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Relationship of the Apolipoprotein E Polymorphism with Carotid Artery Atherosclerosis

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Summary

From the cohort taking part in the Atherosclerosis Risk in Communities (ARIC) study, a multicenter investigation of atherosclerosis and its sequelae in women and men ages 45–64 years, a sample of 145 subjects with significant carotid artery atherosclerosis but without clinically recognized coronary heart disease was identified along with 224 group-matched control subjects. The aim of this paper is to measure the association of the apolipoprotein (apo) E polymorphism with the prevalence of significant carotid artery atherosclerotic disease (CAAD) after considering the contribution of established risk factor variables. The first model used a stepwise selection procedure to define a group of significant physical and lifestyle characteristics and a group of significant plasma lipid, lipoprotein, and apolipoprotein variables that were predictive of CAAD status in this sample. Those variables selected included age (years), body mass index (BMI; kg/m^2), consumption of cigarettes (CigYears; number of cigarettes/d $\times$ the number of smoking years), hypertension status, high-density lipoprotein (HDL)–cholesterol (mg/dl), total cholesterol (mg/dl), and Lp[a] (μg/ml). The second model was built by forcing into the equation an a priori set of demographic, anthropometric, and lipoprotein variables, which were age, BMI, CigYears, hypertensive status, LDL-cholesterol, and HDL-cholesterol. In both models, the apo E genotype ε2/3 was related to CAAD status. For both models, the estimated odds ratio of being a CAAD case associated with the apo E genotype ε2/3 was >2:1. The mechanism of the observed association between the ε2/3 genotype and carotid atherosclerosis is unknown, but it is likely due to the known effects of the E2 isoform in causing delayed clearance of triglyceride-rich lipoproteins.

Introduction

A positive family history of heart disease is a significant risk factor for coronary heart disease (CHD) (ten Kate et al. 1982; Shea and Nichols 1983), and genes are known to contribute to interindividual variation in plasma lipid, lipoprotein, and apolipoprotein levels (Boerwinkle and Hixson 1990). The virtual explosion in our knowledge of the human genome holds the promise of identifying gene sequences that are also risk factors for CHD. However, little is known about the ability of individual genes to predict disease beyond that afforded by traditional risk factors such as gender, plasma cholesterol levels, weight, and smoking. The null hypothesis states that genetic information does not improve prediction of CHD beyond that of traditional risk factors, which are typically less expensive to measure, more accessible to the public, and often better lend themselves to intervention.

Several genes have been implicated in the genesis of atherosclerosis and abnormal lipid metabolism. For example, mutations in the LDL-receptor gene, leading to familial hypercholesterolemia, have a large effect on plasma cholesterol levels and are associated with the occurrence of CHD (Goldstein and Brown 1989), but all individuals with this inborn error of metabolism do not have clinically recognized disease. Furthermore, because of the rare frequency of familial hypercholesterolemia relative to the common occurrence of ischemic heart disease, such mutations account for only a small proportion of the observed familial aggregation of CHD in the general population. In contrast, allelic variation at the apolipoprotein E (apo E) gene locus is common, and its impact on plasma lipid transport has been extensively studied (Davignon et al. 1988; Hallman et al. 1991). Apo E is a structural component of circulating chylomicrons, very-low-density lipoproteins (VLDL), and high-density lipoprotein (HDL) and is a ligand for several classes of lipoprotein receptors. Human apo E is polymorphic with three common alleles, ε2, ε3, and ε4 (Utermann et al. 1977). Numerous studies have shown that the average effect of the ε2 allele is to lower total serum cholesterol, and the average effect of the ε4 allele is to raise total cholesterol levels (Sing and Davignon 1985; Hallman et al. 1991; Boerwinkle and Utermann 1988). Dallongeville et al. (1992) and Brown and Roberts (1991) have reported that the apo E polymorphism also influences fasting plasma triglyceride levels. The observed effect of the

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apo E polymorphism on fasting plasma lipid levels is thought to be attributable to altered receptor-binding affinities of the apo E isoforms, leading to changes in postprandial chylomicron remnant clearance and subsequent up- or down-regulation of hepatic LDL receptors and to an altered distribution of the apo E isoforms among triglyceride-rich lipoproteins and HDLs (Weisgraber et al. 1982; Hui et al. 1984; Gregg et al. 1986; Weintraub et al. 1987; and Boerwinkle et al. 1994).

Even though several reports have described an association between the apo E polymorphism and clinically recognized CHD (Menzel et al. 1983; Cumming and Roberts 1984; Lenzen et al. 1986; Kuusi et al. 1989), only one previous study (Hixson et al. 1991) has examined the contribution of this gene directly to atherosclerosis and simultaneously considered the effects of more-established risk factors. In this paper, we characterize the relationship between the common apo E polymorphism and carotid artery atherosclerosis in subjects taking part in the Atherosclerosis Risk in Communities (ARIC) study (1989), after the contribution of established risk factor variables are considered.

Methods

Sampling

Participants were selected from ARIC, a prospective, multicenter investigation of atherosclerosis and its sequelae in women and men aged 45-64 years. Approximately 16,000 residents were recruited into the ARIC study, 4,000 in each of four communities: Forsyth County, NC; the city of Jackson, MS; the northwestern suburbs of Minneapolis, MN; and Washington County, MD. Each sample was a probability sample designed to be representative of residents living in that community, except for the Jackson sample, which consisted only of black residents. The general design of the ARIC study is described elsewhere (ARIC Investigators 1989).

Carotid artery wall thickness was measured by B-mode ultrasonography (Pignoli et al. 1986). Subjects were designated "cases" if the maximum carotid arterial intima-media far-wall thickness was >2.5 mm at any site or if there was bilateral thickening of >1.7 mm at the internal carotid, >1.8 mm at the bifurcation, or >.6 mm at the common carotid arteries. These cutpoints exceeded the 90th percentile for the ARIC study population at each site (Heiss et al. 1991). These criteria were relaxed for blacks to exact 90th percentile cutpoints in order to obtain more subjects for study. Controls were group-matched to cases by gender, race, field center, 10-year age group, and time of examination. Although vascular-wall thickness measured in this way cannot unequivocally be attributed to atherosclerosis, the relationship between wall thickness and atherosclerosis is well documented (Ross 1986). Selection of cases, using the highest decile of the distribution, as advised by collaborating experts in clinical ultrasound, identifies a sample of individuals likely to develop subsequently carotid artery stenosis. Finally, previous analyses (Heiss et al. 1991) have reported differences in mean level or frequency of well-established risk factors of CHD (e.g., cholesterol and hypertension) between ARIC carotid artery cases and controls. Cases and controls were required to meet minimum visualization criteria of arterial wall boundaries on ultrasound. Other exclusion criteria for case/control selection were evidence of symptomatic cardiovascular or cerebrovascular disease defined by a history of angina on effort, physician-diagnosed heart attack, transient ischemic attack or stroke, or intermittent claudication; use of lipid-altering medication, including beta blockers and thyroid medication; use of oral contraceptives in premenopausal women; insulin-dependent diabetes mellitus; chronic renal or liver disease; and hypertriglyceridemia defined as a fasting triglyceride level >400 mg/dl.

Sitting blood pressure was measured after a 5-min rest, three times, using a random zero sphygmomanometer. The blood pressure value used here was the average of the second and third readings. Hypertension was defined by a systolic or diastolic blood pressure >160 or 95 mmHg, respectively, or by current use of antihypertensive medication. Body mass index (BMI) was calculated from measurements of weight (to the nearest pound) or height (to the nearest centimeter) and is reported in units of kg/m^2.

Postprandial Protocol

To gain further insight into the mechanism by which the apo E polymorphism may be associated with carotid artery atherosclerotic disease (CAAD), the extent of postprandial response was measured in these case-control subjects. On the day of the fat-tolerance test, study participants arrived at the field centers after fasting for 12 h, and a blood specimen was taken before administration of the test meal. For each 2 m^2 of body surface area, a liquid meal was ingested containing 1,237 Kcal, 32.7 g of protein, 47.8 g of carbohydrate, 300 mg of cholesterol, 105 g of fat, and 100,000 IU of vitamin A (Aquasol; Armour Pharmaceutical). Participants were instructed not to take anything by mouth except water, sugarless drinks, or coffee for a period of 8 h after ingestion of the test meal. Postprandial blood specimens were collected into EDTA-containing tubes at fasting, 3.5, and 8 h. Plasma was separated by centrifugation (1,500 $\times$ g, 20 min at 4°C) and stored in the dark at 4°C for 1-3 d. Postprandial "response" was defined as the difference between levels 8 h postprandially and fasting, a measure which was found to be significantly associated with coronary artery disease in a cross-sectional study (Patsch et al. 1992).

Laboratory Methods

Plasma cholesterol and triglyceride concentrations were measured enzymatically on a Cobas-Fara centrifugal

analyzer (Roche Diagnostics) with the appropriate enzymatic kits (catalog numbers 236691 and 701912; Boehringer Mannheim). HDL-cholesterol was determined by measuring cholesterol in the supernatant after precipitation with $MgCl_2$ and dextran sulfate (Warnick et al. 1982; Patsch et al. 1989). Concentrations of HDL_3-cholesterol were determined after reprecipitation of the total HDL-cholesterol supernatant with increasing concentrations of $MgCl_2$ and dextran sulfate (Warnick et al. 1982; Patsch et al. 1989). LDL-cholesterol levels were calculated according to Friedewald et al. (1972). Plasma apo B and A-I levels were measured by radioimmunoassay according to the methods of Schonfeld et al (1974) and Brown et al (1988), respectively. Plasma Lp(a) concentrations were measured by an enzyme-linked immunoassay described elsewhere (Gaubatz et al. 1986). Retinyl palmitate levels in plasma were determined by high-pressure liquid chromatography as described elsewhere (Boerwinkle et al. 1994).

Apo E genotypes were determined by restriction isotyping (Hixson and Vernier 1990). Genomic DNA was extracted from frozen buffy coats, amplified by PCR using the primers reported by Emi et al. (1988), and restricted with *Hha*I. The apo E genotypes were typed directly from ethidium bromide–stained 12% polyacrylamide gels.

Statistical Methods

The one-way analysis of variance (ANOVA) and nonparametric Kruskal-Wallis statistic were applied to test whether the demographic and anthropometric variables and plasma lipid, lipoprotein, and apoliprotein levels differed among apo E genotypes within the two groups defined by CAAD status. A simple contingency χ^2 statistic was first used to test whether apo E genotype and allele frequencies were different among CAAD disease groups. These analyses were performed separately in white and black subjects.

Conditional stepwise logistic regression (Kleinbaum et al. 1982; Hosmer and Lemeshow 1989) was used to identify a set of variables that were significantly related to case-control status. The list of potential variables included age; BMI; CigYears (cigarette consumption, defined as the number of cigarettes smoked/d $\times$ the number of smoking years); hypertension status; ethanol consumption; and fasting glucose, insulin, total cholesterol, triglyceride, LDL-cholesterol, HDL-cholesterol, HDL_2-cholesterol, HDL_3-cholesterol, apoA-I, apoB, and Lp[a] levels. Because this is a cross-sectional study, the dependent variable in the logistic regression equation is the log odds of being a CAAD case at the time of baseline examination, rather then the logs odds of developing CAAD during some fixed time interval. Logistic regression analysis was used rather than discriminant analysis because the model for logistic regression is less restrictive with respect to assumptions than the linear discriminant models (Press and Wilson 1978). Two separate models were used to estimate the relationship between the apo E polymorphism and CAAD status. In the first model, a set of significant demographic and anthropometric variables were initially identified; to this list the set of biochemical measures, such as plasma lipid concentrations variables, were added. We next asked whether information about apo E genotype improved the ability to discriminate CAAD case status beyond that afforded by these more-traditional risk factors. In the second model, an a priori set of demographic, anthropometric, and lipoprotein variables were identified and "forced" into the model. This set of variable was chosen to represent a set of well-accepted risk factors as identified by the American Heart Association and the National Institutes of Health (AHA/NHLBI 1990). Next, we asked whether apo E genotypes contribute to our ability to discriminate CAAD case status beyond that afforded by these established risk factors. A P-value of $<.10$ was considered statistically significant.

Results

Lifestyle Variables and Plasma Lipid, Lipoprotein, and Apolipoprotein Concentrations in Study Subjects

This study examined 145 cases (47 women and 98 men) and 244 controls (89 women and 155 men), for whites, and 24 cases (11 women and 13 men) and 46 controls (26 women and 20 men), for blacks. In whites, there were 337 nonhypertensive subjects (224 controls and 113 cases) and 50 hypertensive subjects (19 controls and 31 cases). In blacks, there were 43 nonhypertensive subjects (29 controls and 14 cases) and 27 hypertensive subjects (17 controls and 10 cases). In whites, cases showed higher average values than controls for age (57 versus 55 years), BMI (27 versus 26 kg/m^2), glucose levels (103 versus 99 mg/dl), cigarette consumption (621 versus 277 CigYears), and ethanol consumption (55 versus 46 g/wk). Significant differences were observed between cases and controls for age (P = .036), BMI (P = .001), glucose level (P = .003), and CigYears (P = .001). In blacks, cases showed higher average values than controls for age (56 versus 55 years) and ethanol consumption (48 versus 22 g/wk). However, these differences were not statistically significant (table 1).

Fasting plasma lipid, lipoprotein, and apolipoprotein levels for the cases and controls are shown in table 2. In whites, cases had higher average values than controls for total cholesterol (221 versus 204 mg/dl), triglycerides (146 versus 107 mg/dl), LDL-cholesterol (145 versus 128 mg/dl), apoB (98 versus 86 mg/dl), and Lp[a] (83 versus 62 μg/ml); while cases had lower average values than controls for HDL-cholesterol (47 versus 55 mg/dl), HDL_2-cholesterol (12 versus 15 mg/dl), HDL_3-cholesterol (35 versus 40 mg/dl), and apoA-I (124 versus 133 mg/dl). These differences between cases and controls were statistically significant for all variables. In blacks,

Table 1

Physical and Lifestyle Characteristics in Control and Case Subjects

Characteristic and Race	Controls			Cases			P^a
	n	Mean	(SD)	*n*	Mean	(SD)	
Age (years):							
White	244	55	(5)	145	57	(5)	.036
Black	46	55	(6)	24	56	(6)	.556
BMI (kg/m^2):							
White	244	26	(4)	145	27	(5)	.001
Black	46	28	(6)	24	28	(5)	.736
Glucose (mg/dl):							
White	244	99	(10)	145	103	(17)	.003
Black	45	107	(33)	24	99	(12)	.268
CigYears[b]:							
White	240	277	(416)	142	621	(525)	.001
Black	43	277	(607)	23	279	(378)	.993
Ethanol (g/wk):							
White	242	46	(84)	145	55	(99)	.679
Black	46	22	(61)	24	48	(118)	.227
Wall Thickness (mm):							
White	244	.63	(.081)	145	1.17	(.257)	.001
Black	46	.66	(.097)	24	1.04	(.187)	.001

[a] Probability of observing the mean difference between cases and controls by chance.
[b] Number of cigarettes per day times the number of smoking years.

cases showed higher average values compared with controls for HDL_2-cholesterol (18 versus 16 mg/dl) and Lp[a] (176 versus 141 μg/ml), while cases showed lower average values compared with controls for total cholesterol (206 versus 212 mg/dl), triglycerides (107 versus 112 mg/dl), HDL-cholesterol (56 versus 57), HDL_3 (38 versus 41 mg/dl), LDL-cholesterol (129 versus 133 mg/dl), apoA-I (132 versus 143 mg/dl), and apoB (84 versus 93 mg/dl). Despite these observed differences in plasma lipid levels among black subjects, they did not reach statistical significance, most probably because of the relaxed CAAD selection criteria for black subjects described in Methods. As can be seen in table 1, the difference in average carotid artery wall thickness between cases and controls is less in the sample of blacks than in the sample of whites.

Apo E Genotype and Allele Frequencies in Cases and Controls

The observed frequencies of the six apo E genotypes in cases and controls are shown in table 3. The most frequent genotype was ε3/3, in both disease groups and races. One white subject in each disease group was identified as an ε2/2, and only a few subjects showed the ε2/4 or ε4/4 genotypes. In blacks, the observed apo E genotypes were ε2/3, ε2/4, ε3/3, and ε3/4. No significant difference was observed in apo E genotype frequencies between cases and controls, when all genotypes were considered ($P = .63$ for whites; $P = .44$ for blacks) or when the analysis was restricted to the three most common genotypes, ε2/3, ε3/3, and ε3/4 ($P = .22$ for whites; $P = .46$ for blacks).

The estimated apo E allele frequencies in the sample of 244 white control subjects were .063, 0812, and .125 for the ε2, ε3, and ε4 alleles, respectively. In the sample of white cases, the estimated apo E alleles frequencies were .090, .783, and .127 for the ε2, ε3, and ε4 alleles, respectively. The estimated frequencies of the ε2, ε3, and ε4 alleles in the sample of black controls were .076, .793, and .130, respectively, and .104, .812, and .0833, respectively, in the sample of black cases. In both race and disease groups, the observed genotype frequencies were in agreement with Hardy-Weinberg expectations. The frequencies of the apo E alleles were not significantly different between CAD cases and controls in either racial group ($P = .39$ for whites; $P = .63$ for blacks).

Effect of the Apo E Polymorphism on Plasma Lipid, Lipoprotein, and Apolipoprotein Levels

The estimated mean and SD of plasma lipid, lipoprotein, and apolipoprotein levels for cases and controls in the sample of whites and blacks are shown in table 4. Two different statistics (ANOVA and Kruskal-Wallis tests) were applied, to test the effect of the apo E genotypes on plasma lipid levels. In the sample of white control subjects, the effect of the apo E genotypes on plasma LDL-cholesterol and apoB was significant by the ANOVA. According to the Kruskal-Wallis test, in the group of white controls, the levels of LDL-cholesterol and

Table 2

Plasma Lipid, Lipoprotein, and Apolipoprotein Levels in Control and Case Subjects

Variable[a] and Race	Controls			Cases			P^b
	n	Mean	(SD)	*n*	Mean	(SD)	
Cholesterol:							
White	244	204	(38)	145	221	(41)	.000
Black	44	212	(42)	24	206	(41)	.579
TRG:							
White	244	107	(60)	145	146	(91)	.000
Black	44	112	(48)	24	107	(52)	.714
HDL-cholesterol:							
White	244	55	(18)	145	47	(15)	.000
Black	44	57	(16)	24	56	(19)	.853
HDL_2:							
White	244	15	(10)	145	12	(7)	.001
Black	44	16	(9)	24	18	(9)	.423
HDL_3:							
White	244	40	(11)	145	35	(11)	.000
Black	44	41	(11)	24	38	(13)	.379
LDL-cholesterol:							
White	243	128	(36)	143	145	(37)	.000
Black	44	133	(40)	24	129	(39)	.679
ApoA-I:							
White	244	133	(30)	145	124	(29)	.005
Black	44	143	(32)	24	132	(34)	.192
ApoB:							
White	244	86	(26)	145	98	(26)	.000
Black	44	93	(34)	24	84	(26)	.263
Lp[a]:							
White	243	62	(73)	144	83	(87)	.012
Black	42	141	(103)	23	176	(132)	.242

[a] All levels expressed as mean (SD) in mg/dl except Lp[a], which is expressed as μg/ml.
[b] Probability of observing the mean difference between cases and controls by chance.

apoB were consistently different among apo E genotypes. Average LDL-cholesterol levels were significantly lower in individuals carrying an ε2 allele, compared with the other genotypes. For example, average LDL-cholesterol levels were 89, 108, and 95 mg/dl for the ε2/2, ε2/3, and ε2/4 white control subjects, respectively, and 131, 129, and 135 mg/dl for the ε3/3, ε3/4, and ε4/4 white control subjects, respectively. The only significant difference among apo E genotypes in white cases was observed on plasma apoB levels (P = .027), using the

Table 3

Comparison of Apolipoprotein E Genotype Frequencies between Controls and Cases

Group and Race	Apolipoprotein E Genotypes						Total
	ε2/2	ε2/3	ε2/4	ε3/3	ε3/4	ε4/4	
Controls (*n* and frequency [%]):							
White	1 (.41)	22 (9.02)	7 (2.87)	165 (67.62)	44 (18.03)	5 (2.05)	244 (100)
Black	0 (0.0)	7 (15.22)	0 (0.0)	27 (58.70)	12 (26.09)	0 (0.0)	46 (100)
Cases (*n* and frequency [%]):							
White	1 (.69)	21 (14.48)	3 (2.07)	89 (61.38)	28 (19.31)	3 (2.07)	145 (100)
Black	0 (0.0)	4 (16.67)	1 (4.17)	16 (66.67)	3 (12.50)	0 (0.0)	24 (100)

Note.—χ^2 = 3.45 with P = .63 for comparison of cases and control within whites. χ^2 = 1.64 with P = .44 for comparison of cases and control within blacks. χ^2 = 3.07 with P = .22 for comparison of cases and control within whites considering only the three common genotypes. χ^2 = 1.54 with P = .46 for comparison of cases and control within blacks considering only the three common genotypes.

Table 4

Fasting Plasma Lipids, Lipoprotein, and Apolipoprotein Levels by ApoE Genotype

Variable[a] and Race	ε2/2	ε2/3	ε2/4	ε3/3	ε3/4	ε4/4	P^b	P^c
Controls:								
Cholesterol								
White	175	186 (40)	176 (17)	207 (39)	206 (35)	204 (40)	.075	.050
Black	...	214 (67)	...	213 (38)	207 (38)	...	.918	.962
TRG								
White	76	118 (83)	85 (33)	105 (60)	111 (56)	105 (19)	.822	.854
Black	...	129 (71)	...	101 (46)	126 (48)	...	.224	.339
HDL-cholesterol								
White	71	55 (22)	64 (17)	55 (18)	54 (15)	47 (12)	.631	.389
Black	...	50 (19)	...	59 (17)	57 (19)	...	.494	.493
HDL_2								
White	16	15 (13)	20 (12)	15 (10)	14 (8)	13 (2)	.860	.805
Black	...	14 (4)	...	17 (10)	16 (8)	...	.738	.947
HDL_3								
White	55	40 (14)	44 (7)	40 (11)	40 (10)	34 (11)	.489	.329
Black	...	36 (9)	...	42 (10)	41 (14)	...	.506	.432
LDL-cholesterol								
White	89	108 (40)	95 (27)	131 (36)	129 (32)	135 (42)	.010	.009
Black	...	138 (58)	...	135 (39)	126 (31)	...	.781	.807
ApoA-I								
White	161	133 (32)	159 (40)	131 (29)	136 (32)	122 (21)	.167	.268
Black	...	128 (23)	...	148 (34)	141 (30)	...	.323	.296
ApoB								
White	73	78 (38)	68 (17)	86 (25)	88 (22)	111 (28)	.047	.021
Black	...	76 (24)	...	94 (30)	103 (46)	...	.275	.381
Lp[a]								
White	9	40 (43)	58 (79)	66 (77)	54 (67)	114 (74)	.250	.073
Black	...	103 (70)	...	150 (120)	143 (81)	...	.581	.494
Cases:								
Cholesterol								
White	222	204 (41)	234 (5)	221 (42)	231 (32)	213 (27)	.314	.304
Black	...	200 (14)	167	211 (46)	199 (48)	...	.739	.518
TRG								
White	184	145 (96)	229 (102)	134 (57)	174 (155)	126 (16)	.225	.455
Black	...	164 (72)	91	92 (43)	120 (31)	...	.082	.150
HDL-cholesterol								
White	34	46 (13)	34 (8)	48 (16)	45 (12)	61 (28)	.277	.351
Black	...	52 (19)	52	59 (21)	45 (9)	...	.692	.814
HDL_2								
White	9	10 (4)	12 (7)	12 (7)	11 (7)	17 (10)	.501	.556
Black	...	14 (4)	18	20 (10)	11 (2)	...	.432	.346
HDL_3								
White	25	36 (9)	22 (12)	37 (11)	35 (8)	44 (19)	.189	.339
Black	...	38 (10)	34	39 (14)	33 (8)	...	.905	.876
LDL-cholesterol								
White	151	129 (33)	154 (17)	146 (38)	155 (35)	127 (2)	.197	.146
Black	...	114 (45)	97	134 (39)	131 (40)	...	.699	.644
ApoA-I								
White	94	114 (23)	104 (30)	126 (30)	124 (23)	154 (36)	.115	.149
Black	...	144 (33)	136	130 (39)	126 (5)	...	.908	.536
ApoB								
White	97	84 (25)	112 (9)	99 (25)	107 (31)	88 (16)	.054	.027
Black	...	78 (22)	70	87 (30)	81 (10)	...	.874	.734
Lp[a]								
White	75	69 (78)	83 (84)	98 (92)	98 (92)	121 (192)	.859	.796
Black	...	73 (43)	238	196 (148)	192 (103)	...	.413	.233

[a] All levels expressed as mean (SD) in mg/dl except Lp[a] which is expressed as μg/ml.
[b] *P*-value from the one-way ANOVA.
[c] *P*-value from the Kruskal-Wallis test.

Table 5

Relationship Between the Apo E Polymorphism and CAAD Status Using a Stepwise Selection Procedure

Variables	Coefficient	Standard Error	*P*	Odds Ratio
Apo E Genotypes:				
ε2/2	1.26	1.506	.40	3.53
ε2/3	.613	.346	.077	1.85
ε2/4	−.045	.733	.951	.956
ε3/4	.240	.303	.427	1.27
ε4/4	.115	.751	.878	1.12
Significant Lifestyle and Plasma Lipid Variables:				
Age (years)	.153	.051	.003	1.17
BMI (kg/m^2)	.0778	.0358	.0297	1.081
CigYears	.0016	.0003	.0001	1.002
Hypertension	.892	.407	.0284	2.440
Cholesterol (mg/dl)	.0079	.0037	.0292	1.008
Lp[a] (μg/ml)	.0039	.0017	.0242	1.004
HDL-cholesterol (mg/dl)	−.0331	.0109	.0025	.967
Significant Lifestyle and Plasma Lipid Variables and Apo E Genotypes:				
Age (years)	.149	.0511	.0036	1.161
BMI (kg/m^2)	.0836	.0366	.0224	1.087
CigYears[a]	.0017	.0003	.0001	1.002
Hypertension	.985	.406	.0155	2.68
Cholesterol (mg/dl)	.0090	.0037	.0159	1.009
Lp[a] (μg/ml)	.0039	.0018	.0248	1.004
HDL-cholesterol (mg/dl)	−.0335	.0112	.0026	.967
ε2/2	2.20	2.56	.391	9.00
ε2/3	.800	.455	.079	2.23
ε2/4	.289	1.060	.785	1.34
ε3/4	−.0083	.381	.983	.992
ε4/4	.646	.790	.414	1.91

[a] CigYears expressed as number of cigarettes per day times number of smoking years.

Kruskal-Wallis test. No significant differences were observed in blacks for any of these measures.

Conditional Logistic Regression with the Apo E Polymorphism

Because of the small size of the black sample, the conditional logistic regression was performed only in the sample of white subjects. When apo E genotypes were considered as the only potential variables, the ε2/3 genotype was associated with CAAD status ($P = .077$). The odds of being a CAAD case were 1.8 times higher for ε2/3 individuals compared with ε3/3 individuals (table 5). It is interesting to note here that, although apo E genotype or allele frequencies were not different between cases and controls (table 3), the ε2/3 genotype was related to case/control status in this conditional logistic regression analysis. We next selected by stepwise logistic regression the lifestyle variables that were significant predictors of the probability of having CAAD (table 5). Age, cigarette consumption, body mass, and hypertension status were the significant risk factors identified by this analysis. Likewise, only those plasma lipid variables that were significantly related to CAAD status were included in the logistic equation. These were total cholesterol ($P = .0292$), Lp[a] ($P = .0242$), and HDL-cholesterol ($P = .0025$). After selecting this set of demographic, anthropometric, and plasma lipid variables, we asked whether any of the apo E genotypes provided additional information for discriminating CAAD case status. Only the ε2/3 genotype ($P = .078$) provided additional information for discriminating CAAD case status at the .10 level of significance (table 5). According to this analysis, the odds of being a CAAD case for ε2/3 individuals is 2.2 times as high as the odds for ε3/3 individuals, after considering the effects of the other variables. Therefore, although the level of statistical significance did not change, the odds ratio for the ε2/3 genotype increased from 1.8 to 2.2, after considering the other variables.

Second, a model with an a priori set of demographic, anthropometric, and lipoprotein variables known to be important in predicting CHD was created. The list of established risk factors included age, cigarett consumption, body mass, hypertension status, LDL-cholesterol, and HDL-cholesterol. The apo E genotypes were then introduced into this model and their significance tested. The results are presented in table 6. As in the first model, the

Table 6

Relationship between the apo E Polymorphism and CAAD Status Using a Set of A PRIORI Predictor Variables

Variables	Coefficient	Standard Error	P	Odds Ratio
Age (years)	.1444	.0502	.0040	1.155
BMI (kg/m²)	.0999	.0366	.0063	1.105
CigYears[a]	.0018	.0003	.0001	1.002
Hypertension	.9363	.4095	.0222	2.551
LDL-cholesterol	.0105	.0040	.0085	1.011
HDL-cholesterol	−.0264	.0114	.0209	.974
ε2/2	1.8639	2.0969	.3741	6.449
ε2/3	.8963	.4364	.0400	2.451
ε2/4	.1969	1.0564	.8520	1.218
ε3/4	−.0227	.3757	.9517	.978
ε4/4	.8492	.7883	.2814	2.338

[a] Number of cigarettes per day times number of smoking years.

genotype ε2/3 proved to be significantly related to CAAD case status (P = .0400) . The estimated odds of being a CAAD case associated with the apo E genotype ε2/3 was 2.45, adjusted for the other variables in the model.

The ε2 allele is associated with elevated postprandial lipemia (Weintraub et al. 1987; Boerwinkle et al. 1994), and these postprandial lipoproteins are atherogenic (Zilversmit 1979; Patsch et al. 1992). Therefore, we next asked whether the significant association between the ε2/3 genotype and CAAD in this sample may be mediated through its known effect on delayed postprandial lipoprotein clearance. Measures of postprandial lipoprotein clearance were added to the list of identified risk factor variables in both models, and then the ability of the apo E polymorphism to discriminate CAAD case status was assessed. The results are shown in tables 7 and 8 for postprandial retinyl palmitate. The conclusions based on postprandial triglyceride levels are the same as those for postprandial retinyl palmitate, so the additional data on triglycerides are not shown here. In both model 1, where stepwise logistic regression was used to select the set of significant risk factor variables (table 7), and model 2, where a priori criteria were used to identify this set of variables (table 8), the ability of ε2/3 genotype to discriminate CAAD case status was only marginally affected by inclusion of a postprandial response variable in the equation.

Discussion

To gain insight into the association of lipid transport and atherosclerosis and its modulation by genetic factors, we tested the relationship between the common apo E polymorphism and the occurrence of CAAD, after considering the contribution of established risk factor variables. Apo E genotype and allele frequencies were not significantly different in this sample of CAAD cases and controls. However, using conditional logistic regression, we showed that the apo ε2/3 genotype is associated with CAAD status after the effects of other risk factors are considered. This result was true when a stepwise variable selection procedure was used to define the set of traditional predictor variables or when a set of a priori predictor variables were used. For both models, the estimated odds ratio of being a CAAD case associated with the apo E genotype ε2/3 was >2. Therefore, we should accept the alternative hypothesis that apo E gene information improves the prediction of CAAD beyond that of traditional risk factors.

Numerous reports have indicated that the apo E polymorphism influences plasma lipid levels. Hallmann et al. (1991) have studied the frequency and effects of the apo E polymorphisms among nine ethnically and geographically diverse populations. They concluded that, although the frequencies of the apo E alleles are heterogeneous among populations, the effects of this gene are relatively consistent: the average effect of the ε2 allele is to lower plasma cholesterol (especially LDL-cholesterol) levels, and the average effect of the ε4 allele is to raise plasma cholesterol (especially LDL-cholesterol) levels. However, the effect of the ε4 allele, as measured by the difference between ε3/4 heterozygotes and ε3/3 homozygotes, is small compared with the effect of the ε2 allele, as measured by the difference between ε2/3 heterozygotes and ε3/3 homozygotes. Similar results have been obtained in studies of U.S. blacks (Eichner et al. 1989). Even though the relative frequency of the ε4 allele is generally higher in blacks than in whites, the same effects of the apo E polymorphism on plasma cholesterol levels are apparent. The data reported here from white cases and controls from the ARIC study are consistent with the results from these other studies (table 4). In the entire sample and in the control group, average apo B and LDL-cholesterol levels in individuals carrying an ε2 allele were significantly lower than in the other subjects. Only average apo B levels were significantly different among apo E genotypes in the sample of white cases. The effects of the apo E polymorphism were also evident in the sample of black cases and controls for the average apo B levels, but they were not statistically significant.

Even though some previous studies of survivors of myocardial infarction have suggested an association of the ε2 allele with atherosclerotic disease (Kameda et al. 1984; Utermann et al. 1984), the majority of epidemiological studies favor an association of the ε4 allele with the occurrence of myocardial infarction or angiographically documented CHD (Menzel et al. 1983; Cumming and Roberts 1984; Lenzen et al. 1986). In general, the frequency of the ε2 allele is reduced among CHD patients, while the frequency of the ε4 allele is increased. In the Pathobiological Determinants of Atherosclerosis in Youth study, Hixson et al. (1991) reported that individuals with the ε2/3 genotype had the lowest degree of

Table 7

Relationship between the apo E Polymorphism and CAAD Status, Using a Stepwise Selection Procedure Plus Postprandial Retinyl Palmitate

Variables	Coefficient	Standard Error	*P*	Odds Ratio
Significant Lifestyle and Plasma Lipids Variables:				
Age (years)	.1398	.0528	.0081	1.150
BMI (kg/m^2)	.0778	.0363	.0326	1.081
CigYears[a]	.0016	.0003	.0001	1.002
Hypertension	1.1117	.4198	.0081	3.040
Cholesterol (mg/dl)	.0101	.0039	.0108	1.010
Lp[a] (μg/ml)	.0036	.0018	.0444	1.004
HDL-cholesterol (mg/dl)	−.0367	.0119	.0020	1.000
Ret8_0d (mg/dl)	.0003	.0002	.2036	1.000
ApoE Genotypes:				
Age (years)	.1318	.0533	.0134	1.141
BMI (kg/m^2)	.0861	.0375	.0217	1.090
CigYears	.0017	.0003	.0001	1.002
Hypertension	1.0974	.4238	.0096	2.996
Cholesterol (mg/dl)	.0114	.0041	.0054	1.011
Lp[a] (μg/ml)	.0037	.0018	.0449	1.004
HDL-cholesterol (mg/dl)	−.0373	.0121	.0021	.963
Ret8_0d (mg/dl)	.0002	.0002	.3365	1.000
ε2/2	1.8680	2.8484	.5119	6.476
ε2/3	.7334	.4935	.1373	2.082
ε2/4	.0498	1.0845	.9633	1.051
ε3/4	−.2502	.3993	.5309	.779
ε4/4	.1658	.8982	.8536	1.180

[a] CigYears expressed as number of cigarettes per day times number of smoking years.

both thoracic and abdominal aorta atherosclerosis and that individuals with the ε3/4 genotype had the greatest degree of involvement. Schachter et al. (1994) reported that the frequency of the ε4 allele is significantly decreased and the frequency of the ε2 allele is significantly increased in centenarians, compared with controls. The most likely cause of the observed association between the ε4 allele and CHD is the role of this gene in raising plasma LDL-cholesterol.

The observed association of the ε2 allele with CAAD may, therefore, appear unexpected. However, the design of our study and the models used for the analysis of association differ from those employed in previous studies. First, our study design excluded subjects with

Table 8

Relationship between apo E Polymorphism and CAAD Status, Using a Set of A Prior Risk Factor Variables with Postprandial Retinyl Palmitate

Variables	Coefficient	Standard Error	*P*	Odds Ratio
Age (years)	.1340	.0524	.0106	1.143
BMI (kg/m^2)	.1002	.0371	.0068	1.105
CigYears[a]	.0017	.0003	.0001	1.002
Hypertension	1.0850	.4270	.0111	2.960
Cholesterol (mg/dl)	.0117	.0043	.0067	1.012
HDL-cholesterol (mg/dl)	−.0293	.0124	.0183	.971
Ret8_0d (mg/dl)	.0002	.0002	.2184	1.000
ε2/2	1.4701	2.3438	.5305	4.350
ε2/3	.8058	.4744	.0894	2.238
ε2/4	.0032	1.0564	.9676	1.003
ε3/4	−.2595	.3928	.5088	.771
ε4/4	.4087	.8947	.6478	1.505

[a] CigYears expressed as number of cigarettes per day times number of smoking years.

prevalent CHD. The purpose of this exclusion was to begin to study the risk factors for atherosclerosis separate from those for the acute thrombolytic event. Because of an association of carotid intima-media thickness with CHD, our selection procedure eliminated three times as many potential CAAD cases as controls from this study. Hence, individuals with more advanced stenotic or occlusive atherosclerotic disease have been excluded, and the associations of the apo E polymorphism with CAAD status may have been modified. Second, the relationship between the ε2/3 genotype and CAAD status increased after the effects of plasma LDL-cholesterol and the other risk factor variables were considered. In addition, apo E genotype and allele frequencies were not significantly different among CAAD cases and controls. In the Framingham study, the relative odds of developing CHD after 12 years for ε2/3 individuals and ε3/4 individuals were 1.36 and 1.32, respectively, after adjusting for age, total cholesterol, and HDL-cholesterol (Wilson et al. 1994). Therefore, an answer to the question of whether or not the apo E polymorphism is related to CAAD is more complex than first perceived. It not only depends on the direct effects of the apo E polymorphism on lipid metabolism and the atherosclerotic process, but it also depends on the population of inference and the other risk factors to be considered. Alternatively, it may be that the predictors of carotid artery atherosclerosis are different from that for the coronary arteries. Specifically, the relationship between the apo E polymorphism and CAAD may be different from that of its relationship with CHD.

Because of its recognized importance in remnant clearance, the apo E gene has been targeted for studying postprandial lipid response (Mahley and Rall 1989). Apo E plays a major role in lipid metabolism through cellular uptake of lipoprotein particles by apo E-specific and apo B/E receptors on the liver and other tissues (Mahley et al. 1990). Reduced binding affinity of the apo E2 isoform and subsequent up-regulation of hepatic LDL receptors is one likely mechanism by which the ε2 allele is associated with lower plasma LDL-cholesterol levels (Weintraub et al. 1987; Boerwinkle and Utermann 1988). In addition, reduced formation of plasma LDL particles may contribute to lower LDL-cholesterol in ε2 individuals, since the conversion of VLDL to LDL in the plasma of patients with familial type III hyperlipoproteinemia is impeded by the apo E2 isoform (Ehnholm et al. 1984). Consistent with the function of the apo E2 isoform, several studies have shown a reduction in the clearance of postprandial lipoproteins in subjects carrying an ε2 allele (Gregg et al. 1986; Brenninkmeijer et al. 1987; Weintraub et al. 1987; Brown and Roberts 1991; Superko and Haskell 1991; Boerwinkle et al. 1994). In a previous publication of 474 subjects from the ARIC study, the ε2 allele was associated with delayed clearance of postprandial retinyl palmitate but had no effect on postprandial triglyceride concentrations (Boerwinkle et al. 1994). Hence, only terminal catabolism of remnants appears to be impaired in subjects carrying the ε2 allele. After adding postprandial retinyl palmitate concentrations into the equation, the log odds of being a CAAD case for individuals with the ε2/3 genotype was reduced (tables 7 and 8). Thus, the association of the ε2/3 genotype with CAAD in our sample can be at least partially attributed to the impairment in terminal remnant catabolism that results from the functionally defective apo E2 isoform. Since the magnitude of the association between the ε2/3 genotype and CAAD status increased after consideration of total (model 1) and LDL- (model 2) cholesterol, the atherogenicity conferred by this genotype may be offset by its simultaneous effect in lowering LDL-cholesterol.

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References

American Heart Association/National Heart, Lung, and Blood Institute (1990) The cholesterol facts. Circulation 81:1721–1733

ARIC Investigators (1989) The atherosclerosis risk in communities study: design and objectives. Am J Epidemiol 129:687–702

Boerwinkle E, Brown S, Sharrett AR, Heiss G, Patsch W (1994) Apolipoprotein E polymorphism influences postprandial retynil palmitate but not triglycerode concentrations. Am J Hum Genet 54:341–360

Boerwinkle E, Hixson JE (1990) Genes and normal lipid variation. Curr Opin Lipidol 1:151–159

Boerwinkle E, Utermann G (1988) Simultaneous effects of the apolipoprotein E polymorphism on apolipoprotein E, apolipoprotein B, and cholesterol metabolism. Am J Hum Genet 42:104–112

Brenninkmeijer BJ, Stuyt PMJ, Demacker PNM, Stalenhoef AFH, van't Laar A (1987) Catabolism of chylomicron remnants in normolipidemic subjects in relation to the apoprotein E phenotype. J Lipid Res 28:361–370

Brown AJ, Roberts DCK (1991) The effect of fasting triacylglyceride concentration and apolipoprotein E polymorphism on postprandial lipemia. Arterioscler Thromb 11:1737–1744

Brown SA, Rhodes CE, Dunn K, Gotto Jr AM, Patsch W (1988) Effect of blood collection and processing on radioimmunoassay results for apolipoprotein A-I in plasma. Clin Chem 34:920–924

Cumming AM, Roberts FW (1984) Polymorphism at the apoprotein-E locus in relation to risk for coronary disease. Clin Genet 25:310–313

Dallongeville J, Lussier-Cacan S, Davignon J (1992) Modulation of plasma triglyceride levels by apo E phenotype: A meta-analysis. J Lipid Res 33:447–454

Davignon J, Gregg RE, Sing CF (1988) Apolipoprotein E polymorphism and atherosclerosis. Arteriosclerosis 8:1–21

Ehnholm C, Mahley RW, Chappell DA, Weisgraber KH, Ludwig E, Witztum JL (1984) Role of apolipoprotein in lipolytic conversion of β-very low density lipoproteins to low density lipoprotein in type III hyperlipoproteinemia. Proc Natl Acad Sci USA 81:5566–5570

Eichner JE, Kuller LH, Ferrell RE, Kamboh MI (1989) Phenotypic effects of apolipoprotein structural variation on lipid profiles. IV. Apolipoprotein polymorphisms in a small group of black women from the Healthy Women study. Genet Epidemiol 6:681–689

Emi M, Wu LL, Robertson MA, Myers RL, Hegele RA, Williams RR, White R, (1988) Genotyping and sequence analysis of apolipoprotein E isoforms. Genomics 3:373–379

Friedewald WT, Levy RI, Fredrickson DS (1972) Estimation of the concentration of low density lipoprotein in plasma, without use of the preparative ultracentrifuge. Clin Chem 18:499–502

Gaubatz JW, Ghanem KI, Guevara J, Nava ML, Patsch W, Morrisett JD (1986) Quantitation, isolation, and characterization of human lipoprotein(a). Methods Enzymol 129:167–186

Goldstein JL, Brown MS (1989) Familial hypercholesterolemia. In: Scriver C, Beaudet A, Sly W, Valle E (eds) The metabolic basis of inherited disease. McGraw-Hill, New York, pp 1215–1250

Gregg RE, Zech LA, Schaefer EJ, Stark D, Wilson D, Brewer Jr HB (1986) Abnormal in vivo metabolism of apolipoprotein E4 in humans. J Clin Invest 78:815–821

Hallmann DM, Boerwinkle E, Saha N, Sandholzer C, Menzel HJ, Czázár A, Utermann G (1991) The apolipoprotein E polymorphism: a comparison of allele frequencies and effects in nine populations. Am J Hum Genet 49:338–349

Heiss G, Sharret AT, Barnes R, Chambless LE, Szklo M, Alzola C, ARIC Investigators (1991) Carotid atherosclerosis measured by B-mode ultrasound in populations: associations with cardiovascular risk factors in the ARIC Study. Am J Epidemiol 134:250–256

Hixson JE, Pathobiological Determinants of Atherosclerosis in Youth (PDAY) Research Group (1991) Apolipoprotein E polymorphisms affect atherosclerosis in young males. Arterioscler Thromb 11:1237–1244

Hixson JE, Vernier DT (1990) Restriction isotyping of human apolipoprotein E by gene amplification and cleavage with *Hha*I. J Lipid Res 31:545–548

Hosmer DW, Lemeshow S (1989) Applied logistic regression. John Wiley and Sons, New York

Hui DY, Innerarity TL, Mahyley RW (1984) Defective hepatic lipoprotein receptor binding of β-very low density lipoproteins from type III hyperlipoproteinemic patients. J Biol Chem 259:860–869

Kameda K, Matsuzawa Y, Kubo M, Ishikawak I, Maejima I, Yamamura T, Yamamoto A (1984) Increased frequency of lipoprotein disorders similar to type III hyperlipoproteinemia in survivors of myocardial infarction in Japan. Atherosclerosis 51:241–249

Kleinbaum DG, Kupper LL, Morgenstern H (1982) Epidemiologic research: principles and quantitative methods. John Wiley & Sons, New York

Kuusi T, Nieminen MS, Ehnholm C, Yki-Jarvinen H, Valle M, Nikkila EA, Taskinen M-R (1989) Apoprotein E polymorphism and coronary artery disease: increased prevalence of apolipoprotein E-4 in angiographically verified coronary patients. Arteriosclerosis 9:237–241

Lenzen HJ, Assman G, Buchwalsky R, Schulte HS (1986) Association of apolipoprotein E polymorphism, low-density lipoprotein cholesterol, and coronary artery disease. Clin Chem 32:778–781

Mahley RW, Innerarity TL, Rall Jr SC, Weisgraber KH, Taylor JM (1990) Apolipoprotein E: genetic variants provide insights into its structure and function. Curr Opin Lipidol 1:87–95

Mahley RW, Rall SC (1989) Type III hyperlipoproteinemia (dysbetalipoproteinemia): the role of apolipoprotein E in normal and abnormal lipoprotein metabolism. In: Scriver C, Beaudet A, Sly W, Valle E (eds) The metabolic basis of inherited disease. McGraw-Hill, New York, pp 1195–1214

Menzel HJ, Kladetzky RG, Assman G (1983) Apolipoprotein E polymorphism and coronary disease. Arteriosclerosis 3:310–315

Patsch W, Brown SA, Morrisett JD, Gotto AM Jr, Patsch J (1989) A dual-precipitation method evaluated for measurement of cholesterol in high density lipoprotein subfractions HDL_2 and HDL_3 in human plasma. Clin Chem 265:265–270

Patsch JR, Miesenbock G, Hopferwieser T, Muhlberger V, Knapp E, Dunn JK, Gotto Am Jr (1992) Relation of triglyceride metabolism and coronary artery disease: studies in the postprandial state. Arterioscler Thromb 12:1336–1245

Pignoli P, Tremoli E, Poli A, Oreste P, Paoletti R (1986) Intimal plus medial thickness of the arterial wall: a direct measurement with ultrasound imaging. Circulation 74:1399–1406

Press SJ, Wilson S (1978) Choosing between logistic regression and discriminant analysis. J Am Stat Assoc 73:699

Ross R (1986) The pathogenesis of atherosclerosis: an update. N Engl J Med 314:488–500

Schachter F, Faure-Delanef L, Guenot F, Rouger H, Froguel P, Lesueur-Genot L, Cohen D (1994) Genetic associations

with human longevity at the APOE and ACE loci. Nat Genet 6:29–32

Schonfeld G, Lees RS, George PK, Pfleger B (1974) Assay of total plasma apolipoprotein B concentration in human subjects. J Clin Invest 53:1458–1467

Shea S, Nichols A (1983) The clinical importance of familial history of ischemic heart disease. Cardiovasc Rev Rep 4:1343–1351

Sing CF, Davignon J (1985) Role of apolipoprotein E genetic polymorphism in determining normal plasma lipid and lipoprotein variation. Am J Hum Genet 37:268–285

Superko HR, Haskell WL (1991) The effect of apolipoprotein E isoform difference on postpandrial lipoprotein in patients matched for triglycerides, LDL-cholesterol, and HDL-cholesterol. Artery 18:315–325

ten Kate LP, Moman H, Daiger SP, Motulsky AG (1982) Familial aggregation of coronary heart disease and its relation to known genetic risk factors. Am J Cardiol 50:945–953

Utermann G, Hees M, Steinmetz A (1977) Polymorphism of apolipoprotein E and occurrence of dysbetalipoproteinemia in man. Nature 269:604–607

Utermann G, Hardewig A, Zimmer F (1984) Apo E phenotypes in patients with myocardial infarction. Hum Genet 65:237–241

Warnick GR, Benderson JM, Albers JJ (1982) Dextran sulfate-Mg^{2+} precipitation procedure for quantification of high-density-lipoprotein cholesterol. Clin Chem 28:1379–1388

Weintraub MS, Eisenberg S, Breslow JL (1987) Dietary fat clearance in normal subjects is regulated by genetic variation in apolipoprotein E. J Clin Invest 80:1471–1477

Weisgraber KH, Innerarity TL, Mahley RW (1982) Abnormal lipoprotein receptor-binding activity of the human E apoprotein due to cystein-arginine interchange at a single site. J Biol Chem 257:2518–2521

Wilson PWF, Larson MG, Ordovas JM, Schaefer EJ (1994) Apolipoprotein E isoforms and CHD prevalence in the Framingham offspring. Circulation Suppl 1:I-810

Zilversmit DB (1979) Atherogenesis: a postprandial phenomenon. Circulation 60:473–485