

Lack of genetic linkage evidence for a *trans*-acting factor having a large effect on plasma lipoprotein[a] levels in African Americans

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Abstract The distribution of plasma lipoprotein[a] (Lp[a]) concentrations, a risk factor for cardiovascular disease, varies greatly among racial groups, with African Americans having values that are shifted toward higher levels than those of whites. The underlying cause of this heterogeneity is unknown, but a role for “*trans*-acting” factors has been hypothesized. This study used genetic linkage analysis to localize genetic factors influencing Lp[a] levels in African Americans that were absent in other populations; linkage results were analyzed separately in non-Hispanic whites, Hispanic whites, and African Americans. As expected, all three samples showed highly significant linkage at the approximate location of the lysophosphatidic acid locus. The white populations also independently had regions of significant linkage on chromosome 19 (LOD 3.80) and suggestive linkage on chromosomes 12 (LOD 1.60), 14 (LOD 2.56), and 19 (LOD 2.52). No linkage evidence was found to support the hypothesis of another single gene with large effects specifically segregating in African Americans that may account for their elevated Lp[a] levels.—Barkley, R. A., A. C. Brown, C. L. Hanis, S. L. Kardia, S. T. Turner, and E. Boerwinkle. Lack of genetic linkage evidence for a *trans*-acting factor having a large effect on plasma lipoprotein[a] levels in African Americans. *J. Lipid Res.* 2003. 44: 1301–1305.

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Plasma lipoprotein[a] (Lp[a]) levels are positively associated with cardiovascular disease (1–5). Structurally, Lp[a] consists of two main components: a core of a LDL particles and a surrounding apolipoprotein[a] (apo[a]) high-molecular-mass glycoprotein. Apo[a] contains a high degree of homology to plasminogen at both the protein

and gene levels. Of specific interest is the homology of parts of apo[a] to the serine protease domain and to the fourth and fifth kringle in plasminogen (5). The kringle-5 domain is present as only a single copy, whereas the kringle-4 domain is present as multiple copies. A varying number of copies of the kringle-4 domain can be detected as size polymorphisms of apo[a]. This size polymorphism in apo[a] has been found to account for the majority of the variability in plasma Lp[a] levels, with apo[a] size generally being inversely associated with plasma Lp[a] concentration (6).

The distribution of Lp[a] levels has been found to vary greatly among populations (7–11). In general, the distributions in non-Hispanic white populations have been found to be shifted toward low Lp[a] levels, whereas the distributions in African American populations have been found to be more bell shaped and shifted toward higher and greater risk-associated levels, (7, 8, 11). The underlying cause of these differences is unknown. Recent studies have demonstrated that the inverse relationship between apo[a] size and the level of Lp[a] in plasma is maintained in the African American population; however, the relationship is more sigmoid in shape, rather than having a clear linear relationship (12). Others have hypothesized that “*trans*-acting” factor(s) must exist that either increase the rate of secretion of apo[a] or decrease Lp[a]’s catabolism in African Americans relative to non-Hispanic whites (13). This study used genetic linkage analysis to test the hypothesis that there is a gene influencing plasma Lp[a] levels in African Americans that is not acting in whites. This other gene would be segregating among family members in the admixed African American population and thus would be detectable by genetic linkage analysis.

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Study participants

Subjects in this study included 1,046 non-Hispanic white individuals in 389 sibships from Rochester, MN (two to 10 individuals/sibship), 1,556 African American individuals in 525 sibships from Jackson, MS (two to 10 individuals/sibship), and 1,061 Hispanic white individuals in 319 sibships from Starr County, TX (two to 10 individuals/sibship). All individuals were participants in the Genetic Epidemiology Network of Arteriopathy study of the Family Blood Pressure Program (14) and provided their informed consent. Exclusion criteria included secondary hypertension, alcoholism or drug abuse, insulin-dependant diabetes mellitus, active malignancy, pregnancy or lactation, and inability to participate.

The mean ages in years for the non-Hispanic white, African American, and Hispanic white samples were 56.06 ± 11.05 , 57.48 ± 10.26 , and 55.87 ± 11.82 , respectively. The percentage of males in each sample was 45.6% in non-Hispanic whites, 31.23% in African Americans, and 40.15% in Hispanic whites. The mean waist-to-hip ratio for each sample was 0.91 ± 0.09 , 0.91 ± 0.08 , and 0.98 ± 0.09 , respectively.

Laboratory techniques

Plasma Lp[a] levels for each individual were obtained using a sandwich radioimmunoassay with the results reported in mg/dl (15, 16). Values from this assay may be influenced by the number of kringle IV repeats, and therefore, the linkage results at the location of the apo[a] gene may not strictly reflect an effect on plasma Lp[a] levels or concentrations. However, linkage results at other locations in the genome will be robust to this uncertainty. Genotyping was obtained using standard methods for a total of 460 microsatellite markers across the autosomes, with an average spacing of 7.2 centiMorgans (cM) between markers.

Linkage analysis

A multipoint linkage analysis was performed using the Sequential Oligogenic Linkage Analysis Routines (SOLAR) computer package, version 1.6.6 (<http://www.sfbr.org/sfbr/public/software/software.html>). This software carries out multipoint-variance component linkage analyses to localize genes influencing a quantitative trait (17). Each cohort was analyzed with the t-distribution option for a robust estimation of means and variances.

To test the null hypothesis of no linkage to a quantitative trait locus (QTL), the likelihood of a model with the additive variance due to the QTL equal to zero was compared with a model with the additive genetic variance due to the QTL equal to that estimated by SOLAR. LOD scores were calculated by converting twice the difference in natural log (ln) likelihoods of the two models into values of \log_{10} . To account for the nonnormalized data, log-transformed Lp[a] values ($\ln[\text{Lp[a]}]$) were used for the linkage scan. LOD scores greater than 1.5 are presented in this report. The complete results of the multipoint linkage analyses across the genome are available by contacting the authors.

The analyses presented here are those not including covariates because their effects were not statistically significant. The main conclusions presented here remained unaltered when re-running the scans forcing hypertension status or diabetes status into the model (data not shown).

RESULTS

The distributions of Lp[a] varied among the three populations, with the distributions of both white cohorts

weighted toward lower values and the distribution of the African American cohort more normally distributed and shifted toward higher values (Fig. 1). In the non-Hispanic whites, Lp[a] levels ranged from 3.6 to 147.0 mg/dl with a mean of 29.36 mg/dl. In the African Americans, Lp[a] levels ranged from 4.9 to 229.0 mg/dl with a mean of 46.15 mg/dl. In Hispanic whites, Lp[a] levels ranged from 4.9 to 190.0 mg/dl with a mean of 24.83 mg/dl. Using a two-sample Kolmogorov-Smirnov test for equality of distributions, each pair of distributions was significantly different ($P < 0.001$).

The overall polygenic heritability for $\ln[\text{Lp[a]}]$ was estimated to be $90.1 \pm 6.6\%$ in non-Hispanic whites, $87.6 \pm 6.1\%$ in African Americans, and $74.2 \pm 6.8\%$ in Hispanics, with all estimates being statistically significant ($P < 0.0001$ in each case). As expected, the highest LOD score in an initial genome-wide scan in all three ethnic samples was found on chromosome 6 at the approximate location of lysophosphatidic acid (LPA) (Fig. 2), the gene encoding apo[a] on 6q26-q27 (18, 19). The peaks in non-Hispanic

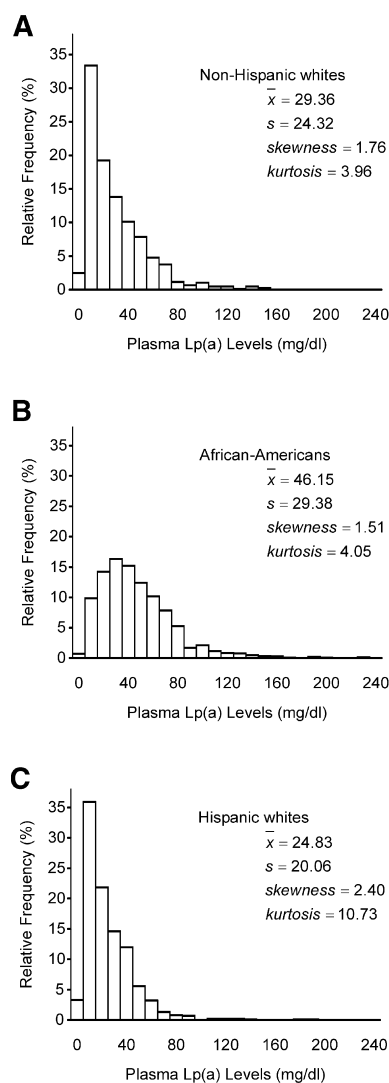


Fig. 1. Histograms of plasma lipoprotein[a] (Lp[a]) levels (mg/dl) in three populations.

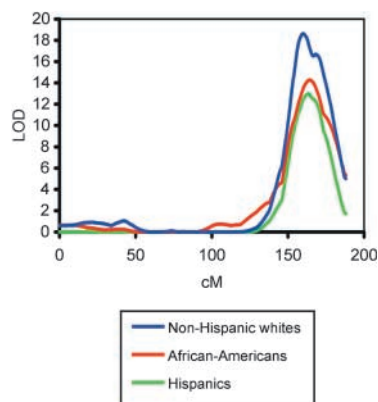


Fig. 2. Linkage of log-transformed plasma Lp[a] levels to chromosome 6.

whites, African Americans, and Hispanics were LOD 18.62 at 160 cM from the tip of the p-arm, LOD 14.27 at 164 cM, and LOD 12.97 at 163 cM, respectively. The heritability estimated to be explained by this apo[a] gene locus in non-Hispanic whites, African Americans, and Hispanic whites is 0.73, 0.57, and 0.53, respectively. Taking into account the identity by descent (IBD) sharing at this peak in each ethnic group, a second genome-wide linkage scan was performed (**Fig. 3**). In the non-Hispanic whites, there were three peaks >1.5 in this scan, including chromosome 19 at 30 cM (LOD 3.80), chromosome 19 at 47 cM (LOD 2.52), and chromosome 12 at 38 cM (LOD 1.60). In African Americans, there were no peaks above a 1.5 LOD score. In Hispanics, there was a peak on chromosome 14 at 17 cM (LOD 2.56).

DISCUSSION

The data presented here provided no evidence for the presence of a gene outside of the LPA locus specifically influencing plasma Lp[a] levels in African Americans that might also account for the elevated Lp[a] levels in this population relative to that of whites (13). The hypothesized gene's product was thought to act to either increase the rate of production of apo[a] or decrease Lp[a] catabolism. As a result of admixture, the variation in this hypothesized transacting factor (between African and white populations) would be segregating in the African American population and would be detectable using genetic linkage analysis (20). Previous studies have estimated the amount of European ancestry present in African Americans in Jackson, MS to be 16.9% (95% CI 14.7–18.8%) (21). The data presented in this study provide no evidence for the existence of a single gene with large effects that are specific to African Americans' Lp[a] levels. The only significant linkage signal in African Americans is the peak found on chromosome 6 (LOD 14.27) at the approximate location of LPA, the gene encoding apo[a]. Performing an analysis taking into account the effects of the LPA locus, LOD scores for the African American sample did not go above LOD 1.5, suggesting no evidence for

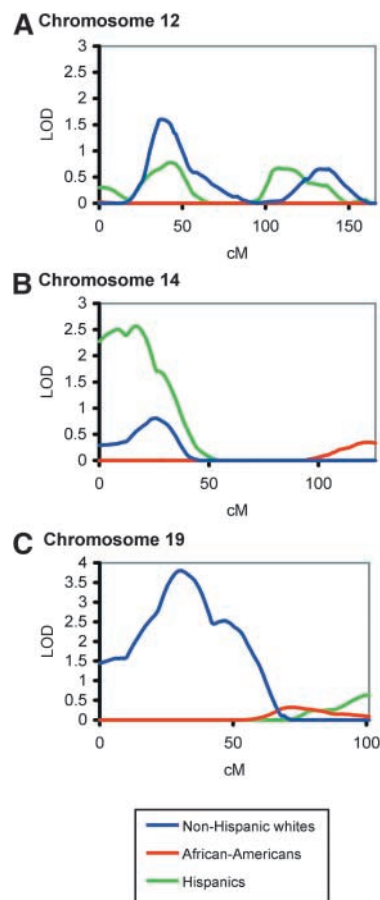



Fig. 3. Linkage of log-transformed plasma Lp[a] levels, taking into account the effects of the lysophosphatidic acid locus. A: Chromosome 12. B: Chromosome 14. C: Chromosome 19.

linkage to an Lp[a]-influencing gene specific to African Americans.

The expected magnitude of a single gene acting to elevate Lp[a] plasma concentrations in African Americans, as compared with the whites, would be large. After accounting for the IBD sharing at the locus encoding apo[a] on chromosome 6, the hypothesized gene acting in African Americans should be detectable through genetic linkage analysis, providing at least a suggestive LOD score. Due to the size of each of the samples (1,046 non-Hispanic whites, 1,556 African Americans, and 1,061 Hispanic whites) and the large number of possible pairings of siblings for IBD calculations, it is not likely that lack of linkage is attributable to lack of statistical power. Also, the group of most interest, the African Americans, contains the highest number of individuals with both Lp[a] levels measured and microsatellite markers genotyped. Therefore, lack of linkage evidence in a region can best be attributed to absence of a major-effect, Lp[a]-influencing gene of interest in the region. The current analysis does not eliminate the possibility of the existence of multiple genes, each with very small effects, influencing Lp[a] plasma levels in African Americans.

Of note are the additional linkage peaks for Lp[a] levels in non-Hispanic whites' and Hispanic whites' samples.

These linkages provide evidence for genes that might be aiding in keeping Lp[a] levels lower in these ethnic groups through either reduced production of Lp[a] or greater catabolism or clearance. The non-Hispanic white samples' linkage peak on chromosome 19 at 30 cM achieves statistical significance with an LOD of 3.80. The gene encoding the LDL receptor lies in this approximate region, and these data may support the receptor's role in the clearance of Lp[a] from circulation. The LDL receptor is known to bind to cholesterol-rich lipoproteins containing apoB-100 and/or apoE and mediate their uptake from circulation (22). The LDL receptor is most widely known for its association with familial hypercholesterolemia, yet its involvement in the control of Lp[a] levels has been debated by previous studies (23–27). The other suggestive linkage peaks seen in non-Hispanic whites (chromosome 19 at 47 cM with LOD 2.52, and chromosome 12 at 38 cM with LOD 1.60) and Hispanics (chromosome 14 at 17 cM with LOD 2.56) may be locating other genes involved in this catabolic pathway. For example, the peak on chromosome 12 is near the location of the gene encoding the LDL lipoprotein receptor-related protein (28). LRP is a 600 kDa cell surface receptor expressed in a variety of tissues, including hepatocytes (29). LRP shows homology to the LDL receptor, but is considered more complex in structure and function, containing 31 ligand binding-type repeats, as compared with the seven found in the LDL receptor (22). A large variety of ligands are known for LRP, including apoB-48, tissue-type plasminogen activator, plasminogen activator inhibitor 1, receptor-associated protein, and α -macroglobulin (22, 30). Lp[a] or an altered form of Lp[a] could also be hypothesized ligands for this multifunctional receptor. Alternatively, these linkage signals may be part of other unrelated mechanisms influencing plasma Lp[a] levels. Regardless, future studies aimed at identifying the mechanism underlying elevated Lp[a] levels in African Americans should focus on 1) sequences *cis* to the apo[a] gene, 2) better understanding of how variations within apo[a] itself influence Lp[a] metabolism in this population, and 3) identification of environmental influences that may be contributing to the observed difference. 

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