High Levels of Lp(a) With a Small Apo(a) Isoform Are Associated With Coronary Artery Disease in African American and White Men

Furcy Paulatre, Thomas A. Pearson, Henry F.C. Weil, Catherine H. Tuck, Merle Myerson, Jill Rubin, Charles K. Francis, Herbert F. Marx, Edward F. Philbin, Roberta G. Reed, Lars Berglund

Abstract—Elevated levels of lipoprotein(a) [Lp(a)] and the presence of small isoforms of apolipoprotein(a) [apo(a)] have been associated with coronary artery disease (CAD) in whites but not in African Americans. Because of marked race/ethnicity differences in the distribution of Lp(a) levels across apo(a) sizes, we tested the hypothesis that apo(a) isoform size determines the association between Lp(a) and CAD. We related Lp(a) levels, apo(a) isoforms, and the levels of Lp(a) associated with different apo(a) isoforms to the presence of CAD (≥50% stenosis) in 576 white and African American men and women. Only in white men were Lp(a) levels significantly higher among patients with CAD than in those without CAD (28.4 versus 16.5 mg/dL, respectively; \( P = 0.004 \)), and only in this group was the presence of small apo(a) isoforms (<22 kringle 4 repeats) associated with CAD (\( P = 0.043 \)). Elevated Lp(a) levels (≥30 mg/dL) were found in 26% of whites and 68% of African Americans, and of those, 80% of whites but only 26% of African Americans had a small apo(a) isoform. Elevated Lp(a) levels with small apo(a) isoforms were significantly associated with CAD (\( P < 0.01 \)) in African American and white men but not in women. This association remained significant after adjusting for age, diabetes mellitus, smoking, hypertension, HDL cholesterol, LDL cholesterol, and triglycerides. We conclude that elevated levels of Lp(a) with small apo(a) isoforms independently predict risk for CAD in African American and white men. Our study, by determining the predictive power of Lp(a) levels combined with apo(a) isoform size, provides an explanation for the apparent lack of association of either measure alone with CAD in African Americans. Furthermore, our results suggest that small apo(a) size confers atherogenicity to Lp(a). (Arterioscler Thromb Vasc Biol. 2000;20:2619-2624.)

Key Words: lipoprotein(a) ■ coronary artery disease ■ African Americans ■ angiography ■ genetic variation

Lipoprotein(a) [Lp(a)] has been identified as a risk factor for cardiovascular disease. In numerous, but not all, prospective studies, mainly in white populations, elevations of plasma Lp(a) levels, usually defined as ≥30 mg/dL, were significantly correlated with coronary artery disease (CAD). Curiously, although mean Lp(a) levels are twice as high in African Americans compared with whites, studies to date have failed to establish a significant association between elevated Lp(a) levels (≥30 mg/dL) and CAD among African Americans. The lack of understanding of this racial difference has made it difficult to conclude with full confidence that Lp(a) is a risk factor for CAD.

In addition to high Lp(a) levels, the presence of small apo(a) isoforms has been associated with CAD in whites. In the majority of studies using high-resolution sizing techniques, small apo(a) size has been defined as <22 kringle 4 (K4) repeats. The majority of whites with high Lp(a) levels possesses at least 1 small apo(a) isoform; however, the majority of African Americans with high Lp(a) levels has no small apo(a) isoform. The high degree of correlation between elevated levels of Lp(a) and small apo(a) isoforms in whites makes it difficult to ascertain whether one is a confounder for the other regarding CAD. The lack of correlation between elevated levels of Lp(a) and small apo(a) isoforms in African Americans indicates that the independent contributions of high Lp(a) levels and small apo(a) isoforms could be tested in this ethnic group. To explore the hypothesis that CAD was associated specifically with the presence of an elevated level of Lp(a) with a small apo(a) isoform, we compared Lp(a) levels, apo(a) sizes, and the level of Lp(a) particles carrying small apo(a) sizes in African American and white patients undergoing coronary angiography.

Methods

Additional information is available in the expanded Methods section available online at http://atvb.ahajournals.org.
Subjects
A total of 648 patients (401 men and 247 women) ethnically self-identified as African American (n=232), white (n=344), or other (n=72), scheduled for diagnostic coronary arteriography at either Harlem Hospital Center in New York City or the Bassett Hospital in Cooperstown, NY, were enrolled. The present report is based on the findings in 572 African Americans and whites.

Measurements of Lipids and Lipoproteins
Serum triglycerides and total and HDL cholesterol were determined by using a standard enzymatic procedure, and LDL cholesterol was calculated. Lp(a) levels were measured by using a method insensitive to size variation in apo(a) (Sigma Diagnostics). Corrected LDL cholesterol levels were calculated by adjusting for Lp(a) levels.26 Elevated Lp(a) levels were defined as ≥72 nmol/L ($\approx$30 mg/dL), as described in Methods. A level of 72 nmol/L corresponds to 30 mg/dL.

Apo(a) Isoform Size Determination
Apo(a) isoforms were analyzed by SDS–agarose gel electrophoresis followed by immunoblotting.27 Apo(a) isoforms were classified as being either of small size (<22 K4 repeats) or large size (≥22 K4 repeats).17–24 To validate the apo(a) isoform size determination, we performed genotyping of apo(a) allele sizes by pulsed field electrophoresis.28 Overall, there was an excellent agreement between the phenotyping and genotyping procedures (r=0.997).

In the majority of patients (n=323, 198 men and 125 women), 2 distinct apo(a) protein bands were detected. No apo(a) protein bands could be detected in 20 patients (13 men and 7 women) with Lp(a) concentrations <2.6 nmol/L (<1.1 mg/dL). In the remaining 229 patients (141 men and 88 women), a single apo(a) protein band was detected. Of the 552 subjects with detectable apo(a) isoforms, 473 (86%) carried exclusively large or exclusively small apo(a) isoforms, and there was no need for apportioning. In the 68 subjects carrying a large plus a small isoform, the relative contribution of small-isofrom Lp(a) and large-isofrom Lp(a) was based on the intensity of staining of the 2 bands. The relative intensity of each apo(a) isoform was multiplied by the total plasma Lp(a) level to compute the Lp(a) level associated with each apo(a) isoform.

Coronary Angiography
The coronary angiograms were read by 2 experienced readers blinded to patient identity, the clinical diagnosis, and the lipoprotein results. The readers recorded the location and extent of luminal narrowing for 15 segments of the major coronary arteries.29 The presence of CAD was defined as the presence of at least 50% stenosis in any 1 of 15 coronary artery segments. Of the patients without CAD, the majority (80.5%) had <25% stenosis, and of the patients with CAD, 81% had >75% stenosis.

Statistical Analysis
Because the distribution of values for Lp(a) and triglycerides were skewed, logarithmic transformations of these data were performed before statistical analysis. Comparisons of means between groups were made by the Student t test. The Fisher exact test was used to calculate the probability value for odds ratios (ORs) of the association of univariate categorical data with case-control status. Conditional logistic regression was used to assess the association with case-control status for multivariate models. SAS was used for all calculations.

Results
Lp(a) levels were approximately twice as high in African Americans as in whites (P<0.0001) (Table 1). Mean Lp(a) levels were higher in white patients with CAD than in those without CAD (69.0±96.9 versus 47.1±59.7 nmol/L, respectively; P=0.01), whereas there was no significant difference among African Americans (141.3±106.0 versus 120.5±90.6 nmol/L for patients with and without CAD, respectively; P=NS). The difference among whites was due to higher Lp(a) levels among men with CAD compared with men without CAD; mean Lp(a) levels were 68.1±91.7 versus 39.7±49.2 nmol/L, respectively (P=0.004). There was no significant difference in Lp(a) levels among white women with or without CAD. Lipid and lipoprotein levels for African Americans and whites with and without CAD are shown in Table 2.

An inverse relationship between apo(a) sizes and plasma Lp(a) levels in both ethnic groups was observed. In African Americans, median Lp(a) levels gradually declined from small to large apo(a) sizes, whereas in whites, median Lp(a) levels declined abruptly for apo(a) sizes of ≥21 K4 repeats (Figure). Large apo(a) isoform sizes were more prevalent among African Americans than among whites with elevated Lp(a) levels (≥72 nmol/L, corresponding to ≥30 mg/dL).

### TABLE 1. Lp(a) Levels in Whites and African Americans Undergoing Coronary Angiography

<table>
<thead>
<tr>
<th></th>
<th>Whites</th>
<th>African Americans</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Men</td>
<td>Women</td>
</tr>
<tr>
<td>N</td>
<td>221</td>
<td>119</td>
</tr>
<tr>
<td>Age, y</td>
<td>56.6±10.3</td>
<td>56.5±10.7</td>
</tr>
<tr>
<td>Disease/no disease, n/n</td>
<td>140/81</td>
<td>49/70</td>
</tr>
<tr>
<td>Mean Lp(a) levels</td>
<td></td>
<td></td>
</tr>
<tr>
<td>nmol/L</td>
<td>57.7±79.7</td>
<td>61.6±88.4</td>
</tr>
<tr>
<td>mg/dL</td>
<td>24.0±33.2</td>
<td>25.7±36.9</td>
</tr>
<tr>
<td>Median Lp(a) levels</td>
<td></td>
<td></td>
</tr>
<tr>
<td>nmol/L</td>
<td>24.3</td>
<td>24.2</td>
</tr>
<tr>
<td>mg/dL</td>
<td>10.1</td>
<td>10.1</td>
</tr>
<tr>
<td>Subjects with Lp(a) ≥72 nmol/L, %</td>
<td>26.2</td>
<td>26.9</td>
</tr>
</tbody>
</table>

Values are mean±SD, except for median levels. Lp(a) levels were measured as nanomoles per liter and converted to milligrams per deciliter by use of a conversion factor of 2.4 nmol/L=1 mg/dL, as described in Methods. A level of 72 nmol/L corresponds to 30 mg/dL.

*P<0.001 for African American men and women compared with white men and women, respectively.
subjects with elevated Lp(a), 80% of whites (72 of 90) but only 26% of African Americans (40 of 157) carried at least 1 small apo(a) isoform (<22 K4 repeats). This pattern was similar among men and women.

We evaluated the association between CAD and elevated Lp(a) levels as well as between CAD and the presence of a small apo(a) isoform in the 4 different sex/ethnicity groups. There was a borderline association (P = 0.057, OR 2.0) between elevated Lp(a) levels and CAD (Table 3) among white men but not women. In contrast, there was no significant association between elevated Lp(a) levels and CAD among African Americans irrespective of sex. The presence of at least 1 small apo(a) isoform was associated with CAD in white men (P = 0.043, OR 2.0; Table 3). Although the OR for this association was similar in African American men (OR 2.0), it did not reach significance. In women, independent of ethnicity, there was no association between the presence of a small size apo(a) and CAD.

We considered how the amount of Lp(a) that was associated with specific sizes of apo(a) isoforms varied with the presence of CAD. As shown in the Figure, the median Lp(a) level associated with smaller apo(a) isoform sizes was markedly higher in patients with CAD than in patients without CAD for African Americans and whites. In contrast, median Lp(a) levels associated with large apo(a) isoform sizes were similar in patients with or without CAD in both ethnic groups. It is clear from the figure that irrespective of ethnicity, median levels for cases and controls diverged for small apo(a) isoforms and converged for larger apo(a) isoforms. Elevated levels of small-isoform Lp(a) were significantly associated with CAD in white and African American men (P < 0.01, ORs 3.0 and 4.3, respectively; Table 3). There was no significant

### Table 2. Lipid and Lipoprotein Levels in African Americans and Whites With and Without CAD

<table>
<thead>
<tr>
<th></th>
<th>CAD</th>
<th>No CAD</th>
<th>CAD</th>
<th>No CAD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>African Americans</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cholesterol, mg/dL</td>
<td>195.1 ± 41.1</td>
<td>188.2 ± 40.4</td>
<td>218.1 ± 53.7*</td>
<td>195.2 ± 43.0</td>
</tr>
<tr>
<td>Triglycerides, mg/dL</td>
<td>126.2 ± 66.3</td>
<td>113.3 ± 70.4</td>
<td>126.3 ± 52.3</td>
<td>111.5 ± 42.4</td>
</tr>
<tr>
<td>LDL cholesterol, mg/dL</td>
<td>123.9 ± 37.9</td>
<td>118.7 ± 36.8</td>
<td>146.4 ± 53.4†</td>
<td>119.7 ± 40.1</td>
</tr>
<tr>
<td>HDL cholesterol, mg/dL</td>
<td>45.9 ± 19.4</td>
<td>46.8 ± 14.9</td>
<td>48.5 ± 12.2</td>
<td>53.3 ± 19.6</td>
</tr>
<tr>
<td>Wholes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cholesterol, mg/dL</td>
<td>199.8 ± 40.9†</td>
<td>184.8 ± 35.5</td>
<td>216.3 ± 45.3*</td>
<td>195.6 ± 42.1</td>
</tr>
<tr>
<td>Triglycerides, mg/dL</td>
<td>195.2 ± 134.6*</td>
<td>161.3 ± 90.2</td>
<td>218.9 ± 127.5†</td>
<td>159.4 ± 87.5</td>
</tr>
<tr>
<td>LDL cholesterol, mg/dL</td>
<td>125.6 ± 37.5*</td>
<td>114.5 ± 30.8</td>
<td>130.1 ± 41.3</td>
<td>117.3 ± 29.5</td>
</tr>
<tr>
<td>HDL cholesterol, mg/dL</td>
<td>37.2 ± 10.8</td>
<td>38.0 ± 9.7</td>
<td>43.7 ± 11.0</td>
<td>47.6 ± 14.8</td>
</tr>
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</table>

Values are mean ± SD.

*P < 0.01 and †P < 0.001 for CAD vs no CAD.

### Table 3. Risk of CAD as Function of Elevated Plasma Lp(a) (>72 nmol/L, >30 mg/dL), Small Apo(a) Isoform Size, or Elevated Small-Isoform Lp(a) (>72 nmol/L, ≥30 mg/dL) in Different Sex/Ethnicity Groups

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>OR</th>
<th>95% CI</th>
<th>P</th>
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<tr>
<td>Risk of CAD as function of elevated plasma Lp(a) (&gt;72 nmol/L, ≥30 mg/dL)</td>
<td></td>
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<td></td>
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<tr>
<td>White men</td>
<td>221</td>
<td>2.0</td>
<td>1.0–3.8</td>
<td>0.057</td>
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<tr>
<td>White women</td>
<td>119</td>
<td>1.1</td>
<td>0.5–2.6</td>
<td>NS</td>
</tr>
<tr>
<td>African American men</td>
<td>131</td>
<td>0.8</td>
<td>0.4–1.6</td>
<td>NS</td>
</tr>
<tr>
<td>African American women</td>
<td>101</td>
<td>1.5</td>
<td>0.6–3.7</td>
<td>NS</td>
</tr>
<tr>
<td>Risk of CAD as function of presence of small apo(a) isoform (&lt;22 K4 repeats)</td>
<td></td>
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<tr>
<td>White men</td>
<td>221</td>
<td>2.0</td>
<td>1.0–3.8</td>
<td>0.04</td>
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<tr>
<td>White women</td>
<td>119</td>
<td>1.0</td>
<td>0.4–2.2</td>
<td>NS</td>
</tr>
<tr>
<td>African American men</td>
<td>131</td>
<td>2.0</td>
<td>0.8–4.7</td>
<td>NS</td>
</tr>
<tr>
<td>African American women</td>
<td>101</td>
<td>1.0</td>
<td>0.4–2.8</td>
<td>NS</td>
</tr>
<tr>
<td>Risk of CAD as function of presence of small-isoform Lp(a) level (&gt;72 nmol/L)</td>
<td></td>
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<tr>
<td>White men</td>
<td>221</td>
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<td>1.3–6.9</td>
<td>0.008</td>
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<tr>
<td>White women</td>
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<td>0.4–2.5</td>
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<tr>
<td>African American men</td>
<td>131</td>
<td>4.3</td>
<td>1.4–13.0</td>
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<tr>
<td>African American women</td>
<td>101</td>
<td>1.4</td>
<td>0.4–4.5</td>
<td>NS</td>
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</table>
association between elevated small-isoform Lp(a) levels and CAD in women. (For details, please see www.ahajournals.org.) These results strongly suggest that the level of Lp(a) particles carrying small apo(a) isoforms predicts the association between Lp(a) and CAD found in men. In contrast, elevated large-isoform Lp(a) levels were not associated with CAD in any sex/ethnicity group (ORs 0.7 to 2.0 in the 4 groups, respectively; P< NS in all).

We next determined which of the 3 factors, ie, elevation of Lp(a) levels, the presence of a small apo(a) isoform, or elevated levels of small-isoform Lp(a), was the best predictor of CAD in men. In contrast, elevated large-isoform Lp(a) levels were not associated with CAD in any sex/ethnicity group (ORs 0.7 to 2.0 in the 4 groups, respectively; P< NS in all).

We next determined which of the 3 factors, ie, elevation of Lp(a) levels, the presence of a small apo(a) isoform, or elevated levels of small-isoform Lp(a), was the best predictor of CAD in white men (the only sex/ethnicity group for which all 3 factors were associated separately with CAD). (For details, please see www.ahajournals.org.) In summary, the elevation of small-isoform Lp(a) levels, ie, the subset of Lp(a) particles carrying a small apo(a) isoform, was a more significant risk factor for CAD than either the elevation of Lp(a) levels or the presence of a small apo(a) isoform. Among African American men, elevated levels of small-isoform Lp(a) was the only one of these variables significantly associated with CAD (Table 3). Thus, independent of race/ethnicity, an elevated small-isoform Lp(a) level was associated with CAD in men. In contrast, small-isoform Lp(a) was not associated with CAD among women. When analyzing all subjects together, we found a significant interaction between elevated small-isoform Lp(a) levels and sex (P<0.03), supporting these findings. In sex-specific analysis, there was no significant interaction between elevated small-isoform Lp(a) levels and ethnicity.

To determine the extent of confounding of the association between CAD and elevated small-isoform Lp(a) levels by other risk factors, we performed conditional logistic regression with a model controlling for age, race/ethnicity, diabetes, hypertension, smoking, and plasma concentrations of small-isoform Lp(a), HDL cholesterol, LDL cholesterol corrected for cholesterol content of Lp(a), and logarithmically transformed triglycerides. When controlling for these factors, we found that elevations of small-isoform Lp(a) remained highly significantly associated with CAD for all men (P<0.0001, OR 4.8). Significant associations of CAD with small-isoform Lp(a) were also found when African American and white men were analyzed separately (OR 4.3 [P<0.03] and OR 5.8 [P<0.001], respectively).

**Discussion**

We demonstrate a significant association between CAD and elevated levels of small-isoform Lp(a), ie, elevated levels of Lp(a) particles with small apo(a) isoforms, in African American and white men. To our knowledge, this is the first study demonstrating a significant association between Lp(a) and
CAD in African Americans. We suggest that the failure to observe an association between Lp(a) and CAD in African Americans in other studies reflects analysis of the relationship between either Lp(a) levels or apo(a) size with CAD, without taking into consideration the level of Lp(a) associated with different apo(a) isoforms.

The possibility that size variation of apo(a) could be associated with cardiovascular disease has been raised in previous studies, although the results have been inconsistent. In several of these studies, only a limited number of apo(a) size isoforms were resolved. Subsequently, a high-resolution technique allowing separation of differently sized apo(a) isoforms was introduced. This was used in the present study, and the distribution of Lp(a) levels across apo(a) sizes was in good agreement with previous findings in normal healthy African Americans and whites. Of note, even with the higher resolution of apo(a) isoforms, we did not see an association of apo(a) size alone with CAD in African Americans.

Our findings indicate that it is important to take apo(a) sizes and the level of Lp(a) associated with each apo(a) isoform into account when assessing CAD risk. Therefore, the presence of 2 circulating apo(a) isoform sizes in the majority of all individuals presents a challenge. We determined the level of Lp(a) associated with specific apo(a) sizes on the basis of independent measures of Lp(a) levels and the intensity of apo(a) bands. We recognize the possibility of bias in apportioning Lp(a) levels. However, our classification of apo(a) sizes as being either of small or large size served to minimize the need for apportioning Lp(a), because this was required in only 14% of the patients.

We found that the level of Lp(a) particles with isoform sizes of <22 K4 repeats was higher in patients with CAD (Figure). In exploratory analyses, as when different cutoff levels were used in the present study (<21, <22, <23, or <24 K4 repeats), the ORs for CAD were not substantially affected (ORs 2.9 to 4.3, P<0.005 for men). Our studies suggest that the level of Lp(a) associated with small-sized apo(a) constitutes a risk factor and, therefore, that small apo(a) is atherogenic. Although our results broadly implicate small-sized apo(a) as being atherogenic, further mechanistic studies are needed to address this issue in more detail.

Although the frequency of subjects carrying at least 1 small isoform was comparable in African Americans and whites (21% and 29%, respectively), ~80% of whites but only ~26% of African Americans with elevated Lp(a) had at least 1 small apo(a) isoform. Because most whites with elevated Lp(a) carry small apo(a) sizes, it is difficult in this ethnic group to ascertain the relative contribution to the risk for CAD of small apo(a) size on one hand and elevated Lp(a) levels on the other. In contrast, African Americans have elevated Lp(a) over a wider range of apo(a) isoforms. Our results are consistent with previous studies demonstrating that in African Americans, neither the elevation of Lp(a) levels nor the presence of small apo(a) isoforms alone was significantly associated with CAD. However, our demonstration that the combination of elevated Lp(a) levels and small apo(a) size was associated with CAD in African American and white men provides the first experimental evidence supporting previous suggestions that ethnic differences in the relationship between apo(a) isoform size and Lp(a) levels may have an impact on the use of Lp(a) level as a risk factor. Thus, an elevated Lp(a) level is a poor surrogate marker for the relevant risk factor, ie, high levels of small isoform Lp(a), in African Americans, but the strong association in whites between elevated Lp(a) and small apo(a) size makes elevated Lp(a) levels a reasonable surrogate marker in this group for elevated small isoform Lp(a). However, also among whites in whom high Lp(a) levels are carried by large apo(a) isoforms, the use of Lp(a) levels to estimate CAD risk may be less accurate. Therefore, a measurement based on Lp(a) levels along with apo(a) sizes may be the optimal way to assess risk for CAD independent of ethnicity.

We recognize the limitations of the present study, which was designed as a cross-sectional and not a prospective study. However, differences between the various sex/ethnicity groups regarding serum lipids and lipoproteins, including Lp(a) levels, were similar to previous findings in large population groups (results not shown). In addition, established risk factors, such as total and LDL cholesterol levels, were significantly higher among patients with CAD in both ethnic groups. Although patients classified as being without CAD in general had a low degree of stenosis, the presence of coronary atherosclerosis to at least some extent cannot be excluded. Therefore, further prospective studies are needed to extend these findings to the general population.

An interesting observation in the present study was the lack of association between elevated Lp(a) levels, small apo(a) size, or elevated small isoform Lp(a) levels with CAD in women. Although studies simultaneously addressing apo(a) size and Lp(a) levels in relation to CAD are scarce in women, an elevated Lp(a) level is a risk factor in women. Several factors could contribute to this sex difference. First, the extent of stenosis in a coronary artery, as judged by angiography, may not be equally representative of CAD in both sexes. Second, because estrogen affects Lp(a) levels, hormonal effects could cloud an association between Lp(a) and CAD.

In conclusion, the present study, the first to evaluate the level of Lp(a) associated with specific apo(a) sizes as a risk factor for CAD, provides new insights into the role of Lp(a). First, our results conclusively demonstrate that the combination of high Lp(a) levels and small-sized apo(a) is a risk factor in white and African American men. Second, our findings suggest that the absence of association between Lp(a) and CAD in African Americans in previous studies is not due to a difference in the atherogenicity of Lp(a) across race/ethnicity but is a consequence of race/ethnicity differences in the distribution of Lp(a) levels across apo(a) sizes. Thus, the present study lays to rest the notion that Lp(a) is not a risk factor for CAD in African Americans, an issue that until now has been controversial. Finally, our results raise the possibility that small isoform Lp(a) is the atherogenic subpopulation of Lp(a) particles that determines the degree of risk associated with any level of Lp(a). Further studies on the atherogenicity of small isoform Lp(a) are needed.

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References

12. Rosengren A, Wilhelmsen L, Eriksson E, Risberg B, Wedel H. Lipoprotein (a) and coronary heart disease: a prospective case-control study in a general population sample of middle aged men. BMJ. 1990; 301:1248–1251.
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Supplementary materials: Paultre et al U2000-0649

EXPANDED MATERIALS AND METHODS

Subjects:
All consecutive patients scheduled for coronary arteriography at the two sites (Harlem Hospital Center, New York City, NY and The Mary Imogene Bassett Hospital, Cooperstown, NY) between June 1993 and April 1997 were approached. Of the patients meeting the inclusion criteria, 87% at Harlem Hospital and 92% at Bassett Health Care agreed to participate in the study. Exclusion criteria were: age >70 years, recent (within 6 months) myocardial infarction or thrombolysis, a history of percutaneous transluminal coronary angioplasty (PTCA), surgery during the previous six weeks, a known communicable disease such as hepatitis or AIDS, or current lipid-lowering medication. Information on diabetes mellitus, hypertension and smoking was obtained by a standardized questionnaire upon entry into the study. The study was approved by the Institutional Review Boards at Harlem Hospital, Bassett Healthcare, and Columbia University College of Physicians and Surgeons.

Measurements of lipids and lipoproteins
Fasting blood samples were drawn approximately 2 to 4 hours before the catheterization procedure, and serum and plasma samples were stored at ~80°C prior to analysis. The Lp(a) assay measures the number of circulating Lp(a) particles and in this assay, an Lp(a) level of 2.4 nmol/L corresponds to 1 mg/dL. In our hands, the interassay coefficient of variation was 8.4% at an Lp(a) level of 19.9 nmol/L (8.3 mg/dL) and 9.0% at an Lp(a) level of 67.1 nmol/L (28.0 mg/dL).
Apo(a) isoform size determination

Apo(a) isoform sizes were analyzed in 572 subjects (352 men and 220 women) by SDS-agarose gel electrophoresis followed by immunoblotting. Briefly, 12.5 µl of plasma was mixed with 30 µl of sample buffer, and 10 µl of the mixture was loaded on a 2% submarine SDS agarose gel. Samples were separated for 15 hours at 100 V at 4°C using 45 mM Tris-borate buffer, pH 8.6, containing 2 mM EDTA. Proteins were transferred onto nitrocellulose (Amersham Hybond-C extra, Arlington Heights, IL) using an electroblotter for 8 hours in the cold, the membrane was blocked using powdered skim milk (Carnation) and then incubated with a primary antibody against apo(a) (Incstar, Stillwater, MN). The apo(a) bands are visualized with the ECL Amersham technique on Kodak X-OMAT films using a second, labeled antibody (Pierce, Rockford, IL). The results were related to standards with known apo(a) isoforms (Immuno AG, Innsbruck, Austria and Intracel, Issaquah, WA) taking into account the inverse logarithmic relation between the number of K4 repeats and isoform mobility during agarose gel electrophoresis (1).

To validate the apo(a) isoform size determination, we performed genotyping of apo(a) allele sizes by pulsed field electrophoresis (2). Briefly, leucocytes were isolated from whole blood using Leuco-Prep tubes (Becton, Dickinson & Co., Lincoln Park, MJ), embedded in agarose plugs, and stored at 4°C in 0.5 M EDTA, pH 8.0. A segment of the agarose-cellular plug was washed in 10 mM Tris-HCl, pH 8.0 containing 1 mM EDTA, and incubated for 2 h with Hpal (New England Biolabs, Beverly, MA). The plugs were subjected to transverse alternating gel electrophoresis with a CHEF-DR II apparatus.
(Bio-Rad Laboratories, Melville, NY), using pulse times of 4 seconds for 30 min followed by 8 and 15 second pulses (180 V/90 mA) for 17.5 h. The size-fractionated DNA was blotted onto a nylon membrane (ICN Biomedicals, Irvine, CA) and hybridized with a human apo(a) K-4 specific single-stranded fragment labeled with fluorescein (Gene Images, Amersham). The probe was a generous gift of Dr. Helen Hobbs, University of Texas Southwestern Medical Center, Dallas, TX. The filters were washed for 1 h and exposed to Kodak X-OMAT film. Apo(a) allele sizes, designated by the estimated number of K-4 encoding sequences per allele, were determined using mid range PFG markers (New England Biolabs) as size standards.

In the majority of patients (n=323, 198 men and 125 women), two distinct apo(a) protein bands were detected. No apo(a) protein bands could be detected in 20 patients (13 men and 7 women) with Lp(a) concentrations <2.6 nmol/L (<1.1 mg/dL). In the remaining 229 patients (141 men and 88 women), a single apo(a) protein band was detected. The occurrence of a single apo(a) band in the phenotyping procedure could be due to (1) homozygosity, (2) presence of a non-expressed apo(a) allele or alternatively an apo(a) allele coding for a non-detectable apo(a) protein isoform, or (3) by the presence of two similar-sized apo(a) alleles, detected as a single, coalescing protein band. To address the latter possibility, we performed apo(a) genotyping in the 183 subjects with a single apo(a) band for whom stored DNA samples were available. The majority of patients with similar-sized apo(a) genes had either two small-size apo(a) alleles (<22 K4 repeats) or two large-size apo(a) alleles (≥22 K4 repeats). In only three cases did we find similar-sized apo(a) alleles in a size range (20-23 K4 repeats) which potentially could affect the
relative distribution of plasma Lp(a) to either small or large apo(a) size, possibly impacting on their association with CAD. To more exhaustively ascertain whether coalescing bands indeed were present in these subjects, the phenotyping procedure was repeated using more stringent conditions, allowing separation of similar-sized apo(a) proteins. However, in every one of these patients could we detect only a single apo(a) protein strongly arguing against the possibility of coalescence. From these analyses, we were able to determine for each subject with detectable apo(a) isoforms whether their Lp(a) plasma level was accounted for wholly by particles carrying a small apo(a) isoform (<22 K4 repeats) [small-isoform-Lp(a)], wholly by particles carrying a large apo(a) isoform (≥22 K4 repeats) [large-isoform-Lp(a)] or by the combination of both small-isoform-Lp(a) and large-isoform-Lp(a).

Distribution of subjects

Altogether 189 Caucasians (140 men and 49 women) and 100 African Americans (53 men and 47 women) were defined as having CAD. The number of patients without CAD were 151 Caucasians (81 men and 70 women) and 132 African Americans (78 men and 54 women). 90 Caucasians (58 men and 32 women) and 157 African Americans (84 men and 73 women) had elevated Lp(a) levels (≥72 nmol/L or ≥30 mg/dL). Of these, 57 Caucasian patients (43 men and 14 women) and 68 African American patients (32 men and 36 women) had CAD. The number of patients with elevated Lp(a) and small isoforms, i.e. elevated small-isoform-Lp(a) (≥72 nmol/L or ≥30 mg/dL) were 67 Caucasians (43 men and 24 women) and 30 African Americans (17 men and 13 women).
RESULTS

As shown in Figure 1 and confirming the results from Table 3 in the manuscript text, Lp(a) levels associated with smaller apo(a) isoform sizes was markedly higher in men with CAD than in men without CAD for both African Americans and Caucasians. In contrast, there was no appreciable difference in Lp(a) levels associated with any apo(a) isoform sizes for women with or without CAD in both ethnic groups.

We determined which of the three factors, elevation of Lp(a) levels, presence of a small apo(a) isoform or elevated levels of small-isoform-Lp(a), was the best predictor of CAD in Caucasian men (the only gender/ethnicity group where all three factors were associated separately with CAD). With elevation of Lp(a) levels and elevation of small-isoform-Lp(a) levels present simultaneously in the analysis, elevation of Lp(a) levels was not associated with CAD (OR 0.8) while the association between elevation of the small-isoform-Lp(a) levels and CAD remained significant (OR 3.8) (Table 1). Similarly, simultaneous analysis of the presence of a small apo(a) isoform and elevation of small-isoform-Lp(a) showed that the presence of a small apo(a) isoform was not associated with CAD (OR 1.0), while the strong association between elevation of small-isoform-Lp(a) levels and CAD remained (OR 3.2)
REFERENCES


FIGURE LEGEND

Figure 1.

Relationship between Lp(a) levels associated with specific apo(a) isoform sizes in African American and Caucasian men (Panel A) patients with (cases) and without CAD (controls). Corresponding results for African American and Caucasian women are given in Panel B. Individual results for plasma Lp(a) levels apportioned to specific apo(a) isoform sizes are shown by filled circles for cases and open circles for controls. Results for apo(a) sizes are presented in isoform groups, e.g. <19 K4 repeats, 19-21 K4 repeats etc. The Lp(a) level of 72 nmol/L (corresponding to 30 mg/dL) is indicated. For African Americans, one case and two controls had Lp(a) levels >400 nmol/L. For Caucasians, two cases had Lp(a) levels >400 nmol/L.
**TABLE 1.**

**Model A.**

Risk of CAD in Caucasian men as a function of both Lp(a) levels ≥72 nmol/L (≥30 mg/dL) and small-isoform-Lp(a) levels ≥72 nmol/L (≥30 mg/dL) determined by logistic regression. Both Lp(a) levels and small-isoform-Lp(a) levels were simultaneously used in the model as independent variables.

<table>
<thead>
<tr>
<th></th>
<th>Odds ratio</th>
<th>95% CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lp(a) ≥72 nmol/L</td>
<td>0.8</td>
<td>0.3-2.2</td>
<td>0.64</td>
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<tr>
<td>small-isoform-Lp(a) ≥72 nmol/L</td>
<td>3.8</td>
<td>1.1-13.7</td>
<td>0.04</td>
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</tbody>
</table>

**Model B.**

Risk of CAD in Caucasian men as a function of both presence of a small apo(a) isoform and small-isoform-Lp(a) levels ≥72 nmol/L (≥30 mg/dL) determined by logistic regression. Both presence of small apo(a) isoform and small-isoform-Lp(a) levels were simultaneously used in the model as independent variables.

<table>
<thead>
<tr>
<th></th>
<th>Odds ratio</th>
<th>95% CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Presence of a small apo(a) isoform</td>
<td>1.0</td>
<td>0.4-2.5</td>
<td>0.92</td>
</tr>
<tr>
<td>Small-isoform-Lp(a) ≥72 nmol/L</td>
<td>3.2</td>
<td>1.0-10.5</td>
<td>0.06</td>
</tr>
</tbody>
</table>
African American and Caucasian Men

Apo(a) Isoform Size (K4 Repeats)

Lp(a) (nmol/L) Associated with Specific Apo(a) Isoform Size

African American and Caucasian Women

Lp(a) (nmol/L) Associated with Specific Apo(a) Isoform Size (K4 Repeats)