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Heritability of apolipoprotein (a) traits in two-generational African-American and Caucasian families[S]

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Abstract Heritability of *LPA* allele, apo(a) isoform sizes, and isoform-associated lipoprotein(a) [Lp(a)] levels was studied in 82 Caucasian and African-American families with two parents and two children (age: 6–74 years). We determined: *1*) Lp(a) levels; *2*) *LPA* allele sizes; *3*) apo(a) isoform sizes; and *4*) isoform-specific apo(a) levels (ISLs), the amount of Lp(a) carried by an individual apo(a) isoform. Trait heritability was estimated by mid-parent-offspring analysis. The ethnicity-adjusted heritability estimate for Lp(a) level was 0.95. Heritability for ISLs corresponding to the smaller *LPA* allele in a given allele-pair was higher than that corresponding to the larger *LPA* allele (0.91 vs. 0.59, *P* = 0.017). Although not statistically different, heritability for both apo(a) isoforms (0.90 vs. 0.70) and *LPA* alleles (0.98 vs. 0.82) was higher for the smaller versus larger sizes. Heritability was generally lower in African-Americans versus Caucasians with a 4-fold difference for the larger *LPA* allele (0.25 vs. 0.94, *P* = 0.001). In Caucasians, an overall higher heritability pattern was noted for the older (≥47 years) versus younger (<47 years) families. In conclusion, Lp(a) level and traits associated with the smaller *LPA* alleles were strongly determined by genetics, although with a varying ethnic influence. Ethnic differences in heritability of the larger *LPA* allele warrant further investigations.—Enkhmaa, B., E. Anuurad, W. Zhang, K. Kim, and L. Berglund. **Heritability of apolipoprotein (a) traits in two-generational African-American and Caucasian families.** *J. Lipid Res.* 2019. **60: 1603–1609.**

Supplementary key words lipoprotein (a) • apolipoprotein (a) isoform • *LPA* allele • heritability estimates • family resemblance • family study

Lipoprotein(a) [Lp(a)], a major genetic cardiovascular risk factor, consists of a LDL-like particle and a unique apolipoprotein, i.e., apo(a), linked to the apoB-100 via a single disulfide bond (1). Although stable intra-individually,

Lp(a) levels vary substantially between individuals and ethnicities. On average, populations of African descent exhibit 2- to 3-fold higher Lp(a) levels compared with populations of European descent (1–3). Twin-pairs and family studies report a consistently high heritability estimate for Lp(a) level, making Lp(a) one of the most heritable quantitative human traits (4–8). Population-based studies have uniformly demonstrated an inverse association of the number of kringle 4 (K4) type 2 repeats with Lp(a) level across ethnic groups (2–4, 8–12). However, in spite of this association, a 2.5-fold variation in Lp(a) level associated with the same size allele, i.e., alleles of identical K4 type 2 repeat numbers, has been reported (13). Notably, the apo(a) size polymorphism-induced effect on Lp(a) levels appears to be less prominent in Africans versus non-Africans (2, 14–16). Reports also indicate a lower heritability estimate for Lp(a) level in Africans versus Caucasians. Thus, the *LPA* locus explained almost all of the total genetic variance of the Lp(a) trait in Caucasians, while accounting for <50% of the variance in Africans (3).

In addition to the size polymorphism, other genetic variants at the *LPA* and non-*LPA* loci contribute to inter-ethnic differences in levels (17). Moreover, a strong gene-environment interaction was reported for Lp(a) among African-Americans and Nigerians (18). Although studies have shown an X-chromosome-linked effect as well as a significant sex-environment interaction (19), the roles of biological characteristics such as sex or the stages of lifespan in modulating Lp(a) heritability are less understood. Due to the extensive *LPA* allele size variability, plasma Lp(a) in most individuals consists of two particle populations with different apo(a) sizes. Limited data are available regarding the heritability of each (smaller vs. larger) *LPA* allele/ isoform of a given allele-pair within or across families of different ethnic backgrounds. In the present study, we

Abbreviations: BP, blood pressure; HDL-C, HDL cholesterol; IBD, identical by descent; K4, kringle 4; ISL, isoform-specific apo(a) level; LDL-C, LDL cholesterol; Lp(a), lipoprotein (a); TC, total cholesterol.
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investigated heritability of apo(a)-associated traits and iso-form-specific apo(a) levels (ISLs) in a family-based cohort of African-Americans and Caucasians and determined age-specific heritability estimates. We also analyzed the effects of multiple *LPA* SNPs reported to be present in both ethnic groups on the same traits.

MATERIALS AND METHODS

Human subjects

The details of human subjects and recruitment criteria for families have been described previously (20). Briefly, 82 (60 Caucasian and 22 African-American) families with two parents and two 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 nonfasting blood draws. Race/ethnicity was self-reported for each individual family member and, based on this information, families were categorized as Caucasian or African-American. Data from two children (one in each ethnic group) and one Caucasian father were not included due to unavailability of blood samples, and the present report is based on the findings in 238 Caucasians and 87 African-Americans. The study was approved by the Institutional Review Board at the University of California Davis, conducted in accordance with the Declaration of Helsinki, and informed consent was 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 (BP) was measured with a random-zero mercury sphygmomanometer. BMI was calculated as body weight (kilograms) divided by squares of height (square meters). For children and adolescents (6–20 years), BMI-for-age growth charts for either boys or girls (Center for Disease Control and Prevention) were used to obtain a percentile ranking. Concentrations of total cholesterol (TC), HDL cholesterol (HDL-C), triglycerides, apoB-100, apoA-1, and glucose were measured using standard procedures. LDL cholesterol (LDL-C) concentrations were calculated with the formula of Friedewald, Levy, and Fredrickson (21). Lp(a) levels were determined with an apo(a)-size insensitive ELISA (Mercodia Inc.) in plasma samples. Analyses were run according to the manufacturer's specifications in duplicate samples with two different quality controls, which were within the recommended precision for each test.

LPA allele and apo(a) isoform size determinations

LPA allele sizes were determined by genotyping using pulsed-field gel electrophoresis of whole DNA from leukocytes embedded in agarose plugs with a protocol adapted from Lackner et al. (22) and Rubin et al. (12) as described previously. 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, Ferrell, and Kottke (23). 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 (Technoclone GmbH, Austria) taking into

account the inverse relation between the number of K4 repeats (i.e., apparent molecular mass) and isoform mobility 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 (12, 24). To determine ISLs, Lp(a) levels were apportioned according to the degree of intensity of the bands on the Western blot as described in detail elsewhere (11, 24, 25).

LPA genetic variant sequencing

DNA samples for SNP genotyping were prepared by QLAamp DNA Blood Midi Kit (Qiagen). One hundred and fifty nanograms of genomic DNA per sample were used for SNP genotyping at the University of California Davis DNA Technologies Core. A total of 384 DNA samples were genotyped for 68 *LPA* SNPs using a custom VeraCode GoldenGate assay on a BeadXpress reader following the recommendations of the manufacturer (both assay and instrument from Illumina, San Diego, CA). The fluorescence images of an array matrix carrying Cy3- and Cy5-labeled beads were generated with the two-channel scanner. Raw fluorescence intensity data processing, clustering, and genotype calling were performed using the genotyping module in the BeadStudio package (Illumina). *LPA* SNPs were selected when the allele frequency was greater than 0.1 and less than 0.9 in both African and European descendants. The SNP frequency estimations can be found at http://pga.gs.washington.edu/data/lpa/lpaxx.csnps.txt. The SNP sequences passed the score were produced by Illumina and data were analyzed by GenomeStudio software (Illumina).

Statistics

Statistical analyses of data were performed with SAS software, version 9.4 (SAS Institute, Cary, NC). Results were expressed as mean ± standard deviation of mean or median with interquartile range for non-normally distributed variables. Lp(a) and isoform-associated Lp(a) level [ISL for larger or smaller allele] were natural log transformed prior to statistical analysis. Group differences were determined by ANOVA. 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 (ROMP) in nuclear families (82 quartets composed of spouse pairs with two biological offspring) with and without race/ethnicity as a covariate for ethnicity-adjusted and -unadjusted heritability, respectively. For phenotypic values for one family where the father was missing, the regression was performed on the mother and the resulting slope was doubled. To determine whether heritability differed between early and late in life, age differences in heritability were assessed by comparing young and old families. To define family age categories, the mean age of parents was calculated for each household and the median age of households was determined (47 years). Families were classified into younger household if the mean age of parents was below 47 years or older household if the mean age of parents was over 47 years. Variant association analyses for each trait were performed using genome-wide efficient mixed model association (GEMMA) (26), which incorporates familial relatedness within a cohort. Multiple testing control was done by the false discovery rate procedure.

RESULTS

Characteristics of study population

Eighty-two parent-offspring quartets (both parents and two offspring) participated in this study, ranging in age from 6 to 74 years. There were slightly more male offspring

than female offspring **(Table 1)**. The mean age of offspring was 16 ± 9 years and 15 ± 5 years for African-Americans and Caucasians, respectively. The mean age of parents was 45 ± 9 years and 47 ± 7 years for African-Americans and Caucasians, respectively. There were no significant interethnic differences among offspring or parents for body weight, BMI, BP, TC, LDL-C, HDL-C, apoB-100, and glucose levels. Triglyceride levels were lower in African-Americans versus Caucasians for both offspring ($P < 0.0001$) and parents ($P = 0.027$). In addition, the apoA-1 concentration was higher in African-American offspring compared with Caucasian offspring ($P = 0.005$). As expected, the median Lp(a) level was approximately 3-fold higher in both African-American offspring and parents compared with Caucasian offspring and parents, respectively (for offspring: 32 mg/dl vs. 9 mg/dl, $P < 0.001$; for parents: 27 mg/dl vs. 7 mg/dl, $P < 0.001$). Similarly, both ISLs corresponding to the smaller or larger apo(a) sizes were elevated in African-Americans versus Caucasians for both parents and offspring ($P < 0.001$ for ethnic difference) (Table 1). Twenty-two participants (6.8%) were homozygotes for *LPA* allele sizes, resulting in a heterozygosity index of 93.2% for *LPA* genotypes in the study cohort. While only 4% of the smaller apo(a) sizes in allele-pairs resulted in an undetectable apo(a) protein, the corresponding rate for the larger apo(a) sizes was 15.7%. Thus, an excellent concordance between the distribution patterns of *LPA* alleles and apo(a) isoforms was seen for the smaller sizes in both ethnic groups. In contrast, slightly different distribution patterns were observed for the larger sizes. For example, among Caucasian parents, the median (interquartile range) *LPA* allele and apo(a) isoform sizes were 32 (28–34) and 31 (27–34), respectively. The median

sizes for the larger and smaller *LPA* alleles were similar between African-Americans and Caucasians.

Heritability of Lp(a) and related traits and interethnic differences

The ethnicity-adjusted overall heritability estimate (h^2) for the Lp(a) level was 0.95, indicating that 95% of individual differences in Lp(a) level were due to hereditary factors (**Table 2**). To test whether Lp(a) heritability was impacted by apo(a) size polymorphism, we assessed the heritability of the isoform-specific Lp(a) level corresponding to smaller or larger apo(a) size separately within a given allele-pair. Notably, the heritability estimate of the isoform-specific Lp(a) level for the smaller apo(a) size was significantly greater than that corresponding to the larger apo(a) size (0.93 vs. 0.64, $P = 0.019$). This observation remained significant after adjusting for ethnicity (0.91 vs. 0.59, $P = 0.017$). Interestingly, no difference was seen for *LPA* genotypes or phenotypes. We then estimated the heritability for each ethnicity separately and compared estimates between Caucasians and African-Americans. In Caucasians, all traits were highly heritable (0.61–0.99, $P < 0.0001$) (**Table 3**). In African-Americans, isoform-specific levels for both apo(a) group sizes as well as smaller isoform and allele sizes were heritable (0.52–0.81, $P < 0.05$). No significant heritability was seen for the larger allele and isoform sizes in African-Americans. Although heritability was generally lower in African-Americans versus Caucasians, no significant differences were seen for ISLs or apo(a) isoforms between these two ethnicities. In contrast, a marked 4-fold lower heritability in African-Americans versus Caucasians was found for the larger *LPA* allele (0.25 vs. 0.94, $P = 0.001$). Notably, the

TABLE 1. Characteristics of study population

Traits	African-Americans		Caucasians	
	Offspring (n = 43)	Parent (n = 44)	Offspring (n = 119)	Parent (n = 119)
Anthropometric				
Female [n (%)]	20 (45%)	22 (50%)	46 (38%)	60 (50%)
Age (years)	16 ± 9	45 ± 9	15 ± 5	47 ± 7
Body weight (kg)	57 ± 23	93 ± 20	59 ± 23	87 ± 20
BMI (kg/m^2)	22 ± 7	31 ± 6	22 ± 6	29 ± 6
Systolic BP (mmHg)	115 ± 13	130 ± 16	113 ± 11	128 ± 14
Diastolic BP (mmHg)	68 ± 8	81 ± 13	68 ± 8	79 ± 9
Metabolic				
TC (mg/dl)	168 ± 37	191 ± 43	170 ± 37	205 ± 48
LDL-C (mg/dl)	96 ± 31	115 ± 38	94 ± 28	120 ± 38
HDL-C (mg/dl)	57 ± 16	46 ± 16	54 ± 15	50 ± 18
Triglycerides (mg/dl)	64 (52–79)[b]	125 (79–174)[a]	94 (70–133)	155 (100–235)
apoB-100 (mg/dl)	71 ± 24	92 ± 27	68 ± 16	94 ± 28
apoA-1 (mg/dl)	153 ± 26[b]	147 ± 32	140 ± 25	149 ± 25
Glucose (mg/dl)	86 ± 19	106 ± 47	90 ± 15	101 ± 30
Lp(a)/apo(a)-related				
Lp(a) level (mg/dl)	32 (15–66)[c]	27 (9–56)[c]	9 (2–35)	7 (2–37)
ISL, larger (mg/dl)	9 (5–21)[c]	13 (5–25)[c]	3 (1–9)	3 (1–10)
ISL, smaller (mg/dl)	20 (8–41)[c]	21 (6–39)[c]	5 (2–30)	5 (2–27)
apo(a) isoform, larger (kringles)	30 (27–33)	30 (29–33)	32 (29–34)	31 (27–34)
apo(a) isoform, smaller (kringles)	25 (21–28)	25 (23–28)	25 (22–30)	25 (23–30)

Data are expressed as mean ± standard deviation or median (interquartile range) for non-normally distributed variables. ISL, larger, ISL associated with the larger allele in a given *LPA* allele-pair; ISL, smaller, ISL associated with the smaller allele in a given *LPA* allele-pair.

[a] $P < 0.05$ versus Caucasian parent.

[b] $P < 0.01$ versus Caucasian offspring.

[c] $P < 0.001$ versus Caucasian parent or offspring.

TABLE 2. Heritability estimates from offspring-mid-parent regression for Lp(a) and related traits in all families before and after adjustment for ethnicity

Traits	Unadjusted		Adjusted for Ethnicity	
	$h^2 \pm$ SE	P^a	$h^2 \pm$ SE	P^a
Plasma				
Lp(a) level	0.97 ± 0.07		0.95 ± 0.07	
ISL, larger	0.64 ± 0.08		0.59 ± 0.09	
ISL, smaller	0.93 ± 0.08	0.019	0.91 ± 0.09	0.017
apo(a) phenotype[b]				
Larger isoform (kringles)	0.71 ± 0.12		0.70 ± 0.11	
Smaller isoform (kringles)	0.92 ± 0.09	0.157	0.90 ± 0.09	0.179
LPA genotype				
Larger allele (K4 repeats)	0.82 ± 0.09		0.81 ± 0.09	
Smaller allele (K4 repeats)	0.99 ± 0.09	0.182	0.97 ± 0.09	0.236

h^2, heritability estimate; ISL, larger, ISL associated with the larger allele in a given *LPA* allele-pair; ISL, smaller, ISL associated with the smaller allele in a given *LPA* allele-pair.
[a]Significance for differences between variables associated with larger versus smaller apo(a) sizes.
[b]Participants with *LPA* alleles that did not give rise to apo(a) protein, i.e., null alleles, were not considered in the analyses.

heritability estimates seen in African-Americans showed large variabilities (2- to 3-fold higher standard errors).

Qualitative age effects on heritability of Lp(a) and related traits and interethnic differences

We stratified families into two groups based on the median age of parents in the household (47 years) and compared age-specific heritability for younger (<47 years) versus older (≥47 years) families to examine whether heritability would differ by age of family parenthood. There were no significant differences in age of parenthood between Caucasians and African-Americans for either younger (41 ± 4 years vs. 40 ± 4 years, respectively) or older (52 ± 5 years vs. 53 ± 8 years, respectively) families. In Caucasians, all traits were highly heritable in both age groups (0.51–1.00, P < 0.001) (**Table 4**). Although not significantly different, heritability estimates were consistently higher in older versus younger Caucasian families for all tested traits. In African-Americans, the age-specific heritability pattern was heterogeneous. Heritability estimates were lower for some traits, e.g., larger *LPA* allele and apo(a) isoform sizes, but not for others in older versus younger families (Table 4). Particularly, the heritability estimate of the Lp(a) level was 2-fold lower in older versus younger African-American families (0.51 vs. 1.00, P < 0.05). In younger families, heritability did not differ significantly between Caucasians and African-Americans (Table 4). In contrast,

in older families, age effects on heritability estimates between Caucasians and African-Americans differed for Lp(a) level and the larger *LPA* allele size. For both instances, heritability was considerably lower in African-Americans versus Caucasians [Lp(a) level: 0.51 vs. 1.00, respectively, P < 0.05 for ethnic difference; the larger *LPA* allele size: 0.05 vs. 0.97, respectively, P < 0.001 for ethnic difference).

Associations between *LPA* SNPs and Lp(a)/apo(a)-related traits by ethnicity

In order to identify *LPA* genetic variants associated with isoform-associated Lp(a) level for smaller and larger apo(a) sizes separately, we performed an association analysis. Out of 68 *LPA* SNPs included in the analyses, 12 SNPs were associated with Lp(a) level and/or ISL for smaller or larger apo(a) sizes (P < 0.05); although they were not significant after multiple testing correction at false discovery rate <0.05 (supplemental Table S1). In Caucasians, five SNPs were identified as associated with Lp(a) level, the less common allele being associated with a decreased level of Lp(a). Of these five SNPs, four and three were also found to be associated with ISLs for smaller and larger apo(a) sizes, respectively, the less common allele being associated with declines in the trait levels (supplemental Table S1). Notably, none of these SNPs was significantly associated with any of the phenotypes in African-Americans. Thus, in

TABLE 3. Heritability estimates from offspring-mid-parent regression for Lp(a) and related traits by ethnicity

Traits	Caucasians		African-Americans		
	$h^2 \pm$ SE	P	$h^2 \pm$ SE	P	P for Ethnic Difference
Lp(a) level	0.99 ± 0.09	<0.0001	0.79 ± 0.14	<0.0001	NS
ISL, larger	0.61 ± 0.10	<0.0001	0.52 ± 0.22	0.028	NS
ISL, smaller	0.93 ± 0.10	<0.0001	0.80 ± 0.21	0.001	NS
apo(a) isoform,[a] larger	0.77 ± 0.13	<0.0001	0.34 ± 0.26	NS	NS
apo(a) isoform,[a] smaller	0.92 ± 0.10	<0.0001	0.78 ± 0.26	0.007	NS
LPA allele, larger	0.94 ± 0.08	<0.0001	0.25 ± 0.23	NS	0.001
LPA allele, smaller	0.99 ± 0.09	<0.0001	0.81 ± 0.24	0.003	NS

h^2, heritability estimate; ISL, larger, ISL associated with the larger allele in a given *LPA* allele-pair; ISL, smaller, ISL associated with the smaller allele in a given *LPA* allele-pair.
[a]Participants with *LPA* alleles that did not give rise to apo(a) protein, i.e., null alleles, were not considered in the analyses.

TABLE 4. Age-specific heritability estimates of Lp(a) and related traits

Traits	Caucasians				African-Americans			
	Younger Family (<47 years)		Older Family (≥47 years)		Younger Family (<47 years)		Older Family (≥47 years)	
	h² ± SE	P	h² ± SE	P	h² ± SE	P	h² ± SE	P
Lp(a) level	0.88 ± 0.15	<0.0001	1.00 ± 0.11[a]	<0.0001	1.00 ± 0.19[b]	0.0001	0.51 ± 0.20	0.043
ISL, larger	0.51 ± 0.14	0.0013	0.73 ± 0.15	<0.0001	0.32 ± 0.37	NS	0.61 ± 0.23	0.041
ISL, smaller	0.69 ± 0.16	0.0002	1.04 ± 0.13	<0.0001	0.88 ± 0.24	0.003	0.78 ± 0.40	NS
apo(a) isoform,[c] larger	0.73 ± 0.12	<0.0001	0.83 ± 0.19	0.0002	0.42 ± 0.47	NS	0.28 ± 0.30	NS
apo(a) isoform,[c] smaller	0.80 ± 0.16	<0.0001	0.98 ± 0.12	<0.0001	0.69 ± 0.39	NS	0.93 ± 0.31	0.025
LPA allele, larger	0.86 ± 0.12	<0.0001	0.97 ± 0.11[d]	<0.0001	0.99 ± 0.47	0.057	0.05 ± 0.22	NS
LPA allele, smaller	0.91 ± 0.18	<0.0001	1.00 ± 0.11	<0.0001	0.74 ± 0.35	0.057	0.95 ± 0.32	0.024

The median age (47 years) of adults in the household was used to stratify families to younger and older age groups. h², heritability estimate; ISL, larger, ISL associated with the larger allele in a given LPA allele-pair; ISL, smaller, ISL associated with the smaller allele in a given LPA allele-pair.
[a]P < 0.05 for ethnic difference (Caucasian vs. African-American).
[b]P < 0.05 for age difference between younger versus older family.
[c]Participants with LPA alleles that did not give rise to apo(a) protein, i.e., null alleles, were not considered in the analyses.
[d]P < 0.001 for ethnic difference (Caucasian vs. African-American).

African-Americans, a different set of SNPs (a total of seven) was found to be associated with the phenotypes. Out of these SNPs, four (rs9457951, rs9457986, rs41269884, and rs41271032) had the same directional effect on both Lp(a) level and ISL for smaller apo(a) sizes. Within each ethnic group, it appeared that SNP-induced effects were concordant direction-wise, regardless of significance level, for all traits. After adjustments for age and sex, the findings remained unaltered (data not shown).

DISCUSSION

In this two-generation family study, we investigated the heritability of isoform-specific Lp(a) level for each apo(a) size as well as larger and smaller LPA alleles and apo(a) isoforms in relation to ethnicity and age. In addition to confirming the highly heritable nature of the Lp(a) level, our findings demonstrated that traits associated with the smaller apo(a) size of a given allele-pair are among the most genetically determined highly heritable quantitative human traits. Thus, a higher proportion of phenotypic variance in ISL could be explained by genetics for the smaller versus larger apo(a) sizes. Although our findings demonstrated some differences in ethnicity- and age-specific heritability for Lp(a)/apo(a) traits in Caucasians and African-Americans, for the majority of cases the findings were similar. In addition, several LPA SNPs were associated with Lp(a) and ISLs in Caucasians and African Americans, but none of these SNPs were common between these two ethnicities.

High heritability estimates (0.51 to 0.98) for total Lp(a) level in twin-pairs and family studies have established Lp(a) as one of the most heritable quantitative human traits (4–8). In line with these reports, families in our study presented a high heritability estimate (0.95) for the Lp(a) level. However, less is known about the heritability of ISLs. Notably, ethnicity-specific heritability estimates were uniformly lower in our African-Americans versus Caucasians. Consistent with these findings, family and sib-pair studies have reported a lower heritability for Lp(a) level in Africans (0.51 in South-African Blacks and 0.61 in Khoi San)

versus Caucasians (0.71); and while the LPA locus explained almost all of the total genetic variance in Lp(a) level in Caucasians, it accounted for <50% of the variance in Africans (3). A sib-pair analysis in African-American families estimated that 78% of variation in Lp(a) level was attributable to polymorphisms at either the LPA locus or sequences close to it (8). Schmidt et al. (27) found a nearly identical heritability estimate (0.76) for Lp(a) in an autochthonous African population without admixture, i.e., Gabonese families from Western Central Africa. In Gabonese Africans, the effect of the K4 type 2 repeats (expressed as the sum from both alleles) on the total variability of Lp(a) level was estimated to be 44% (27).

In our previous studies, we demonstrated the usefulness of assessing ISLs in CVD risk assessment (12, 25, 28–31). Others have also used a similar approach (13, 32, 33); however, heritability data derived from this type of approach is largely absent. The study by Schmidt et al. (27) represents one of the first efforts to use these levels to estimate heritability and the effects of LPA locus on Lp(a) levels. The authors estimated heritability from the correlation of allele-associated Lp(a) level for alleles identical by descent (IBD) in parent-offspring and full- or half-sib pairs. The heritability estimates from correlation of allele-associated Lp(a) for IBD alleles (0.78–0.80) were similar to those obtained by a classical mid-parent-offspring regression (0.76). There are some differences between our study and that of Schmidt et al. (27). First, our African-American families had a distinct family structure (quartets with both parents and two offspring), while Schmidt et al. (27) studied Gabonese African families with a heterogeneous structure and size. Second, we included both African-Americans and Caucasians, enabling direct data comparisons by ethnicity. Third, for the isoform-specific Lp(a) level, we estimated heritability separately for the larger or smaller apo(a) size of a given allele-pair. This approach allowed an individual assessment of phenotypic variance explained by each inherited IBD allele based on the slope of the regression of offspring on mid-parental value in families. The heritability estimate for ISL for smaller apo(a) sizes in our African-American families was in the same range (0.80) as those seen in the Gabonese African families. In contrast, the

heritability of ISL for larger apo(a) sizes was generally lower in both African-Americans (0.59) and Caucasians (0.61).

Several factors may contribute to explain heritable differences for larger versus smaller apo(a) isoform-specific Lp(a) levels. In both human and non-human primate carriers of *LPA* alleles with two different sized apo(a)s, the one with smaller apo(a) size commonly represents the quantitatively dominating Lp(a) variant for the majority of cases (34). Studies in baboon hepatocytes suggested that low Lp(a) levels associated with large sized apo(a) could be, at least in part, due to a higher posttranslational degradation of large apo(a) isoforms (35). It is tempting to speculate that synthesis of larger apo(a) proteins may be more susceptible to nongenetic environmental effects, resulting in a lower degree of heritability as seen in our study. Emphasizing the potential for gene-environment interactions, a population study of US Blacks and Nigerians with a common genetic background but two different environments showed significantly higher Lp(a) levels in US Blacks for the same sized apo(a) compared with Nigerians (18). We have recently shown that infection with human immunodeficiency virus suppresses Lp(a) levels and initiation of antiretroviral therapy normalizes these levels, underscoring the potential of an environmental impact (36).

We further noted different heritability estimates between younger and older families. In Caucasians, all traits were highly heritable in both age groups with a uniform higher estimate in older versus younger families. In contrast, in African-Americans, a marked 2-fold lower heritability was noted in older versus younger families for Lp(a). Although these results should be interpreted with caution, they suggest two possibilities. First, a degree of heritability could have changed over time or generations. Second, heritability might differ in early versus late stages of lifespan. Both imply that the amount of variation in genetic effects on Lp(a) traits, relative to variation in environmental effects, differs by age and ethnicity, mostly due to changes in variation attributed to environment factors and possibly aging processes. Overall, these findings suggest that a variable set of genetic (heritable) and/or environmental factors plays roles at different stages of lifespan in diverse ethnic groups. However, we acknowledge that these findings should be confirmed in larger scale studies and that the additional approach of using the age of offspring to categorize families may be useful to shed further insight into the variability in heritability estimates across the lifespan.

In our study, the frequency distributions of apo(a) sizes were similar between African-Americans and Caucasians. Hence, the 3- to 4-fold higher Lp(a) and ISLs in African-Americans versus Caucasians are likely due to differences in other *LPA* variants. We focused our SNP association analyses on genetic variants previously reported for both ethnic groups in a reasonable frequency and evaluated differences in terms of directionality of association and level of significance by ethnicity. As expected, when assessed within each ethnic group, the SNP effects were concordant direction-wise for all related traits, regardless of their significance levels. In a study by Deo et al. (17), the SNP rs9457951 expressed the strongest association among all SNPs and explained 5% of Lp(a) level variation. In line with this report, rs9457951 was associated with total Lp(a) level as well as Lp(a) level carried by the smaller apo(a) sizes in our African-Americans. In addition, we found minor allele-frequencies of 0.171 and 0.0063, respectively, in our African-Americans and Caucasians, for rs9457951, which were very similar to those noted by Deo et al. (17). Overall, two distinct sets of *LPA* SNPs displayed associations with Lp(a) levels in the two ethnic groups, confirming the presence of a heterogeneity in the genetic architecture accountable for Lp(a) variability across human populations. Given the moderate size of our study cohort, these SNPs were no longer significantly associated after correcting for multiple testing.

There are strengths and limitations in this study. Our cohort with two-generation families had an overall modest sample size. However, this allowed us to apply the gold standard methods for genotyping (pulsed-field gel electrophoresis) and phenotyping (agarose gel electrophoresis with immunoblotting) of apo(a) and to determine larger and smaller apo(a) sizes. Due to technical challenges, these approaches have been used sparingly in large-scale genetic studies. In contrast, methods based on the sum of the number of K4 type 2 repeats provide an average size. We recognize that very low Lp(a) levels, which tend to be associated with larger *LPA* alleles, are inherently more subject to experimental errors. Inclusion of a single type family unit (quartets only) allowed a uniform assessment, reducing the potential impact of a multi-structural design. Lack of information on potential genetic admixture may introduce limitations as this study was carried out for a single specific gene rather than whole genome-wide association. In validation of our approach, we did not find any evidence of discordance in apo(a) genetic data between parents and offspring for any given family. Due to our unique study design, it is difficult to firmly conclude whether *LPA* SNPs linked to a minor allele are in association with either larger or smaller allele size of a given allele-pair. Finally, lack of data on the two well-studied *LPA* SNPs (rs10455872 and rs3798220) may present limitation regardless of our approach to focus on ethnic-differences in the effects.

In conclusion, traits associated with the smaller *LPA* allele were strongly determined by genetics, although with a varying degree of ethnic influence. The findings also suggested that the same alleles may potentially induce diverging effects across African-American-Caucasian ethnicities. Future studies focused on ethnicity-, age-, and sex-specific differences in heritability and in the degree of genetic control by various polymorphisms are needed to improve our understanding of the regulation of Lp(a)/apo(a) in diverse world populations and may shed light on the evolutionary aspects of this cardiovascular risk factor.

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