Protective effect of apolipoprotein E2 on coronary artery disease in African Americans is mediated through lipoprotein cholesterol

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Abstract We studied the relationship of apolipoprotein E (apoE) isoforms and coronary artery disease (CAD) in 224 African Americans and 326 Caucasians undergoing diagnostic coronary angiography. The presence of CAD was defined as >50% stenosis in at least one artery. ApoE allele frequencies were 0.12, 0.62, and 0.26 for ε2, ε3, and ε4, respectively, in African Americans and 0.08, 0.78, and 0.14 for ε2, ε3, and ε4, respectively, in Caucasians. Among African Americans, CAD was present in 9 of 34 ε2 carriers (26%), significantly smaller ($P < 0.05$) in proportion compared with 39 of 82 ε3 carriers and 43 of 92 ε4 carriers (48% and 47%, respectively), suggesting a protective effect of the ε2 allele. No such difference was seen in Caucasians. In African Americans but not Caucasians, LDL cholesterol was lower in ε2 carriers than in ε3 and ε4 carriers (106 vs. 127 and 134 mg/dl, respectively; $P < 0.005$). After adjusting for lipid levels, the association between apoE2 and CAD was no longer significant. Thus, the protective effect of apoE2 seen in African Americans could be explained by a favorable lipid profile in ε2 carriers, whereas in Caucasians, the absence of such a protective effect could be attributable to the lack of effect of apoE2 on the lipid profile.—Anuurad, E., J. Rubin, G. Lu, T. A. Pearson, S. Holleran, R. Ramakrishnan, and L. Berglund. **Protective effect of apolipoprotein E2 on coronary artery disease in African Americans is mediated through lipoprotein cholesterol. *J. Lipid Res.* 2006.** 47: **2475–2481.**

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Apolipoprotein E (apoE) plays a key role in the metabolism of cholesterol and triglycerides: apoE mediates the catabolism of remnants of chylomicrons and VLDL via a "remnant" receptor and also the binding of chylomicron remnants, VLDL, and intermediate density lipoproteins to the LDL receptor (1). Three different apoE alleles (ε2, ε3, and ε4) on chromosome 19, each allele encoding one apoE isoform (apoE2, apoE3, and apoE4), result in six different genotypes (E2/2, E3/2, E4/2, E3/3, E4/3, and E4/4) (2, 3). It has been estimated that apoE polymorphism may account for 2–16% of the variability of LDL cholesterol levels, a contribution incomparable with any other gene in the general population (4–7). Compared with ε3 homozygotes, carriers of the ε2 allele have lower circulating cholesterol and higher triglyceride levels, whereas the ε4 allele is associated with higher levels of total and LDL cholesterol (8). Numerous epidemiological studies have investigated the relationship between apoE polymorphism and coronary artery disease (CAD) risk (9–12), demonstrating a significant association of apoE genotypes with susceptibility to CAD (11–13).

Most studies of the impact of apoE on lipid levels have been carried out in Caucasian and Asian populations. These studies have demonstrated that apoE allele frequency varies across race/ethnicity as well as within race/ethnic groups; the ε4 allele frequency varies among Europeans, with a high frequency among Northern Europeans (12, 14–16), whereas certain Asian (17–21) and American Indian (22, 23) groups appear to have reduced frequencies of the ε2 and ε4 alleles. Furthermore, the ε4 allele frequency is considerably higher among African Americans (24–27). In Caucasians, the ε4 allele has been associated with high LDL cholesterol levels, premature atherosclerosis, and decreased longevity, whereas the ε2 allele has been suggested to be protective. There is a lack of data regarding the influence of apoE genotype on CAD among African Americans. In addition, the impact of apoE polymorphism on lipid levels is less well documented in this race/ethnic group. As part of the Harlem-Bassett Study, an angiographic study in Caucasians and African

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Americans, we evaluated apoE polymorphism and lipid levels in relation to the presence of cardiovascular disease across race/ethnicity.

MATERIALS AND METHODS

Subjects

Subjects were recruited from a patient population scheduled for diagnostic coronary arteriography either at Harlem Hospital Center in New York City or at the Mary Imogene Bassett Hospital in Cooperstown, NY. The study design has been described (28, 29). Briefly, 648 patients, 401 men and 247 women, self-identified as Caucasian (n = 344), African American (n = 232), or other (n = 72) were enrolled. Exclusion criteria for this study included the use of lipid-lowering drugs. This report is based on findings in 550 subjects (224 African Americans, 326 Caucasians) for whom lipid levels and apoE genotypes were available. The study was approved by the Institutional Review Boards at Harlem Hospital, the Mary Imogene Bassett Hospital, Columbia University College of Physicians and Surgeons, and the University of California, Davis, and informed consent was obtained from all subjects.

Biochemical analyses

Fasting blood samples were drawn ~2–4 h before the catheterization procedure, and serum and plasma samples were stored at −80°C before analysis. Concentrations of triglycerides (Sigma Diagnostics, St. Louis, MO), total and HDL cholesterol, and glucose (Roche, Sommerville, NJ) were determined using standard enzymatic procedures (30–32). HDL cholesterol levels were measured after precipitation of apoB-containing lipoproteins with dextran sulfate (32). LDL cholesterol levels were calculated in subjects with triglyceride levels of <400 mg/dl with the formula of Friedewald, Levy, and Fredrickson (33). Of 550 subjects, 10 had triglyceride levels of ⩾400 mg/dl and were excluded from analysis for LDL cholesterol. Insulin assay was performed using the Coat-A-Count RIA kit (DPC Diagnostic Products Co., Los Angeles, CA). Homeostasis model assessment-insulin resistance was calculated using a formula available from http://www.dtu.ox.ac.uk/index.html?maindoc=/homa/index.html (34).

Determination of apoE isoforms

ApoE isoforms were determined at the DNA level by amplification using the polymerase chain reaction and specific oligonucleotides as described (35). The amplified fragments were then digested with the enzyme *Hha*I and separated on a polyacrylamide gel as described by Hixson and Vernier (36).

Coronary angiography

Two readers, blinded to patient identity, clinical diagnosis, lipoprotein, and genotype results recorded the localization and extent of luminal narrowing for 15 segments of the major coronary arteries. The presence of CAD was defined as at least 50% stenosis in any of 15 coronary artery segments. A composite cardiovascular score (0–75) was calculated based on determination of the presence of stenosis on a scale of 0–5 from 15 predetermined coronary artery segments.

Statistics

Analysis of data was done with SPSS statistical analysis software (SPSS, Inc., Chicago, IL). Results are expressed as means ± SEM. Triglyceride and insulin levels and cardiovascular score were logarithmically transformed to achieve normal distributions. Proportions were compared between groups using Chi-square analysis and Fisher's exact test where appropriate. Group means were compared using Student's *t*-test. General linear measurement multivariate analyses for CAD groups and apoE groups were used for anthropometric and metabolic parameters after adjustment for gender and drug use, and post hoc analyses were performed with the Bonferroni test for two independent samples. Multiple logistic regression analysis was applied to predict the variables that independently and significantly contributed to the dependent variable: the presence of cardiovascular disease. Unless noted otherwise, a nominal two-sided $P < 0.05$ was used to assess significance.

RESULTS

Clinical characteristics of the subjects are shown in **Table 1**. In both race/ethnic groups, patients with CAD were older and had significantly higher levels of total and LDL cholesterol, triglyceride, and apoB compared with patients without CAD. In African Americans, patients with CAD had significantly higher levels of glucose and insulin compared with patients without CAD, and apoA-I levels were lower. As expected, CAD patients had significantly ($P < 0.001$) higher cardiovascular scores compared with those without CAD across race/ethnicity.

African Americans had a significantly higher frequency of the ε4 allele (0.27 vs. 0.14; Chi-square = 28.9, $P < 0.001$) and a significantly lower frequency of the ε3 allele (0.61 vs. 0.78; Chi-square = 35.2, $P < 0.001$) compared with Caucasians (**Table 2**). In further analysis, subjects were divided into three genotype groups: ε2 carriers (genotypes E2/2 and E3/2), ε3 carriers (genotype E3/3), and ε4 carriers (genotypes E3/4 and E4/4). Subjects with the E2/4 genotype were excluded from analysis because of the known opposite effect of ε2 and ε4 on lipid levels. Clinical characteristics of African Americans and Caucasians across apoE genotype are shown in **Fig. 1**. In African Americans, levels of LDL cholesterol were significantly lower among ε2 carriers compared with non-ε2 carriers (Fig. 1A); in Caucasians, however, there were no such differences in LDL cholesterol level across apoE genotype. Caucasians had substantially higher levels of triglyceride compared with African Americans (Fig. 1B); furthermore, triglyceride levels were significantly higher among Caucasian ε2 carriers compared with ε3 and ε4 carriers. Among African Americans, but not among Caucasians, apoB levels were significantly lower among ε2 carriers compared with ε3 and ε4 carriers (Fig. 1C). Although glucose and insulin levels were generally higher in ε4 carriers compared with ε2 and ε3 carriers across race/ethnicity, the differences did not reach statistical significance (Fig. 1E, F).

Among African Americans, CAD was present in 9 of 34 ε2 carriers (26%), significantly smaller ($P < 0.05$) in proportion compared with 39 of 82 ε3 carriers and 43 of 92 ε4 carriers (48% and 47%, respectively) (**Table 3**). In contrast, among Caucasians, the CAD proportions were 23 of 39 ε2 carriers, 120 of 203 ε3 carriers, and 39 of 74 carriers ε4 carriers (59, 59, and 53%, respectively), not significantly

TABLE 1. Clinical characteristics of Caucasians and African Americans undergoing coronary angiography

Characteristic	Caucasians			African Americans		
	CAD (n = 185)	Without CAD (n = 141)	*P*	CAD (n = 101)	Without CAD (n = 123)	*P*
Men/women	136/49	72/69		54/47	73/50	
Diabetes mellitus medication (%)	26%	10%		43%	17%	
Hypertension medication (%)	69%	39%		73%	70%	
Postmenopausal (%)	86%	71%		76%	56%	
Anthropometric						
Age (years)	59.2 ± 0.8	52.4 ± 0.9	<0.001	57.8 ± 0.9	52.2 ± 0.8	<0.001
Body mass index (kg/m^2)	29.2 ± 0.5	29.8 ± 0.6	NS	27.9 ± 0.6	29.0 ± 0.5	NS
Waist-hip ratio	0.97 ± 0.01	0.96 ± 0.01	NS	0.92 ± 0.01	0.93 ± 0.01	NS
Metabolic						
Total cholesterol (mg/dl)	206 ± 3	189 ± 4	0.001	204 ± 5	190 ± 4	0.023
LDL cholesterol (mg/dl)	130 ± 3	116 ± 3	0.002	132 ± 4	118 ± 4	0.017
HDL cholesterol (mg/dl)	40 ± 1	41 ± 1	NS	47 ± 2	51 ± 2	NS
Triglyceride (mg/dl)	176 ± 6	156 ± 7	0.045	126 ± 5	104 ± 5	0.002
Glucose (mg/dl)	128 ± 4	121 ± 5	NS	124 ± 4	112 ± 4	0.049
Insulin (μU/ml)	23 ± 2	22 ± 3	NS	24 ± 2	17 ± 2	0.015
Homeostasis model assessment-insulin resistance	3.23 ± 0.31	3.05 ± 0.36	NS	3.09 ± 0.25	2.25 ± 0.23	0.016
ApoA-I (mg/dl)	122 ± 2	121 ± 2	NS	124 ± 3	134 ± 3	0.011
ApoB (mg/dl)	143 ± 3	129 ± 3	0.002	140 ± 4	125 ± 4	0.005
Cardiovascular score	25.6 ± 0.8	3.9 ± 0.9	<0.001	28.6 ± 0.9	3.5 ± 0.9	<0.001

ApoA-I, apolipoprotein A-I; CAD, coronary artery disease; NS, nonsignificant ($P \geq 0.05$). Data are means ± SEM. General linear measurement multivariate analyses were used for anthropometric and metabolic parameters after adjustment for gender and drug use, and post hoc analyses were performed with the Bonferroni test for two independent samples. Values for triglyceride, insulin, homeostasis model assessment-insulin resistance, and cardiovascular score were logarithmically transformed before analyses. A composite cardiovascular score (0–75) was calculated based on determination of the presence of stenosis on a scale of 0–5 from 15 predetermined coronary artery segments.

different from one another. The cardiovascular score in African Americans was significantly ($P = 0.030$) lower among ε2 carriers (8.0 ± 2.6) compared with ε3 and ε4 carriers (15.8 ± 1.7 and 15.8 ± 1.6, respectively) (Fig. 1D). In contrast, there was no such difference in cardiovascular score in Caucasians across apoE genotype.

We performed stepwise multiple logistic regression analysis to identify the variables that independently and significantly contributed to the presence of cardiovascular disease (**Table 4**). In univariate analysis, carrying ε2 was significantly associated with protection from CAD (odds ratio = 0.40, $P = 0.03$) in African Americans. In a multiple logistic regression model, after adjusting for LDL cholesterol, the association between apoE2 and CAD was no longer significant ($P = 0.10$). The *P* value remained nonsignificant after further adjustment for age, gender, drug use, HDL cholesterol, and presence of diabetes mellitus. These findings suggest that the protective effect of apoE2 observed in African Americans could be explained by a favorable effect of the ε2 allele on lipid profile, whereas in Caucasians, the absence of such a protective effect could be attributable to the lack of effect of apoE2 on the lipid profile.

DISCUSSION

In this study, we observed that the ε2 allele was associated with a lower frequency of angiographically determined heart disease in African Americans. Furthermore, the protective effect of ε2 in African Americans was mediated through lower LDL cholesterol levels. The ε4 allele, implicated in other studies as a risk factor for CAD, was not associated with CAD in either African Americans or Caucasians.

TABLE 2. ApoE polymorphisms and allele frequencies in relation to CAD

ApoE Variable	Caucasians			African Americans		
	All (n = 326)	CAD (n = 185)	Without CAD (n = 141)	All (n = 224)	CAD (n = 101)	Without CAD (n = 123)
Genotype						
E2/E2	4 (1%)	3 (2%)	1 (1%)	2 (1%)	0 (0%)	2 (1%)
E2/E4	10 (3%)	3 (2%)	7 (5%)	16 (7%)	10 (10%)	6 (5%)
E3/E2	35 (11%)	20 (10%)	15 (10%)	32 (17%)	9 (9%)	23 (19%)
E3/E3	203 (62%)	120 (65%)	83 (59%)	82 (37%)	39 (38%)	43 (35%)
E3/E4	68 (21%)	36 (19%)	32 (23%)	80 (35%)	40 (40%)	40 (33%)
E4/E4	6 (2%)	3 (2%)	3 (2%)	12 (5%)	3 (3%)	9 (7%)
Allele						
ε2	0.081	0.078	0.085	0.116	0.094	0.134
ε3	0.780	0.800	0.755	0.616	0.628	0.605
ε4	0.138	0.121	0.159	0.267	0.277	0.260

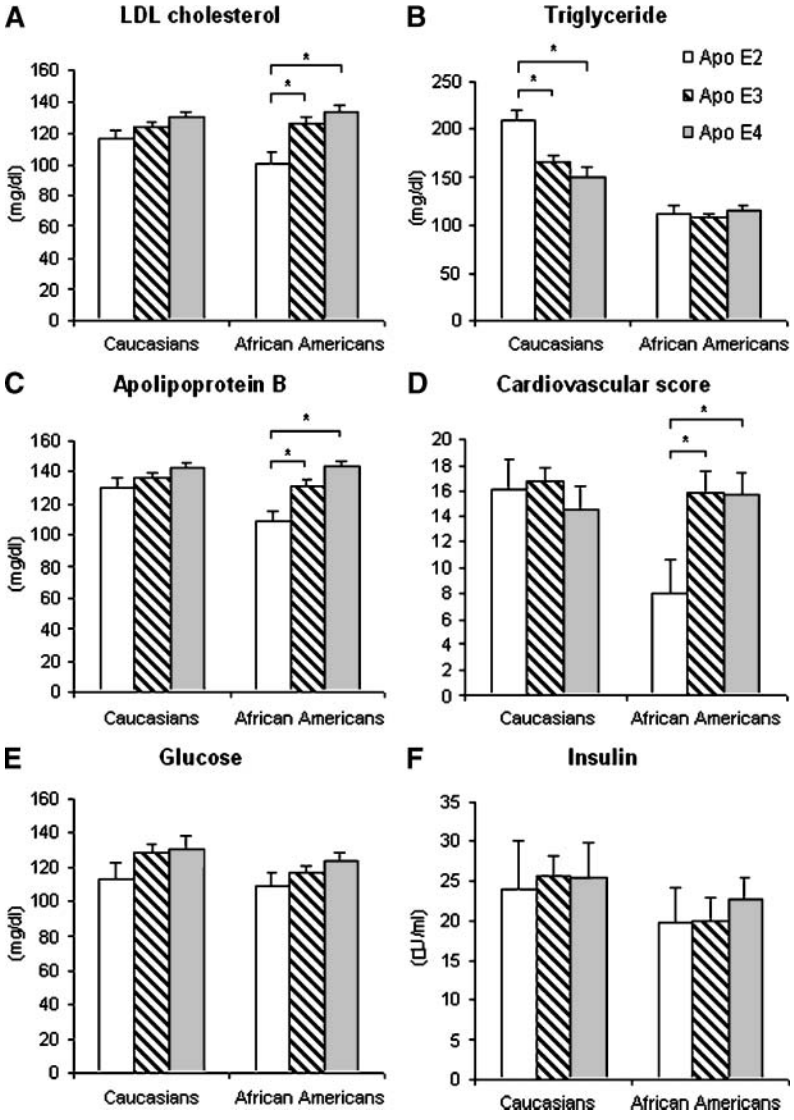

Fig. 1. Levels of LDL cholesterol (A), triglyceride (B), and apolipoprotein B (apoB) (C) and cardiovascular score (D), glucose (E), and insulin (F) across apoE genotypes in African Americans and Caucasians. Data are means ± SEM. * $P < 0.05$. General linear measurement multivariate analyses for three apoE groups were used for metabolic parameters after adjustment for gender and drug use, and post hoc analyses were performed with the Bonferroni test for two independent samples. Values for triglyceride score and insulin were logarithmically transformed before analyses. Symbols are as shown in B.

Numerous studies have investigated the contributory role of variability at the apoE locus on cholesterol and lipoprotein levels (9–12, 37) as well as its impact on CAD (11–13). Still, the underlying mechanisms connecting the apoE polymorphism to CAD risk are not completely understood. The role of apoE polymorphism on plasma levels of LDL cholesterol, apoB, and apoE is well documented, and in general, apoE2 is associated with lower total and LDL cholesterol levels and apoE4 is associated with higher levels (38). However, the relationship between apoE polymorphism and plasma levels of triglycerides, HDL cholesterol, and apoA-I is less well explored (4, 8, 39). Although African Americans have a higher ε4 allele frequency than Caucasians, there is less information available on the association between apoE and lipid levels or CAD among the former. In this study, we observed a significant effect of apoE polymorphism on total and LDL cholesterol and apoB among African Americans, but not among Caucasians, with lower levels among ε2 carriers compared with non-ε2 carriers. It has been suggested that the potential antiatherogenic effects of the ε2 allele involving lower LDL cholesterol levels may be offset by the accumulation of atherogenic large VLDL cholesterol and remnant triglyceride-rich lipoproteins (4, 8, 39). In agreement with previous population studies (25, 40, 41), Caucasians had substantially higher levels of plasma triglyceride compared with African Americans. Therefore, we hypothesize that the relatively lower triglyceride levels in African Americans may enhance a favorable effect of the apoE genotype, whereas a protective effect of ε2 in Caucasians might be offset by higher triglyceride levels.

TABLE 3. Cardiovascular disease across apoE genotypes

	Caucasians		African Americans	
Allele	CAD	Without CAD	CAD	Without CAD
ε2	23 (59%)	16 (41%)	9 (26%)	25 (74%)[a]
ε3	120 (59%)	83 (41%)	39 (48%)	43 (52%)
ε4	39 (53%)	35 (47%)	43 (47%)	49 (53%)

Values shown are n and (%).
[a] $P < 0.05$, ε2 carriers versus non-ε2 carriers in African Americans.

TABLE 4. Multiple logistic regression analysis relating case status in African Americans to risk factors

Model	Odds Ratio	95% Confidence Interval	*P*
Model 1: apoE only in model			
ε2 carrier	0.40	0.17–0.91	0.030
Model 2: apoE and LDL cholesterol in model			
ε2 carrier	0.49	0.21–1.15	0.103
LDL cholesterol (per 40 mg/dl)	1.37	1.03–1.82	0.031
Model 3: full model			
ε2 carrier	0.53	0.20–1.42	0.211
Age (per 10 years)	1.08	1.04–1.13	<0.001
Gender	0.65	0.33–1.25	0.201
Medication	0.49	0.21–1.13	0.096
Diabetes mellitus	3.08	1.46–6.49	0.003
LDL cholesterol (per 40 mg/dl)	1.40	1.01–1.95	0.042
HDL cholesterol (per 20 mg/dl)	0.59	0.37–0.94	0.028

As lipid levels were predictive of CAD in both race/ethnic groups, we performed multiple logistic regression analysis to determine whether the ε2 allele had a protective effect beyond its effect on lipid levels. In univariate analysis, we observed a significant association of the ε2 allele with the presence of CAD in African Americans but not in Caucasians. The ε2 allele has been substantially related to a reduced risk for CAD in studies conducted in Europe (11–13, 42), whereas there is a lack of such studies relating apoE polymorphism to coronary risk conducted among African Americans (25). In a previous population-based study (24), we showed that the ε2 allele was associated with lower LDL cholesterol levels in elderly Hispanics, Caucasians, and African Americans (>65 years). In this study, taking other risk factors into account, the ε2 allele was no longer associated with the presence of CAD. As the odds ratio and *P* value for ε2 were not substantially different in the multiple logistic regression model compared with univariate analysis, we cannot rule out the possibility that the ε2 allele may confer protection through other mechanisms. Thus, beyond an effect on lipid metabolism, apoE genotypes may also play additional roles in the development of CAD through inflammatory, antioxidant, and immune activities (4, 43, 44).

In this study, we did not find any association of the ε4 allele with the presence of CAD in either African Americans or Caucasians. The ε4 allele has been implicated in some studies as a risk factor for CAD (11, 42, 45–47), although others have failed to find such an association, including a prospective angiographic study (37, 48–50). In the Scandinavian Simvastatin Survival Study, Gerdes et al. (12) concluded that patients carrying the ε4 allele have an 80% increased risk of coronary death compared with patients not carrying the allele. Furthermore, we did not observe any difference in ε4 frequency between CAD and non-CAD patients in either race/ethnic group.

This study has several limitations. Subjects in our study were recruited from patients scheduled for coronary angiography, and for some genotypes, the number was relatively small. As the apoE4 genotype has been associated with CAD, a potential source of error might be a differing distribution of apoE genotypes among our subjects compared with the population at large. Arguing against this possibility, the apoE allele frequency pattern was similar to that described previously for African-American and Caucasian populations (51, 52). Furthermore, the ε4 allele frequency was similar in subjects with and without CAD for both African Americans (47% vs. 53%; NS) and Caucasians (53% vs. 47%; NS).

In conclusion, the results presented in this study indicate an antiatherogenic effect of the ε2 allele among African Americans and that the apparent protective effect of the ε2 allele is likely mediated through lipoprotein levels. Further studies are needed to explore the underlying mechanisms for this finding.

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