Apolipoprotein [a] genotype influences isoform dominance pattern differently in African Americans and Caucasians

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Abstract **Plasma lipoprotein [a] (Lp[a]) concentrations are inversely associated with, and largely determined by, apolipoprotein [a] (apo[a]) gene size, a highly polymorphic trait. We studied if, within an individual, the smaller apo[a] isoform always dominated, whether there was interaction between the two alleles, and whether these features differed between Caucasians and African Americans. We determined apo[a] gene sizes, apo[a] protein sizes and relative amounts, and plasma Lp[a] levels in 430 individuals (263 Caucasians and 167 African Americans). Of the 397 heterozygotes with at least one detectable apo[a] isoform (238 Caucasians and 159 African Americans), the larger allele dominated in 28% of Caucasians and 23% of African Americans, while the smaller allele dominated in 56% of Caucasians and 45% of African Americans. In Caucasians, dominance of the smaller allele increased with Lp[a] levels, from 44% at Lp[a] ≤30 nM to 81% at Lp[a] >100 nM ($P < 0.0001$). Dominance by the smaller allele increased with increasing size of the larger allele in both groups but with the smaller allele only in African Americans. There was no interaction between apo[a] alleles within genotypes; one apo[a] isoform level was not associated with the other isoform level, and isoform levels were not affected by the difference in size. More of the dominance pattern was explained by Lp[a] level and apo[a] genotype in African Americans than in Caucasians (29% vs. 13%). Thus, genotype influences isoform-specific Lp[a] levels and dominance patterns differently in African Americans and in Caucasians.**—Rubin, J., F. Paultre, C. H. Tuck, S. Holleran, R. G. Reed, T. A. Pearson, C. M. Thomas, R. Ramakrishnan, and L. Berglund. **Apolipoprotein [a] genotype influences isoform dominance pattern differently in African Americans and Caucasians.** ***J. Lipid Res.*** **2002.** 43: **234–244.**

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Lipoprotein[a] (Lp[a]) is an independent risk factor for cardiovascular disease (1–11). Lp[a] consists of a cholesterol-rich lipid particle analogous to LDL, where apolipoprotein B-100 (apoB-100) is linked to apolipoprotein [a] (apo[a]) by a disulfide bond. Each Lp[a] particle contains one molecule of apo[a], which has a number of size isoforms due to a variable number of kringle 4 (K4) repeats, so called because of their resemblance to the K4 domain of plasminogen (12). The size variation of the apo[a] protein, i.e., the number of K4 repeats, arises from a size variation in the apo[a] gene. In each individual, plasma Lp[a] is the sum of Lp[a] carried by the two apo[a] isoforms. It has been well established that plasma Lp[a] levels are largely genetically determined, and dependent on apo[a] size (13, 14). Thus, more than 90% of the variation in plasma Lp[a] levels has been attributed to the apo[a] gene (14). In general, there is an inverse relation between apo[a] allele (or isoform) size and plasma levels; small apo[a] sizes, with few K4 repeats, are usually associated with high levels (13–17).

Using primary hepatocytes from baboons, White and co-workers demonstrated that larger size apo[a] isoforms are secreted from hepatocytes at a slower rate than smaller size apo[a] isoforms, and that a greater proportion of larger apo[a] isoforms is targeted for intracellular degradation (18–21). Similar results have been obtained by other investigators using transfected cells (22, 23). These in vitro results suggest that intracellular processing, such as the efficiency of apo[a] transport out of the endoplasmic reticulum, is a major determinant of plasma Lp[a] levels. This molecular mechanism could potentially explain the general inverse association between apo[a]

Abbreviations: apo [a], apolipoprotein [a]; Lp [a], lipoprotein [a].

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size and plasma levels (24). However, the importance of this mechanism in humans remains to be determined. For a given apo[a] size, African Americans commonly have higher plasma levels than do Caucasians. Even within an ethnic group, there is considerable variation in levels for a given apo[a] size, suggesting additional regulatory factors (25–27). In addition to the size polymorphism, there are other genetic variants in the apo[a] gene, some of which are in linkage disequilibrium with the apo[a] size polymorphism (28–31).

Direct study of apo[a] allele size requires pulsed field gel electrophoresis, a relatively cumbersome technique. Therefore, in most studies, apo[a] allele size has been inferred indirectly from the apo[a] protein isoform size determined by Western blotting techniques (32–39). This approach is useful in cases where two distinct apo[a] isoform sizes can be detected. However, the quite substantial number of cases where only one apo[a] protein isoform can be detected raises a problem. In these cases, it is generally assumed that the detected apo[a] isoform represents the smaller of the two apo[a] allele sizes (33). However, to confirm or refute this, parallel determinations of apo[a] allele and isoform sizes are needed, and this has rarely been done.

In the present study, we measured apo[a] allele sizes, protein isoform sizes, and plasma Lp[a] levels in Caucasians and African Americans. The combined determination of apo[a] genotype as well as isoform-specific Lp[a] levels, not done previously, allowed us to test whether the smaller apo[a] protein isoform was always predominant over the larger apo[a] protein, and whether there were different dominance patterns among Caucasians and African Americans. Further, we looked for interaction between the two apo[a] alleles within an individual.

MATERIALS AND METHODS

Subjects

Subjects were recruited from a patient population scheduled for diagnostic coronary arteriography either at Harlem Hospital Center in New York City or at the Mary Imogene Bassett Hospital in Cooperstown, NY. The study has been described previously (40, 41). Briefly, a total of 648 patients, 401 men and 247 women, ethnically self-identified as Caucasian (n = 344), African American (n = 232) or Other (n = 72) were enrolled. The present report is based on the findings in 430 subjects (263 Caucasians, 167 African Americans) in whom Lp[a] levels, apo[a] allele sizes, and circulating apo[a] isoforms were available. The study was approved by the Institutional Review Boards at Harlem Hospital, the Mary Imogene Bassett Hospital, and Columbia University College of Physicians and Surgeons, and informed consent was obtained from all subjects.

Apo[a] allele size determination

To determine apo[a] allele sizes, we performed genotyping using pulsed field electrophoresis of DNA from leucocytes embedded in agarose plugs, essentially as described by Lackner et al. (42). The size-fractionated DNA was blotted onto a nylon membrane (ICN Biomedicals, Irvine, CA) and hybridized with a human apo[a] K4-specific single-stranded fragment labeled with fluorescein (Gene Images, Amersham, Piscataway, NJ). The probe was a generous gift of Dr. Helen Hobbs, University of Texas Southwestern Medical Center, Dallas, TX.

Each gel had samples spanning a wide range of apo[a] sizes known from the immunoblots, as described below. Migration distances were regressed against isoform sizes to calibrate each gel. Bands whose points fell >1 K4 repeat from the calibration line were re-examined to determine and resolve the discrepancy between the Western blot and the pulsed field gel. The calibration line was then used to determine all gene sizes, including undetected alleles, i.e., alleles with no detectable protein isoforms (see below). Midrange PFGE markers (24.5 to 291 kb) (New England Biolabs, Beverly, MA) covering the entire range of possible DNA sizes were run on each gel to confirm the linearity of the size-distance relationship. The apo[a] alleles were classified according to the estimated total number of K4 repeats contained within their sequences (43). To verify the methodology, duplicate measurements were carried out for eight subjects on different pulsed field gels. Each duplicate was within 1 K4 repeat of the original reading.

Apo[a] isoform size determination

Apo[a] isoform sizes were analyzed by SDS-agarose gel electrophoresis of plasma samples, followed by immunoblotting (40, 44). The apo[a] bands were visualized with the ECL Amersham technique on Kodak X-OMAT films using a second, labeled antibody (Pierce, Rockford, IL). The results were related to standards with known apo[a] isoforms (Immuno AG, Innsbruck, Austria and Intracel, Issaquah, WA) taking into account the inverse logarithmic relation between the number of K4 repeats and isoform mobility during agarose gel electrophoresis (45).

Single apo[a] protein bands

In some subjects with two distinct apo[a] allele sizes, we detected only a single apo[a] isoform. A possible source of error is coalescence of two similar sized but separate apo[a] protein bands appearing as a single isoform in the phenotyping procedure. Repeated phenotyping, with a longer electrophoresis time in order to increase the migration distance, in subjects with apo[a] alleles 1 or 2 K4 repeats apart, failed to reveal two apo[a] bands, indicating that in all subjects with a single apo[a] protein band, the other apo[a] allele corresponded to a protein band with undetected levels. For detectable apo[a] proteins, there was agreement between apo[a] allele and isoform sizes, as mentioned above. Where two alleles were detected by genotyping, but only one protein by phenotyping, the missing allele on the Western blot was termed the "undetected allele."

Measurement of plasma Lp[a] levels

Fasting blood samples were drawn approximately 2 to 4 h before the catheterization procedure, and serum and plasma samples were stored at −80°C prior to analysis. Lp[a] levels were measured using a sandwich ELISA (Sigma Diagnostics, St Louis, MO). In our hands, the interassay coefficient of variation was 8.4% at an apo[a] level of 19.9 nM and 9.0% at an apo[a] level of 67.1 nM (40).

Determination of allele-specific Lp[a] levels and dominance

We determined protein dominance by optical analysis of the apo[a] protein bands on the Western blots. The visual estimations were validated by computerized scanning. For each of the apo[a] protein bands, levels were apportioned according to the degree of intensity of the bands on the Western blot, using 10% increments. For example, an individual with an apo[a] level of 100 nM, carrying apo[a] proteins with 25 and 35 K4 repeats, with the

smaller protein dominating by 80%, had 80 nM apportioned to the 25 and 20 nM to the 35 K4 repeat protein. By serial dilutions of individual samples, it was ascertained that the relative intensities of the two apo[a] isoform bands remained the same over the dilution range.

Each subject was classified as: *a*) larger band dominating; *b*) smaller band dominating; or *c*) neither band dominating. To be sufficiently confident that one band dominated, we assigned 70% as the criterion for dominance. Thus, an apo[a] protein band was defined as dominating if it carried ⩾70% of the total level. Two apo[a] proteins were defined as co-dominating if each band carried ⩾40% but ⩽60% of the total Lp[a]. When using the classification of smaller or larger apo[a] proteins, we are referring to the two apo[a] protein isoforms in a given individual. Thus, for two individuals with the genotypes 24/27 and 20/24, the apo[a] allele (and apo[a] protein) with 24 K4 repeats would be the smaller in the first case and the larger in the second case. This terminology differs from the frequently used terms small (LMW) and large (HMW) apo[a] protein sizes, where both apo[a] alleles in any individual could be of small or, alternatively, large size.

Data analysis

The study of apo[a] genotypes and their relationship with Lp[a] levels is complex. With over 35 possible apo[a] allele sizes, the number of possible genotypes exceeds 500; even with several hundred subjects, any genotype is found in very few subjects. For this reason, it is necessary to group subjects into a small number of genotype categories that can then be compared with respect to allele-specific Lp[a] levels or relative dominance. Therefore, allele size and allele size differences were categorized into ranges. Phenotypes were categorized as no, one, or two bands, as well as larger dominating, smaller dominating, or codominating.

Statistics

Proportions were compared between groups using χ^2 analysis, and Fisher exact test where appropriate. TG levels were log transformed and Lp[a] levels were square root transformed to achieve normal distributions. Group means were compared using Student's *t*-test. Influence of multiple factors on the proportion of total Lp[a] attributed to the smaller allele was analyzed by multiple regression. All statistical summaries and tests were done using SAS software (SAS Institute, Cary, NC).

RESULTS

The lipid and lipoprotein patterns, presented in **Table 1**, are similar to those reported in other population studies (13, 25, 26, 46, 47). The variability in plasma Lp[a] levels was greater for small isoforms than for large isoforms; the variance in square root apportioned Lp[a] levels for sizes with 11–21 K4 repeats was 20.6 and 35.3 nM in Caucasians and African Americans, respectively, compared to 5.1 and 14.1 nM for sizes with >21 K4 repeats ($P < 0.01$). In addition, for each apo[a] size range, the variance was greater for African Americans than for Caucasians ($P < 0.001$).

Apo[a] allele distribution

The frequency distributions of apo[a] allele and isoform sizes are graphed separately for Caucasian and African American subjects in **Fig. 1**. The African American distribution had a narrower and taller peak between 22 and 31 repeats, while the Caucasian distribution was much broader, ranging from 19 to 34. The curves suggest a bimodal distribution for African Americans (peaks at 25 and 29 K4 repeats) and a trimodal one for Caucasians (peaks at 20, 23, and 27/28 K4 repeats) with the last peak very broad. The gap between the allele and isoform curves represents undetected alleles. Among Caucasians, undetected alleles were most common in the mid range, whereas among African Americans they were fairly evenly distributed across apo[a] sizes. The range of apo[a] allele sizes was 13–48 in Caucasians and 11–48 in African Americans. We detected two different-sized apo[a] alleles in 415 of the 430 subjects; 15 subjects, 8 Caucasians (3%) and 7 African Americans (4.2%), had a single apo[a] allele, indicating homozygosity (**Table 2**). Since a homozygote has only a single detectable protein band, it is not possible to determine whether such a band corresponds to one or both alleles, nor is it possible to apportion the plasma Lp[a] level to the two alleles. Hence, the 15 homozygotes were excluded from further analysis.

TABLE 1. Apolipoprotein [a] and lipid levels in study subjects

	Caucasians		African Americans	
	Men n = 171	Women n = 92	Men n = 93	Women n = 74
Age (years)	57.1 ± 10.1	56.1 ± 10.7	54.2 ± 9.7	54.7 ± 9.7
Apo[a] (nM)				
Median	25	26	102	130.5
25th percentile	7	10	61	68
75th percentile	79	87.5	165	210
Square root apo[a] levels	6.3 ± 4.5	6.8 ± 4.8	10.5 ± 4.3[a]	11.2 ± 4.5[a]
Cholesterol (mmol/l)	4.98 ± 1.01	5.29 ± 1.16	4.96 ± 1.05	5.39 ± 1.30
TGs (mmol/l)	1.74 ± 52%	1.75 ± 57%	1.19 ± 45%[a]	1.27 ± 37%[a]
LDL cholesterol (mmol/l)	3.14 ± 0.87	3.20 ± 0.95	3.12 ± 0.98	3.45 ± 1.23
HDL cholesterol (mmol/l)	0.97 ± 0.26	1.19 ± 0.36	1.24 ± 0.47[a]	1.32 ± 0.43

Results are expressed as mean ± SD, except for median levels and percentiles. Lp[a] levels were measured as nM. TG levels are expressed as the exponential of the mean of the individual natural logs. For cholesterol, TGs, LDL and HDL cholesterol levels, 1 mmol/l corresponds to 38.7 and 88.5 mg/dl for cholesterol and TGs, respectively.

[a] $P < 0.0001$, Lp[a] and TG levels in African American men and women compared to Caucasian men and women, respectively, and for HDL cholesterol levels in African American men compared to Caucasian men.

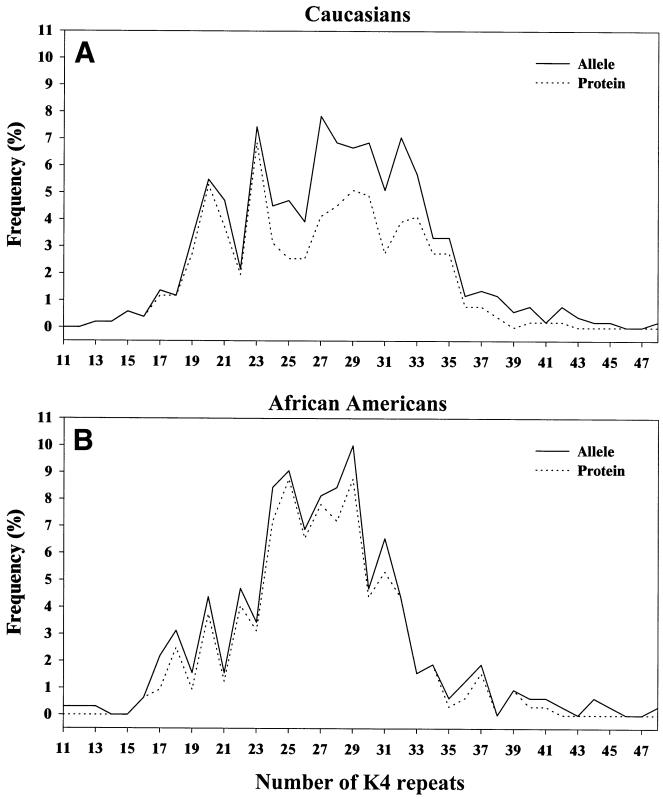

Fig. 1. Frequency distribution of apo[a] alleles and isoforms in Caucasians (A) and African Americans (B). Alleles are represented by the solid lines and apo[a] protein isoforms by the dashed lines (the dashed lines are not shown where they coincide with the solid lines). The isoform distribution was calculated by dividing the total number of protein bands detected by the total number of alleles, separately for each population. Homozygotes (n = 15) were excluded as it was not possible to determine if the single apo[a] protein band corresponded to one or two proteins.

Undetected apo[a] alleles

Table 2 shows the number of heterozygous subjects with zero, one, or two protein isoform bands. Two distinct apo[a] protein isoforms were detected in 232 of the 415 individuals heterozygous for the apo[a] allele. No apo[a] protein bands could be detected in 18 subjects (17 Caucasians and 1 African American), who all had Lp[a] concentrations <2.6 nM. A single apo[a] protein band was detected in 165 subjects, and this was more common among Caucasians than among African Americans (47% vs. 29%, $P < 0.005$). Consequently, the number of undetected bands in relation to the total number of alleles was higher among Caucasians than African Americans (30% vs. 15%, $P < 0.0001$).

In each of the 165 subjects with a single apo[a] isoform, the genotype information allowed us to determine which one of the two apo[a] size isoforms was not detected. We examined the proportion of undetected alleles of distinct apo[a] sizes in **Table 3**. As seen in the table, the frequency of apo[a] alleles corresponding to undetected apo[a] proteins increased steadily in Caucasians over the apo[a] size range, from 8% in the smallest apo[a] size range to 64% in the largest apo[a] size range ($P < 0.0001$). Among African Americans, there was no such trend. The distribution was U-shaped: the lowest frequency of undetected apo[a] alleles was seen in the 21–35 K4 repeat range when compared to both the smallest (<21 K4 repeats) and largest apo[a] sizes (≥36 K4 repeats, $P < 0.005$).

TABLE 3. Distribution of undetected apo[a] alleles over the apo[a] size range in Caucasians and African Americans

Apo[a] Allele Size (K4 Repeats)	Apo[a] Alleles in Caucasians (n = 510)		Apo[a] Alleles in African Americans (n = 320)	
	Undetected apo[a] Alleles	Relative Frequency	Undetected apo[a] Alleles	Relative Frequency
		%		%
11–20	5/65	8	13/41	32
21–25	27/120	23	9/87[a]	10
26–30	56/164	34	11/122	9
31–35	42/125	34	5/48	10
≥36	23/36[b]	64	10/22	45

Undetected alleles are given in relation to total alleles in each size range.

[a] $P < 0.005$, proportion of undetected African American apo[a] alleles in the 21–35 K4 repeat range compared with small (11–20 K4 repeats) or large sized apo[a] alleles (≥36 K4 repeats).

[b] $P < 0.0001$, proportion of undetected Caucasian apo[a] alleles in the 11–20 K4 repeat range compared with ≥36 K4 repeats.

TABLE 2. Distribution of subjects with single and double apo[a] alleles and protein isoforms

	Caucasians n = 263	African Americans n = 167	All n = 430
Genotyping (apo[a] alleles)			
Homozygous	8	7	15
Heterozygous	255	160	415
Phenotyping[c] (apo[a] protein isoforms)			
No protein isoforms	17 (7%)	1[a] (0.6%)	18 (4%)
One protein isoform (% of individuals with one protein)	119 (47%)	46[a] (29%)	165 (40%)
Two protein isoforms (% of individuals with two proteins)	119 (47%)	113[b] (71%)	232 (56%)
Undetected isoforms/total number of alleles	153/510 (30%)	48/320[b] (15%)	201/830 (24%)

[a] $P < 0.005$, African Americans compared with Caucasians.

[b] $P < 0.0001$, African Americans compared with Caucasians.

[c] Homozygotes were not included in the phenotype data.

TABLE 4. Distribution of subjects with single or double apo[a] protein band(s) across levels

	Lp[a] Levels			
	Caucasians (n = 238)		African Americans (n = 159)	
	<30 nM n = 125	≥30 nM n = 113	<30 nM n = 18	≥30 nM n = 141
Single band	73 (58%)	46 (41%)	9 (50%)	37 (26%)
Double band	52 (42%)	67[a] (59%)	9 (50%)	104[b] (74%)

Single or double band refers to the presence of one or two bands on the Western blots, respectively. Relative frequencies are given in parentheses.

[a] $P < 0.01$ proportion of Caucasians with double bands with Lp[a] levels <30 nM compared to ≥30 nM.

[b] $P < 0.05$, double bands in African Americans compared with Caucasians with Lp[a] ≥30 nM.

Next, we investigated whether the number of subjects with one versus two detectable apo[a] protein bands changed with total Lp[a] levels. We excluded the 18 subjects with no apo[a] protein bands, and the results in **Table 4** are therefore based on the 397 heterozygous subjects with at least one apo[a] protein band. Comparing Lp[a] levels <30 nM with those ≥30 nM, the proportion of subjects with double bands increased significantly with Lp[a] levels in Caucasians (42% to 59%, $P < 0.01$). Further, when directly comparing subjects with Lp[a] levels ≥30 nM, African Americans had more double bands than did Caucasians ($P < 0.05$).

Dominance pattern

We next determined whether the smaller or larger (or neither) apo[a] protein dominated in each subject. The dominance pattern is shown in **Table 5** for three ranges of Lp[a] levels. In Caucasians, the proportion of subjects with a dominant smaller isoform increased from 44% for levels <30 nM to 81% for levels >100 nM ($P < 0.0001$). In contrast, the dominance pattern was stable in African Americans over the Lp[a] plasma level range. Overall, the smaller apo[a] isoform dominated in 56% of Caucasians and in 45% of African Americans, while the larger apo[a] isoform dominated in approximately one quarter of all subjects. Among African Americans, there was a higher degree of codominance of the two protein isoforms than among Caucasians (33% vs. 16%, $P < 0.0005$).

We then explored whether the size difference between the two apo[a] alleles in an individual determined whether the larger or the smaller protein dominated in that person. It might be expected that in subjects with a large apo[a] allele size difference, dominance of the smaller allele would be more common. As seen in **Table 6**, dominance by the smaller allele was less frequent with a 1 or 2 K4 repeat difference between allele sizes compared to larger size differences among Caucasians ($P < 0.01$). Otherwise, there was no relationship between allele size difference and dominance in either ethnic group.

Next, we divided the apo[a] allele sizes into four ranges, 11–21, 22–27, 28–34, and >34 K4 repeats, to analyze the dominance pattern in terms of absolute allele sizes. The distribution of smaller allele dominance in these ranges is shown in **Table 7**. Focusing on the bottom row and the right column for each ethnic group, it can be seen that the bottom row tabulates the dominance over four size ranges of the larger allele, while the right column tabulates the dominance over four size ranges for the smaller allele. Among African Americans, the dominance of the smaller allele increased with increasing size of both the larger and the smaller apo[a] allele (from 27% to 96%, $P < 0.001$, and 24% to 100%, $P < 0.001$, respectively). In contrast, for Caucasians, the dominance for the smaller apo[a] allele decreased with an increase in size from small to medium for both the larger and smaller allele (from 71% to 49%, $P < 0.02$, and 74% to 44%, $P = 0.02$, respectively). Thus, the dominance pattern expressed in relation to genotypes differed markedly between African Americans and Caucasians.

Combined effects of genotype and level on dominance pattern

The analyses performed so far have looked at the association of the dominance pattern with a number of factors, one at a time. We next investigated the combined effects of all these factors to determine which were independently associated with the dominance pattern. The proportion of the total Lp[a] level attributed to the smaller allele (percentage smaller allele) was regressed as the dependent variable on the smaller allele size, the larger allele size and the total Lp[a] level, square-root transformed for normality. The size variables are given in number of K4 repeats. The best relationship found for these variables for Caucasians was:

TABLE 5. Distribution of apo[a] size dominance across levels

	Lp[a] Levels							
	Caucasians				African Americans			
	<30 nM n = 125	30–100 nM n = 61	>100 nM n = 52	All n = 238	<30 nM n = 18	30–100 nM n = 53	>100 nM n = 88	All n = 159
Larger dominating	52 (42%)	12 (20%)	2 (4%)	66 (28%)	6 (33%)	14 (26%)	16 (18%)	36 (23%)
Codominating	18 (14%)	12 (20%)	8 (15%)	38 (16%)	2 (11%)	16 (30%)	34 (39%)	52[a] (33%)
Smaller dominating	55 (44%)	37 (60%)	42[b] (81%)	134 (56%)	10 (56%)	23 (43%)	38 (43%)	71 (45%)

Apo[a] isoform dominance was determined as described in Materials and Methods. Relative frequencies are given in parentheses.

[a] $P < 0.0005$, frequency of codominance among Caucasians compared to that among African Americans.

[b] $P < 0.0001$, frequency of Caucasian subjects with the smaller apo[a] isoform dominating at apo[a] <30nM compared with apo[a] levels >100 nM.

TABLE 6. Distribution of apo[a] size dominance across allele size differences

	K4 Repeat Difference							
	Caucasians n = 238				African Americans n = 159			
	1–2 n = 37	3–4 n = 58	5–10 n = 99	>10 n = 44	1–2 n = 18	3–4 n = 41	5–10 n = 77	>10 n = 23
Larger dominating	15 (41%)	18 (31%)	26 (26%)	7 (16%)	4 (22%)	8 (20%)	18 (23%)	6 (26%)
Codominating	9 (24%)	7 (12%)	18 (18%)	4 (9%)	7 (39%)	15 (37%)	26 (34%)	4 (17%)
Smaller dominating	13[a] (35%)	33 (57%)	55 (56%)	33 (75%)	7 (39%)	18 (44%)	33 (43%)	13 (57%)

Relative frequencies are in parentheses.

[a] $P < 0.01$, frequency of smaller allele dominance among Caucasian with a 1–2 K4 repeat difference in allele sizes compared to all other allele size differences.

$$\% \text{ smaller allele} = -8.7 + 1.54 \text{ (larger allele size)} + 3.4 \text{ (square-root Lp[a])}$$

The multiple R^2 was 0.13 and the residual error was 37.8%. The coefficients were statistically significant ($P < 0.01$ for larger allele size and $P < 0.0001$ for Lp[a] level). Notably, in this group, smaller allele size was not statistically significant.

The best relationship found for African Americans was:

$$\% \text{ smaller allele} = -112.7 + 3.31 \text{ (smaller allele size)} + 2.25 \text{ (larger allele size)} + 2.2 \text{ (square-root Lp[a])}$$

The multiple R^2 was 0.29 and the residual error was 28.6%. The coefficients were all statistically significant ($P < 0.001$). While the coefficients for the larger allele size and transformed Lp[a] level were numerically different between the two groups, the differences were not statistically significant ($P > 0.3$ for each). The smaller-allele-size coefficient of 3.31 in African Americans was significantly different from the non-significant value in Caucasians ($P < 0.002$). Also, the intercept differed between the two groups ($P < 0.01$), expressing the finding that there was less dominance overall by the smaller allele in African Americans. Further, it should be noted that the multiple regression model explained more of the variability in smaller allele dominance in African Americans (29%) compared with Caucasians (13%) and that the residual error around the model was smaller in African Americans compared with Caucasians.

In Caucasians, dominance of the smaller allele increased with size of the larger allele and Lp[a] level. For African Americans, the size of the larger and smaller allele, as well as Lp[a] level, were independent predictors of dominance by the smaller allele, confirming the univariate results in Table 7. However, the results from the multiple regression model modified the results from the univariate analyses in two significant ways. First, there was indeed an association between the total Lp[a] level with dominance pattern in African Americans, as in Caucasians, once the genotype effects were taken into account. Second, in Caucasians, the negative effect of increasing allele sizes on smaller allele dominance, seen in Table 7, was not found when taking Lp[a] level into account; the effect of the larger allele size on dominance pattern was in fact quite similar to the findings in African Americans.

TABLE 7. Smaller apo[a] allele dominance in different allele size groups

	Larger Alleles				
Smaller Alleles	11–21	22–27	28–34	>34	Total
Caucasians (n = 238)[a]					
11–21	2/6 (33%)	23/32 (72%)	27/34 (79%)	7/8 (86%)	59/80[b] (74%)
22–27		12/17 (71%)	22/64 (34%)	9/17 (53%)	43/98 (44%)
28–34			18/39 (46%)	14/21 (67%)	32/60 (53%)
>34				0/0	
Total	2/6 (33%)	35/49 (71%)	67/137[d] (49%)	30/46 (65%)	134/238 (56%)
African Americans (n = 159)					
11–21	1/3	4/19 (21%)	3/18 (17%)	2/2 (100%)	10/42 (24%)
22–27		5/15 (33%)	28/69 (41%)	11/11 (100%)	44/95 (46%)
28–34			8/12 (67%)	8/9 (89%)	16/21[c] (76%)
>34				1/1 (100%)	1/1 (100%)
Total	1/3 (33%)	9/34 (27%)	39/99 (39%)	22/23[e] (96%)	71/159 (45%)

The numbers represent the proportion of dominance by the smaller allele in the given size range out of the total number of subjects with genotypes in the range and are expressed as percentages in parentheses. The smaller allele is in the row, and the larger allele appears in the column.

[a] Association of smaller allele dominance with allele size different between African Americans and Caucasians ($P < 0.0001$ for smaller allele size and $P < 0.001$ for larger allele size).

[b] $P = 0.02$, proportion of smaller apo[a] allele dominance greater in Caucasians when smaller allele size is <22 K4 repeats compared to 22–27 ($P < 0.0001$) or >27 K4 repeats.

[c] $P < 0.001$, for increasing trend in smaller apo[a] allele dominance as smaller allele size increases in African Americans.

[d] $P < 0.02$, proportion of smaller apo[a] allele dominance smaller in Caucasians when larger allele size is 28-34 K4 repeats, compared to others.

[e] $P < 0.001$ for increasing trend in smaller apo[a] allele dominance as larger allele size increases in African Americans.

Interaction between the two isoforms

Finally, we investigated whether there was any interaction between the two apo[a] isoforms within an individual. Since undetected bands were likely to be due to allele-specific genetic factors, subjects with single bands were excluded from this correlation analysis. First, we tested whether the level of one apo[a] isoform was associated with that of the other isoform, using the same groups as in Table 7. Among Caucasians, the 28–34 K4 repeat group contained the most subjects and was used as the reference group, while the 22–27 K4 repeat group was used as the reference group for African Americans. Square-root transformed Lp[a] levels were used. As seen in **Fig. 2**, levels of one apo[a] isoform were not correlated with levels of the other isoform in any of the size ranges in either ethnic group (results are shown for the genotype ranges with the largest number of subjects). Altogether, only about 10% of the variation in levels for one isoform was explained by the other isoform.

Next, building on the results in Table 6 where dominance pattern in relation to allele size difference was determined, we explored if, in any individual, isoform-specific Lp[a] levels were affected by the difference in size between the two apo[a] alleles. There was no correlation between isoform-specific Lp[a] levels and allele size difference in any of the size ranges for either ethnic group (**Fig. 3**). The two correlation analyses suggest that the Lp[a] level for a given apo[a] isoform size is not affected by the other isoform.

DISCUSSION

In the present study, by determining apo[a] genotypes, isoform-specific Lp[a] levels and apo[a] isoform dominance pattern, we addressed the hypothesis that the smaller apo[a] isoform was always dominant. The novel approach of comparing the two alleles and isoforms within an individual made it possible to control for other genes as well as environmental factors, which could confound comparisons between individuals.

We demonstrated that the dominance pattern was not simply a function of apo[a] gene size or the difference in size between apo[a] alleles. In any one individual, in contrast to the widespread notion that the smaller apo[a] allele corresponds to the dominant plasma apo[a] protein isoform, this was the case in only 56% of Caucasians and 45% of African Americans, and in fact, the larger allele dominated in about 25% of all subjects. Dominance by the smaller allele did not increase with allele size difference. In both groups, smaller allele dominance increased with Lp[a] levels. Further, we did not find any evidence that the concentration of one apo[a] isoform affected the concentration of the other isoform, nor that the size difference between the two isoforms affected isoform-specific levels, arguing both against an interaction between alleles and also against a common regulator of both apo[a] isoform levels. These findings were similar among African Americans and Caucasians.

However, differences between the two ethnic groups were also found: *a*) undetected apo[a] alleles were more common among Caucasians, particularly with larger allele sizes; *b*) dominance by the smaller apo[a] isoform was more common among Caucasians, increasing with Lp[a] levels, whereas codominance was more common among African Americans; *c*) dominance by the smaller allele increased with increasing size of the larger allele in both groups but with the smaller allele only in African Americans. Altogether, this suggests different regulatory mechanisms between the two ethnic groups, in agreement with previous studies (48).

The exact causes for these differences have not been clarified, and they may involve both regulation of the apo[a] gene, and factors involved in translation and/or secretion. Previous in vitro findings have suggested that the length of the apo[a] protein, i.e., the number of K4 repeats, could be a major determinant of the efficiency of apo[a] secretion in vivo (18–24). However, our results argue against the possibility that apo[a] size is the sole predictor of dominance, suggesting that additional mechanisms are involved. In addition to post-translational modifications, apo[a] secretion is affected by apo[a] gene transcription and apo[a] mRNA stability (15, 49). DNAse-sensitive sites have been found upstream of the apo[a] gene and in the intergenic region separating human plasminogen and apo[a] genes, and they may play a role in the regulation of apo[a] transcription (50, 51). In addition, there are other apo[a] gene polymorphisms in linkage disequilibrium with the size polymorphism (28–31). Thus, a careful analysis of promotor sequence variants may help explain the basis for our observations. Further, it cannot be excluded that intracellular chaperone proteins, involved in secretion, may differ between African Americans and Caucasians (27).

We demonstrated that for both ethnic groups, the apo[a] allele size difference within any genotype did not affect isoform levels. Also, one apo[a] isoform level was not associated with that of the other isoform. Thus, overall, we did not find evidence for an interaction between the two apo[a] alleles in an individual, irrespective of ethnicity. One can envision different interaction possibilities: a common regulatory factor that would affect a person's two apo[a] alleles in the same fashion would result in a positive correlation between the two apo[a] protein isoforms, while an increase in secretion of one apo[a] isoform with a parallel reduction in the secretion of the other would result in a negative correlation. While the absence of either a positive or negative correlation in Fig. 2 does not definitively rule out the presence of any interaction, it does not provide any support for this possibility.

Similar to previous studies (17, 25, 48, 52–54), we found that the variation in plasma levels for a given apo[a] isoform was greater for small sizes, and greater among African Americans than among Caucasians. The question arises as to whether this variation is due to variations in catabolism or in synthesis. If variation in catabolism is the primary determinant of Lp[a] level differences between individuals, we would expect the levels of the two isoforms to go up or down together, leading to a positive correla-

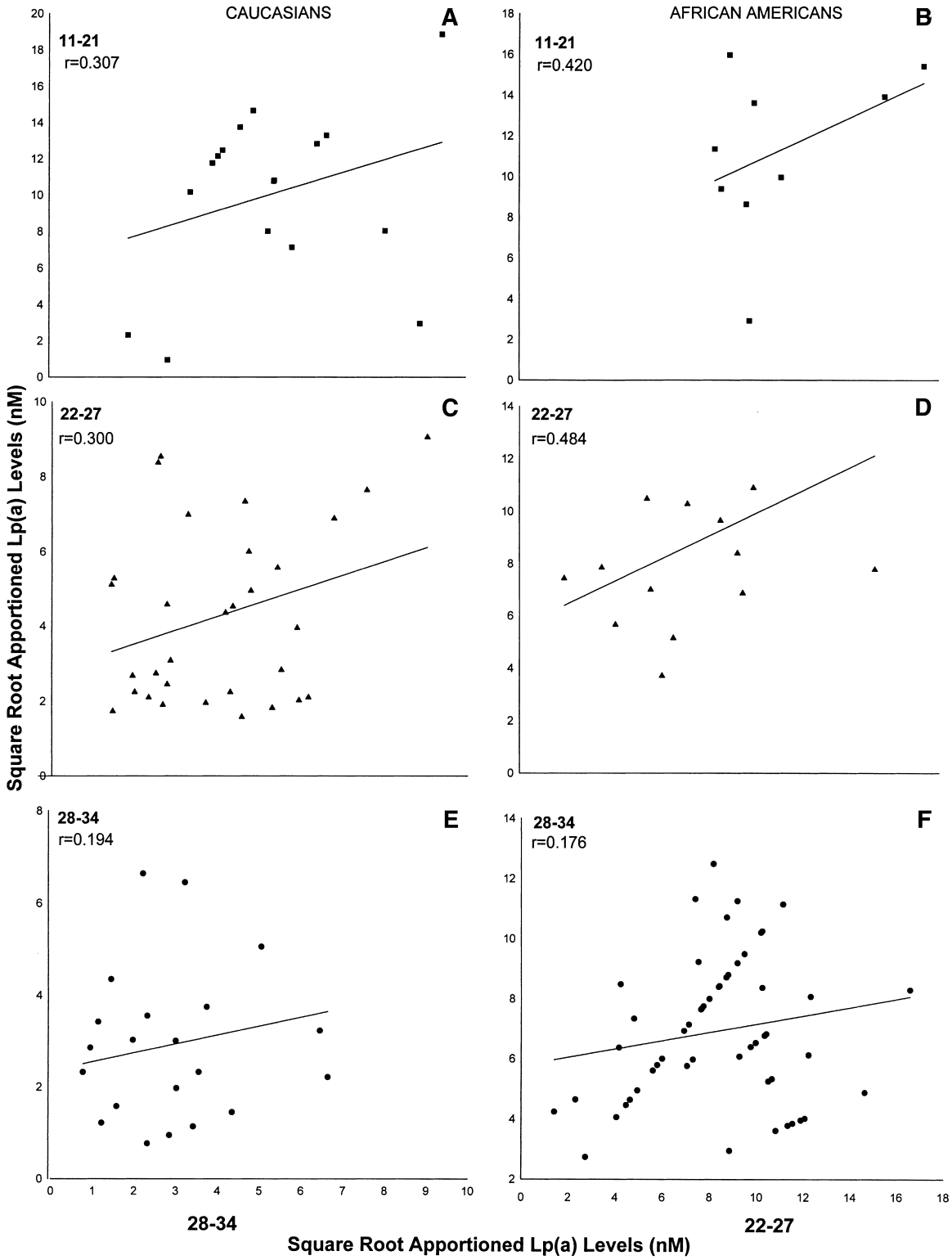

Fig. 2. Relationship between the two apo[a] isoform levels within individuals. In this comparison, individuals with only one apo[a] protein isoform were excluded. Apo[a] isoforms were arranged into three groups: 11–21, 22–27, and 28–34 K4 repeats. Isoforms containing 22–27 and 28–34 repeats were the most common groups among African Americans and Caucasians, respectively, and used as reference groups, graphed on the x-axis. For Caucasians, the 28–34 apo[a] isoform levels were compared with those of 11–21 (A), 22–27 (C), and 28–34 (E) apo[a] K4 repeats, graphed on the y-axis. The corresponding comparisons for African Americans are shown in B, D, and F. Isoform-specific apo[a] levels were square root transformed. The correlation coefficients are given in the top left of each graph.

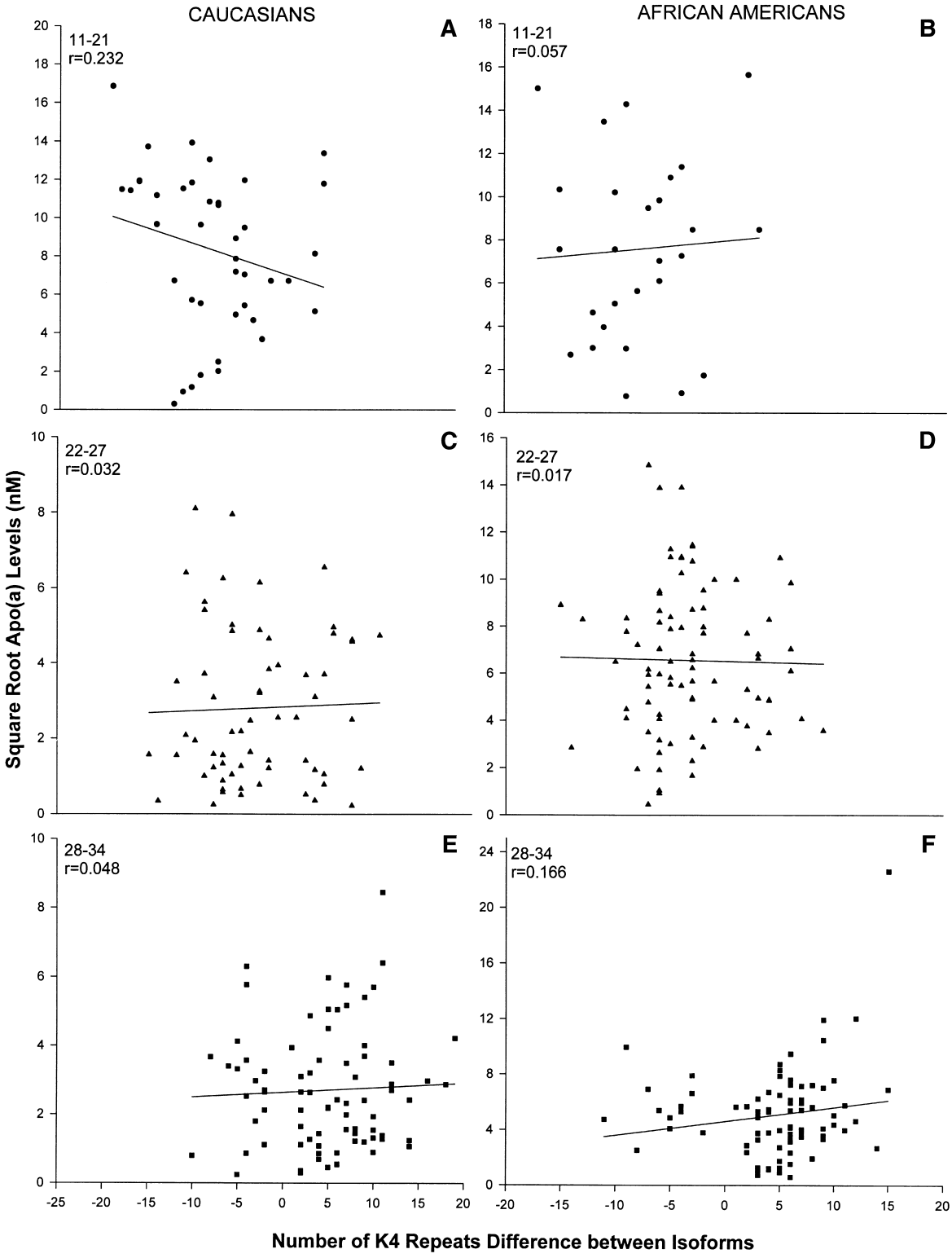

Fig. 3. Relationship between apo[a] isoform levels and the size difference (in K4 repeats) between the two isoforms within an individual. In this comparison, individuals with only one apo[a] protein isoform were excluded. For each individual, two data points were entered, corresponding to the two isoforms. Each apo[a] isoform was plotted against the size difference to the other isoform in the same individual. The size difference could be positive or negative; in each individual, the level for the larger isoform was graphed against a positive difference, and the level for the smaller isoform against a negative difference. Thus, for an individual with a 35/25 K4 repeat genotype, the 35 K4 allele level was graphed at a +10 size difference and the 25 K4 allele level at a −10 size difference. Apo[a] isoforms were arranged into three groups: 11–21, 22–27, and 28–34 K4 repeats, given in A, C, and E for Caucasians and B, D, and F for African Americans, respectively. Isoform-specific apo[a] levels were square root transformed. The correlation coefficients are given in the top left of each graph.

tion in Fig. 2. However, if variation in synthesis is the primary determinant, we would not expect to see any correlation in Fig. 2 since the synthetic rates of the two isoforms are likely to vary independently. Thus, the lack of correlation seen in Fig. 2 is more consistent with the hypothesis that Lp[a] levels vary due to synthetic differences. Rader et al. have come to a similar conclusion from turnover studies (55, 56). Within an individual, Lp[a] particles with large and small isoforms are likely to differ in synthetic rate but have the same fractional catabolic rate (55–57). A limitation of the interpretation of the results in Fig. 2 is the assumption that within an individual, the fractional catabolic rates of Lp[a] particles derived from different apo[a] alleles are likely to be the same. With the limitation in mind, it is interesting that measuring the two isoforms in a number of individuals with similar apo[a] size genotypes allows us to attempt to answer, in the absence of metabolic turnover studies, whether catabolism or synthesis is the main regulator of plasma Lp[a] levels.

Our study has to be interpreted with caution as there are some important limitations. We are basing our conclusions on measurements of apo[a] allele size and on the distribution pattern of circulating apo[a] isoforms. Therefore, we cannot address possible mechanisms influencing steps between the gene and the plasma compartment, specifically intracellular synthetic and secretory pathways. However, it should be noted that our study is one of few where apo[a] allele sizes, as well as the concentration of each apo[a] isoform, have been determined across ethnicity, and the questions asked in this study have not been addressed previously.

In summary, we demonstrate that there are differences with regard to apo[a] allele distribution and frequency of non-detected alleles in African Americans and Caucasians. In about 25% of our subjects, irrespective of ethnicity, the larger apo[a] allele corresponded to the dominating apo[a] protein isoform. Further, apo[a] genotype influenced dominance pattern differently in African Americans and Caucasians. Finally, the concentration of one apo[a] protein isoform did not affect the concentration of the other, nor did the size difference between the two alleles affect isoform levels, arguing against an interaction between the alleles. We conclude that the pattern of association between plasma Lp[a] levels and allele sizes is complex and only in part determined by apo[a] size. Further studies are needed to determine other factors, besides size, that are involved in the complex regulation of apo[a] isoform levels, and to determine the basis for the differences among the two ethnic groups.

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