Genetic studies of human apolipoproteins. XI. The effect of the apolipoprotein C-II polymorphism on lipoprotein levels in Nigerian blacks

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Abstract The human apolipoprotein C-II locus exhibits genetically determined structural polymorphism in United States and African blacks. In the present study, we have investigated the effect of the apoC-II polymorphism on quantitative serum levels of total cholesterol, total high density lipoprotein (HDL) cholesterol, cholesterol in high density lipoprotein subfractions, low density lipoprotein (LDL) cholesterol, and triglycerides (TG) in a sample of 368 unrelated Nigerian blacks. The frequencies of the *APOC-II*1* and *APOC-II*2* alleles in the samples were 0.947 and 0.053, respectively. In males, the effect of the *APOC-II*2* allele was to lower the total serum cholesterol and LDL-cholesterol levels by 13.28 mg/dl and 10.55 mg/dl, respectively, relative to the common allele, *APOC-II*1*. In females, the effect was to lower total plasma cholesterol by 4.49 mg/dl and LDL-cholesterol by 3.21 mg/dl. The effect of apoC-II on quantitative lipoprotein levels is shown to be independent of variation at the linked apoE locus, but the products of the two loci interact in determining overall quantitative phenotypes. — **Sepehrnia, B., M. I. Kamboh, L. L. Adams-Campbell, C. H. Bunker, M. Nwankwo, P. P. Majumder, and R. E. Ferrell.** Genetic studies of human apolipoproteins. XI. The effect of the apolipoprotein C-II polymorphism on lipoprotein levels in Nigerian blacks. *J. Lipid Res.* 1989. **30:** 1349–1355.

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Apolipoproteins are the protein components of the plasma lipoproteins. They play an important role in lipid metabolism in the transport of lipids in the plasma, as recognition molecules in the receptor-mediated transport of lipids across plasma membranes, and as allosteric affectors of enzymes of lipid metabolism. Apolipoprotein C-II (apoC-II) is a constituent of chylomicrons, very low density lipoproteins (VLDL), and high density lipoproteins (HDL). Human apoC-II is a single polypeptide chain of 79 amino acid residues and an estimated molecular weight of 8,900 (1). Mature apoC-II acts as an obligate activator of lipoprotein lipase (LPL) which catalyzes the hydrolysis of triglycerides in chylomicrons and VLDL. The cofactor activity of apoC-II for LPL has been established in patients with genetically determined deficiency of apoC-II, who are severely hypertriglyceridemic, and have a functional deficiency of LPL (2). It has been shown that the enzyme LPL interacts with the COOH-terminal amino acid residues 56–79 (3) of apoC-II. Residues 44–55 possess phospholipid-binding activity that enhances LPL activation in the presence of phospholipid. Deletion of residues 77–79 abolishes the ability of the COOH-terminal fragment to activate LPL and it has been suggested that the two COOH-terminal glutamic acid residues are involved in ionic interaction between LPL and apoC-II (3). The apoC-II gene has been cloned and mapped to the apoC-I/C-II/E linkage group on human chromosome 19 (4). Humphries et al. (5) have used a cDNA clone for apoC-II to study two families with apoC-II deficiency and found no evidence of deletion or rearrangement of the apoC-II gene in these families. Restriction fragment length polymorphisms have been identified in the apoC-II region, but detailed studies of their possible association with disorders of lipid metabolism are limited.

Menzel et al. (6) first reported a protein polymorphism in apoC-II in U.S. blacks. This polymorphism involved the substitution of glutamine for lysine at residue 55 of the

Abbreviations: apo, apolipoprotein; IEF, isoelectric focusing; VLDL, very low density lipoproteins; LDL, low density lipoproteins; HDL, high density lipoproteins; HDL-C, total high density lipoprotein cholesterol; TC, total cholesterol; LPL, lipoprotein lipase; TG, triglyceride; BMI, body mass index.

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apoC-II polypeptide chain. They reported a phenotype frequency of 12% for this variant in a sample of 50 normolipemic U.S. blacks. Sepehrnia, Kamboh, and Ferrell (7) confirmed the presence of this polymorphism in U.S. blacks and further demonstrated its presence in Nigerian blacks, and established its autosomal codominant pattern of segregation in families. This study presents a detailed analysis of the effect of the apoC-II polymorphism on quantitative variation in serum lipids and lipoproteins of 368 unrelated, normal individuals from Benin City, Nigeria.

MATERIALS AND METHODS

Materials

Acrylamide and bis acrylamide were purchased from Bio-Rad Laboratories (Richmond, CA). Pharmalytes pH 4–6.5 were from Pharmacia (Uppsala, Sweden), and Ampholine pH 3.5–5.0 was from LKB (Bromma, Sweden). Nitrocellulose was obtained from Schleicher & Schull (Keene, NH). Goat anti-human apoC-II antiserum was purchased from Daiichi Chemicals (Tokyo, Japan); alkaline phosphatase-conjugated rabbit anti-goat IgG was from Pel-Freeze (Rogers, AR), and ultrapure urea was from Bethesda Research Laboratories (Gaithersburg, MD). All other chemicals were reagent grade.

Samples

The Nigerian population sample consisted of two groups. The first group (n = 172) were medical students, nursing students, and mid-wifery students at the University of Benin, Benin City. The second group was a random sample of civil servants and teachers from the Oroyo College Secondary School, Benin City (n = 196). The total sample contained 368 unrelated subjects (193 males; 175 females) with an average height and weight of 168 ± 7.6 cm and 62.95 ± 9.4 kg in males and 159 ± 6.2 cm and 57.9 ± 9.9 kg in females. The age range was from 17 to 54 years with an average age of 29.80 years. A detailed description of the study population has been published elsewhere (8).

All individuals were fasted at the time of blood sampling. Venous blood samples were drawn into siliconized glass tubes. The blood samples were kept at room temperature for 2 h. Then the samples were centrifuged and serum was pipetted into glass tubes and frozen at −47°C for 2 weeks. A styrofoam ice chest with frozen cold packs was used to transport samples from Nigeria to Pittsburgh. Total serum cholesterol (TC) and triglycerides (TG) were measured manually using an enzymatic method (9,10). TC and total HDL cholesterol (HDL-C) were determined by heparin–manganese chloride precipitation methods, whereas subfraction HDL_3 cholesterol (HDL_3-C) concentration was measured after the precipitation of HDL_2 cholesterol (HDL_2-C) subfraction by dextran sulfate (11,12). LDL cholesterol (LDL-C) was calculated using the equation of Friedewald, Levy, and Fredrickson (13).

A 2-ml aliquot of serum from each individual was transferred to the Human Genetics Laboratory and stored at −80°C prior to being typed.

Isoelectric focusing

Thin-layer polyacrylamide gels (5%: monomer 4.85%, bis 0.15%) containing 6 M urea were prepared as follows: 10.8 g urea was dissolved in 5 ml monomer (29.1% w/v), 5 ml bis (0.9% w/v) and the final volume was adjusted to 30 ml with deionized water (7). The pH gradient was generated by 1% (w/v) ampholyte concentration, pH 4–6.5. The polymerization agent, 20 μl of a 0.1% riboflavin solution, was added to the acrylamide solution with constant stirring, then poured between two glass plates (122 × 260 mm) separated by a 0.5-mm plastic gasket. Polymerization was achieved by continuous overnight exposure to fluorescent light.

The gel was loaded with serum samples absorbed on 5 × 4 mm Whatman 3 MM filter wicks placed 1 cm from the cathode. Electrode strips were saturated with 1 M H_3PO_4 for the anode and 1 M NaOH for the cathode. The gel was placed into an LKB 2217 Ultrophor electrofocusing unit connected with a Lauda RM6 cooling unit operating at 10–13°C and an LKB powere supply. After 20 min focusing, the sample wicks were removed and the cathode electrode was blotted with filter paper. The total isoelectric focusing time was 3 h.

Immunoblotting

After isoelectric focusing was completed, a 24 × 8.5 cm nitrocellulose membrane of 0.2 μm pore size, presoaked in TBS buffer (0.25 M NaCl; 0.03 M Tris-HCl, pH 8.0), was placed on the gel for 45 min as described by Kamboh and Ferrell (14). After the passive transfer of proteins, unoccupied protein-binding sites were blocked by exposure to 5% (w/v) nonfat powdered milk in deionized water for 45 min. The membrane was then incubated for 45 min with 50 ml TBS solution containing 100 μl of goat anti-human apoC-II antiserum followed by 3 10-min washings in TBS buffer. Following the washing steps, the membrane was exposed to 50 ml TBS solution containing 10 μl rabbit anti-goat IgG conjugated with alkaline phosphatase, for 45 min, followed by 3 10-min washings in TBS buffer. Finally, the membrane was stained for alkaline phosphatase using 20 mg β-naphthylphosphate, 20 mg Fast Blue BB salt, and 50 mg magnesium sulfate in 50 ml of buffer (1.8 g NaOH, 3.7 g boric acid/liter).

Statistical methods

Allele frequencies were estimated by gene counting. A contingency chi-square test was used to test for homogeneity

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between the two population samples. For each lipid and lipoprotein variable, adjustments were done to remove the effects of concomitant variables which were: age, age^2, height, height^2, weight, and body mass index [BMI]. The adjustment was perfomed in two steps. First, significant concomitant variables were identified by stepwise regression analysis. Second, the multiple regression equation using the significant concomitant variables was estimated for each lipid and lipoprotein variable, and was used to compute the residuals (i.e., the adjusted values of the variable).

The adjusted lipid and lipoprotein variables were then examined for closeness to the normal distribution by computing skewness and kurtosis coefficients. Suitable transformations of the adjusted variables were performed to induce normality to the extent possible.

To test whether the mean lipid/lipoprotein profiles were equal between sexes and among apoC-II phenotypes, a multivariate analysis of variance was performed by considering jointly the vectors of mean values of the lipid/lipoprotein variables.

Analyses of variance were used to test differences in mean values of adjusted variables between sexes and/or among apoC-II phenotypes. The standard statistical analyses were performed using SAS, BMDP, and MINITAB program packages. Computational formulae used for the average excess of the apoC-II allele have been given elsewhere (15).

RESULTS

The phenotype and allele frequency distribution of apoC-II in male and female Nigerians is presented in **Table 1**. Both sexes show comparable apoC-II values and no significant departure from Hardy-Weinberg equilibrium was detected in either sex. Stepwise regression analyses were performed on each quantitative variable to identify significant concomitant variables. Age and [BMI] were found to be the two most important concomitant variables. After adjustment for the effects of these two concomitant variables, the effects of the other concomitant variables were not significant. The multiple linear regression equations obtained from the stepwise regression analyses were then used for adjusting the values of lipid variables of interest.

TABLE 1. Distribution of common apoC-II phenotypes and allele frequencies in Nigerian males and females

Sex	Number Tested	Phenotypes			Allele Frequencies	
		1-1	2-1	2-2	*APO C-II*1*	*APO C-II*2*
Male	193	173	18	2	0.943	0.057
Female	175	158	17	0	0.951	0.049
Pooled	368	331	35	2	0.947	0.053

The adjusted mean values of all serum lipid variables are given in **Table 2**. Note that the average adjusted TC and LDL-C among individuals with apoC-II 2-1 phenotype are, respectively, 12.13 mg/dl and 9.66 mg/dl lower than the average adjusted values of individuals of the apoC-II 1-1 phenotype. The differences are significant at the 5% level. The differences for the other quantitative lipoprotein variables between 1-1 and 2-1 phenotypes are not statistically significant. (The 2-2 phenotype was excluded from these comparisons because of small sample size, 2 out of 368 individuals).

A two-way analysis of variance was performed separately for each lipid variable to test whether the adjusted mean values of lipid variables differed significantly between sexes and/or among apoC-II phenotypes. However, prior to performing the analyses of variance, the skewness and kurtosis coefficients of the adjusted lipid variables were computed. The adjusted values were also transformed logarithmically and the skewness and kurtosis coefficients of the transformed values were also computed to find out whether a logarithmic transformation resulted in bringing the distribution closer to the normal distribution. The logarithmic transformation was useful for HDL-C, HDL_3-C, and TG, but not for TC and LDL-C. Two-way analyses of variances were, therefore, performed on the untransformed adjusted values of TC and LDL-C and on the

TABLE 2. Adjusted mean values of serum lipid variables in the sample of 368 Nigerian Blacks

Lipid Variable	ApoC-II Phenotype			Pooled Mean
	2-2	2-1	1-1	
	mg/dl ± SE[a]			
TC	145.61 ± 11.03	150.32 ± 1.84	162.45 ± 0.07	161.21 ± 1.82
HDL-C	35.82 ± 7.65	44.30 ± 0.39	47.02 ± 0.02	46.64 ± 0.61
HDL_3-C	22.06 ± 5.55	28.37 ± 0.26	30.12 ± 0.00	29.91 ± 0.38
LDL-C	95.56 ± 3.81	92.29 ± 1.46	101.95 ± 0.05	100.96 ± 1.67
TG	75.06 ± 4.99	68.76 ± 1.19	67.94 ± 0.00	68.06 ± 1.64

[a]Adjusted mean value is unadjusted grand mean plus mean of the adjusted residual.

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$\log_{10}$ transformed values of HDL-C, HDL_3-C, and TG. The analysis of variance models included sex and apoC-II phenotype as the main effects and also the interaction between the two. For each variable, the analysis was performed including and excluding individuals of the apoC-II 2-2 phenotype because the number of individuals with the 2-2 phenotype was small.

For purposes of testing the significance of the main and interaction effects, we first performed a multivariate analysis of variance using the mean vectors of the lipid variables. The Wilks' Λ values were computed and the corresponding approximate F-ratios (16) are given in the first row of **Table 3**. It is seen that while the difference between sexes is significant, the differences among apoC-II phenotypes are not significant. We have also performed univariate analysis of variance for each of the lipid variables separately (Table 3). It is seen that neither of the main effects is significant for HDL-C or HDL_3-C. Only the effect of sex is significant for TG, while the effects of both sex and phenotype are significant for TC and LDL-C. However, it should be noted that the effect of phenotype was significant only when individuals of the 2-2 phenotype were excluded. We attribute this effect to the small number of apoC-II 2-2 individuals (2/368). The interaction effects are nonsignificant for all variables. Because of the significant differences between sexes, separate adjusted mean lipid values were computed for males and females (**Table 4**).

We estimated the effects of the apoC-II polymorphism on TC, LDL-C, and TG levels separately for each sex (**Table 5**). The average excesses of the *APOC-II*2* allele on TC and LDL-C are − 13.28 mg/dl and − 10.55 mg/dl, respectively, in males. In females, the average excess of *APOC-II*2* allele on TC is − 4.49 mg/dl and on LDL-C is − 8.33 mg/dl. In the pooled sample the average excess of the *APOC-II*2* allele on TC is − 11.37 mg/dl and on LDL-C is − 8.33. The average excess of the common allele, *APOC-II*1*, on either TC or LDL-C is negligible in the pooled sample as also in both sexes. Although the effect of phenotype was nonsignificant for TG, the effect of sex was significant, therefore we computed the average excesses of the alleles on TG. While among males the average excess of both alleles was negligible, among females the average excess of *APOC-II*2* was 4.35 mg/dl.

A complementary DNA probe and somatic cell hybrids have been used to map the apoC-II gene to human chromosome 19 (4,17). Family studies using apoE protein polymorphism and RFLP alleles near the apoC-II gene show that the apoC-II and apoE loci are closely linked (18). Linkage disequilibrium has also been reported between the apoE and apoC-II loci (18,19). Because the allelic effect of the *APOC-II*2* allele is similar to that reported for the *APOE*2* allele, we wished to rule out the possibility that strong disequilibrium between these loci was responsible for the estimated effects. We have examined the extent of linkage disequilibrium between the apoE and apoC-II loci in our data using Bennett's (20) estimation procedure. The apoE phenotypes were determined on the sample of 369 individuals by the method of Kamboh, Ferrell, and Kottke (21). The maximum likelihood estimate of the coefficient of linkage disequilibrium, D, in the present data set turns out to be D = − 0.0056. The value of ln-likelihood at D is − 614.91. At D = 0, the value of ln-likelihood is − 615.77. The χ^2 value (with 1 d.f) for test-

TABLE 3. Two-way analysis of variance for testing significances and the effects of sex, apoC-II phenotype, and interaction between sex and phenotype

	F-Ratio for Effect of				
	Gender		Phenotype		Gender X Phenotype
Lipid Variable	Including 2-2 Phenotype	Excluding 2-2 Phenotype	Including 2-2 Phenotype	Excluding 2-2 Phenotype	Excluding 2-2 Phenotype
All (multivariate)[a]	12.08[b] (5,360)[c]	11.98[b] (5,358)	0.90 (10,720)	1.23 (5,358)	0.30 (5,358)
TC	9.99[b] (1,364)	9.69[b] (1,362)	2.58[b] (2,364)	4.65[b] (1,362)	0.49 (1,362)
HDL-C	1.48 (1,364)	1.25 (1,362)	1.86 (2,364)	1.87 (1,362)	0.19 (1,362)
HDL_3-C	0.50 (1,364)	0.67 (1,362)	2.17 (2,364)	1.91 (1,362)	1.12 (1,362)
LDL-C	9.83[b] (1,364)	9.73[b] (1,362)	1.74 (2,364)	3.41[b] (1,362)	0.57 (1,362)
TG	8.30[b] (1,364)	8.25[b] (1,362)	0.12 (2,364)	0.14 (1,362)	0.78 (1,362)

[a]F-ratios for the multivariate test are approximate.
[b]Significant at 5% level.
[c]Figures in parentheses indicate degrees of freedom.

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TABLE 4. Adjusted mean values of serum lipid variables in the sample of Nigerian black males (n = 193) and females (n = 175)

Lipid Variable	Sex	ApoC-II Phenotype 1-1 (Male = 173) (Female = 158)	2-1 (Male = 18) (Female = 17)	2-2 (Male = 2) (Female = 0)	Pooled Mean
		mg/dl ± SE[a]			
TC	Male	160.80 ± 2.35	143.34 ± 6.03	150.83 ± 2.65	159.07 ± 2.57
	Female	164.05 ± 2.63	159.03 ± 6.09		163.56 ± 2.60
HDL-C	Male	46.13 ± 0.89	43.38 ± 3.09	36.41 ± 2.92	45.77 ± 0.86
	Female	47.92 ± 0.91	44.61 ± 2.07		47.60 ± 0.85
HDL_3-C	Male	30.87 ± 0.58	29.37 ± 1.82	22.31 ± 0.68	30.64 ± 0.55
	Female	29.29 ± 0.53	27.43 ± 1.87		29.11 ± 0.52
LDL-C	Male	99.27 ± 2.25	84.85 ± 5.33	98.69 ± 5.99	97.92 ± 2.35
	Female	104.65 ± 2.33	101.06 ± 6.44		104.30 ± 2.37
TG	Male	76.99 ± 2.35	75.50 ± 6.12	78.61 ± 31.31	76.87 ± 2.50
	Female	57.86 ± 1.81	62.68 ± 5.09		58.33 ± 1.81

[a]Adjusted mean value in unadjusted males and females grand mean of the adjusted residual.

ing the significance of difference between these likelihood values is 1.716, which is not significant at the 5% level. In other words, we conclude that there is no evidence of significant linkage disequilibrium between the apoE and the apoC-II loci in the present data set. Thus, the detected effect of the apoC-II polymorphism on cholesterol and triglyceride levels in this sample is not due to the linkage disequilibrium of this locus with the apoE locus.

DISCUSSION

Polymorphism of apoC-II in blacks was first suggested by Menzel et al. (6). They showed that this variation was due to the substitution of glutamine for lysine at residue 55 of the apoC-II polypeptide chain. Comparison of the isoprotein pattern and allele frequency in U.S. and African blacks (7) convinced us that variant allele characterized by Menzel et al. (6) and the *APOC-II*2* allele observed in Nigerians are identical. The biochemical findings already discussed (1,3) suggest that an amino acid substitution at residue 55 might have an impact on phospholipid binding, LPL activation, or both. In this study we have found that individuals heterozygous for *APOC-II*2* allele have lower total cholesterol and LDL-C levels, and increased TG levels.

Menzel et al. (6) reported that there is no significant effect of the apoC-II 2-1 phenotype on quantitative levels of lipids and lipoproteins in a sample of 50 U.S. blacks. These apparent differences may be due to two factors. First, the sample size in Menzel et al. (6) was perhaps too small (44 subjects with the apoC-II 1-1 phenotype and 6 subjects with apoC-II 2-1 phenotype) to allow the detection of the effect of the apoC-II polymorphism on lipid levels. Second, the most important factor is that the lipid and lipoprotein variables in Menzel et al. (6) were not adjusted for concomitant variables that may have obscured the phenotype effect. A paired *t*-test was performed to test for significant differences between the unadjusted lipid and lipoprotein levels for apoC-II 1-1 and apoC-II 2-1 individuals in U.S. blacks (Menzel's study) and Nigerian blacks (this study). **Table 6** shows that TC, LDL-C, HDL-C, and TG levels are significantly different in apoC-II 1-1 individuals for the two populations. In apoC-II 2-1 individuals, only TC and HDL-C are significantly different for U.S. blacks and Nigerian blacks. These re-

TABLE 5. Average excess of the apoC-II alleles on TC, LDL-C, and TG in Nigerian males and females

	Male		Female		Pooled	
	*APOC-II*1*	*APOC-II*2*	*APOC-II*1*	*APOC-II*2*	*APOC-II*1*	*APOC-II*2*
			mg/dl			
TC	0.87	−13.28	0.23	−4.49	0.63	−11.37
LDL-C	0.64	−10.55	0.17	−3.21	0.50	−8.33
TG	0.05	−0.80	−0.22	4.35	−0.08	1.34

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TABLE 6. Comparison of unadjusted lipid and lipoprotein levels (± SD) in United States blacks (data from 6) and Nigerian blacks for apoC-II 1-1 and apoC-II 2-1 phenotypes

Population	No.	ApoC-II Phenotype	TC	LDL-C	HDL-C	TG
				mg/dl		
U.S. blacks	44	1-1	199 ± 36	111 ± 33	62 ± 14	90 ± 84
Nigerian blacks	331	1-1	162 ± 35 (6.42)[a]	101 ± 32 (1.89)[a]	46 ± 11 (3.31)[a]	67 ± 31 (1.80)[a]
U.S. blacks	6	2-1	189 ± 32	106 ± 27	62 ± 13	92 ± 49
Nigerian blacks	35	2-1	154 ± 32 (2.47)[a]	95 ± 29 (0.91)	44 ± 12 (3.17)[a]	72 ± 26 (0.98)

Values in parentheses are *t*-test values.
[a]Significant at 5% level.

sults indicate that despite the shared African ancestry of these populations, differences in cultural practices and/or dietary habits have a significant influence on lipid and lipoprotein levels. It is known that the Nigerian blacks use little animal fat but have a diet high in saturated fat derived from palm oil.

Studies on the apoE polymorphism in Nigerian blacks (22) and U.S. blacks (23) demonstrate that the frequency of the *APOE*4* allele is significantly higher in blacks than in other reported ethnic groups. The effect of the *APOE*4* allele in all the reported populations, including Nigerian blacks, is to raise TC and to lower TG. We assigned 35 individuals of the apoC-II 2-1 phenotype with their corresponding apoE phenotypes (**Table 7**), and then estimated the average effects of apoE polymorphism on these subsamples. The results show that the effect of the *APOE*4* allele in individuals carrying the *APOC-II*2* allele is to lower the total cholesterol and LDL-C by 4.70 mg/dl and 1.76 mg/dl, respectively. This effect is significantly different from the average excess of the *APOE*4* allele in the total Nigerian sample, where the *APOE*4* allele was found to raise total cholesterol and LDL-C by 6.15 mg/dl and 6.77 mg/dl, respectively (22). These differences indicate that there is an interaction between alleles at the apoE and apoC-II loci in determining the overall cholesterol levels in Nigerians. Unfortunately, we did not observe any individuals carrying both the *APOE*2* and *APOC-II*2* alleles to estimate the simultaneous effect of these two alleles, both of which are associated with low cholesterol and high triglyceride levels. However, on the basis of our observation of the simultaneous effect of the *APOE*4* and *APOC-II*2* alleles we would predict that the combined effect of the *APOE*2* and *APOC-II*2* alleles would result in an additive decrease in total cholesterol levels. It is postulated that the presence of the *APOC-II*2* allele in conjunction with E 3-2 phenotype may mimic the quantitative effects seen in apoE 2-2 homozygotes, because of cumulative single gene dose effects contributed by the *APOC-II*2* and *APOE*2* alleles. More than 90% of patients with type III hyperlipidemia have the apoE 2-2 phenotype (24). However, we suggest that individuals with E 3-2 phenotypes who are also carriers of the *APOC-II*2* allele may also be susceptible to developing type III hyperlipidemia or other related hyperlipidemia phenotypes. In this context, Menzel et al. (6) noted in their study that all four hyperlipidemic patients were double heterozygous for the E and C-II genes. The presence of the apoE 3-2 and apoC-II 2-1 phenotypes in all four hyperlipidemic patients is significantly different from what one would expect in normolipidemic individuals based on the respective frequencies of these two alleles in the black population. Although the number of patients observed by Menzel et al. (6) is too small (n = 4) for any conclusions to be reached, the study opens further avenues to investigate the role of the apoC-II locus in Type III hyperlipidemia and other lipid disorders.

TABLE 7. The apoC-II phenotypes and corresponding apoE phenotypes in 337 Nigerian blacks

ApoE Phenotype	ApoC-II Phenotype 1-1	2-1	2-2	Total
3-3	150	21	2	173
4-3	112	12	0	124
4-4	38	2	0	40
Total	300	35	2	337

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