# Apo[a] size and PNR explain African American-Caucasian differences in allele-specific apo[a] levels for small but not large apo[a]

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**Abstract** **Apolipoprotein [a] (apo[a]) gene size is a major predictor of lipoprotein [a] level. To determine genetic predictors of allele-specific apo[a] levels beyond gene size, we evaluated the upstream C/T and pentanucleotide repeat (PNR) polymorphisms. We determined apo[a] sizes, allele-specific apo[a] levels, and C/T and PNR in 215 Caucasians and 139 African Americans. For Caucasians, apo[a] size affected allele-specific levels substantially greater in subjects with apo[a] < 24 K4; for African Americans, the size effect was smaller than in Caucasians, <24 K4, but did not decrease at higher repeats. In both groups, the level decreased with increasing size of the other allele. Controlling for apo[a] sizes, PNR decreased allele-specific apo[a] levels in Caucasians with increasing PNR > 8. In a multiple regression model, apo[a] allele size and size and expression of the other apo[a] allele (and PNR > 8 for Caucasians) significantly predicted allele-specific apo[a] levels. For a common PNR 8 allele, predicted values were similar in the two ethnicities for small size apo[a]. Allele-specific apo[a] levels were influenced by the other allele size and expression. Observed differences between Caucasians and African Americans in allele-specific apo[a] levels were explained for small apo[a] sizes by the other allele size and PNR; the ethnicity differences remain unexplained for larger sizes.**—Rubin, J., H. J. Kim, T. A. Pearson, S. Holleran, R. Ramakrishnan, and L. Berglund. **Apo[a] size and PNR explain African American-Caucasian differences in allele-specific apo[a] levels for small but not large apo[a].** ***J. Lipid Res.*** **2006.** 47: **982–989.**

**Supplementary key words** lipoprotein [a] • African Americans • genotyping • polymorphism • apolipoprotein [a] • pentanucleotide repeat

An increased lipoprotein [a] (Lp[a]) level is an independent risk factor for cardiovascular disease (1–12). In Lp[a], an LDL-like particle, apolipoprotein B-100, is covalently bound to apolipoprotein [a] (apo[a]). Apo[a] has many size isoforms as a result of the variable number of kringle K4 repeats (13, 14). The size variation of the apo[a] protein (i.e., the number of K4 repeats) corresponds to a size variation in the apo[a] gene National Center for Biotechnology Information Online Mendelian Inheritance in Man accession number 152200. Plasma apo[a] levels are genetically determined and partially regulated by apo[a] size; apo[a] level and apo[a] size are inversely related, although the relationship is not linear and differs between populations (15–17).

Although the apo[a] size polymorphism is an important predictor of apo[a] level, the latter vary considerably among individuals carrying apo[a] isoforms of the same size (16, 18, 19). In several studies, small apo[a] sizes have been associated with cardiovascular disease (12, 20–29), and associations between small apo[a] and cardiovascular disease have remained significant after adjustment for apo[a] level (12, 24–28). This suggests that atherogenicity associated with Lp[a] could depend on apo[a] size. We recently reported that the smaller apo[a] size is not always the dominant isoform (30). In view of the strong genetic basis of apo[a] level (15), it is likely that other genetic factors contribute to apo[a] level.

Besides the size variation, a C/T polymorphism in the promoter region of the gene (+93) and an upstream pentanucleotide repeat (PNR) polymorphism, with 5–12 TTTTA repeats starting at −1,373, have been described in addition to other single-nucleotide polymorphisms (31–38). In transfection experiments, the T nucleotide was associated with lower apo[a] formation as a result of the introduction of a novel start codon (31). In vitro studies of the PNR polymorphism have demonstrated less transcriptional activity for constructs containing nine compared with eight TTTTA repeats (39). In studies in

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Caucasians and African blacks, the C/T polymorphism had an independent effect on plasma Lp[a] levels among Africans but not among Caucasians (32). Individuals carrying the PNR 11 allele had low Lp[a] levels, even after controlling for apo[a] size (40). However, as plasma Lp[a] levels associated with each of the two apo[a] size alleles in a genotype are highly variable, there is a need to address allele-specific apo[a] levels. In this study, we assessed, for the first time, the association of the C/T, PNR, and apo[a] gene size polymorphisms with allele-specific apo[a] levels in African Americans and Caucasians.

## METHODS

### Study population

DNA samples from African American and Caucasian subjects participating in the Harlem-Bassett Study were included in this study. The design of the Harlem-Bassett Study, the recruitment procedure, and the clinical characteristics of the two ethnic groups have been described previously (12, 30, 41). Briefly, 648 patients, 401 men and 247 women, ethnically self-identified as African Americans (n = 232), Caucasians (n = 344), and other (n = 72), were recruited from a patient population scheduled for diagnostic coronary arteriography at either Harlem Hospital Center in New York City or the Mary Imogene Bassett Hospital in Cooperstown, NY. Exclusion criteria were as follows: age > 70 years, recent (within 6 months) myocardial infarction or thrombolysis, a history of percutaneous transluminal coronary angioplasty, surgery during the previous 6 weeks, a known communicable disease such as hepatitis or acquired immunodeficiency syndrome, and current lipid-lowering medication. The study was approved by the Institutional Review Boards at Harlem Hospital, the Mary Imogene Bassett Hospital, Columbia University College of Physicians and Surgeons, and the University of California, Davis. Informed consent was obtained from all participants.

### C/T and PNR polymorphism determination

The +93 C/T polymorphism was detected by applying an Amplification Refractory Mutation System method (42). Leukocyte DNA was extracted and amplified by PCR in a DNA Thermal Cycler (GeneAmp PCR System 9600; Perkin-Elmer, Norwalk, CT). In addition to the buffers and nucleotide components recommended by the Taq polymerase distributors (GIBCO-BRL, Grand Island, NY), each amplification reaction contained 40 pmol of upper primer, 4 pmol of lower primer (which amplify a 335 bp region containing the mutation), 12.5 pmol of either the C- or T-specific primer (which amplify the specific mutation), and 1 μg of DNA. Each reaction mixture was heated to 94°C for 3 min for initial denaturation, after which time 1 unit of Taq polymerase was added and the final reaction volume was reached. This was followed by 10 cycles of 94°C for 30s, 65°C for 30s, and 72°C for 60s; 20 cycles of 94°C for 30s, 55°C for 30s, and 72°C for 60s; and 1 cycle of 94°C for 30s, 55°C for 30s, and 72°C for 10 min. Three microliters of 5× loading dye (0.25% xylene cyanol and 25% glycerol) was added to 15 μl of the final PCR product and run on a 1.5% DNA typing-grade agarose gel (GIBCO-BRL) at 200 V for 1 h. A DNA ladder spanning the DNA sizes of the PCR products was run on each gel. The gel was stained with ethidium bromide, and DNA bands were visualized on an ultraviolet transilluminator (FOTO/PREP; Fotodyne, Inc., Hartland, WI).

The 1.4 kb upstream PNR polymorphism was detected using a fluorescence-based detection system described in detail elsewhere (43).

### Apo[a] allele size determination

Apo[a] allele sizes were determined for 430 of the 576 Caucasian and African American subjects (263 Caucasians and 167 African Americans) using pulsed-field electrophoresis (30, 44), with complete data on the C/T and PNR polymorphisms for 264 subjects (160 Caucasians and 104 African Americans). 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 from Dr. Helen Hobbs (University of Texas Southwestern Medical Center, Dallas, TX).

### Measurement of plasma apo[a] levels

Fasting blood samples were drawn, and serum and plasma samples were stored at −80°C before analysis. Apo[a] levels were measured as described previously using a sandwich ELISA (Sigma Diagnostics, St. Louis, MO), and apo[a] allele-specific levels (i.e., levels associated with alleles defined by size) were determined as described in detail previously (12, 30).

### Statistics

Proportions were compared between groups using Chi-square analysis and Fisher's exact test where appropriate (45). Apo[a] levels were not normally distributed, although the distribution was somewhat more symmetric in African Americans than in Caucasians. A square-root transformation resulted in normal distributions and was therefore used (30). Group means were compared using Student's *t*-test.

The influence of multiple factors on allele-specific apo[a] levels was analyzed by multiple regression. Only expressed apo[a] alleles were analyzed by multiple regression. The small number of subjects homozygous for the size allele (eight Caucasians and seven African Americans) were treated as if the two alleles had equal apo[a] levels; the analyses were repeated excluding those subjects with no change in the results. In the multiple regression model, we included the variables slope of size if ≤24 or >24 K4 repeats, because of the suggestion of a change in slope around a size of 24 K4 repeats in our earlier report on this population (12). The influence of the other allele size was studied with two variables: the size of the other allele and whether or not it was expressed (i.e., correspondence with a protein isoform).

In studying the effect of C/T and PNR polymorphisms on allele-specific levels, it is necessary to know for each apo[a] size allele which C/T and PNR alleles are on the same chromosome. For subjects who are homozygous at the C/T or PNR locus, there is no ambiguity for that locus. However, subjects heterozygous at either locus are ambiguous: for instance, an individual with PNR 9/10 and apo[a] size 23/28 may have genotypes 9–23/10–28 or 9–28/10–23. We estimated haplotype frequencies separately in Caucasians and African Americans and applied those frequencies to calculate the probability of each PNR (or C/T) allele for each apo[a] size allele. For instance, if the haplotype frequencies for a given PNR-apo[a] size combination in the above example are p1 for 9–23, p2 for 10–28, p3 for 9–28, and p4 for 10–23, then for the 23 K4 allele, the probability of the PNR 9 allele is $p = p1p2/(p1p2 + p3p4)$, and the probability of the PNR 10 allele is $q = 1 - p = p3p4/(p1p2 + p3p4)$. The value of PNR > 8 for the 23 K4 allele is $9 - 8 = 1$ with probability p and $10 - 8 = 2$ with probability q, so the value used is $1 + q$. This estimate takes into account the known linkage disequilibrium



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TABLE 1. Distribution of PNR genotypes and alleles

| PNRs | Caucasians | | | | | African Americans | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | <8 | 8 | 9 | 10 | 11 | <8 | 8 | 9 | 10 | 11 |
| <8 | 3 | 6 | 2 | 0 | 0 | 5 | 28 | 4 | 2 | 0 |
| 8 | | 68 | 56 | 41 | 5 | | 49 | 29 | 7 | 4 |
| 9 | | | 10 | 10 | 5 | | | 6 | 1 | 1 |
| 10 | | | | 6 | 1 | | | | 1 | 0 |
| 11 | | | | | 2 | | | | | 2 |
| Allele frequencies | 3%[a] (n = 14) | 57% (n = 244) | 22% (n = 93) | 15%[a] (n = 64) | 3% (n = 15) | 16%[a] (n = 44) | 60% (n = 166) | 17% (n = 47) | 4%[a] (n = 12) | 3% (n = 9) |

PNR, pentanucleotide repeat.
[a] $P < 0.0001$, Caucasians versus African Americans.

between the PNR and the size loci and therefore is superior to assuming that PNR 9 and PNR 10 are equiprobable, so that PNR > 8 would be 1.5. All statistical analyses were done using SAS software (SAS Institute, Cary, NC). Statistical significance was set at $P < 0.05$.

## RESULTS

### C/T genotype distributions

The C allele was more common than the T allele in both ethnic groups, and C/C was the most common genotype, 71% among Caucasians and 95% among African Americans. Among Caucasians, 16% of the alleles were T alleles. Among African Americans, the T allele frequency was even lower, at only 2.5% ($P < 0.0001$ compared with Caucasians), and there were no T/T homozygotes in this group.

### PNR polymorphism genotype and allele distributions

The PNR allele frequencies and the genotype distributions are given in **Table 1**, with some of the less common PNR alleles combined for clarity. We detected a range of 5–11 PNRs in African Americans and 6–11 PNRs in Caucasians, and the 8 PNR allele was the most common (57% in Caucasians and 60% in African Americans). As seen in the table, 16% of all alleles among African Americans were small PNRs (<8 PNR), whereas only 3% of the alleles among Caucasians were in this range ($P < 0.0001$). On the other hand, the proportion of large PNR alleles (>9 PNR) was 18% among Caucasians but only 7% among African Americans ($P < 0.0001$).

### Apo[a] gene polymorphisms and allele-specific apo[a] levels

We next examined whether the C/T and PNR polymorphisms affected total and allele-specific apo[a] levels (i.e., the amount of plasma apo[a] in each individual associated with a specific apo[a] size) (30). For African Americans, the T allele frequency was too low for study. In Caucasians, total apo[a] levels were lower in T carriers compared with C/C homozygotes ($4.8 \pm 3.8$ vs. $6.8 \pm 4.4$ $\sqrt{nM}$; $P < 0.01$), but it is necessary to take apo[a] size into account. **Table 2** shows allele-specific apo[a] levels (expressed in square root form to achieve a normal distribution) for the different C/T and PNR genotypes separately for small and large apo[a] sizes, dichotomized at the median apo[a] size of 27 K4 repeats. Adjusting for apo[a] size, no effect of the T allele was observed (square root apo[a] levels of 4.4 vs. 5.5 $\sqrt{nM}$ for 11–26 K4 repeats and 2.4 vs. 3.5 $\sqrt{nM}$ for higher repeats). The difference in total apo[a] levels was attributable to the T allele frequency being higher among large apo[a] sizes. The carriers of the 9, 10, and 11 PNR alleles appeared to have lower allele-specific apo[a] levels compared with PNR 8/8 homozygotes in Caucasians but not in African Americans.

To take both of the upstream polymorphisms and apo[a] sizes into account, we analyzed allele-specific apo[a] levels with a multiple regression model. We included the respective apo[a] allele size, the size of the other apo[a] allele, the presence of an isoform corresponding to the other allele (i.e., an expressed allele), the PNR number, and the presence of the T allele in the model. Because Table 2

TABLE 2. Allele-specific apo[a] levels across C/T and PNR genotypes

| Genotypes | C/C | T/* | <8/* | 8/8 | 8/9, 9/9 | 10/8–10 | 11/* |
|---|---|---|---|---|---|---|---|
| Caucasians | | | | | | | |
| 11–26 K4 repeats | 5.5 ± 4.8 (n = 107) | 4.4 ± 5.2 (n = 23) | 4.9 ± 3.2 (n = 5) | 6.9 ± 5.3 (n = 37) | 4.9 ± 4.9 (n = 22) | 4.7 ± 4.6 (n = 56) | 3.7 ± 4.4 (n = 10) |
| 27–48 K4 repeats | 2.5 ± 2.4 (n = 99) | 2.4 ± 2.2 (n = 63) | 4.2 ± 2.9 (n = 9) | 2.6 ± 2.4 (n = 53) | 2.2 ± 1.9 (n = 60) | 2.5 ± 2.6 (n = 32) | 1.4 ± 1.4 (n = 8) |
| African Americans | | | | | | | |
| 11–26 K4 repeats | 8.1 ± 5.0 (n = 92) | n.d. | 8.0 ± 4.5 (n = 28) | 8.1 ± 4.8 (n = 38) | 9.2 ± 5.3 (n = 16) | 7.0 ± 5.6 (n = 7) | 6.1 ± 7.0 (n = 5) |
| 27–48 K4 repeats | 5.8 ± 4.0 (n = 96) | n.d. | 6.6 ± 5.1 (n = 28) | 5.0 ± 3.8 (n = 40) | 5.1 ± 3.2 (n = 22) | 5.4 ± 3.1 (n = 9) | 6.2 ± 3.6 (n = 7) |

Apolipoprotein [a] (apo[a]) levels are expressed as square root transformed nM ± SD. n.d., not determined.



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TABLE 3. Multiple regression of the square root of allele-specific apo[a] levels

| Variable | Caucasians | | | | | | African Americans | | |
|---|---|---|---|---|---|---|---|---|---|
| Number of alleles | 357 | 357 | 357 | 357 | 215 | 215 | 272 | 272 | 272 |
| Root mean squared error | 3.21 | 2.93 | 2.90 | 2.86 | 2.68 | 2.62 | 3.34 | 3.25 | 3.21 |
| Intercept if size ≤ 24 | 16.5 | 31.3 | 31.9 | 34.1 | 34.9 | 34.7 | 18.2 | 22.1 | 23.3 |
| Intercept if size > 24 | | 7.3 | 7.8 | 11.1 | 10.9 | 13.1 | | | |
| Slope of size if ≤24 | −0.42 | −1.12 | −1.12 | −1.09 | −1.11 | −1.04 | −0.38 | −0.39 | −0.38 |
| Slope of size if >24 | | −0.12 | −0.12 | −0.13 | −0.10 | −0.14 | | | |
| Other allele size | | | | −0.09 | −0.12 | −0.13 | | −0.14 | −0.14 |
| Presence of other allele isoform | | | −0.95[a] | −1.22 | −1.53 | −1.55 | | | −1.53[a] |
| PNR > 8 | | | | | | −0.48[a] | | | |

The analysis was carried out separately for African Americans and Caucasians. All coefficients were significant at $P < 0.001$, except as indicated. Presence of other allele isoform is a dichotomous variable indicating whether the other apo[a] allele in a given genotype corresponds to a detectable protein isoform. PNR > 8 represents the impact of PNR alleles of more than eight repeats. The coefficients shown for slope of size if >24 represent the sum of the coefficients for size and size > 24.

[a] $P < 0.002$.

suggested a steady decrease in levels with increasing total number of PNR repeats, a single factor, PNR > 8, was used. **Table 3** shows the results of fitting a variety of models with increasing complexity. In this analysis, undetected alleles were excluded. The residual or root mean squared error shows the quality of the fit; a smaller value means a better fit. For each model shown, every coefficient was highly significant. The left part of the table shows results for Caucasians. The first column shows a single straight line with size. In the second column, the two-line model with a break at 24 K4 repeats is shown to improve the fit. The fit was further improved by including whether or not the other allele in a genotype was expressed, as shown in the third column. The fourth column shows additional improvement by including the size of the other allele. To study the effect of the PNR polymorphism, we first repeated the analysis in column 4 in the subset of subjects with available PNR data in column 5, with essentially unchanged findings. Column 6 shows additional improvement by inclusion of the PNR polymorphism. All of the factors tested except for the T allele were significant predictors of allele-specific apo[a] levels in Caucasians. In African Americans, the T allele was not used because of its low frequency, and the PNR number was not a significant factor. Furthermore, the relationship with allele size was not different above versus below 24 K4 repeats for African Americans. The other significant predictors were similar in the two ethnic groups. The regression analyses were done separately for African Americans and Caucasians, but the regression coefficients for other allele size and the presence of other allele isoforms were nearly identical for the two groups.

**Figure 1** shows the primary data on allele-specific apo[a] levels, adjusted for the effects of the other allele and PNR, along with the regression lines for the two groups. The regression line was straight for African Americans, but there was a break in the slope for Caucasians, with a larger slope than for African Americans at <24 K4 repeats and a smaller slope at >24 K4 repeats. The maximum difference between the two ethnic groups was found at 24 K4 repeats. The regression lines crossed at 17 K4 repeats, with the predicted levels very similar for African Americans and Caucasians at small allele sizes. The effects of the other allele and of the expression of the other allele are shown in **Fig. 2** to be similar between African Americans and Caucasians. The size effect similarity is seen in the regression lines being parallel; the expression effect similarity is seen in the spacing between the regression lines being the same.

### Effects of apo[a] size, genotype, and PNR polymorphism on allele-specific apo[a] levels

**Table 4** illustrates the simultaneous impact of the three polymorphisms on allele-specific apo[a] levels using the multiple regression model. As seen in the table, for both

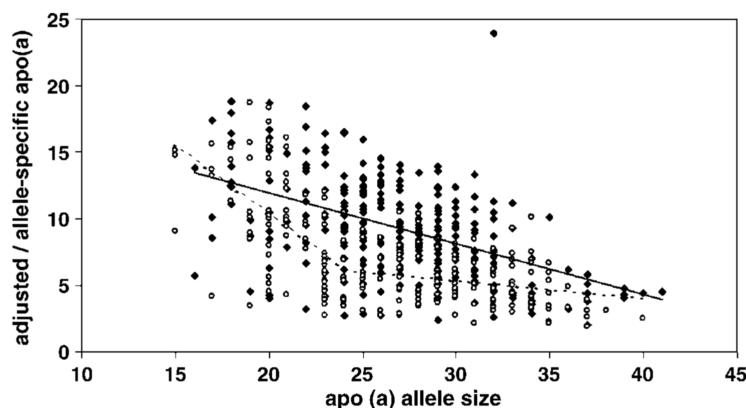


**Fig. 1.** Adjusted allele-specific apolipoprotein [a] (apo[a]) levels, expressed as square root, graphed against apo[a] allele size for African Americans (closed diamonds) and Caucasians (open circles). The solid line represents the regression line for African Americans, and the dashed line represents the regression line for Caucasians. Adjustment was to 8/8 pentanucleotide repeat (PNR) and an unexpressed other allele of size 27, representing the median allele size, using the regression coefficients in Table 3 [adding 0.14(OS-27) + 1.53OX for African Americans and 0.12(OS-27) + 1.56OX + 0.86PNR8 for Caucasians, where OS is the other allele size, OX is 1 if the other allele is expressed and 0 if not, and PNR8 is the excess of PNR > 8].



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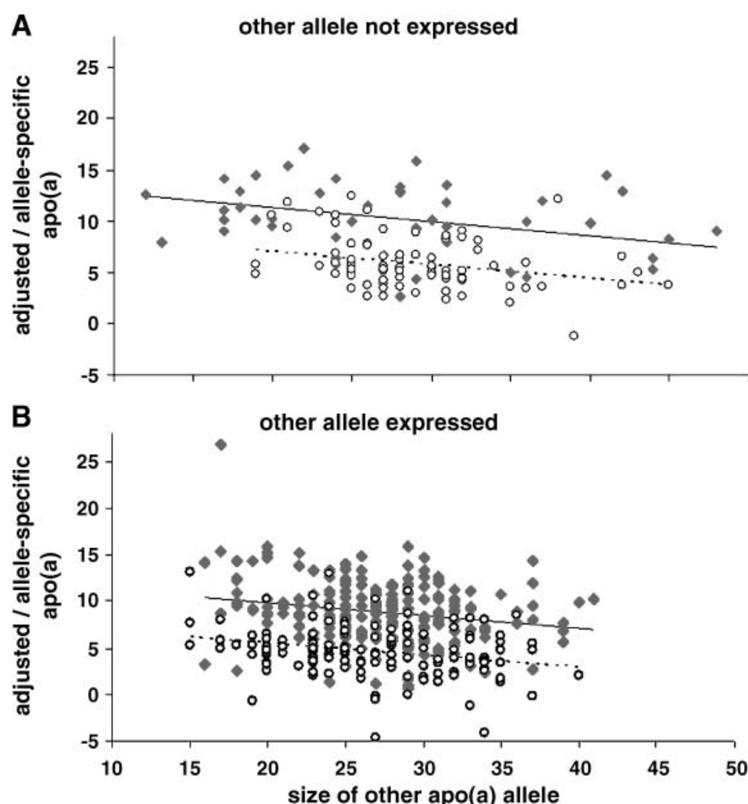


**Fig. 2.** Adjusted allele-specific apo[a] levels, expressed as square root, graphed against the size of the other apo[a] allele in a genotype for African Americans (closed diamonds) and Caucasians (open circles). The data in A are from subjects in whom the other allele was not expressed, and the data in B are from subjects in whom the other allele was expressed. The solid lines represent the regression lines for African Americans, and the dashed lines represent the regression lines for Caucasians. Adjustment was to allele size 24, representing the break in the line for Caucasians, and 8/8 PNR, using the regression coefficients in Table 3 [adding 0.38(AS-24) for African Americans and 1.04(AS-24) − 0.9AS-24 + 0.48PNR8 for Caucasians, where AS is the allele size, AS-24 is allele size < 24 if positive and 0 if not, and PNR8 is the excess of PNR > 8].

African Americans and Caucasians, predicted allele-specific apo[a] levels decreased with increasing apo[a] allele size from 18 to 36 K4 repeats. We then explored the effect of the other allele with variation in size and expression. Allele-specific levels were lower when the other allele in a genotype had a larger size (i.e., 38 vs. 20 K4 repeats), even if the other allele did not produce a circulating apo[a] isoform. In addition, if the other allele was expressed (i.e., a second protein isoform was detected), apo[a] levels of the given allele were even lower. This pattern, suggesting a size- and expression-dependent impact of the two alleles in a genotype, did not differ between African Americans and Caucasians. For Caucasians but not for African Americans, an additional variable, PNR genotype, influenced allele-specific levels. Allele-specific apo[a] levels were lower for PNR genotype 9/10 compared with genotype 8/8. Regarding differences between the two ethnic groups, for an apo[a] size of 18 K4 repeats, apo[a] levels were essentially similar for African Americans and the Caucasian subgroup with an 8/8 PNR genotype for a range of values for the other allele size, regardless of whether the other allele was expressed. However, for larger allele sizes, African Americans had significantly higher levels than Caucasians.

## DISCUSSION

We report two novel findings here. First, taking the PNR polymorphism and the other apo[a] size allele into

TABLE 4. Allele-specific apo[a] levels predicted by multiple regression for selected genotypes

| Variable | African Americans | | | Caucasians | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Allele size | 18 | 27 | 36 | 18 | 18 | 27 | 27 | 36 | 36 |
| PNR | | | | 8/8 | 9/10 | 8/8 | 9/10 | 8/8 | 9/10 |
| Other allele | | | | | | | | | |
| 20 | | | | | | | | | |
| Undetected | 187 | 105 | 47 | 178 | 141 | 44 | 27 | 28 | 15 |
| Expressed | 147 | 76 | 28 | 139 | 107 | 26 | 13 | 14 | 5 |
| 38 | | | | | | | | | |
| Undetected | 124 | 60 | 19 | 121 | 91 | 18 | 8 | 9 | 2 |
| Expressed | 92 | 38 | 8 | 89 | 64 | 8 | 2 | 2 | 0 |

Values shown are in nM. The calculations were made using the most significant equation (coefficients in the rightmost column) for each ethnic group. Within each ethnic group, the value in each cell is significantly different from the value in any other cell that differs in any one of the four independent variables (allele size, PNR, other allele size, and presence of other allele isoform, undetected or expressed). Levels for African Americans and Caucasians were significantly different for the same apo[a] size genotype except for apo[a] size 18, PNR 8/8.



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account, levels of small isoform apo[a] were quite similar for African Americans and Caucasians, apparent from the coefficients provided in Table 3 and seen in Fig. 1. Second, the size of the other allele and whether the allele is expressed (i.e., has a corresponding protein isoform) influence allele-specific levels, and furthermore, this pattern is similar for African Americans and Caucasians, as seen in Fig. 2. This finding suggests a relationship between the two alleles that appears to be independent of the difference in allele-specific levels between African Americans and Caucasians.

The apo[a] gene, particularly apo[a] size, is a major regulator of plasma apo[a] levels (14, 15, 46). However, there is wide variation in apo[a] levels in individuals with a given apo[a] size (18, 30, 46). Furthermore, African Americans have median apo[a] levels two to three times those of Caucasians, independent of apo[a] size variations (12, 16, 19, 46). As Lp[a] levels are regulated largely by genetic factors in both African Americans and Caucasians, exploration of genetic factors beyond apo[a] size is important and could contribute to our understanding of the atherogenicity associated with Lp[a] (12, 21, 47). In this study, we determined the C/T, PNR, and apo[a] gene size distributions and the simultaneous effects of these polymorphisms on allele-specific apo[a] levels. Although these polymorphisms have been evaluated previously (46–50), ours is the first study to examine all three polymorphisms simultaneously in Caucasians and African Americans and to address their association with allele-specific apo[a] levels. As part of this approach, we controlled for apo[a] gene size when evaluating the impact of the upstream polymorphisms on apo[a] levels.

Based on previous in vitro studies, both the C/T and PNR polymorphisms are candidates to influence plasma apo[a] levels (31–33). Using an in vitro transfection system, the presence of the T allele at the polymorphic C/T site in the apo[a] promoter region resulted in decreased translation and formation of the apo[a] protein (31). In addition, in vivo studies have demonstrated lower apo[a] levels among African T carriers (33) and in Caucasian carriers of the 11 PNR allele (40).

In this study, we addressed the impact of the C/T and PNR polymorphisms on allele-specific plasma apo[a] levels. The effects of these polymorphisms on total plasma apo[a] levels have been addressed in a Caucasian population (50). In addition to extending our analysis to allele-specific apo[a] levels, we included all subjects for whom we had information on the apo[a] size and PNR and C/T polymorphisms, compared with the previous study, which used a selected subset of subjects with one small and dominating apo[a] size allele (50). The estimation of PNR alleles for allele-specific apo[a] levels was carried out using haplotype frequency estimations.

Because of the low T allele frequency in African Americans, we were not able to assess any impact of the T allele on apo[a] levels in this group. Although Caucasian T carriers had lower apo[a] levels than did C/C homozygotes, we found that this was attributable to the association of the T allele with large allele sizes. As seen in Table 2, apo[a] levels were not different in T carriers with similar allele sizes. Multiple regression analysis confirmed that the C/T polymorphism did not independently influence plasma apo[a] levels. Because we did not observe any effect of the T allele on apo[a] levels in Caucasians, it is possible that the association found by Kraft et al. (33) in African blacks may be attributable to linkage disequilibrium with another polymorphism. The low T allele frequency in our African American group suggests that the presence of the T allele in African Americans may be a result of Caucasian admixture; therefore, one could speculate that the T allele developed in the South African black group studied by Kraft et al. (33) independent of the T allele seen in Caucasians.

The PNR polymorphism had no effect on apo[a] levels in African Americans, whereas in Caucasians, a high PNR allele count (PNR $>$ 8) decreased allele-specific apo[a] levels independent of apo[a] size. This finding is in agreement with previous results from Trommsdorff et al. (32), although in that study a different approach was used, with a calculated difference from expected apo[a] levels based on apo[a] size among subjects with increasing PNR size. One interpretation of the ethnic difference is that the effect of the PNR locus is indirect: in Caucasians, but not in African Americans, the PNR locus may be in linkage disequilibrium with another locus affecting apo[a] levels. In agreement with this, Bopp et al. (51) did not detect any difference in promoter activity for the PNR 8 and 9 variants in a study using 5′′ flanking fragments of the apo[a] gene.

A novel finding in our study was the similar impact on allele-specific apo[a] levels of the other allele in a genotype across ethnicity. We observed a similar effect in the two ethnic groups both by size and expression effect of the other allele, as seen in Fig. 2, suggesting that these effects are independent of the difference in plasma Lp[a] levels between African Americans and Caucasians. Although we do not have any data to implicate any underlying mechanism, it is possible that this may be attributable to a number of steps from apo[a] synthesis to secretion. Thus, our results suggest the importance of a more complete understanding of Lp[a] synthetic pathways.

To facilitate the evaluation of the results of the multiple regression carried out in Table 3, we calculated the expected apo[a] levels for certain allele size-genotype-PNR combinations (Table 4). The polymorphism combinations chosen span much of the range of allele sizes and the more common PNR polymorphisms. As seen in the table, each of the three factors (apo[a] allele size, PNR genotype, and size of the other allele in a given genotype) influenced apo[a] levels quite markedly. Thus, among Caucasians, keeping apo[a] allele sizes constant, the presence of the PNR genotype 9/10 versus 8/8 resulted in considerably lower apo[a] levels, as has been noted by others (32). Also, for both ethnic groups, the impact of allele size combinations in a genotype on apo[a] levels is clear. Although allele-specific apo[a] levels were similar for African Americans and Caucasians at smaller apo[a] sizes, we still found significant differences in levels between the two



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groups at larger sizes, suggesting as yet undetermined factors. It is conceivable that studies addressing other genetic apo[a] variants or potential genetic differences beyond the apo[a] gene may shed further light on the difference in allele-specific apo[a] levels for larger apo[a] sizes.

We recognize that our study has a number of limitations. For example, we recruited patients scheduled for coronary angiography, and for some genotypes the number of subjects was relatively small. Therefore, further studies are needed to confirm our findings. However, in conclusion, we found a differential distribution of the C/T and PNR polymorphisms between Caucasians and African Americans. Furthermore, in a multiple regression model, we found that significant predictors of allele-specific Lp[a] levels differed between African Americans and Caucasians. For Caucasians, the respective allele size, whether the allele was >24 K4 repeats, the size of the other apo[a] allele in a given genotype, whether this allele was expressed, and PNR > 8 were significant predictors. For African Americans, PNR number and whether allele size was >24 repeats were not significant. Thus, multiple genetic components of the apo[a] gene influence apo[a] level, with different effects across ethnicity. Notably, the apparent interethnic difference for small apo[a] alleles could be explained by the genetic variants analyzed here. In contrast, adjusting for the known genetic factors, we still observed significant differences in allele-specific apo[a] levels for large apo[a] sizes between the two ethnic groups. Further studies are needed to explain the differences in apo[a] levels between African Americans and Caucasians for large apo[a] alleles.

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