ARIC, Atherosclerosis Risk in Communities.

† Overall *p* value for *ApoE* genotypes and *ApoE* alleles.

remained associated with carotid IMT measures in Whites ( $\epsilon 2 p = 0.003$ ,  $\epsilon 4 p = 0.003$ ) and African Americans ( $\epsilon 2 p = 0.01$ ,  $\epsilon 4 p = 0.004$ ). For model 3 analyses, further adjustments were made for total triglycerides, HDL cholesterol, and LDL cholesterol in addition to the model 2 adjustment covariates to determine whether carotid IMT measures were mediated by alterations in lipid metabolism. Neither the  $\epsilon 2$  nor the  $\epsilon 4$  allele group remained associated with carotid IMT measures in Whites for model 3 ( $\epsilon 2 p = 0.2$ ,  $\epsilon 4 p = 0.1$ ). The association between the  $\epsilon 4$  allele and higher carotid IMT measures in African Americans remained after adjustments for lipid parameters (model 3) ( $\epsilon 4 p = 0.04$ ).

For neither Whites nor African Americans were *ApoE* genotype and allele frequencies significantly different be-

tween CHD cases and noncases (table 2). Although frequency of the  $\epsilon 4$  allele was observed to be higher in African-American incident CHD cases (0.26) compared with noncases (0.22), this difference was not statistically significant ( $p = 0.08$ ). Results from Cox proportional hazards models used to estimate the hazard rate ratios of incident CHD for persons with the  $\epsilon 2$  and  $\epsilon 4$  alleles are presented in table 3 by racial group. After adjustment for age and gender, neither the  $\epsilon 2$  nor the  $\epsilon 4$  allele groups were significant predictors of incident CHD in Whites or African Americans. Likewise, no associations were detected for Whites or African Americans for either the  $\epsilon 2$  or the  $\epsilon 4$  allele group after further adjustments for additional covariates and CHD risk factors. Analyses were also conducted

**TABLE 3. Cox regression models and hazard rate ratios for incident CHD\* in the ARIC\* Study population, by race, United States, 1987–2001**

<i>ApoE</i> * allele	Model 1†			Model 2‡		
	HRR*	95% CI*	<i>p</i> value	HRR	95% CI	<i>p</i> value
Incident CHD in Whites§						
ε2	1.00	0.84, 1.19	1.0	1.11	0.93, 1.33	0.2
ε4	1.07	0.94, 1.21	0.3	1.01	0.89, 1.14	0.9
Incident CHD in African Americans§						
ε2	0.89	0.63, 1.26	0.5	0.97	0.68, 1.38	0.9
ε4	1.13	0.91, 1.40	0.3	1.09	0.87, 1.36	0.5

\* CHD, coronary heart disease; ARIC, Atherosclerosis Risk in Communities; *ApoE*, apolipoprotein E gene; HRR, hazard rate ratio; CI, confidence interval.

† Adjusted for age and gender.

‡ Adjusted for age; gender; smoking, diabetes, and hypertension status; body mass index; low density lipoprotein cholesterol; high density lipoprotein cholesterol; and total triglycerides.

§ Referent group for analysis:  $\epsilon 3$ .

separately for males and females within each racial group, with no changes found in the results (data not shown).

## DISCUSSION

Although multiple published studies have analyzed associations of ApoE genetic variation, lipid levels, and CHD, the majority of these studies are small and at best comprise populations less than a third the size of our large cohort of over 12,000 participants. The present investigation showed that neither the  $\epsilon 2$  nor the  $\epsilon 4$  alleles of the *ApoE* gene are risk factors for incident CHD in Whites or African Americans. *ApoE* allele and genotype frequencies were similar to those previously reported in both racial groups (12, 32–35). Allele frequencies were significantly different between racial groups; the  $\epsilon 2$  and  $\epsilon 4$  alleles were observed more frequently in African Americans than in Whites.

We corroborated the well-established association between *ApoE* genotype and LDL cholesterol levels in both our White and African-American study populations, such that the  $\epsilon 2$  allele was associated with lower levels and the  $\epsilon 4$  allele with higher levels relative to the  $\epsilon 3/\epsilon 3$  genotype. We also observed a trend for increasing LDL cholesterol levels with increasing number of  $\epsilon 4$  alleles in both racial groups. Previous studies investigating variability in HDL cholesterol levels with *ApoE* genotype have led to mixed results (21, 32, 35, 36). In the present study, we observed lower levels of HDL cholesterol in the  $\epsilon 4$  allele group and higher levels of HDL cholesterol in the  $\epsilon 2$  allele group, compared with the  $\epsilon 3/\epsilon 3$  genotype, for both Whites and African Americans.

The potential link between *ApoE* genotype and carotid artery wall thickness has been the subject of debate, with some studies showing associations of the  $\epsilon 4$  allele with increased IMT and/or the  $\epsilon 2$  allele with decreased IMT measures (14, 36), some showing the  $\epsilon 2/\epsilon 3$  genotype to be associated with increased IMT measures (37), and others detecting no association between IMT and *ApoE* genotype (21, 33). One factor potentially influencing these discordant study results is the inclusion of lipid parameters in the analysis models, especially considering that *ApoE* genotypes are predictors of LDL cholesterol levels, and LDL cholesterol has been shown to be an independent predictor of IMT. For example, studies by Ilveskoski et al. (21) and Beilby et al. (33), both of which found no association between IMT and *ApoE* genotype, included LDL cholesterol in their multivariate analyses. Conversely, the association between IMT and *ApoE* genotype detected by Slooter et al. (36) resulted from regression models that did not include LDL cholesterol as a covariate.

In our investigation, the  $\epsilon 4$  allele was associated with carotid IMT measures in multiple analysis models for African Americans only. For Whites, thinner carotid IMT measures were associated with the  $\epsilon 2$  allele compared with the  $\epsilon 3/\epsilon 3$  genotype, and increased carotid IMT measures were associated with the  $\epsilon 4$  allele relative to the  $\epsilon 3/\epsilon 3$  genotype, but these associations disappeared after lipid parameters were included in the analysis model. As a result, the effects of the  $\epsilon 2$  and  $\epsilon 4$  alleles on carotid IMT levels in Whites are likely mediated by alterations in lipid metabolism. Similar

to the Whites, African Americans with the  $\epsilon 2$  allele had thinner carotid IMT measures compared with those with the  $\epsilon 3/\epsilon 3$  genotype, while the  $\epsilon 4$  allele was associated with increased carotid IMT measures. However, for African Americans, the  $\epsilon 4$  allele association remained after adjustments for lipid parameters; no association was detected with the  $\epsilon 2$  allele after adjusting for lipids. Therefore, in African Americans, the *ApoE*  $\epsilon 4$  allele may have an independent effect on carotid artery wall thickness. This finding is consistent with previous reports showing a decreased effect of lipoprotein parameters on cardiovascular disease risk in African Americans (38, 39).

Results of the present study show that genetic variation within the *ApoE* gene is not a risk factor for incident CHD in Whites or African Americans. This study also confirms that *ApoE* genotypes predict LDL cholesterol levels in both Whites and African Americans, and it determined the *ApoE*  $\epsilon 4$  allele to be a potential predictor of carotid IMT measures in African Americans. Differences in degree of control for cardiovascular risk factors (i.e., LDL cholesterol) may partially explain the inconsistent associations reported between *ApoE* genotypes and carotid IMT in previous studies. Furthermore, the majority of studies investigating the relation between *ApoE* genotype and carotid IMT have either focused on predominantly Caucasian populations or failed to evaluate the association independently by racial group.

In light of the documented relation between LDL cholesterol and CHD, it is important to reconcile the statistically significant effect of the *ApoE* polymorphism on LDL cholesterol levels and the reported absence of a statistically significant effect of the *ApoE* polymorphism on incident CHD. Three interacting explanations are likely. The first is insufficient statistical power. In these data, the *ApoE* polymorphism accounted for 2–3 percent of the interindividual variation in LDL cholesterol. Because the relation between LDL cholesterol and CHD is not absolute, the effect of the gene on CHD risk will be diluted. In other words, the power of the incident CHD analyses will be lower than the power of the LDL cholesterol analyses. Considering the frequency of the  $\epsilon 4$  allele observed here and a hazard ratio of 1.2 for the  $\epsilon 4$  allele relative to the  $\epsilon 3$  allele, the ARIC Study provides only 80 percent power to detect a significant relation at the  $p < 0.05$  level.

The second likely explanation is the dual association of the  $\epsilon 2$  allele with CHD. On the one hand, the  $\epsilon 2$  allele is associated with low LDL cholesterol, as observed again here. On the other hand, the same  $\epsilon 2$  allele is associated with type III hyperlipidemia (40). As a result, the expected risk-lowering effect of the  $\epsilon 2$  allele may be dampened in a large sample, such as ARIC.

The third likely explanation is the possibility of survival bias in the ARIC cohort. If the  $\epsilon 4$  allele only predisposed carriers to early CHD and the early, fatal CHD cases were not represented in the ARIC cohort, then the effect of the *ApoE* polymorphism on CHD would have already occurred before eligibility. To test this hypothesis, we examined the relation between the *ApoE* polymorphism and incident CHD before the age of 65 years ( $n = 571$  cases with genotype data). Indeed, when logistic regression was used in the sample of Whites, with age and sex as covariates, the  $\epsilon 4$  allele

was a significant predictor of early CHD (hazard rate ratio = 1.18, 95 percent confidence interval: 1.01, 1.39;  $p = 0.04$ ).

Strengths of the current study include its large cohort size, its average subject follow-up time of 13 years, and its inclusion of both Whites and African Americans. Limitations concern multiple testing; therefore, we were cautious in interpreting the results and propose a trend for association between the ApoE  $\epsilon 4$  allele and carotid IMT measures in African Americans. Future studies should further explore the relation between subclinical measures of atherosclerosis and the ApoE  $\epsilon 4$  allele in African Americans.

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