Lipoprotein(a) in Childhood: Relation with Other Atherosclerosis Risk Factors and Family History of Atherosclerosis

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The relation between lipoprotein(a) [Lp(a)], apolipoprotein (apo) E phenotypes, cholesterol, triglycerides, apo A-I, apo B, and a family history of atherosclerosis or risk factors was studied in 2- and 4-year-old French Caucasian children (n = 499). Lp(a) concentrations were distributed in a typical skewed manner and were found to be an independent lipid variable. The distribution of apo E phenotypes did not differ by gender. Cholesterol and apo B were under apo E phenotype control; Lp(a) was not. A significant positive relation was found between Lp(a) concentrations and the number of parental risk factors. Children whose grandparents had a history of cardiovascular disease had Lp(a) concentrations shifted towards higher values. Measurement of Lp(a) in children may help to identify those at an increased risk of atherosclerotic disease, especially when their parents have at least two relevant risk factors.

Indexing Terms: reference values/apolipoproteins/cholesterol/pediatric chemistry/heritable disorders

Lipoprotein(a) [Lp(a)] is a cholesterol-rich lipoprotein composed of a low-density lipoprotein (LDL) particle in which apolipoprotein (apo) B-100 is covalently linked to apo(a), a highly glycosylated protein that exhibits considerable size heterogeneity (molecular mass range 200–800 kDa).5 The molecular mass of Lp(a) is genetically controlled and determines, at least in part, the concentrations of Lp(a) in plasma. Apo(a) serves to quantify Lp(a) (I, 2).

Epidemiological studies have established a link between high concentrations of Lp(a) and an increased risk of early coronary heart disease (3, 4) and stroke (5).

The mechanism by which Lp(a) is atherogenic is not known; however, it appears to involve not only the lipoprotein character of Lp(a) but also a possible effect on fibrinolysis. Indeed, Lp(a) has antithrombotic properties, competing directly with plasminogen for the plasminogen receptor and thereby interfering with clot lysis.

Apo E plays a critical role in the metabolism of triglyceride-rich lipoproteins and cholesterol homeostasis. The genetic polymorphism of apo E results from the existence of three codominant alleles (e2, e3, e4), which code for three apo isoforms: E2, E3, and E4. Epidemiological studies have found the e2 allele to be associated with low concentrations of cholesterol and LDL cholesterol, whereas e4 is associated with high concentrations of both species and early onset of coronary heart disease and atherosclerosis (6). The E3/E3 phenotype is less frequent in subjects who have had a complete stroke or transient ischemic attack than in healthy blood donors (7).

Because the atherosclerotic process begins early in life, the aims of this study were: (a) to determine, during a routine health check, the frequencies of apo E phenotypes and Lp(a) concentrations, together with cholesterol, triglyceride (TG), apo A-I, and apo B concentrations in 499 randomly selected French 2- and 4-year-old children; (b) to identify a possible link between Lp(a), apo E phenotypes, other lipid variables, and a family history of atherosclerosis; and (c) to assess the significance of a parental history of atherosclerosis or risk factors for identifying children at risk of early atherosclerosis.

Materials and Methods

Patients. Venous blood was obtained after a 12-h fast from 499 randomly selected French Caucasian children, ages 2 and 4 years. Each child underwent a complete physical examination by a pediatrician, and the parents responded to a questionnaire about the family history of atherosclerosis (coronary heart disease, stroke, and intermittent claudication) and risk factors such as obesity, diabetes, hypertension, and hyperlipoproteinemia. These children were seen at the Centre de Bilans de Santé de l’Enfant in Paris (founded by La Caisse primaire d’Assurance Maladie) for a check-up at ages 10, 24, and 48 months. The majority of the families were thus being seen for the second or third time. Control samples were collected at the Hôpital Tenon blood bank from healthy French adults and were selected according to internationally accepted criteria. In particular, they were free of hypertension, diabetes, obesity, personal and family history of cardiovascular and neurovascular disease, and primary and secondary dyslipidemia. These studies were performed in accordance with the Helsinki Declaration of 1975, as revised in 1983.

Untreated and EDTA-treated blood samples were collected. EDTA-treated plasma was used only for apo E phenotyping; all the other determinations were performed on untreated plasma.
formed with serum. Serum was stored at 4°C. EDTA-treated plasma was obtained by low-speed centrifugation at 4°C; the antiproteases phenylmethylsulfonyl fluoride (final concentration 0.1 mmol/L; Sigma Chemical Co., St. Louis, MO) and D-phenylalanine-L-proline-L-arginine chloromethyl ketone (10 mM/L, Calbiochem, La Jolla, CA) were added, and samples were stored at -20°C until apo E phenotyping.

**Laboratory methods.** Lipid variables [except for Lp(a) and apo E phenotyping] were analyzed in Le Centre de Bilans de Santé de l'Enfant. Cholesterol and triglycerides were assayed enzymatically in a Cobas Fara-II® centrifugal analyzer (Hoffmann-La Roche, Basel, Switzerland). Apo A-I and B were measured by immunoturbidimetry in the same analyzer.

Lp(a) was measured immunonephelometrically in the Beckman Array® Protein system (Beckman Instruments, Brea, CA) at Hôpital Tenon. Standard and control Lp(a) were purchased from ImmunoAG (Vienna, Austria) and anti-Lp(a) antiserum from Dako (Copenhagen, Denmark).

Apo E phenotypes were determined by direct isoelectric focusing of plasma, followed by immunoblotting (8). Briefly, plasma apo E was dissociated from lipoprotein with Tween 20 and treated with neuraminidase (Boehringer Mannheim, Mannheim, Germany) before analysis, and revealed by immunoblotting with an anti-apo E antibody (Daichi Pure Chemicals, Tokyo, Japan).

**Statistical methods.** Qualitative data are reported as proportions. Quantitative variables are reported as means ± SD and in histogram form. If distributions were gaussian and the variances equal, values were compared by Student's t-test or analysis of variance. When the distribution was not gaussian, (a) data were reported as median, range, and percentiles; and (b) the Mann–Whitney and Kruskal–Wallis nonparametric tests were used to compare two or more groups. Logarithmic transformation of Lp(a) data was done to verify the results obtained by the nonparametric methods.

All the tests were two-tailed and the level of significance was set at P < 0.05. The statistical analyses were done with the Statview II computer program (Abacus Concepts, Berkeley, CA).

### Results

We determined the concentrations of cholesterol, TG, apo A-I and B, and Lp(a) in these 499 children (Table 1). TG concentrations differed significantly according to the children's ages (P <0.001), but not by sex.

In 2-year-olds, Lp(a) concentrations differed significantly between girls and boys (respective median, 37 and 42 mg/L; P <0.05). In 4-year-olds, only cholesterol concentrations differed significantly between boys (4.41 mmol/L) and girls (4.62 mmol/L; P <0.05).

Lp(a) concentrations in the children and adults were distributed in the typical skewed manner (Fig. 1), reflecting the difference between the mean (176 mg/L) and the median (48 mg/L); overall, the Lp(a) concentration varied from 10 to 1820 mg/L. About 20% of the children had Lp(a) >300 mg/L, a concentration above which cardiovascular risks are enhanced in adults (9). The difference between the mean Lp(a) in 2-year old boys and girls (Table 1) has been averaged by the reciprocal difference between 4-year-old girls and boys. No correlation between Lp(a) concentrations and the other lipid variables was found.

The observed apo E phenotype frequencies and the relationship between apo E polymorphism and concentrations of cholesterol, apo B, and Lp(a) in the children are shown in Table 2. Only the three predominant phenotypes are given here, i.e., E4/E3, E3/E3, and E3/E2, with the E3/E3 phenotype being the most common. The distribution of apo E phenotypes was not different between boys and girls and was similar to that in the adult population (8). Apo E polymorphism influenced cholesterol and apo B concentrations, these values being markedly lower in E3/E2 subjects (P <0.05). The median Lp(a) concentrations were also different: 34 mg/L in E4/E3, 52 mg/L in E3/E3, and 49 mg/L in E3/E2 subjects; however, these differences were not significant by the Kruskal–Wallis test.

Risk factors (hypertension, hyperlipoproteinemia, diabetes, and obesity) were identified in the parents, and a history of atherosclerotic disease (coronary heart disease, intermittent claudication, or stroke) was sought in the grandparents, the parents being too young to have accumulated sufficient atherosclerotic

### Table 1. Serum lipid and lipoprotein concentrations (mean ± SD) in 2- and 4-year-old children.

<table>
<thead>
<tr>
<th></th>
<th>Age 2 years</th>
<th>Age 4 years</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M</td>
<td>F</td>
</tr>
<tr>
<td>No. of subjects</td>
<td>137</td>
<td>93</td>
</tr>
<tr>
<td>Cholesterol, mmol/L</td>
<td>4.45 ± 0.84</td>
<td>4.50 ± 0.83</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>0.81 ± 0.32b</td>
<td>0.88 ± 0.36p</td>
</tr>
<tr>
<td>Apo A-1, g/L</td>
<td>1.15 ± 0.30</td>
<td>1.21 ± 0.34</td>
</tr>
<tr>
<td>Apo B, g/L</td>
<td>0.92 ± 0.29</td>
<td>0.98 ± 0.25</td>
</tr>
<tr>
<td>Lp(a), mg/L</td>
<td>232 ± 120</td>
<td>120</td>
</tr>
<tr>
<td>Range</td>
<td>10-1820</td>
<td>10-1165</td>
</tr>
<tr>
<td>Median</td>
<td>42</td>
<td>37</td>
</tr>
</tbody>
</table>

* Significantly different from female children (P <0.05).

b Significantly different from 4-year-old children of the same sex (P <0.05).
Table 2. Serum lipid, apo B, and Lp(a) concentrations according to apo E phenotypes in children.*

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>E4/E3</th>
<th>E3/E3</th>
<th>E3/E2</th>
</tr>
</thead>
<tbody>
<tr>
<td>% (and no.) of subjects</td>
<td>20.2 (98)</td>
<td>70.6 (343)</td>
<td>9.2 (n = 45)</td>
</tr>
<tr>
<td>Cholesterol, mmol/L</td>
<td>$4.68 \pm 0.95^c$</td>
<td>$4.51 \pm 0.82^c$</td>
<td>$4.04 \pm 0.73$</td>
</tr>
<tr>
<td>Apo B, g/L</td>
<td>$0.97 \pm 0.32^c$</td>
<td>$0.92 \pm 0.25^c$</td>
<td>$0.82 \pm 0.19$</td>
</tr>
<tr>
<td>Lp(a), mg/L</td>
<td>Median: 139 (10–1037); 183 (10–1820); 185 (10–1374)</td>
<td>34</td>
<td>52</td>
</tr>
</tbody>
</table>

* Only data related to the three predominant phenotypes are given here.

** Mean ± SD.

Table 3. Serum concentrations of Lp(a) in children according to atherosclerotic risk factors in their parents.

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>n</th>
<th>Mean (mg/L)</th>
<th>Median (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>133</td>
<td>149</td>
<td>41</td>
</tr>
<tr>
<td>Obesity</td>
<td>138</td>
<td>198</td>
<td>64.5</td>
</tr>
<tr>
<td>Hyperlipoproteinemia</td>
<td>48</td>
<td>201</td>
<td>82.5</td>
</tr>
<tr>
<td>Diabetes</td>
<td>8</td>
<td>152</td>
<td>95.5</td>
</tr>
<tr>
<td>Hypertension</td>
<td>25</td>
<td>209</td>
<td>63</td>
</tr>
</tbody>
</table>

n = number of children in each category. Each subject could have parents with several risk factors.

parental risk factors had higher Lp(a) than did those with no parental risk factors: Medians were 63 mg/L for two risk factors and 60 mg/L for three risk factors vs 41 mg/L for no risk factors and 43.5 mg/L for one risk factor (Fig. 2). The other lipid variables were not significantly associated with parental risk factors.

No history of atherosclerotic disease was found in the grandparents of 377 of the children, while at least one grandparent of 95 of the children had had cardiovascular disease. A smaller group of children (n = 13) had at least one grandparent with cerebrovascular disease. Children with a grandparental history of cardiovascular disease had higher TG, apo B, and apo A-I concentrations than did the other children. Children whose grandparents had a history of cardiovascular disease had Lp(a) concentrations shifted towards higher values (Table 4), although these differences were not significant ($P < 0.15$, Mann–Whitney test). Of children with a grandparental history of cardiovascular disease, 26% had Lp(a) >300 mg/L, a concentration above which cardiovascular risks are enhanced in adults, compared with 18% of children with no relevant grandparental history.

Discussion

Because Lp(a) concentrations are markedly higher in black children than in white children, and because the frequency distribution of Lp(a) differs markedly ac-

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Fig. 1. Lp(a) distribution in children and adults (mean age: 35 ± 10 years): (A) 2- and 4-year-old girls [one subject with 1452 and one with 1261 mg/L. Lp(a) not shown], median: 46 mg/L; (B) 2- and 4-year-old boys [three subjects with 1216, 1374, and 1820 mg/L. Lp(a) not shown], median: 52.5 mg/L; (C) adults, median: 82 mg/L.
Bogalusa Heart Study showed that 8–17-year-old females had slightly higher Lp(a) values than 8–17-year-old males (10). Rifai et al. documented a progressive increase in Lp(a) concentrations during the first 2 years of age (12). We found that 20% of 2-year-old and 16% of 4-year-old boys had Lp(a) <10 mg/L (the detection limit in the immunephelometric assay we used). Previous studies (12) have shown that 50% of neonates and 20% of adults have no detectable Lp(a). Our study confirms the progression of Lp(a) concentrations with age, which probably reflects a maturation phenomenon that may be influenced by gender.

It is well known that increased Lp(a) is associated with a higher risk of myocardial infarction (3), coronary heart disease (4), vein graft restenosis (16), and cerebrovascular disease (5). Children whose grandparents had a history of cardiovascular disease tended to have higher concentrations of Lp(a) than the other children. Srinivasan et al. (10) previously reported increased Lp(a) in Caucasian children with a parental history of myocardial infarction; further, the prevalence of parental myocardial infarction was higher in the children with Lp(a) >250 mg/L than in those with values ≤250 mg/L (10). In our study, the children were too young for many of their parents to have had a myocardial infarction. The distribution of Lp(a) concentration in the children differed according to whether or not one grandparent had had a coronary event. This difference remained under the limit of significance, however, probably because of the high number of ties and the highly skewed distribution of the Lp(a) values. Given the possibility of inheriting high Lp(a) concentrations, and their association with cardiovascular disease, it is not surprising that children with a positive grandparental history of cardiovascular disease had a higher median Lp(a) value than did those with no relevant grandparental history. These data emphasize the importance of hereditary factors in high Lp(a).

In both 2- and 4-year-old children with a grandparental history of early myocardial infarction (case subjects), high apo B and TG were significant determinants of such case subjects vs controls. Surprisingly, apo A-I concentration, which is considered to protect somewhat against coronary heart disease, was also higher in case subjects than in controls. Apo A-I was significantly lower in 11-year-old children with a parental history of early myocardial infarction in the study by Marquez et al. (17), but was not significantly lower in children 2–13-year-olds with a positive parental history in the studies by Bayle et al. (18) and Wilcken et al. (19). This discrepancy may due to differences in age ranges of studied children.

We also found that children whose parents reported having two risk factors had higher concentrations of Lp(a) than those whose parents had no risk factors and, the greater the number of risk factors, the higher the Lp(a) concentration. To our knowledge, no such relationship has previously been reported. Moreover, apo B and cholesterol concentration were, and Lp(a)

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**Table 4. Lp(a) percentiles in children according to atherosclerotic diseases in their grandparents.**

<table>
<thead>
<tr>
<th>Disease</th>
<th>n</th>
<th>25</th>
<th>50</th>
<th>75</th>
<th>90</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>377</td>
<td>19*</td>
<td>45</td>
<td>189</td>
<td>480</td>
</tr>
<tr>
<td>Coronary artery disease</td>
<td>95</td>
<td>20</td>
<td>67</td>
<td>335</td>
<td>772</td>
</tr>
<tr>
<td>Cerebrovascular disease</td>
<td>13</td>
<td>10</td>
<td>60</td>
<td>416</td>
<td>613</td>
</tr>
</tbody>
</table>

* Lp(a) concentration, mg/L.

cording to the ethnic group (10, 11), we report in this study results only for children with French Caucasian parents and grandparents.

To our knowledge, there have been no reports of the distribution of Lp(a) concentrations in 2- and 4-year-old children. Infantile values of Lp(a) have been reported (12, 13), and one study has established that the gene regulating apo(a) is fully expressed by age 1 year (14). We thus decided to study only children at ages 2 and 4 years, and not those 10 months old. Another reason for not studying the younger group was the near impossibility of examining them in a fasting state [Lp(a) concentrations correlate with triglyceride in the postprandial period (15)].

Children showed a highly skewed frequency distribution of Lp(a) concentrations, as has been previously reported in Caucasian populations (11). There are few studies on the influence of gender and age on Lp(a) in children. Rifai et al. (12) reported no difference between males and females at birth. In our study, the 2-year-old boys had higher Lp(a) concentrations than the 2-year-old girls, and Lp(a) concentrations were not significantly different between 4-year-old boys and girls. The
concentration levels were not, related to the presence of hyperlipidemia in the parents or grandparents. These results suggest that Lp(a) and LDL might contribute to atherogenesis through different mechanisms.

Lp(a) consists of an LDL particle linked to apo(a). In contrast to the apo B concentration, the Lp(a) concentration is not influenced by apo E polymorphism. Variations in apo B and cholesterol concentrations dependent on apo E polymorphism were observed in these children, as has been previously reported in adults. However, when we evaluated the percentiles of Lp(a) according to apo E phenotypes, the E4/E3 phenotypes were associated with lower Lp(a) values than were the E3/E3 and E3/E2 phenotypes, especially in males (data not shown), even though this effect was modulated by the highly skewed distribution. Conflicting findings about the influence of apo E phenotypes on Lp(a) have been previously reported in adults (20, 21). To our knowledge, there is no other study in children. Data from our study reinforce the hypothesis that concentrations of Lp(a) and LDL (apo B) in serum may be under separate metabolic control (22).

In conclusion, this study provides sex- and age-specific values for serum Lp(a) in French Caucasian children and emphasizes the need to measure Lp(a) in childhood screening, especially when parents have at least two risk factors for atherosclerotic disease. The detection of high concentrations of serum Lp(a) during childhood signals the requirement to manage the atherosclerotic risk factors that can be reduced by diet, lifestyle, and, in certain cases, drugs.

References
2. Howard GC, Pizzo SV. Lipoprotein(a) and its role in atherothrombotic disease. Lab Invest 1993;69:373–86.