Integrated Role of Two Apolipoprotein E Polymorphisms on Apolipoprotein B Levels and Coronary Artery Disease in a Biethnic Population

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Abstract

Background: Apolipoprotein E (ApoE) plays a major role in lipoprotein metabolism and genetic variability of ApoE confers susceptibility to coronary artery disease (CAD). Beyond variability in the coding region, promoter polymorphisms in the ApoE gene impact on ApoE transcription.

Methods: We determined the ApoE – 491 A/T promoter polymorphism, ApoE isoforms, lipid and lipoprotein levels, and CAD risk factors in 313 Caucasians and 215 African Americans.

Results: Caucasians had a lower ApoE T allele frequency compared to African Americans (18.1% vs. 32.3%, $P < 0.05$). Among T/* carriers, ApoB levels were significantly lower in Caucasians, but significantly higher among African Americans, in both cases compared to A/A homozygotes ($P = 0.017$, and $P = 0.012$). For a given –491A/T genotype, levels of atherogenic lipoproteins differed across ApoE2/E3/E4 isoforms among African Americans, but not Caucasians, as T/* carriers with ApoE4 had significantly higher ApoB levels compared to T/* carriers with ApoE2 ($P = 0.010$). Among patients with CAD, Caucasian A/A homozygotes and African American T/* carriers had higher ApoB levels compared to the same genotype without CAD ($P = 0.007$, $P = 0.049$, respectively).

Conclusions: We observed an ethnicity-specific variability in ApoB levels across the ApoE – 491 A/T polymorphism and a modulatory impact on this pattern by ApoE2/E3/E4 isoforms.

Introduction

APOLIPOPROTEIN E (ApoE) PLAYS a key role in lipid metabolism and cardiovascular disease (CVD). A common polymorphism in the ApoE gene, coding for three common isoforms (E2, E3, and E4) has been studied extensively.^{1–3} Largely reflecting its presence in atherogenic lipoprotein fractions, there has been a significant increased interest in ApoB levels as a predictor of CVD. This has been underscored in several recent large prospective studies.^{4–6} ApoB levels are impacted by genetic and environmental factors.^{7,8} In particular, genetic variation in the coding region of the ApoE gene (ApoE2/ε3/ε4) plays a critical role in modulating atherogenic ApoB-containing lipoproteins.⁹ Commonly, the ε2 allele is associated with lower ApoB levels than the ε3 allele, while the converse is true for the ε4 allele.⁹ Numerous

epidemiological studies, largely among Caucasians, have investigated the relationship between the ApoE2/E3/E4 polymorphism, plasma concentrations of total cholesterol, low-density lipoprotein cholesterol (LDL-C), ApoB, and coronary artery disease (CAD) risk, demonstrating a significant association of ApoE2/E3/E4 genotypes with susceptibility to CAD.^{9–12}

Previously, we reported on the association of ApoE2/E3/E4 genotypes and lipid levels with CAD in African Americans, and Caucasians, and found a protective effect of ApoE2 in African Americans due to lipoprotein cholesterol levels.¹³ Beyond variability in the coding region, a number of other polymorphisms in the ApoE gene have been described.¹⁴ The ApoE promoter region is complex, hosting numerous regulatory elements located in the proximal 5'-flanking region and in the first intron.^{14,15} Thus, polymorphisms within this

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region carry the potential of a functional impact mediated by regulation of ApoE transcription.¹⁴ Although there is a wealth of information on lipids and cardiovascular risk for the ApoE2/E3/E4 isoforms, there is scarce information for the ApoE –491 A/T polymorphism in the promoter region.^{14,16,17} In one study, subjects homozygous for the T allele had more pronounced atherosclerosis than subjects with A/A and A/T genotypes.¹⁶ Another study showed an association between A/A homozygosity and atherothrombotic stroke.¹⁷ Recently, we demonstrated that polymorphisms in the ApoC-I gene, located in close proximity to the ApoE gene, were associated with cardiovascular disease phenotypes.¹⁸ In the present study, we assessed the relationship between the ApoE promoter –491 A/T polymorphism, ApoE2/E3/E4 isoforms, and lipoprotein phenotypes, and whether the former was associated with a cardiovascular risk profile.

Materials and Methods

Subjects

Subjects were recruited from a patient population scheduled for diagnostic coronary angiography 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.^{13,18–20} Briefly, 576 patients, self-identified as Caucasian (222 male and 121 female), African American (132 male and 101 female), and 72 others were enrolled. Exclusion criteria for this study included the use of lipid-lowering drugs. This report is based on findings in 528 subjects (313 Caucasians, and 215 African Americans) in whom lipid levels and ApoE isoforms and ApoE –491 A/T polymorphism data 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.

Clinical and biochemical assessments

Blood pressure was measured with a random-zero mercury sphygmomanometer. Waist circumference was calculated as the average of two measurements taken after inspiration and expiration at the midpoint between the lowest rib and iliac crest. Fasting blood samples were drawn $\approx$ 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 cholesterol, high-density lipoprotein cholesterol (HDL-C), glucose (Roche, Somerville, NJ), and insulin (DPC Diagnostic Products Co., Los Angeles, CA) were determined using standard enzymatic procedures.^{21–23} HDL-C levels were measured after precipitation of ApoB-containing lipoproteins with dextran sulfate,²³ and LDL-C levels were calculated. ApoA-I and B were measured by rate immunonephelometry (Array, Beckman, Brea, CA).²⁴ High-sensitivity C-reactive protein (hsCRP) levels were measured using an enzyme-linked immunoabsorbent assay, standardized according to the World Health Organization (WHO) First International Reference Standard.^{25,26} Fibrinogen levels were measured by the clot-rate method of Clauss.²⁷ Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated using the updated model available from the Oxford Centre for Endocrinology and Diabetes.²⁸

Determination of ApoE polymorphisms

ApoE isoforms were determined at the DNA level by amplification using the polymerase chain reaction and specific oligonucleotides as described.²⁹ The amplified fragments were then digested with the enzyme *HhaI* and separated on a polyacrylamide gel as described by Hixson and Vernier.³⁰ The ApoE –491 A/T polymorphism was determined by nested polymerase chain reaction (PCR) and digestion as previously described.¹⁴ Subjects with ApoE A/T and T/T genotypes were classified as ApoE T/* carriers.

Coronary angiography

Two readers, blinded to patient identity, clinical diagnosis, lipoprotein levels, and genotype results recorded the localization and extent of luminal narrowing for 15 segments of the major coronary arteries.³¹ The presence of CAD was defined as at least 50% stenosis in any of 15 coronary artery segments. Of the patients without CAD, the majority (80.5%) had <25% stenosis, and of the patients with CAD, 81% had >75% stenosis. 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 $\pm$ standard deviation (SD). Triglyceride, CRP, insulin, HOMA-IR levels, and the cardiovascular score were logarithmically transformed to achieve normal distributions. Proportions were compared between groups using chi-squared analysis and the Fisher exact test where appropriate. Group means across A/A and T/* genotypes were compared using the Student *t*-test. Group means across ApoE4/E3/E4 isoforms within a given ApoE A/A and T/* genotypes were compared using one-way analysis of variance (ANOVA), and *post hoc* analyses were performed by Bonferroni test for two independent samples. Unless noted otherwise, a nominal two-sided $P < 0.05$ was used to assess significance.

Results

The ApoE –491 A allele was the most common allele for both Caucasians and African Americans. However, as seen in Table 1, the A allele frequency was significantly lower among African Americans (0.677 vs. 0.819, $\chi^2 = 28.6$, $P < 0.001$), which resulted in a higher frequency of T/* carriers in this group. There was no significant difference in A or T allele frequency for subjects with or without CAD within each ethnic group (Table 1).

Clinical characteristics across the A/T polymorphism for the two ethnic groups are shown in Table 2. Among Caucasians, T/* carriers had significantly lower ApoB levels compared to A/A homozygotes (128 ± 32 vs. 138 ± 37 mg/dL, $P = 0.017$). Although a similar pattern was seen for total and LDL-C levels, the differences between the two genotypes did not reach statistical significance for these parameters. The opposite pattern was observed for African Americans; ApoB levels were significantly higher among T/* carriers compared to A/A homozygotes (139 ± 45 vs. 126 ± 34 mg/dL,

TABLE 1. ApoE -491 A/T POLYMORPHISM AND ALLELE FREQUENCIES IN RELATION TO CORONARY ARTERY DISEASE

	Caucasians			African Americans		
	All (n = 313)	CAD (+) (n = 173)	CAD (-) (n = 132)	All (n = 215)	CAD (+) (n = 92)	CAD (-) (n = 117)
Genotype						
A/A	217 (69%)	117 (68%)	93 (70%)	101 (47%)	48 (52%)	50 (43%)
A/T	79 (25%)	48 (28%)	30 (23%)	89 (41%)	33 (36%)	53 (45%)
T/T	17 (6%)	8 (4%)	9 (7%)	25 (12%)	11 (12%)	14 (12%)
Alleles						
A	0.819	0.815	0.818	0.677	0.701	0.654
T	0.181	0.185	0.182	0.323	0.299	0.346

Values represent numbers (percentage) for genotypes and allele frequencies of the given genotypes. There was missing data on CAD among 14 subjects (8 for Caucasians; 6 for African Americans).

ApoE, apolipoprotein E; CAD, coronary artery disease.

$P = 0.012$). Significant differences were also seen for total and LDL-C levels ($P = 0.046$ and $P = 0.022$, respectively) (Table 2). No significant differences between the A and T genotypes within each ethnic group were noted for systolic or diastolic blood pressure, BMI, waist-to-hip ratio (WHR), diabetes status, HDL-C, triglyceride, ApoA-I, glucose, insulin resistance parameters, CRP, fibrinogen, or the cardiovascular score.

We next compared clinical characteristics for ApoE -491 A/T across ethnicity (Table 2). Beyond the well-established interethnic differences in HDL-C and triglycerides, Caucasian subjects with A/A homozygosity had significantly higher levels of ApoB, glucose, insulin, HOMA-IR, and WHR and lower levels of ApoA-I, CRP, fibrinogen, and diastolic

blood pressure compared to their African American counterparts. Among T/* carriers, Caucasians had significantly lower levels of ApoB, total cholesterol, LDL-C, ApoA-I, fibrinogen, and higher WHR compared to African Americans.

Because we observed an ethnically distinct, inverse pattern for ApoB levels across the ApoE -491 A/T polymorphism, we next analyzed apolipoprotein levels in subjects with and without CAD across the ApoE A/T genotypes. As seen in Fig. 1, ApoB levels were significantly higher in Caucasian A/A subjects with CAD compared to those without CAD ($P = 0.007$). No corresponding difference was observed for T/* carriers. Among African Americans, higher ApoB levels were seen in subjects with CAD compared to subjects without CAD, irrespective of A/T genotype

TABLE 2. CLINICAL CHARACTERISTICS OF STUDY POPULATION ACROSS THE ApoE -491 A/T POLYMORPHISM

Characteristics	Caucasians			African Americans		
	A/A (n = 217)	T/* (n = 96)	P value	A/A (n = 101)	T/* (n = 114)	P value
Age (years)	57.3 ± 10.3	55.4 ± 10.5	N.S.	54.9 ± 8.9 ^a	54.3 ± 10.0	N.S.
SBP (mm Hg)	125 ± 19	125 ± 15	N.S.	131 ± 18	128 ± 19	N.S.
DBP (mm Hg)	74 ± 10	76 ± 10	N.S.	79 ± 12 ^a	79 ± 11	N.S.
BMI kg/m ²	29.7 ± 6.2	29.8 ± 5.6	N.S.	28.5 ± 6.2	28.6 ± 5.8	N.S.
WHR	0.97 ± 0.08	0.97 ± 0.09	N.S.	0.93 ± 0.06 ^a	0.93 ± 0.06 ^b	N.S.
Diabetic (%)	45 (21%)	18 (19%)	N.S.	31 (31%)	34 (30%)	N.S.
Total cholesterol (mg/dL)	198 ± 41	191 ± 39	N.S.	191 ± 39	203 ± 50 ^b	0.046
LDL-C (mg/dL)	123 ± 35	116 ± 33	N.S.	118 ± 37	131 ± 45 ^b	0.022
HDL-C (mg/dL)	41 ± 13	40 ± 11	N.S.	49 ± 18 ^a	49 ± 17 ^b	N.S.
Triglyceride (mg/dL)	152 (106–221)	145 (119–220)	N.S.	106 (80–147) ^a	105 (80–142) ^b	N.S.
ApoA-I (mg/dL)	122 ± 23	121 ± 24	N.S.	131 ± 30 ^a	130 ± 29 ^b	N.S.
ApoB (mg/dL)	138 ± 37	128 ± 32	0.017	126 ± 34 ^a	139 ± 45 ^b	0.012
Glucose (mg/dL)	134 ± 67	131 ± 69	N.S.	117 ± 51 ^a	123 ± 56	N.S.
Insulin (μU/mL)	15.1 (9.8–28.4)	15.8 (8.6–25.9)	N.S.	14.2 (8.6–23.1) ^a	14.1 (8.9–22.1)	N.S.
HOMA-IR	2.0 (1.3–3.9)	2.1 (1.2–3.4)	N.S.	1.9 (1.2–3.2) ^a	1.9 (1.1–2.8)	N.S.
CRP (mg/L)	2.6 (1.4–7.3)	3.2 (1.6–7.5)	N.S.	3.9 (2–11.6) ^a	4.3 (2.1–9.5)	N.S.
Fibrinogen (mg/dL)	327 ± 91	321 ± 83	N.S.	391 ± 125 ^a	384 ± 106 ^b	N.S.
Cardiovascular score	12.9 (1.6–28)	12.9 (1.4–24.3)	N.S.	12.6 (1.5–26)	7.1 (1.4–21.8)	N.S.

Data are means ± standard deviation (SD) or for nonnormally distributed variables as median (interquartile range). Group means were compared using a *t*-test. Values for triglyceride, insulin, homeostasis model assessment of insulin resistance (HOMA-IR), CRP and the composite score, were logarithmically transformed before analyses. Apo T/* represents apoE A/T and T/T.

^aCaucasians versus African Americans within A/A homozygotes.

^bCaucasians versus African Americans within T/* carrier.

Apo E, apolipoprotein E; SBP, systolic blood pressure; DBP, diastolic blood pressure; BMI, body mass index; WHR, waist-to-hip ratio; CRP, C-reactive protein; N.S., not significant ($P \geq 0.05$).

FIG. 1. Apolipoprotein B (ApoB) and ApoA-I levels in subjects with (+) and without (-) coronary artery disease (CAD) across ApoE -491 A/T genotypes in Caucasians and African Americans. (*) $P < 0.05$.

($P = 0.015$ and $P = 0.049$, for A/A and T/*, respectively). A somewhat different picture was observed for ApoA-I levels among African American A/A homozygotes, ApoA-I levels were significantly lower in subjects with CAD compared to subjects without CAD ($P = 0.001$), while there was no such difference among African American T/* carriers or among Caucasians, irrespective of A/T genotype.

We next analyzed the distribution of ApoE -491 A/T polymorphism across ApoE2/E3/E4 isoforms in two ethnic groups. As seen in Table 3, we observed an ethnically distinct linkage disequilibrium between these two genotypes ($\chi^2 = 36.6$, $P < 0.001$ for Caucasians and $\chi^2 = 17.7$, $P < 0.001$ for African Americans). Whereas A/A homozygotes dominated among ApoE3 and ApoE4 genotypes in Caucasians (68% and 91% respectively), T/* carriers dominated for the ApoE4 genotype in African Americans (70%). When comparing the distribution of ApoE2, ApoE3, or ApoE4 genotypes across ethnicity within ApoE A/A and T/* genotypes, there was a pronounced interethnic difference ($\chi^2 = 19.8$, $P < 0.001$ for A/A homozygotes and $\chi^2 = 57.1$, $P < 0.001$ for T/* carriers) (Fig. 2). Notably, for the ApoE4 genotype, the

relative ratio of African American to Caucasian T/* carriers was 9.5/1.0, whereas the corresponding ratio for A/A homozygotes was close to unity (1.1/1.0). Conversely, for the ApoE2 genotype, the relative ratio of African American to Caucasian T/* carriers was 0.4/1.0, whereas the ratio was reversed for A/A homozygotes (4:1).

In view of the pronounced interethnic difference in distribution of ApoE2/E3/E4 isoforms and the variable atherogenic pattern across A/T genotypes in African Americans, we next compared lipid and apolipoprotein parameters across the ApoE A/T polymorphism for the individual ApoE2/E3/E4 isoforms in this group. Among the ApoE2 genotype, African American subjects with A/A homozygosity had significantly higher HDL-C levels compared to T/* carriers ($P = 0.047$). Although ApoA-I levels were also higher among A/A homozygotes, the difference did not reach statistical significance. We did not observe any differences in lipid, lipoprotein, or apolipoprotein levels between these A/T genotypes in subjects with ApoE3. Among subjects with the ApoE4 genotype, African American T/* carriers had significantly higher levels of total, LDL-C, HDL-C,

TABLE 3. ApoE2/E3/E4 ISOFORMS IN RELATION TO ApoE -491 A/T POLYMORPHISM ACROSS ETHNICITY

Genotype	Caucasians			African Americans		
	A/A	T/*	χ^2/P value	A/A	T/*	χ^2/P value
Total	211	93		93	107	
ApoE2	13 (35%)	24 (65%)	36.6/<0.001	22 (65%)	12 (35%)	17.7/<0.001
ApoE3	134 (68%)	63 (32%)		45 (57%)	34 (43%)	
ApoE4	64 (91%)	6 (9%)		26 (30%)	61 (70%)	

Values represent numbers and percentages (%) of subjects in each genotype across A/A and T/* carriers. ApoE2 represents E2/E2 and E2/E3, ApoE3 represents E3/E3, ApoE4 represents E3/E4 and E4/E4. ApoE T/* represents ApoE A/T and T/T. ApoE, apolipoprotein E.

ApoB, and ApoA-I compared to A/A homozygotes ($P=0.004$, $P=0.024$, $P=0.031$, $P=0.035$, $P=0.002$, respectively) (Table 4). We then compared apolipoprotein levels for both A/A homozygotes and T/* carriers within a given ApoE2/E3/E4 isoform. ApoA-I levels were significantly higher for subjects with the ApoE2 genotype compared to the ApoE4 genotype in A/A homozygotes ($P=0.003$). For T/* carriers, total, LDL-C and ApoB levels were significantly

higher for subjects with the ApoE4 genotype compared to the ApoE2 genotype ($P=0.005$, $P=0.011$, $P=0.010$, respectively).

Discussion

Genetic variations of the ApoE locus have an important impact on lipoprotein metabolism, and the ApoE2/E3/E4

FIG. 2. Distribution of the apolipoprotein E (ApoE) -491 A/T polymorphisms on ApoE2/E3/E4 isoforms across Caucasian and African American ethnicities. Distribution of the A/A homozygotes (A), and T/* carriers (B) among the ApoE2/E3/E4 isoforms across the ethnicity.

TABLE 4. LIPID AND LIPOPROTEIN PARAMETERS ACROSS ApoE –491 A/T POLYMORPHISM AND ACROSS ApoE4/E3/E4 ISOFORMS IN AFRICAN AMERICAN SUBJECTS

ApoE genotypes	ApoE –491 A/T polymorphism		
	A/A	T/*	P value
Lipoproteins			
ApoE2			
Total cholesterol (mg/dL)	188 ± 36	167 ± 41	N.S.
LDL-C (mg/dL)	107 ± 36	101 ± 38	N.S.
HDL-C (mg/dL)	56 ± 20	44 ± 14	0.047
Triglyceride (mg/dL)	104 (86–142)	102 (75–128)	N.S.
ApoA-I (mg/dL)	144 ± 33	127 ± 25	N.S.
ApoB (mg/dL)	114 ± 33	110 ± 38	N.S.
ApoE3			
Total cholesterol (mg/dL)	199 ± 40	192 ± 41	N.S.
LDL-C (mg/dL)	126 ± 37	125 ± 33	N.S.
HDL-C (mg/dL)	50 ± 18	46 ± 16	N.S.
Triglyceride (mg/dL)	102 (76–147)	94 (75–130)	N.S.
ApoA-I (mg/dL)	135 ± 31	128 ± 33	N.S.
ApoB (mg/dL)	130 ± 33	132 ± 39	N.S.
ApoE4			
Total cholesterol (mg/dL)	185 ± 38	216 ± 52 ^b	0.004
LDL-C (mg/dL)	120 ± 36	141 ± 48 ^b	0.024
HDL-C (mg/dL)	43 ± 12	50 ± 17	0.031
Triglyceride (mg/dL)	111 (70–153)	110 (84–143)	N.S.
ApoA-I (mg/dL)	116 ± 17 ^a	131 ± 26	0.002
ApoB (mg/dL)	130 ± 36	150 ± 45 ^b	0.035

Data are expressed as means ± standard deviation (SD) or for nonnormally distributed variables as median (interquartile range). ApoE2 represents E2/E2 and E2/E3, ApoE3 represents E3/E3, ApoE4 represents E3/E4 and E4/E4. ApoE T/* represents ApoE A/T and T/T.

Group means across A/A and T/* genotypes within a given ApoE2/E3/E4 isoforms were compared using the Student *t*-test. Values for triglyceride were logarithmically transformed before analyses.

Group means across ApoE4/E3/E4 isoforms within a given ApoE A/A and T/* genotypes were compared using one-way analysis of variance (ANOVA), and *post hoc* analyses were performed by Benferroni test for two independent samples.

^a*P* < 0.01, compared to ApoE2 group within A/A homozygotes.

^b*P* < 0.01, compared to ApoE2 group within T/* carriers.

Apo, apolipoprotein; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; N.S., not significant.

genotype constellation has been shown to predict cardiovascular risk.^{4,5,9–12} It is well established that allele frequencies at this locus differ across different ethnic groups, in particular across Caucasian–African American ethnicity.^{13,32–34} In our previous study, we showed that the Apo ε2 allele was associated with a lower frequency of angiographically determined CAD in African Americans, whereas the Apo ε4 allele was associated with higher total cholesterol and LDL-C levels. The protective effect of ApoE2 in African Americans was attributed to lower LDL-C levels.¹³ Although much attention has been focused on the association of the ApoE polymorphism with lipid levels, there is less information on apolipoproteins, although we observed a significant effect of the ApoE polymorphism on ApoB levels among African Americans, but not among Caucasians.¹³ Notably, although the lipid content can vary substantially among atherogenic lipoprotein subfractions, ApoB levels essentially reflect particle number.

Besides the ApoE2/E3/E4 isoforms, promoter polymorphisms at sites –491 and –427 upstream of the ApoE locus have been demonstrated to impact transcriptional activity of the ApoE gene, potentially through differential binding of nuclear proteins.^{14,16,17} Using hepatoma cells, Artiga et al. demonstrated that an A-to-T base substitution at nucleotide –491 caused a significant decrease in ApoE promoter activity and suggested that sequences located around this nucleotide are involved in the regulation of ApoE transcription.¹⁴

Our study is to the best of our knowledge the first to show a relationship between the ApoE –491 A/T promoter polymorphism and an atherogenic lipoprotein profile across ethnicity. We noted significant differences in the distribution of A and T alleles in the two ethnic groups. Caucasians had a higher frequency of the A allele and, thus, homozygosity for the A allele was more common in this group, whereas African Americans had a higher T allele and T heterozygote (T/*) frequency. Although a number of cardiovascular risk factors did not differ across A/T genotypes, we noted an interesting pattern with regard to atherogenic lipoproteins, in particular ApoB and LDL-C levels. In Caucasians, ApoB levels were lower for T/* carriers but higher for A/A homozygotes, whereas an opposite pattern was seen among African Americans. We also observed differences in ApoB levels across CAD phenotypes within the respective genotypes. Thus, Caucasian A/A-homozygotes with CAD had higher ApoB levels compared to subjects without CAD. A similar pattern was seen for African American T/* carriers. We also observed higher ApoA-I levels in African American A/A homozygotes without CAD compared to their counterparts with CAD.

The differences in ApoB levels, and in some cases ApoA-I levels, across the ApoE –491 A/T polymorphism in African Americans and Caucasians are intriguing because recent studies have suggested that apolipoprotein levels may be more informative risk markers than conventional lipids.^{5,6,35} The large prospective Apolipoprotein-related MORTality

RISk (AMORIS) study suggested that ApoB and ApoA-I were highly predictive in evaluating cardiac risk and pointed to the use of these markers as valuable for diagnosis and treatment for coronary heart disease.⁵ Although the differences in ApoB levels observed in our study would suggest a potential impact on cardiovascular risk, we did not find any association of the ApoE –491 A/T polymorphism with CAD in either ethnic group. This apparent paradox may indicate that the difference observed did not in isolation sufficiently modulate risk to impact on disease level. Notably, there was a similar distribution pattern of other cardiovascular risk factors such as blood pressure, HDL-C, glucose and insulin across the A/T polymorphism.

A question that arises is whether the ApoE –491 A/T polymorphism would reflect phenotype differences across the ApoE2/E3/E4 isoforms. We noted differences in the distribution of A and T alleles on ApoE2, ApoE3, or ApoE4 genotypes across ethnicity. Thus, homozygosity for the A allele was more common on an ApoE2 genotype in African Americans, whereas the opposite was seen in Caucasians. Further, among E4 carriers, heterozygosity for the T allele was more frequent in African Americans when compared to Caucasians. We also found significant changes in lipid profiles for the ApoE2 and ApoE4 genotypes across the A/T genotype in African Americans. Thus, within the ApoE2 genotype, African American subjects with A/A homozygosity had higher HDL-C levels, indicating a protective effect.

We did not observe any differences in atherogenic lipoprotein levels between A/A homozygotes and T/* carriers for the ApoE3 genotype. However, for the ApoE4 genotype, African-American T/* carriers had higher levels of atherogenic lipoproteins compared to A/A homozygotes. However, HDL-C and ApoA-I levels were also higher in this group, which may suggest a more complex net effect. It has previously been demonstrated that ApoE4 was more effective than ApoE3 in modulating LDL-C levels,^{36,37} and that ApoE deficiency results in decreased LDL ApoB synthesis and accumulation of intermediate-density lipoprotein (IDL).³⁸ Furthermore, the ApoE4 genotype has been reported to be associated with decreased levels of ApoA-I and HDL-C cholesterol across various groups.^{33,39}

However, ApoE isoforms seems to explain only a small proportion of the interethnic variation for plasma lipoproteins.³⁹ Our initial observation suggests that the protective effect of the ApoE2 genotype in African Americans was due to lipoprotein cholesterol levels¹³ could be further refined by analysis of additional ApoE genotypes. Thus, the protective effect of the ApoE2 genotype could be further dissected through the ApoE A/T genotype, because A/A homozygotes had higher levels of ApoA-I, indicating a higher anti-atherogenic potential, while T/* carriers had lower levels of atherogenic lipoproteins (ApoB and LDL-C). Although the relative contribution of promoter T polymorphism and ApoE isoforms on plasma ApoB levels remains to be determined, the results nevertheless suggest that a more detailed genotype analysis could inform phenotypic characteristics.

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. Because the ApoE4 genotype has been associated with CVD, a potential source of error might be a differing distribution of ApoE genotypes in this group 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.^{40,41}

In conclusion, the results presented in this study demonstrated variability in the atherogenicity of the ApoE –491 A/T polymorphism across ethnicity. Furthermore, our results suggest that changes in a limited number of cardiovascular risk factors may not be of a sufficient magnitude to impact on disease expression in the absence of a more pronounced high-risk cardiovascular phenotype.

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