ApoE genotype affects allele-specific apo[a] levels for large apo[a] sizes in African Americans: the Harlem-Basset Study

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Abstract The genetic variability of apolipoprotein E (apoE) influences plasma lipoprotein levels, and allele frequencies differ between African Americans and Caucasians. As African Americans have higher lipoprotein [a] (Lp[a]) levels than Caucasians, we investigated the effects of the apoE gene on allele-specific apolipoprotein [a] (apo[a]) levels across ethnicity. We determined apo[a] sizes, allele-specific apo[a] levels (i.e., levels associated with alleles defined by size), and the apoE gene polymorphism in 231 African Americans and 336 Caucasians. African Americans, but not Caucasians, with the apo E2 genotype had lower levels of Lp[a] compared with those with the apo E4 genotype (9.6 vs. 11.2 nmol/l; P = 0.034, expressed as square root levels). Distribution of apo[a] alleles across apoE genotypes were similar between African Americans and Caucasians. Among African Americans with large apo[a], the allele-specific apo[a] level was significantly lower among ε2 carriers compared with ε3 or ε4 carriers (5.4 vs. 6.6 and 7.4 nmol/l, respectively; P < 0.005, expressed as square root levels). In contrast, there was no significant difference in allele-specific apo[a] levels across apoE genotypes among Caucasians. For large apo[a] sizes, apoE genotype contributed to the observed African American-Caucasian differences in allele-specific apo[a] levels.—Anuurad, E., G. Lu, J. Rubin, T. A. Pearson, and L. Berglund. ApoE genotype affects allele-specific apo[a] levels for large apo[a] sizes in African Americans: the Harlem-Basset Study. J. Lipid Res. 2007. 48: 693–698.

Supplementary key words lipoprotein [a] • polymorphisms • apolipoprotein E • genetics • ethnicity • apolipoprotein [a]

A high plasma level of lipoprotein [a] (Lp[a]) is an independent risk factor for cardiovascular disease (1–3). Lp[a] levels are to a major extent regulated by genetic factors (4). Like LDL, Lp[a] has one molecule of apolipoprotein B-100, which is covalently bound to a carbohydrate-rich protein, apolipoprotein [a] (apo[a]) (5, 6). The size of the apo[a] gene is highly variable, resulting in a size variation of the apo[a] protein, as manifested in a variable number of kringle 4 (K4) repeats (1, 7). There is an inverse relationship between apo[a] size and Lp[a] levels, as smaller apo[a] sizes in general are associated with higher plasma Lp[a] levels. However, for a given size, there is considerable interindividual variation in Lp[a] levels (4, 8, 9). Furthermore, Lp[a] levels vary across ethnicity, with the most profound differences between populations of African descent and non-African populations, including Asians and Caucasians (10–14). This difference is not explained by a difference in apo[a] size frequency. Importantly, Africans as well as African Americans have substantially higher Lp[a] levels than do Caucasians or Asians, adjusting for apo[a] sizes (15, 16). Recently, we demonstrated that for small apo[a] sizes, differences in allele-specific apo[a] levels between African Americans and Caucasians were explained by allelic interactions and by an upstream pentanucleotide repeat polymorphism (17). However, differences in allele-specific apo[a] levels remain unexplained for larger apo[a] sizes.

Overall, genetic factors have a major impact on Lp[a] levels (15, 18, 19). Furthermore, as described above, we and others have identified several genetic variations that affect allele-specific apo[a] levels (8, 16, 17, 20). Genetic variability of apolipoprotein E (apoE) is a major determinant of plasma lipoprotein levels (21), and furthermore, apoE genotype frequencies differ between African Americans and Caucasians (22, 23). As the African American-Caucasian differences in allele-specific apo[a] levels for large apo[a] sizes remain unexplained, we focused on apoE as a possible genetic factor potentially contributing to this difference. Earlier studies have investigated the impact of apoE genotypes on Lp[a] levels, but the results have been
contradictory. Thus, no impact of apoE genotypes on Lp[a] levels were reported in several studies (24–30), whereas others reported significant differences in plasma Lp[a] levels across apoE phenotypes (31–36). Among the latter studies, de Knijff et al. (31) found high plasma levels of both Lp[a] and LDL in healthy Caucasians with the apo e4 allele, and the authors suggested that Lp[a] is cleared by the LDL receptor. Later, Lindahl et al. (36) reported a similar effect of the apoE genotype on Lp[a] levels in patients with familial hypercholesterolemia, with E2 decreasing and E4 increasing plasma Lp[a] levels.

However, most of these studies measured plasma Lp[a] levels, and these levels are affected by apo[a] size variability, so we analyzed allele-specific apo[a] levels to explore the effects of the apoE gene on Lp[a]. We report that African American apo e2 carriers, compared with e3 or e4 carriers, had lower allele-specific apo[a] levels for large apo[a] sizes.

MATERIALS AND METHODS

Subjects

Subjects 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. The study design has been described previously (17, 37, 38). Briefly, a total of 648 patients were enrolled, 401 men and 247 women, of which 344 were ethnically self-identified as Caucasians and 252 were self-identified as African Americans. This report is based on the findings from 567 subjects (291 African Americans and 336 Caucasians) in whom Lp[a] levels and allele-specific apo[a] levels were available. Apo[a] allele sizes, circulating apo[a] isoforms, and allele-specific apo[a] levels were available for 426 subjects (167 African Americans and 259 Caucasians). This study was approved by the Institutional Review Boards at Harlem Hospital, the Mary Imogene Bassett Hospital, the Columbia University College of Physicians and Surgeons, and the University of California Davis, and informed consent was obtained from all subjects.

Measurement of lipids, lipoproteins, and Lp[a]

Fasting blood samples were drawn ~2–4 h before the catheterization procedure, and serum and plasma samples were stored at ~80°C before analysis. Serum triglycerides and total and HDL cholesterol were determined using standard enzymatic procedures. LDL cholesterol levels were calculated with the formula of Friedewald, Levy, and Fredrickson (39). Lp[a] levels were measured in nmol/l by a sandwich ELISA (Sigma Diagnostics, St. Louis, MO) (37, 38).

Apo[a] allele and isoform size determination

To determine apo[a] allele sizes, we performed genotyping using pulsed-field electrophoresis of DNA from leukocytes embedded in agarose plugs, essentially as described previously (40, 41). Apo[a] isoform sizes were analyzed by SDS-agarose gel electrophoresis of plasma samples, followed by immunoblotting. Briefly, the apo[a] bands were visualized with the Amersham ECL technique using a second, labeled antibody (Pierce, Rockford, IL) (37, 42, 43). The protein dominance was determined by optical analysis of the apo[a] protein bands on the Western blots, and the visual estimations were validated by computerized scanning. For each apo[a] protein band, levels were apportioned according to the degree of intensity of the bands on the Western blot, using 10% increments (41).

Determination of apoE isoforms

ApoE isoforms were determined at the DNA level by amplification using the polymerase chain reaction and specific oligonucleotides as described by Hixson and Vernier (44).

Statistics

Analysis of data was done with SPSS statistical analysis software (SPSS, Inc., Chicago, IL). Results are expressed as means ± SD. Triglyceride levels were log-transformed, and Lp[a] levels and allele-specific apo[a] levels were square root-transformed to achieve normal distributions. Proportions were compared between groups using Chi-square analysis and Fisher’s exact test, where appropriate. Group means were compared using Student’s t-test. The distribution of apo[a] alleles for each apoE genotype was analyzed using the Kolmogorov-Smirnov test. General linear measurement multivariate analyses for two ethnic groups were used for lipid and apolipoprotein levels after adjustment for drug use, and post hoc analyses were performed by Bonferroni test for two independent samples. One-way ANOVA was used for apoE distribution analysis, and post hoc analyses were performed by Bonferroni test for two independent samples. Unless noted otherwise, a nominal two-sided P < 0.05 was used to assess significance.

RESULTS

Lipid and lipoprotein characteristics are shown in Table 1. African Americans had significantly higher levels of HDL cholesterol, Lp[a], and apoA-I and lower levels of Apolipoprotein B (mg/dl) 132 α 130 (6) 65 (9) 0.6 0.6 0.138

| Clinical Characteristics | African Americans | Caucasians |
|--------------------------|-------------------|------------|
|                          | (n = 231)         | (n = 336)  |
| Men/women                | 132/99            | 218/118    |
| Age (years)              | 54.7 ± 6          | 56.7 ± 0.6 |
| Total cholesterol (mg/dl)| 196 ± 3           | 198 ± 2    |
| LDL cholesterol (mg/dl)  | 125 ± 2           | 125 ± 2    |
| HDL cholesterol (mg/dl)  | 49 ± 1            | 41 ± 1     |
| Triglyceride (mg/dl)     | 112 ± 4           | 170 ± 4    |
| Lp[a] (nmol/l)           | 130 ± 6           | 59 ± 5     |
| Apolipoprotein A-I (mg/dl)| 130 ± 2            | 122 ± 1    |
| Apolipoprotein B (mg/dl) | 132 ± 2           | 137 ± 2    |

TABLE 1. Characteristics of the study population

Lp[a], lipoprotein [a]. Data are means ± SEM. Group means were compared using Student’s t-test. General linear measurement multivariate analyses for two ethnic groups were used for lipid and apolipoprotein levels after adjustment for drug use, and post hoc analyses were performed by Bonferroni test for two independent samples. Values for triglyceride were logarithmically transformed, and values for Lp[a] were square root-transformed before analyses.

α P < 0.05.

β P < 0.001.
triglycerides compared with Caucasians. There was no difference in the levels of total and LDL cholesterol as well as in the levels of apoB between the two ethnic groups.

African Americans had a significantly higher frequency of the e2 (0.12 vs. 0.08; Chi-square = 4.8, P = 0.028) and e4 alleles (0.26 vs. 0.14; Chi-square = 26.4, P < 0.001) and a significantly lower frequency of the e3 allele (0.61 vs. 0.78; Chi-square = 34.6, P < 0.001) compared with Caucasians (Table 1). In a further analysis, subjects were divided into three genotype groups: e2 carriers (genotypes E2/2, E2/3, and E2/4), e3 carriers (genotype E3/3), and e4 carriers (genotypes E3/4 and E4/4).

We next studied the effect of apoE gene variability on plasma Lp[a] levels in each ethnic group. As shown in Fig. 1, among African Americans, there was a stepwise increase in Lp[a] levels from e2 to e3 to e4 carriers. Thus, African American e2 carriers had significantly lower levels of Lp[a] compared with e4 carriers (9.6 vs. 11.2 nmol/l; P = 0.034, expressed as square root levels). However, there were no such differences in Lp[a] levels across apoE genotypes among Caucasians, and the levels were similar across apoE genotypes. We hypothesized that this different pattern of Lp[a] levels in African Americans across apoE genotypes might be attributable to either 1) a different distribution of apo[a] alleles or 2) a difference in allele-specific apo[a] levels. To test the first possibility, we compared the distribution of apo[a] alleles for each apoE genotype using the Kolmogorov-Smirnov test. As shown in Fig. 2, there were no significant differences in cumulative frequency distribution curves of apo[a] alleles in African Americans and Caucasians across apoE genotypes.

As we did not observe any difference in apo[a] allele distribution across apoE genotypes, we next compared the corresponding distribution of allele-specific apo[a] levels. In agreement with the overall increased Lp[a] levels among African Americans compared with Caucasians, the mean allele-specific apo[a] level was significantly higher in African Americans than in Caucasians (10.6 vs. 6.2 nmol/l; P < 0.001, expressed as square root levels). To take apo[a] size into account, we dichotomized smaller versus larger apo[a] sizes using the median apo[a] size (26 K4 repeats), as in our previous study (17). As seen in Fig. 3A, allele-specific apo[a] levels for smaller apo[a] were similar across apoE genotypes among both African Americans and Caucasians. However, for African Americans with larger apo[a], the allele-specific apo[a] level was significantly lower in subjects with the apo e2 genotype compared with apo e3 and e4 carriers (P < 0.05 and P < 0.001, respectively) (Fig. 3B). For specific numbers, see supplementary Table 1. No such difference was observed for Caucasians, although larger allele-specific apo[a] levels were still significantly lower compared with African Americans across all apoE groups. Furthermore, we extended our approach and analyzed gender differences in allele-specific apo[a]
levels for large apo[a] sizes across apoE genotypes, and levels in men and women were similar (see supplementary Fig. I).

As described above, we used an apo[a] size threshold of 26 K4 in our analysis, as it represented the median value. However, as a cutoff of 22 K4 commonly has been used to define small apo[a], we repeated our analysis using 22 K4 to define large versus small size. The results were similar: the allele-specific large apo[a] levels in African American e2 and e4 carriers were significantly different (6.0 vs. 8.2 nmol/l, respectively; P < 0.001, expressed as square root levels) (see supplementary Fig. II). No such difference was observed among Caucasians.

DISCUSSION

The main novel findings in our study are as follows: 1) African American apo e2 carriers, compared with e3 or e4 carriers, had lower allele-specific apo[a] levels for large apo[a] sizes; and 2) apoE genotype contributed to the observed African American-Caucasian differences for large apo[a] sizes.

We previously reported on the association of apoE genotypes and lipid levels in this population (38). This study was undertaken to address a possible impact of apoE genotypes on allele-specific apo[a] levels. Previous studies have shown the following: 1) apoE genotypes have a wide impact on plasma lipoprotein levels (21, 38); 2) apoE allele frequency differs between African Americans and Caucasians (22, 23, 38); 3) plasma Lp[a] levels differ between African Americans and Caucasians (10–14); and 4) the difference in allele-specific apo[a] levels for larger apo[a] size between African Americans and Caucasians is unexplained (17). In our population, we have shown that African Americans have higher allele-specific apo[a] levels compared with Caucasians. Furthermore, we found that apoE genotypes influenced Lp[a] levels in African Americans but not in Caucasians; African American subjects with the apo E2 genotype had lower Lp[a] levels compared with apo e3 or e4 carriers. Previous studies investigating the influence of apoE gene variation on Lp[a] levels have shown contradictory results, although the reasons for these differences have not been clarified (24–36). Most of these studies have been carried out in Caucasians, and furthermore, total Lp[a] levels have been assessed. As the apo[a] gene is highly polymorphic, the total plasma Lp[a] level is commonly a summary of two different apo[a] size alleles, with likely differing allele-specific levels. To be able to take apo[a] size into account, we used allele-specific apo[a] levels to address possible differences in the impact of apoE gene variation on Lp[a] in more detail.

Apart from our study, Klausen et al. (32) reported that the effect of apoE gene polymorphism on Lp[a] levels was dependent on the molecular size of apo[a]. In their study, limited to Caucasian males and based on total plasma Lp[a] levels, they determined apo[a] as four molecular size groups and reported that for large apo[a] (apo[a]-S4), Lp[a] levels were high in e2 e3 and e3 e4 genotypes and low in e3 e4 genotype. The authors speculated that this might be attributable to a high affinity of large apo[a], apo[a]-S4, as well as apo e4 for triglyceride-rich lipoprotein, which preferentially would be taken up and degraded by remnant receptors (32, 45). Although we did not find any differences across apoE genotypes in Caucasians, our results for African Americans were in the opposite direction, with lower allele-specific apo[a] levels for apo e2 carriers. For reasons of convenience, we categorized apo[a] levels as large or small, although it should be emphasized that we determined allele-specific apo[a] levels representing the entire spectrum of apo[a] sizes. Also, we did not find any effect of apoE genotypes on triglyceride levels in the African American population, arguing against any measurable effects of apoE on total plasma triglycerides (38).

Overall, reduced Lp[a] levels in African American apo e2 carriers could result from at least two different mechanisms. First, the results could reflect a different distribu-
tion of apo[a] alleles across apoE genotypes, where apo e2 carriers could preferentially have larger apo[a] sizes, more commonly associated with lower Lp[a] levels. Alternatively, the finding could be attributable to a difference in allele-specific apo[a] levels across apoE genotypes. Arguing against the first possibility, we did not find any differences in the frequency of apo[a] alleles in our study population across apoE genotypes (Fig. 2). We next extended our analysis to allele-specific apo[a] levels and their distribution across apoE genotypes. Interestingly, allele-specific apo[a] levels showed a different spectrum across apoE carriers among African Americans but not among Caucasians. Thus, we found that African Americans with larger apo[a] size had significant differences in allele-specific apo[a] levels across apoE genotypes: allele-specific levels were lower among e2 carriers compared with apo e3 and e4 carriers. In contrast, no difference was seen for small apo[a] sizes.

Although the mechanism for the size-specific effect of apoE variation on apo[a] levels in African Americans remains to be determined, several possibilities exist. First, the possibility of a direct interaction at the chromosome level, a “gene effect,” seems remote, as the apo[a] and apoE genes are located on different chromosomes, 6q27 and 19q13.31, respectively (National Center for Biotechnology Information Online Mendelian Inheritance in Man accession numbers 132200 and 107741). However, it is well established that the apoE gene plays important roles in the metabolism of lipoproteins: apoE mediates the catabolism of chylomicron and VLDL remnants via a “remnant” receptor and also mediates the binding of chylomicron remnants, VLDL, and intermediate density lipoproteins to the LDL receptor (21). Thus, in many studies, the e4 allele has been associated with increased levels of cholesterol, LDL cholesterol, and apoB, whereas the e2 allele has been associated with lower total and LDL cholesterol concentrations. Furthermore, an intracellular action of apoE on lipoprotein secretion has been demonstrated (46, 47). As Lp[a] levels are largely influenced by synthetic pathways, it might be tempting to suggest an interaction between apoE and apo[a] on the synthetic level. However, further studies are needed to verify such an interaction.

Potential limitations of this study need to be discussed. Subjects in our study were recruited from patients scheduled for coronary angiography, and for some genotypes, the number was relatively small. As the apo E4 genotype has been associated with cardiovascular disease, a potential source of error might be a differing distribution of apoE genotypes in this group compared with the population at large. Arguing against this possibility, the apoE allele frequency pattern was similar to that described previously for African American and Caucasian populations (22, 23). Furthermore, the apo e4 allele frequency was similar in subjects with and without coronary artery disease for both African Americans (46.7% vs. 53.3%; NS) and Caucasians (51.3% vs. 48.7%; NS).

In conclusion, this study indicates that allele-specific levels are lower among African American e2 carriers for large apo[a] sizes, whereas no such differences were observed among Caucasians. Our findings need to be confirmed in other African American samples, and further studies are needed to explore underlying mechanisms.

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