ORIGINAL RESEARCH

Lp(a)-Associated Oxidized Phospholipids in Healthy Black and White Participants in Relation to apo(a) Size, Age, and Family Structure

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BACKGROUND: Lp(a) (lipoprotein(a)) is the major lipoprotein carrier of oxidized phospholipids (OxPL) and this function mediates Lp(a) atherogenicity. However, the relationship between OxPL, Lp(a), and genetic and biological characteristics remains poorly understood. We assessed the relationship between Lp(a)-bound OxPL, apolipoprotein(a) (apo(a)) size, age, and family structure in 2 racial groups.

METHODS AND RESULTS: Healthy Black and White families were recruited from the general population (age: 6–74 years, n=267). OxPL and Lp(a) levels were assayed enzymatically; apo(a) isoform, *LPA* allele sizes, and allele-specific Lp(a) levels were determined. Lp(a)-OxPL levels did not differ significantly by racial and age groups. Lp(a)-OxPL levels were associated with total plasma Lp(a) in all participants and in race-specific analyses. Further, OxPL levels were significantly associated with allele-specific Lp(a) levels carried by the smaller apo(a) size in all participants (β=0.33, P=0.0003) as well as separately for Black (β=0.50, P=0.0032) and White (β=0.26, P=0.0181) participants. A significant association of OxPL with allele-specific Lp(a) levels for larger apo(a) sizes was seen only in Black participants (β=0.53, P=0.0076). In this group, Lp(a)-OxPL levels were also heritable (h^2=0.29, P=0.0235), resulting in a significant interracial difference in heritability between Black and White people (P=0.0352).

CONCLUSIONS: Lp(a)-OxPL levels were associated with allele-specific Lp(a) level carried on smaller apo(a) sizes and among Black participants also for larger apo(a) sizes. The heritability estimates for Lp(a)-bound OxPL differed by race.

Key Words: children ■ general population ■ oxidized lipids ■ parents ■ race

L p(a) (lipoprotein(a)) is a causal independent risk factor for cardiovascular disease, recognized in clinical guidelines.[1–4] Lp(a) is characterized by an LDL (low-density lipoprotein)-like core where the apo (apolipoprotein) B-100 component is linked by a single disulfide bond to apo(a), a protein with a variable number of repeated kringle (K) structures. The defining properties of Lp(a) rest with its apo(a) component as circulating levels of Lp(a) are largely determined by the number of K4 type 2 repeats of the apo(a) protein.

Although much uncertainty remains regarding any physiological role for Lp(a), its carrier role for proinflammatory and proatherogenic oxidized phospholipids (OxPL) has offered some insights.[5] As the majority of OxPL circulates as a complex with Lp(a),[6] a better understanding of the relationship between Lp(a) and OxPL has the promise to shed light on both any potential function as well as its risk factor properties. Although plasma Lp(a) levels are stable within individuals, there is a high degree of variability between

Nonstandard Abbreviations and Acronyms

K	kringles
Lp(a)	lipoprotein(a)
Lp(a)-OxPL	Lp(a)-bound oxidized phospholipids
OxPL	oxidized phospholipids

individuals as well as a well-characterized interracial difference where individuals of African descent have on average 3-fold higher levels.[7] Studies with a large number of African descendants have found a significant link between Lp(a) and cardiovascular disease.[8] The extent to which Lp(a)-bound OxPL levels might vary across the lifespan and racial groups under a normal physiological environment and whether such a variation would be related to differences in Lp(a) levels is not fully understood. In this study, we investigated the associations between OxPL and Lp(a) in a healthy general population cohort, enrolling White and Black children and adults. We conducted in-depth analyses focusing on the relation of allele-specific Lp(a) levels to Lp(a)-bound OxPL, taking both genotypic and phenotypic characteristics of apo(a) into account as well as compared Lp(a) and OxPL levels across generations.

METHODS

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Human Subjects

The details of human subjects, recruitment criteria, and clinical characteristics of the families have been described previously.[9,10] Briefly, 82 (60 White and 22 Black) families with 2 parents and 2 biological children were recruited from the general population residing in the greater Sacramento area. Families were invited to the University of California Davis Clinical and Translational Science Center for collection of demographic and medical history information using standardized questionnaires, physical examinations, and for blood draws. Race/ethnicity was self-reported for each individual family member, and 182 individuals self-identified as White and 87 individuals as Black. Data from 2 children (1 in each racial group) were not included because of unavailability of blood samples, and the present report is based on findings in 181 White and 86 Black participants. The study was approved by the University of California Davis Institutional Review Board and informed consent obtained from all subjects. Minors were asked to give their assents (Assent form for 12–17 years old; or Letter of information for 8–11 years old), and one of the parents signed the consent forms for their children.

Clinical and Biochemical Assessment

Blood pressure was measured with a random-zero mercury sphygmomanometer. Body mass index was calculated as body weight (kg) divided by squares of height (m^2). For children and adolescents (6–20 years), body mass index-for-age growth charts for either boys or girls (Centers for Disease Control and Prevention) were used to obtain a percentile ranking.[11] Concentrations of HDL-C (high-density lipoprotein cholesterol), triglycerides, and apoB-100 were measured using standard procedures. LDL-C (LDL-cholesterol) concentrations were calculated with the formula of Friedewald et al.[12] Lp(a) levels were determined with an apo(a)-size insensitive ELISA (Mercodia Inc.) in plasma samples.[13] Analyses were run according to the manufacturers' specifications in duplicate samples with 2 different quality controls, which were within the recommended precision for each test. Lp(a)-bound oxidized phospholipids (Lp(a)-OxPL) were quantified with the OxiSelect™ Human Oxidized LDL ELISA (STA-358, Cell Biolabs, Inc.) and the Lp(a) ELISA kits (Mercodia, Inc.). Briefly, using whole plasma, total OxPLs were captured by an anti-OxPL antibody in coated plates, and then incubated with 2 monoclonal antibodies directed against

separate nonrepeating antigenic determinants on the apo(a) molecule. Apo(a) in the plasma sample reacts with anti-apo(a) antibodies bound to microtitration wells and peroxidase-conjugated anti-apo(a) antibodies in the solution. Although apo(a) in Lp(a) is bound to apoB-100, no cross-reactions with plasminogen or apoB-100 were detected for these antibodies. A stop solution was added, following a 15-minute incubation with substrate tetramethylbenzidine, and Lp(a)-OxPL are measured by absorbance at 450 nm, and the data were expressed as unit per liter (U/L). The coefficient of variation was <10%. To further analyze the relationship between OxPL and Lp(a), we calculated a ratio of OxPL/Lp(a) by dividing Lp(a)-bound OxPL levels by plasma Lp(a) levels.

LPA Allele and Apo(a) Isoform Size Determinations

LPA allele sizes were determined by genotyping using pulsed-field gel electrophoresis of whole DNA from leucocytes embedded in agarose plugs with a protocol adapted from Lackner et al[14] and Rubin et al[15] as described previously.[10] Apo(a) isoform sizes were determined by Western blotting with sodium dodecyl sulfate-agarose gel electrophoresis of plasma samples, followed by immunoblotting using a slightly modified protocol of Kamboh et al.[16] Briefly, apo(a) bands were visualized with the colorimetric substrates NBT/BCIP (Roche Diagnostics GmbH, Mannheim, Germany) using alkaline-phosphatase conjugated rabbit anti-goat IgG (Fc) antibody (Thermo Scientific, Rockford, IL). The results were related to human apo(a) isoform standard with known apo(a) isoforms taking the inverse relation between the number of K4 repeats (ie, apparent molecular mass) and isoform mobility into account during agarose gel electrophoresis. The protein isoform dominance pattern was assessed by optical analyses of the apo(a) protein expression on the Western blots, followed by a computerized analysis of scans as described previously.[15,17] To determine allele-specific Lp(a) levels, Lp(a) levels were apportioned according to the degree of intensity of the protein bands on the Western blot as described in detail elsewhere.[17–19]

Statistical Analysis

Statistical analyses of data were performed with SAS software, version 9.4 (SAS Institute, Cary, NC). Results were expressed as mean±SE of mean, or median with interquartile range for nonnormally distributed variables. Before statistical analysis, values of LDL-C, triglycerides, and Lp(a)/apo(a)-related variables were logarithmically transformed to reduce the influence of extreme values and to meet the homogeneity of variance assumption as appropriate. For categorical variables, proportions were compared between groups using χ^2 test or Fisher's exact test as appropriate. Group differences in means for quantitative variables were determined by ANOVA by age and race or ANCOVA with covariates. Associations between Lp(a)-OxPL and other variables were assessed using multiple regression analysis with covariates, including age, sex, race (omitted in race-specific analyses), current smoker, lipid-lowering medication use, and antihypertensive medication use (Model 1) and additionally adjusted for the dominating apo(a) size in plasma Lp(a) concentration-related variables and for the plasma Lp(a) concentration in apo(a) size-related variables, respectively (Model 2). Interracial differences were determined by adding an interaction term in multiple regression models. Heritability of each trait was estimated by the slope of the regression of offspring on mid-parental value using the regression of offspring on mid-parent model in nuclear families. The heritability analysis was restricted to 61 families with at least 3 biological family members. For families with a missing member, the missing phenotypic values were imputed using the respective spouse or offspring counterpart's value within the family. To determine whether heritability differed between White and Black participants, interracial differences in heritability were assessed by adding an interaction term in the regression of offspring on mid-parent models. Two-tailed $P<0.05$ were considered statistically significant as appropriate.

RESULTS

OxPL and Lp(a)/apo(a)-Related Variables by Race and Age

Characteristics of the study population have been reported previously.[9,10] Table 1 shows data for selected clinical and laboratory measurements by race and age groups. There were slight differences in the prevalence of medication use and current smoker between White and Black adults. As expected, total and allele-specific Lp(a) levels were elevated in both Black adults and children compared with the corresponding White counterparts. However, apo(a) isoform distributions were similar across race despite the marked differences in Lp(a) levels. Notably, Lp(a)-OxPL concentrations did not differ significantly by race (Black versus White) among adults or children (Table 1).

Relationships of OxPL With Lp(a)/apo(a)-Related and Other Variables in All Participants and by Race

In all participants, Lp(a)-OxPL concentrations were significantly positively associated with total plasma Lp(a) and allele-specific Lp(a) levels carried on the smaller or dominating apo(a) sizes (Table 2). In contrast, and

Table 1. Clinical Characteristics of the Study Population

Characteristics	White Participants*		Black Participants*	
	Children	Adults	Children	Adults
Number, n	65	116	36	50
Female sex, n (%)	25 (38%)	56 (48%)	14 (39%)	25 (50%)
Age, y	14±0	44±1	13±1	43±2
Body mass index, kg/m^2	20±0.5	30±0.6	21±0.7	31±1.0
Current smoker	0 (0%)	12 (10%)	0 (0%)	7 (14%)
Systolic blood pressure, mm Hg	111±1	126±1	112±2	130±2
Medication use, n (%)				
Lipid-lowering	0 (0%)	8 (7%)	0 (0%)	3 (6%)
Antihypertensive	0 (0%)	10 (9%)	0 (0%)	8 (16%)
LDL cholesterol, mg/dL	94±4	117±4	91±4	117±6
HDL cholesterol, mg/dL	53±2	49±1	58±3	47±2
Triglycerides, mg/dL	85 (70–119)	149 (96–216)	64 (48–79)[†]	120 (71–150)[‡]
ApoB-100, mg/dL	68±2	92±2	65±3	93±4
Lipoprotein(a), mg/dL	13 (3–47)	7 (2–32)	32 (14–63)[§]	27 (10–56)[‡]
Lp(a)-OxPL, U/L	4.50 (1.73–6.74)	5.44 (1.93–9.45)	5.41 (2.60–8.91)	4.12 (1.37–7.04)
ASL, larger, mg/dL	5 (2–13)	3 (1–11)	8 (5–18)	12 (2–25)[‡]
ASL, smaller, mg/dL	7 (2–31)	5 (2–24)	22 (7–41)[†]	20 (7–42)[‡]
ASL, dominating, mg/dL	9 (2–31)	6 (2–26)	23 (7–40)	19 (7–39)[‡]
Apo(a) size, larger (K)	30±4	31±4	30±3	31±3
Apo(a) size, smaller (K)	25±4	26±4	25±3	24±4[‡]
Apo(a) size, dominating (K)	26±1	27±0	25±1	26±1

Data are expressed as mean±SE of mean or median (interquartile range) for laboratory values. Data for LDL cholesterol, triglycerides, and Lp(a)/apo(a)-related variables were logarithmically transformed before statistical inferential analyses. Children were defined as ≤18 years of age. Apo(a) indicates apolipoprotein(a); ASL, allele-specific Lp(a) level; HDL, high-density lipoprotein; K, kringles; LDL, low-density lipoprotein; Lp(a), lipoprotein(a); and Lp(a)-OxPL, oxidized phospholipids bound to Lp(a).

*Data are from 181 White and 86 Black participants, who identified themselves as White or Black in the questionnaire and provided blood samples. All variables, excluding HDL-C and Lp(a)/apo(a)-related variables, were significantly higher in adults vs children. HDL-C levels were significantly lower in adults vs children in Black (P=0.002), but not in White participants.

[†]$P<0.001$ vs White children.

[‡]$P<0.05$ vs White adults.

[§]$P=0.058$ vs White children.

expectedly, Lp(a)-OxPL concentrations were significantly inversely associated with the smaller or dominating apo(a) sizes. When adjusted for the covariates, including age, sex, race, current smoker, and medication uses (Table 2, Model 1), these associations in all participants remained significant. Although no significant association was seen between Lp(a)-OxPL and LDL-C or ApoB-100 levels, there was a significant negative association with triglyceride level in all participants, which remained significant when adjusted for the covariates (Table 2, Model 1).

In race-specific analyses, Lp(a)-bound OxPL concentrations were associated positively with plasma Lp(a) and allele-specific Lp(a) levels carried on the smaller or dominating apo(a) sizes and negatively with the dominating isoform sizes within each racial group (Table 2). Figure 1 shows the relationship between Lp(a)-OxPL and allele-specific Lp(a) level carried on the dominating apo(a) size in Black and White participants. Further, in Black participantss, Lp(a)-bound

OxPL concentration was significantly associated with allele-specific Lp(a) level carried on the larger apo(a) size, which resulted in a significant interracial difference (β=0.53±0.19 versus 0.07±0.11 for Black and White participants, respectively, P=0.0445) (Table 2). When adjusted for the covariates (Table 2, Model 1), all these associations excluding only those with the isoform sizes remained significant in both Black and White participants. Interestingly, in White participants, the triglyceride level was significantly negatively associated with the Lp(a)-OxPL level regardless of the covariate adjustments (Table 2).

To better understand whether OxPL is influenced more by its carrier level (ie, Lp(a) level) or by the apo(a) size polymorphism through in-depth analyses, we further adjusted for the dominating apo(a) isoform size or Lp(a) level, separately, in addition to the covariates in Model 1 (Table S1, Model 2). Notably, in all participants including in Black participants, further adjustment for the dominating apo(a) isoform size did not alter the

Table 2. Regression Analysis for Lp(a)-OxPL With Other Variables in All Participants and by Race

Variables	Unadjusted							Model 1*						
	Overall		P (W vs B)	Black Participants		White Participants		Overall		P (W vs B)	Black Participants		White Participants	
	β±SE	P Value		β±SE	P Value	β±SE	P Value	β±SE	P Value		β±SE	P Value	β±SE	P Value
Total plasma Lp(a) level	0.35±0.10	0.0002	0.3320	0.49±0.17	0.004	0.29±0.12	0.0119	0.32±0.09	0.0008	0.3556	0.42±0.16	0.0104	0.27±0.12	0.0182
Allele-specific Lp(a) level														
Larger isoform	0.18±0.10	0.0690	0.0445	0.53±0.19	0.0076	0.07±0.11	0.5313	0.14±0.10	0.1363	0.0567	0.47±0.19	0.0162	0.06±0.11	0.625
Smaller isoform	0.33±0.09	0.0003	0.2212	0.50±0.16	0.0032	0.26±0.11	0.0181	0.31±0.09	0.0004	0.2332	0.48±0.15	0.0023	0.25±0.11	0.0202
Dominating isoform	0.38±0.09	<0.0001	0.3360	0.52±0.17	0.003	0.32±0.11	0.0049	0.34±0.09	0.0002	0.4290	0.44±0.17	0.0089	0.31±0.11	0.0062
Apo(a) size														
Larger isoform	-1.04±1.07	0.3332	0.4128	-2.57±2.14	0.2335	-0.54±1.24	0.6669	-1.07±1.03	0.2977	0.6067	-1.42±2.06	0.4938	-0.83±1.21	0.4928
Smaller isoform	-1.81±0.81	0.0270	0.1614	-3.59±1.50	0.0195	-1.08±0.97	0.2659	-1.56±0.78	0.0466	0.5645	-2.69±1.53	0.0834	-1.40±0.95	0.1418
Dominating isoform	-0.08±0.03	0.0041	0.4189	-0.11±0.05	0.0224	-0.07±0.03	0.0545	-0.06±0.03	0.0172	0.8084	-0.07±0.05	0.1336	-0.06±0.03	0.0594
LDL cholesterol	-0.43±0.38	0.2614	0.8899	-0.51±0.66	0.4465	-0.39±0.47	0.404	-0.14±0.39	0.7255	0.9372	0.30±0.69	0.6617	-0.18±0.48	0.7135
Apolipoprotein B-100	-0.01±0.01	0.1938	0.2482	0.001±0.01	0.878	-0.01±0.01	0.0843	-0.0003±0.005	0.9633	0.3720	0.01±0.01	0.1659	-0.004±0.007	0.5497
Triglycerides	-0.78±0.21	0.0002	0.0561	-0.21±0.38	0.5794	-1.06±0.25	<0.0001	-0.62±0.23	0.0081	0.0670	0.04±0.44	0.9218	-0.95±0.27	0.0007

Regression analysis was performed using logarithmically transformed values of LDL cholesterol, triglyceride, total and allele-specific Lp(a) levels. Apo(a) indicates apolipoprotein(a); LDL, low-density lipoprotein; Lp(a), lipoprotein(a); and Lp(a)-OxPL, oxidized phospholipids bound to Lp(a).

*Model 1 is adjusted for the covariates, including age, sex, current smoker, lipid-lowering medication use, and hypertension medication use and race for all overall analyses. P values for differences between Black (B) and White (W) participants in overall models are based on an interaction term added to the regression models.
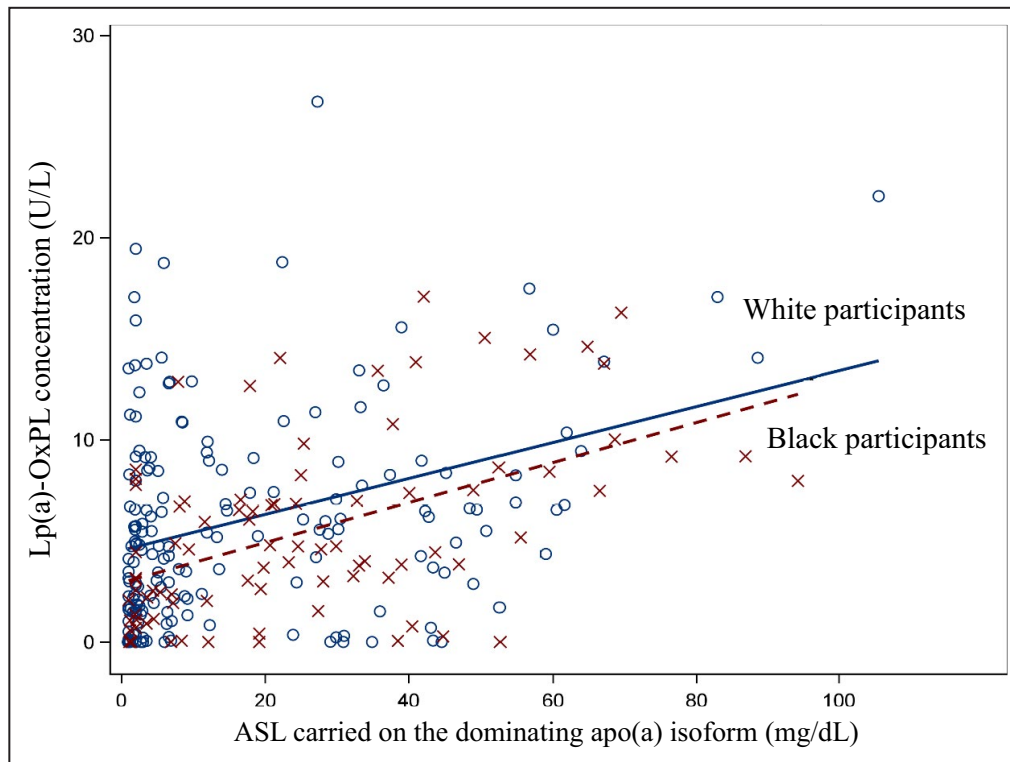
Figure 1. The relationship between Lp(a)-OxPL and allele-specific Lp(a) level carried on the dominating* apo(a) size in Black and White participants.
*Dominating apo(a) is determined as carrying ≥70% of total plasma Lp(a) level for carriers of double apo(a) protein isoforms as well as the only expressed isoform for carriers of a single apo(a) protein isoform as previously described.[15,19] Apo(a) indicates apolipoprotein(a); ASL, allele-specific Lp(a) level; and Lp(a)-OxPL lipoprotein(a)-bound oxidized phospholipids.

significant associations of Lp(a)-OxPL with the total plasma Lp(a) level or allele-specific Lp(a) levels carried on the smaller or dominating isoform. In contrast, when further adjusted for Lp(a) level, the association of Lp(a)-OxPL with the smaller or dominating apo(a) isoform size in all participants lost its significance. In White participants, Lp(a)-OxPL concentration remained significantly associated with the allele-specific Lp(a) level carried on the dominating apo(a) isoform (β=0.27±0.13, P=0.0409) as well as with the triglyceride level (β=−0.99±0.27, P=0.0003) in this model (Table S1).

As the major difference in allele-specific Lp(a) levels between Black and White participants is because of differences in midrange size isoforms, we compared allele-specific Lp(a) levels with Lp(a)-OxPL levels across 3 apo(a) size ranges (small: ≤22 K4 repeats, medium: 23–27 K4 repeats, and large: >28 K4 repeats) in each race for the dominant apo(a) size of each allele-pair. In line with the findings of total plasma Lp(a) levels (Figure 2A), Black participicants had significantly higher allele-specific Lp(a) levels for the apo(a) size range of 23 to 27 K4 repeats (P=0.0009) and also to some extent for large apo(a) sizes compared with White participants(Figure 2B). Despite these differences, levels of Lp(a)-bound OxPL were

similar between Black and White participants across the 3 ranges of apo(a) sizes (Figure 2C). After adjusting for age and sex, the interracial difference in Lp(a)-OxPL for the large apo(a) size range was significant (Figure 2C) (P=0.0038). Next, we determined OxPL abundance on Lp(a) with a ratio (OxPL/Lp(a)) by dividing OxPL carried on Lp(a) by total plasma Lp(a) level and assessed its relation to apo(a) size and race (Figure 2D). In White participants, the distribution of OxPL/Lp(a) ratio differed significantly across the 3 apo(a) size ranges (P=0.0002) with higher levels for the medium and large size ranges compared with those for the small size range. In contrast, this difference was not significant in Black participants (P=0.1073). This heterogeneity across the apo(a) size spectrum in OxPL/Lp(a) ratio between White and Black participants resulted in a significant interracial difference for the medium (P=0.0090) and large (P=0.0017) apo(a) size ranges (Figure 2D). Further, Lp(a)-OxPL level was negatively associated with the dominating apo(a) isoform size in both White (R^2=0.073, P=0.003) and Black (R^2=0.203, P<0.0001) participants (Figure S1A). The positive association of the ratio of OxPL/Lp(a) with the dominating apo(a) isoform size was significant only in White participants (R^2=0.034, P=0.0138) (Figure S1B).
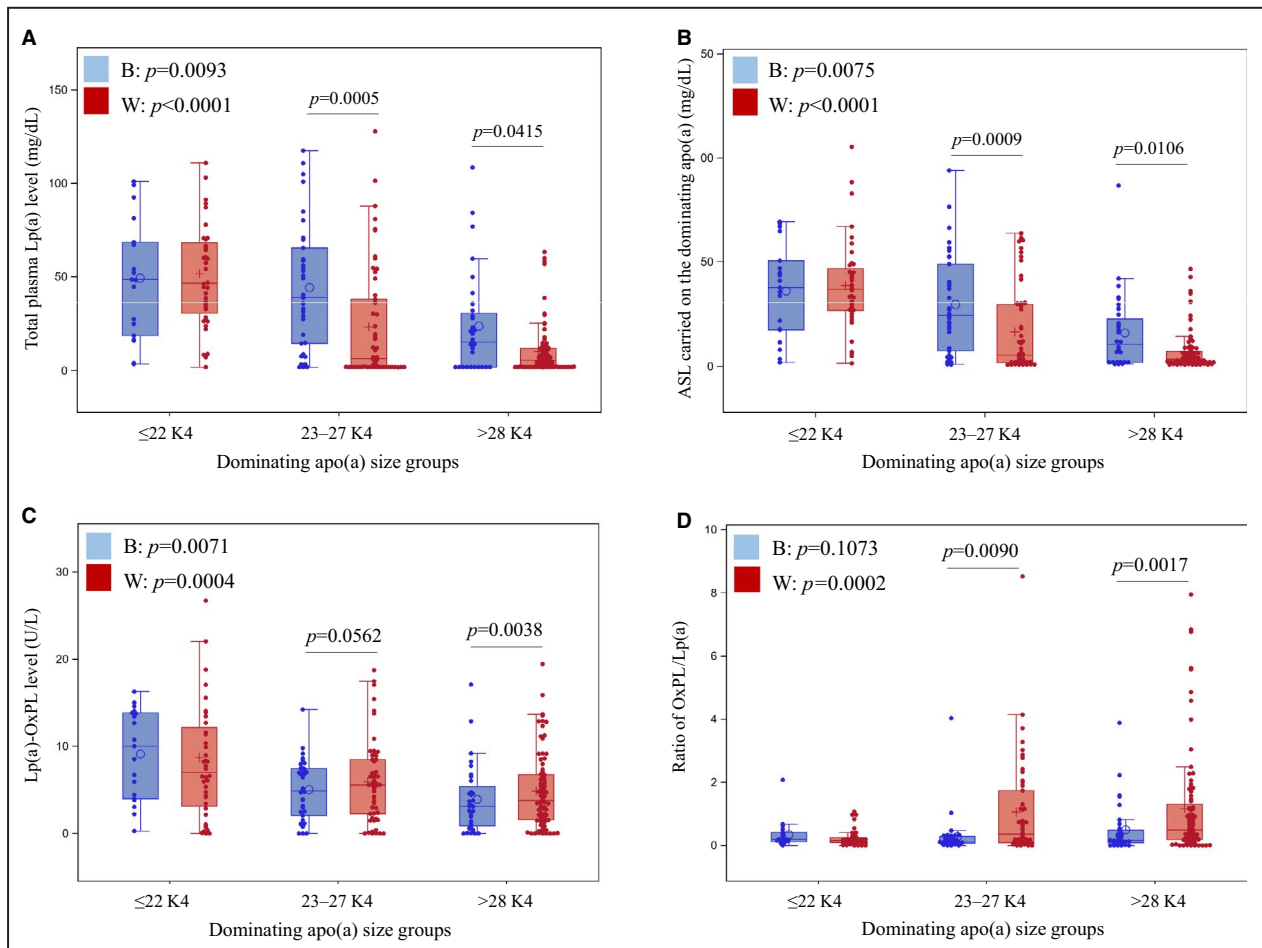
Figure 2. Total plasma Lp(a) level (A), allele-specific Lp(a) level for dominating apo(a) sizes (B), Lp(a)-bound OxPL (C) and OxPL/Lp(a) ratio (D) across 3 apo(a) size ranges in Black and White participants.
Box plots represent interquartile ranges and whiskers mark the upper and lower limits. Median and mean values are denoted with straight lines and "o" (for Black participants) or "+" (for White participants) symbols in the boxes, respectively. Please refer to Figure 1 footnote for determination of dominating apo(a). *P* values for interracial difference in level or ratio for the specified apo(a) size group represent results after adjusting for age and sex. *P* values following each race indicate heterogeneity in the distribution of level or ratio across the 3 groups of dominating apo(a) within the racial group after adjusting for age and sex. Apo(a) indicates apolipoprotein(a); B, Black participants; K, kringle repeats; Lp(a)-OxPL, lipoprotein(a)-bound oxidized phospholipids; and W, White participants.

Lp(a)-OxPL Phenotypic Correlation Between Parents and Offspring Values

Capitalizing on the family study setting, we next studied the degree of heritability for levels of Lp(a)-OxPL between parents and offspring by race (Table 3). To demonstrate the results in a context, first we illustrate the degree of heritability for plasma Lp(a) level (Figure 3A), and then for Lp(a)-OxPL level (Figure 3B). In contrast to the findings for Lp(a) levels where there is a great degree of heritability in both racial groups ($h^2 = 0.86 \pm 0.12$, $P < 0.0001$ in the Black group, $h^2 = 1 \pm 0.11$, $P < 0.0001$ in the White group, $P = 0.2863$ for interracial difference),

Table 3. Heritability Estimates From Offspring-Mid-Parent Regression for Lp(a) and Lp(a)-Bound OxPL in All Families by Race

Variables	Black Participants (n=22 Families)		White Participants (n=39 Families)		Difference Between Black and White Participants	
	$h^2 \pm SE$	*P* Value	$h^2 \pm SE$	*P* Value	Effect Size±SE	*P* Value
Lp(a)	0.86±0.12	<0.0001	1.00±0.11	<0.0001	0.18±0.17	0.2863
Lp(a)-bound OxPL	0.29±0.12	0.0235	−0.07±0.10	0.5093	0.36±0.17	0.0352

Lp(a) indicates lipoprotein(a); and Lp(a)-OxPL, oxidized phospholipids bound to Lp(a).
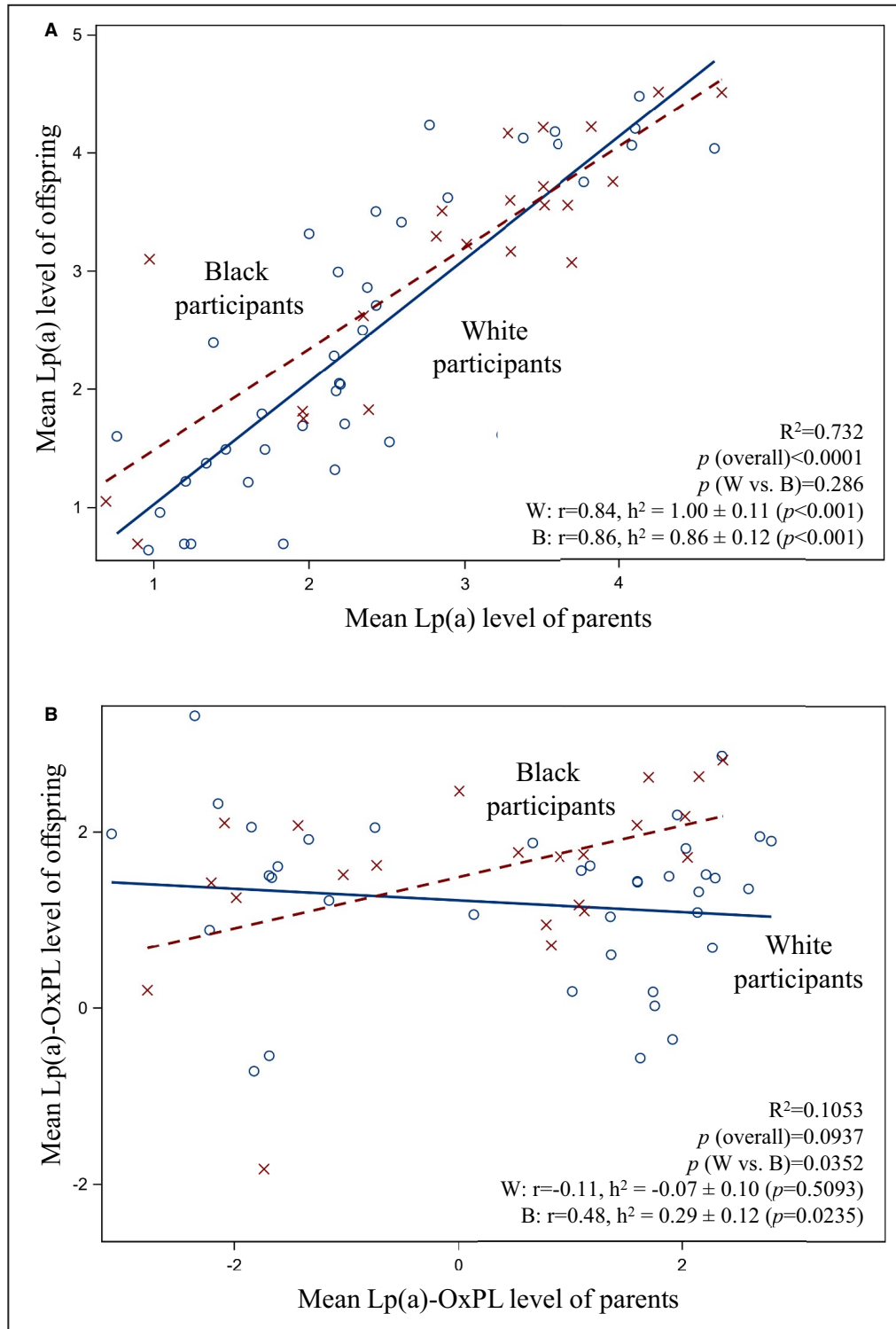
Figure 3. **Degree of heritability in total plasma Lp(a) (A) and Lp(a)-bound OxPL (B) levels between parents and offspring by race.**

Regression plots were created using the mean values of parents or offspring in a given family for White families (W) (n=39 families) and Black families (B) (n=22 families). Lp(a) and Lp(a)-OxPL values were logarithmically transformed before inferential analyses. *P* (overall) indicates significance of the overall association between parents and offspring. *P* (B vs W) indicates significance of the interracial difference in heritability between White and Black participants. Lp(a)-OxPL indicates lipoprotein(a)-bound oxidized phospholipids.

we found a significant interracial difference in heritability between parents and offspring values for Lp(a)-OxPL levels (h^2=0.29±0.12, *P*=0.0235 in the Black group, h^2=−0.07±0.10, *P*=0.5093 in the White group, *P*=0.0352 for interracial difference) (Table 3). In line with the latter observation, there was a significant positive association for levels of Lp(a)-OxPL between Black parents and Black offspring (*r*=0.48, *P*=0.0235) (Figure 3B).

DISCUSSION

A physiological function of Lp(a), if any, remains a puzzle. Although its long suspected role as a cardiovascular risk factor has been firmly confirmed and recognized in clinical guidelines,[1–4] any function ascribed to it still remains to be established. The discovery that Lp(a) serves as a carrier for OxPL[6] has opened exciting possibilities as this potentially could both contribute to its risk factor role as well as shed light on a possible metabolic/physiological role. Although the molecular motif responsible for the binding of OxPL has been ascribed to the apo(a) component,[20] the relationship between OxPL and apo(a) properties remains largely unexplored. Furthermore, given the potential risk factor contribution of OxPL as well as its wide roles in immune defense, inflammation, and homeostasis,[21–24] any impact of age and heredity on Lp(a)-associated OxPL is largely unknown and warrants investigation. In this study among healthy Black and White families where participants had normal lipid levels and were largely free of lipid-lowering therapy (>90%) and chronic diseases,[9,10] we found a significant positive association of OxPL levels with Lp(a) levels as well as with allele-specific Lp(a) levels carried on the smaller or dominating apo(a) isoforms in both Black and White participants. In contrast, although a similar pattern was seen for large apo(a) isoforms in the Black group, there was no such significant association of Lp(a)-OxPL with allele-specific large apo(a) levels in the White group. Notably, adjustments for the covariates did not alter the significant association of Lp(a)-OxPL with allele-specific Lp(a) level carried on the dominating apo(a) isoform in neither racial group. The association between OxPL and allele-specific Lp(a) level carried on the dominating apo(a) isoform in all participants and separately in Black and White participants still remained significant when the size of the dominating apo(a) was taken into account for further adjustment. These results emphasized the importance of the allele-specific Lp(a) level, which takes both the genotypic and phenotypic Lp(a) characteristics into account, in determining OxPL level. Furthermore, compared with Black subject, our

White subjects had significantly lower levels of Lp(a) and allele-specific apo(a), but similar levels of Lp(a)-OxPL, for the medium to large apo(a) size ranges. This is reflected in the higher ratio of OxPL/Lp(a) in the White group compared with the Black group for the medium to large size apo(a) ranges, indicative of a higher burden among White carriers of these isoforms. We also noted a significant heritability for Lp(a)-bound OxPL values in Black but not in White participants. Illustrating this interracial difference, average levels of Lp(a)-OxPL for parents versus offspring were significantly correlated in Black but not in White participants. This finding contrasted with the well-known corresponding correlation for Lp(a) levels across generations and races. Taken together, the relationship between Lp(a)-OxPL, Lp(a), apo(a) size and race is complex and warrants further investigation for potential roles of environmental as well as additional genetic factors.

It is well established that oxidized LDL particles play a role in the development of atherosclerotic cardiovascular disease.[25] Much attention has been placed on understanding the underlying mechanisms and pathways where oxidation of LDL leads to progression of atherosclerotic cardiovascular disease.[26] Notably, oxidized lipids present in LDL are also found in tissues undergoing apoptosis and constitute a signaling pattern involved in development of inflammation, immune activation, and aging.[27,28] Collectively, OxPLs interact with numerous receptors, including scavenger receptors and receptors involved in lipid metabolism, and have been ascribed protective functions.[27,29] Given this yin-yang pattern of protective versus risk factor roles, the finding of a strong association between OxPL and Lp(a) opened exciting opportunities but also new questions. As Lp(a) levels largely remain stable over the lifespan, would an increased metabolic burden of chronic diseases and an age-associated increase in systemic inflammation, as demonstrated in our earlier studies,[9,30] affect any role of Lp(a) as carrier of OxPL? It should be emphasized that such a role should be seen in the context of understanding the relationship between apo(a), apoB and OxPL in Lp(a). Further, given the well-established difference in allele-specific Lp(a) levels across Black-White race,[7,19] any such impact on Lp(a)-bound OxPL levels remains to be determined. Our findings of an association between OxPL and allele-specific Lp(a) levels for smaller apo(a) sizes underscore the risk factor potential of OxPL as Lp(a) particles carrying smaller apo(a) sizes have been shown to be more atherogenic.[19,31] For allele-specific Lp(a) levels of larger apo(a) sizes, we found an association with OxPL only among Black participants. It is in this context interesting to note the interracial difference in allele-specific Lp(a) levels for this range.[15,19] Although we replicated the previously reported higher

allele-specific Lp(a) levels for midsize apo(a) isoforms among Black people in our cohort, we could not detect any corresponding interracial difference for OxPL. In this context, it is important to underscore that we focused specifically on OxPL carried by Lp(a).

Among other lipids and lipoproteins, only triglycerides showed a consistent negative association with Lp(a)-OxPL in the White group but not in the Black group. It is tempting to speculate that the higher triglyceride and lower Lp(a) levels in White participants may underlie the significant negative association of Lp(a)-carried OxPL with triglyceride levels. This interesting observation merits further investigation and a broader focus on OxPL in relation to apoB-containing lipoproteins.

The strong heritability of apo(a) sizes and allele-specific Lp(a) levels is well documented.[10,32] Although allele-specific Lp(a) levels can vary across families for a given apo(a) size, there is generally a strong resemblance from 1 generation to another within families.[32] We confirmed this robust relationship in allele-specific Lp(a) levels in our families across race. However, we found a divergent pattern for Lp(a)-OxPL, where resemblance was significantly greater among Black participants. To our knowledge, this study is one of the first reporting data on the extent to which variations in Lp(a)-bound OxPL levels in offspring can be explained by parental values as well as on interracial differences. Among twins, largely of White origin (>83%), OxPL on apoB-containing lipoproteins were heritable.[33] This observation contrasts with our White findings and may be owing to differences between the 2 studies for cohort characteristics (twins versus families; adults only versus inclusive of children, etc), approaches (measurement of OxPL contained on all apoB-containing lipoproteins versus OxPL specifically bound to Lp(a)) and test methods (heritability estimation based on monozygotic/dizygotic twins versus phenotypic correlation between parents and children). Of note, Lp(a) levels in the twin-pair study[33] were very low (median: 3.1 mg/dL) compared with those in our White participants (≈10 mg/dL), which were in line with other reports for White people.[34] Additionally, beyond genetic factors, members of our healthy families also shared a similar intrafamily environment with at least to some extent shared exposure to such factors. In previous studies, we reported an age-associated increase in systemic inflammatory markers among these individuals.[9] As OxPL levels were quantitatively similar in parents and their children, our results might argue against an increase in OxPL levels in parallel with such an increased inflammatory burden over age. Nevertheless, the higher physiological resemblance of Lp(a)-bound OxPL between Black parents and children needs to be confirmed in larger studies.

This study has limitations. The cross-sectional study design limits our ability to evaluate any causative and longitudinal effects of factors that might influence levels of OxPL and/or Lp(a). There are unmeasured factors such as diet and alcohol consumption that may potentially affect OxPL levels. However, the present family-based study setting reduces the potential impact of variable genetic as well as environmental factors. Another strength is the inclusion of 2 racial groups; although, we recognize the need for a larger study in Black people representative of a broader general population. In addition, although our studies on allele-specific Lp(a) levels made it possible to relate OxPL results more closely to a range of apo(a) sizes, assessing allele-specific OxPL is beyond the scope of the paper and that we recognize this as a limitation.

CONCLUSIONS

In conclusion, among healthy individuals, circulating levels of Lp(a)-bound OxPL were significantly and positively associated with Lp(a) levels. Furthermore, we demonstrated a similar association in both Black and White participants for allele-specific Lp(a) levels with smaller apo(a) sizes shown to have a high atherogenic potential.[19,31] We also found an interracial difference in the heritability of Lp(a)-OxPL, with a significantly higher physiological resemblance between parents' and offspring' values among Black participants. Taken together, the findings illustrate a strong relationship of OxPL with allele-specific Lp(a) levels and contribute to a better understanding of the relationship between OxPL and Lp(a), a highly heritable trait, under normal physiological conditions.

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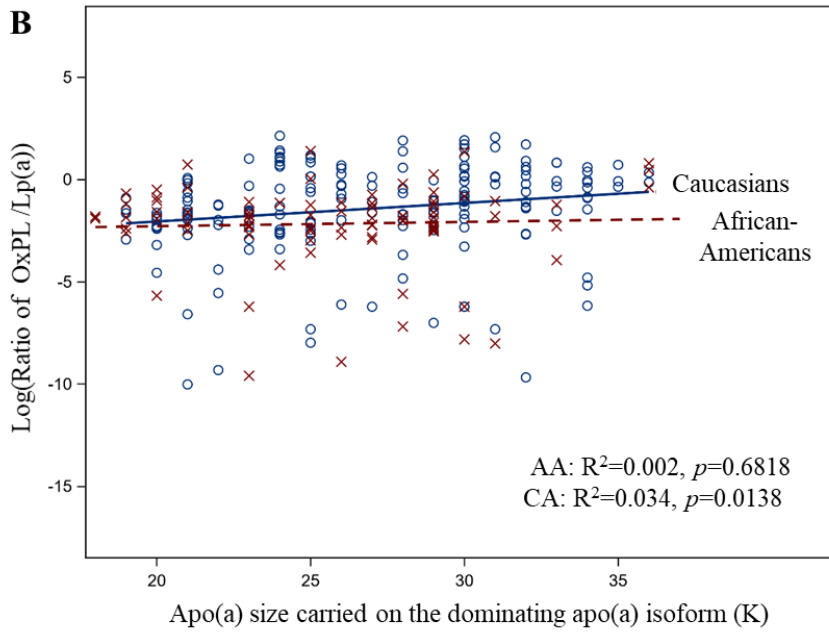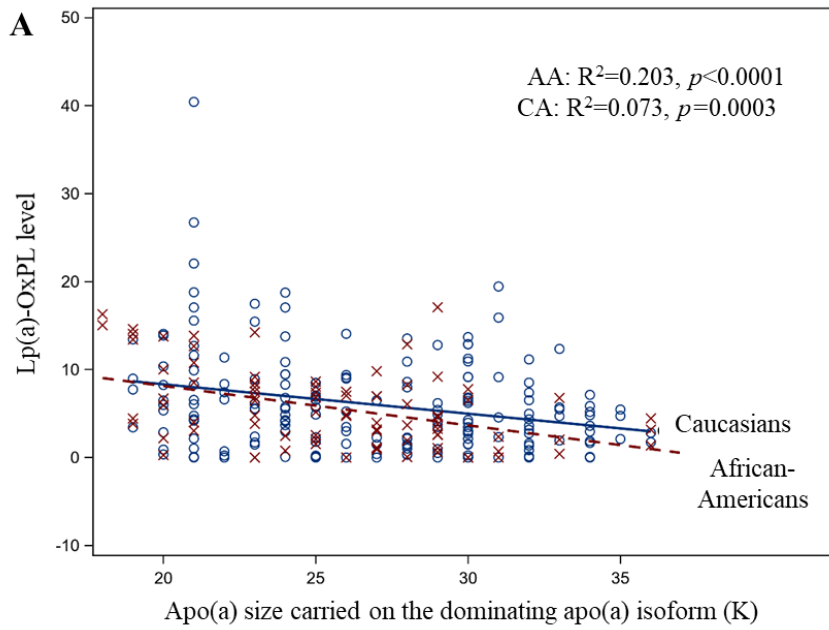
SUPPLEMENTAL MATERIAL

Table S1. Regression analyses for Lp(a)-OxPL with further adjustments (Model 2) in all participants and by ethnicity.

Variables	Overall			African-Americans		Caucasians	
	β ± SE	*p*	*p* (CA vs AA)	β ± SE	*p*	β ± SE	*p*
Total plasma Lp(a) level	**0.27 ± 0.11**	**0.0125**	0.3434	**0.39 ± 0.19**	**0.0369**	0.22 ± 0.13	0.1017
Allele-specific Lp(a) level:							
Larger isoform	0.11 ± 0.10	0.2797	0.0614	**0.45 ± 0.20**	**0.0276**	0.02 ± 0.11	0.8871
Smaller isoform	**0.28 ± 0.11**	**0.0105**	0.2064	**0.44 ± 0.18**	**0.0147**	0.20 ± 0.14	0.1358
Dominating isoform	**0.31 ± 0.11**	**0.0044**	0.4164	**0.41 ± 0.19**	**0.0310**	**0.27 ± 0.13**	**0.0409**
Apo(a) size:							
Larger isoform	0.05 ± 1.20	0.9694	0.6658	0.97 ± 2.16	0.6554	-0.03 ± 1.29	0.9845
Smaller isoform	0.23 ± 1.42	0.8734	0.3179	-1.69 ± 1.53	0.2736	-0.29 ± 1.11	0.7977
Dominating isoform	-0.03 ± 0.03	0.3999	0.7647	-0.02 ± 0.05	0.7527	-0.03 ± 0.04	0.4147
LDL cholesterol	-0.36 ± 0.39	0.3491	0.8709	-0.13 ± 0.69	0.8561	-0.35 ± 0.48	0.4582
Apolipoprotein B-100	-0.004 ± 0.005	0.4475	0.3951	0.006 ± 0.009	0.4807	-0.008 ± 0.007	0.2420
Triglycerides	**-0.63 ± 0.23**	**0.0064**	**0.0412**	0.16 ± 0.43	0.7192	**-0.99 ± 0.27**	**0.0003**

The model is further adjusted for the dominating apo(a) size for the plasma Lp(a) concentration-related variables and for Lp(a) concentration for

the apo(a) size-related variables, respectively, in addition to the same covariates (age, sex, current smoker, lipid-lowering medication use, and

hypertension medication use) and ethnicity for all overall analyses used in Model 1. For non-Lp(a) variables (LDL-C, ApoB, and triglycerides), correlation coefficients adjusted for Lp(a) concentration are given. Regression analysis was performed using logarithmically transformed values of LDL cholesterol, triglyceride, total and allele-specific Lp(a) levels. *P*-values for differences between African-Americans (AA) and Caucasians (CA) in the overall model are based on an interaction term added to the regression model.

Figure S1. Scatter plots of Lp(a)-OxPL level (A) and the ratio of OxPL/Lp(a) (B) as a function of the dominating apo(a) isoform size and ethnicity/race.

AA: $R^2=0.203$, $p<0.0001$
CA: $R^2=0.073$, $p=0.0003$

Caucasians

African-Americans

Caucasians

African-Americans

AA: $R^2=0.002$, $p=0.6818$
CA: $R^2=0.034$, $p=0.0138$

AA, African-Americans; CA, Caucasians;