Parental History of Early Myocardial Infarction Is Associated With Decreased Levels of Lipoparticle AI in Adolescents

Philippe Amouyel, Dominique Isorez, Jean-Marie Bard, Marc Goldman, Pascal Lebel, Gecel Zylberberg, Jean-Charles Fruchart

A family history of coronary heart disease (CHD) is a known risk factor for CHD. To investigate the possible role of lipoprotein particles in the relationship between family history of CHD and risk of CHD, we performed a case-control study in a sample of adolescents. The case group consisted of 97 adolescents whose parents had suffered a verified myocardial infarction before the age of 55 years. The control group was composed of 194 subjects without any family history of CHD. One case patient was matched to two control subjects for gender, age, and body mass index. In both groups, plasma lipid variables were measured, including total cholesterol, triglycerides, high-density lipoprotein cholesterol (HDLC), low-density lipoprotein cholesterol, apolipoprotein (apo)AI, apoB, apoAI-containing lipoprotein particles without apoAII (LpAI) and with apoAII, and apoB-containing lipoprotein particles with apoE and with apoCIII. Adolescents with a family history of early myocardial infarction had lower plasma levels of HDLC (P<.0001), apoAI (P<.01), and LpAI (P<.0001) than control subjects (adjusted for gender, age, body mass index, smoking habits, and oral contraceptive use). No other differences were statistically significant between case and control subjects. The analysis was repeated separately for male and female subjects. In young men, the best predictor of family history of early myocardial infarction was the LpAI plasma level, whereas in young women it was the HDL-C plasma level. Decreased levels of HDL-C and LpAI lipoprotein particles explain part of the relationship between parental history of early myocardial infarction and CHD risk. (Arterioscler Thromb. 1993;13:1640-1644.)

Key Words • lipoproteins • LpAI • family history • coronary heart disease • adolescents • risk factors

A family history of coronary heart disease (CHD) predicts CHD in adults, which suggests that family history may be a useful tool for identifying high-risk subjects at a young age. This increased CHD risk in those with a positive family history is partly related to the familial clustering of known risk factors such as cigarette smoking, obesity, and plasma lipid levels. Among these risk factors, abnormalities in lipid metabolism have been extensively studied. Alterations in plasma lipoprotein and apolipoprotein (apo) levels are variously associated with parental history of CHD in children, adolescents, young adults, and adults: plasma low-density lipoprotein cholesterol (LDL-C) and apoB levels are higher and high-density lipoprotein cholesterol (HDL-C) and apoAI levels are lower in subjects with a family history of CHD.

Biochemical and immunological analyses show that these lipoprotein subfractions are heterogeneous and include subpopulations with different lipid and apolipoprotein compositions. By using combinations of monoclonal and polyclonal antibodies directed against apolipoproteins, it is possible to identify HDL subpopulations of apoAI-containing lipoprotein particles without apoAII (LpAI) or with apoAII (LpAl:AI); LDL subpopulations can be separated as well into apoB-containing lipoparticles with apoE (LpE:B) and with apoCIII (LpCIII:B). This approach emphasizes the functional as well as biochemical differences of particles within the same lipoprotein subpopulation. Case-control studies indicate that these lipoprotein subfractions are associated with CHD; low plasma levels of LpAI and LpAl:AI and high plasma levels of LpE:B and LpCIII:B are strongly associated with increased risk of myocardial infarction.

To investigate the possible relations among alterations of LpAI, LpAl:AI, LpE:B, and LpCIII:B plasma levels and family history of early myocardial infarction, we designed a matched case-control study in a population of adolescents. We compared the plasma lipid, lipoprotein, and lipoparticle levels, strictly adjusted for environmental confounders, between adolescents whose parents had suffered a verified myocardial infarction and adolescents without any family history of CHD.

Methods

Population

Adolescents were recruited in a center for preventive medicine of the Lille Pasteur Institute that has devel-
opened a general examination program for adolescents of high-school age in the surrounding region. Each year about 3500 adolescents ranging from 15 to 20 years of age are examined. Medical histories are taken, and complete physical examinations and laboratory evaluations are performed.

Selection of the Case Subjects

From February 1989 through April 1990, 7476 adolescents were screened. Among them, 138 adolescents gave a plausible history of myocardial infarction in one or the other parent. The affected parent of each adolescent was asked to fill out a questionnaire giving the details of his or her illness and to authorize the inspection of all relevant inpatient and outpatient medical records. The diagnosis of myocardial infarction was established according to the WHO-MONICA study criteria. All participants and their families were fully informed of the aims of the study.

Thirty-eight subjects were excluded: those for whom a parental history of myocardial infarction was not confirmed with the criteria defined and those whose parents had suffered a myocardial infarction after the age of 55 years. The final sample of 100 consisted entirely of adolescents whose mother or father had suffered a confirmed myocardial infarction before the age of 55. Finally, 3 of them refused to participate.

Selection of the Control Subjects

Each case subject was matched with two control subjects (n=194) who were all selected from families in which there was no known or suspected cardiac or cerebrovascular disease in first- and second-degree relatives. They were matched for the following variables: sex, age, body mass index (BMI), and affected parent’s gender and age. This last variable was added after observing that the mean age of parents in the case group was significantly lower than the mean age of parents in the case group. To avoid eventual bias occurring as a result of this difference, parental ages were matched. No refusals to participate occurred within the control group.

General Examinations

Each adolescent was asked to fast for 12 hours before the examination. Compliance was verified through personal interviews at the time of examination. Venous blood samples were obtained by venipuncture. The samples were collected in Vacutainer tubes and allowed to clot for 1 hour at room temperature. Serum was recovered by centrifugation. All analyses were performed on fresh serum. The laboratory was blind to the status of the samples tested.

Each participant underwent a medical examination at the beginning of the study. Specific data that were collected included family history, parental age, height, weight, smoking history, and drug and medication use (including oral contraceptives). Smoking was measured by the number of cigarettes smoked daily. BMI was expressed as weight over height squared (kilograms/square meters). Tanner’s stage was determined to control for the effect of sexual maturation on serum lipids and apolipoproteins.12,13

Serum Lipid, Lipoprotein, and Lipoprotein Particle Analyses

Serum total cholesterol and triglycerides were measured by enzymatic methods (Boehringer Mannheim, FRG) adapted to a Hitachi 705 analyzer. Cholesterol was measured in the HDL-containing supernatant after sodium phosphotungstate/magnesium chloride precipitation (Boehringer Mannheim). An estimate of the LDL-C level was computed according to Friedewald’s formula.14 ApoAI and apoB were quantified by immunonephelometry (Behringwerke, Marburg, FRG). LpAI was quantified by differential electroimmunoassay on ready-to-use plates: lipoprotein particles containing both apoAI and apoAII were retained close to the wells when an excess of anti-apoAII was used, whereas the LpAI lipoprotein particles migrated and reacted with anti-apoAI.15 LpAI:A1, LpAI:B, and LpAII:B were measured by two-site immunoenzymatic assay as described elsewhere.16

Statistical Analysis

The descriptive results and univariate relations were analyzed using the SAS statistical software release 6.04 (SAS Institute Inc, Cary, NC). To take advantage of the matched study, the univariate relations were computed on the differences in laboratory values between each case group member and the mean value of the two matched control subjects. The mean of the differences was tested with a Student’s t test under the hypothesis that this mean equaled zero. A multivariate analysis was developed with a computer program of multiple logistic regression for matched data.17

The distributions of the different variables were first analyzed. Log-normal distributions such as triglycerides and LpE:B were transformed. Conditional multiple logistic regression was used to predict the probability of myocardial infarction in the parents according to serum levels of the variables assessed in their offspring. In an attempt to improve the power of the analysis, an a posteriori adjustment was made by introducing the age and BMI of the adolescent into the equations. Models were constructed to assess whether additional information supplied by the different explanatory variables, after the covariates were taken into account, significantly increased the predicted probability of parental myocardial infarction in a stepwise procedure. The log values of the likelihood for two models, one of which was a special case of the other, were calculated, and a log-likelihood ratio test was performed with degrees of freedom equal to the number of variables differing between the two models.18 χ² test and analysis of percentiles were also used. Significant differences were accepted when statistics resulted in a two-tailed probability of <.01. Because of the different levels of the lipid and lipoprotein variables in adolescent males and females a separate analysis by sex was performed.19

Results

Profiles of the case group and the control group are shown in Table 1. There were no significant differences between the groups on the matching variables. Sex ratios (male frequency to female frequency) were equal in the two groups and almost equal to 1. The sex ratio of affected parents showed a predominance of myocardial
infarction in fathers (n=91) compared with mothers (n=6), precluding a separate analysis. Age, BMI, smoking, and oral contraceptive use were identical in the two groups. Mean age of affected parents did not differ and was below 55.

Total cholesterol and triglyceride plasma levels (Table 2) were compared in univariate matched analysis and did not differ between the two groups. A significant difference was observed for HDL-C level (P<.0001) and for apoAI levels (P<.01). LDL-C fraction and apoB levels did not show any significant difference between case and control subjects, nor did the apoAI/apoB ratio differ. A significant difference between the two groups was observed for the LpAI level (P<.0001). No differences between the case and control groups were observed for LpAlhAI, LpE:B, or LpCIII:B levels.

For all the variables, the two groups were compared in a separate conditional multiple logistic regression for matched data that took into account age, BMI, smoking habits, and oral contraceptive use. For cases with family history of myocardial infarction, n=97; for controls, n=194.

Two models were then fitted: one to the lipid and lipoprotein variables, the other to the apolipoprotein and particle variables. In the first model, when total cholesterol, triglycerides, HDL-C, and LDL-C were introduced simultaneously, only HDL-C was found significantly different between the case and control groups (P<.00003). In the second model, when apoAI, apoB, LpAI, LpAlhAI, LpCIII:B, and LpE:B were introduced simultaneously, only LpAI had a significant coefficient (P<.000006).

We analyzed male and female serum levels separately (Table 4). HDL-C and LpAI serum levels were higher in the adolescent women than in the adolescent men in both groups. When computed separately by gender, two logistic models gave a higher coefficient for LpAI (-8.33, P<.01) than for HDL-C (-7.91, P<.01) in men, and a higher coefficient for HDL-C (-9.89, P<.001) than for LpAI (-5.45, P<.01) in women.

**Discussion**

Atherosclerosis is a lifelong disease process that begins in childhood and culminates in clinical disease in middle age or later. Atherosclerosis first appears as deposits of lipids (mainly cholesterol and cholesteryl ester), called fatty streaks, in the intima of muscular and elastic arteries as early as the second decade of life. Associations are described between the extent of fatty.

### Table 1. Clinical Data in Cases With Family History of Early Myocardial Infarction and in Control Subjects

<table>
<thead>
<tr>
<th>Clinical Data</th>
<th>Case Group (n=97)</th>
<th>Control Group (n=194)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex ratio, M/F</td>
<td>41/56</td>
<td>82/112</td>
</tr>
<tr>
<td>Age, y</td>
<td>17.45 (1.16)</td>
<td>17.31 (1.18)</td>
</tr>
<tr>
<td>Age of parents, y</td>
<td>48.32 (6.43)</td>
<td>47.64 (6.10)</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>20.96 (2.39)</td>
<td>20.91 (2.31)</td>
</tr>
<tr>
<td>Regular smokers, %</td>
<td>25.8</td>
<td>21.1</td>
</tr>
<tr>
<td>Oral contraceptive use, %</td>
<td>28.6</td>
<td>18.8</td>
</tr>
</tbody>
</table>

M indicates male and F, female. Values are mean (SD) where appropriate. There were no significant differences among any of the listed variables. Age of parents in the control group were matched with age of coronary heart disease-affected parents in the case group.

### Table 2. Comparison of Plasma Lipid, Lipoprotein, and Lipoparticle Levels in Adolescents With Family History of Early Myocardial Infarction and in Control Subjects

<table>
<thead>
<tr>
<th>Variable</th>
<th>Coefficient</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>-0.36</td>
<td>-0.96</td>
<td>NS</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>-0.63</td>
<td>-1.62</td>
<td>NS</td>
</tr>
<tr>
<td>HDL-C</td>
<td>-6.72</td>
<td>-4.32</td>
<td>&lt;.0002</td>
</tr>
<tr>
<td>LDL-C</td>
<td>-0.20</td>
<td>-0.58</td>
<td>NS</td>
</tr>
<tr>
<td>ApoAI</td>
<td>-1.84</td>
<td>-2.61</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>ApoB</td>
<td>-0.28</td>
<td>-0.43</td>
<td>NS</td>
</tr>
<tr>
<td>LpAI</td>
<td>-5.37</td>
<td>-4.05</td>
<td>&lt;.0005</td>
</tr>
<tr>
<td>LpAlhAI</td>
<td>-1.12</td>
<td>-1.61</td>
<td>NS</td>
</tr>
<tr>
<td>LpE:B</td>
<td>-0.05</td>
<td>-0.21</td>
<td>NS</td>
</tr>
<tr>
<td>LpCIII:B</td>
<td>-2.00</td>
<td>-0.38</td>
<td>NS</td>
</tr>
</tbody>
</table>

NS indicates not significant; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; ApoAI, apolipoprotein AI; ApoB, apolipoprotein B; LpAI, LpAI-containing lipoparticles without apoAI; LpAlhAI, LpAI-containing lipoparticles with apoAI; LpAlhAI, LpAI-containing lipoparticles with apoAI; LpE:B, apoB-containing lipoparticles with apoE; and LpCIII:B, apoB-containing lipoparticles with apoCIII. Values are mean (SD).
streaks in the coronary arteries of young persons and that of fibrous plaques in adults, which are closely associated with clinical CHD.20,21 Furthermore, the Pathobiological Determinants of Atherosclerosis in Youth (PDAY) research group22 has demonstrated that the percentage of intimal surface involved with atherosclerotic lesions is positively associated with serum very-low-density lipoprotein cholesterol + LDL-C concentrations and negatively associated with serum HDL-C concentrations in young adults, suggesting that plasma lipoprotein levels play an important role in the early onset of atherosclerosis.

The results of the present study demonstrated an association between plasma HDL and LpAI lipoprotein levels in adolescents with a risk factor of atherosclerosis, ie, a family history of early myocardial infarction. These lipoprotein levels were lower in serum of individuals at risk. These results were in good accordance with previous studies that demonstrate abnormalities in lipid and apolipoprotein levels in individuals with a parental history of myocardial infarction. Freedman et al8 reports a statistically significant association between low plasma levels of apoAI and parental history of myocardial infarction in children. Rosseneu et al7 obtained similar results in university students. The offspring of patients who suffered a myocardial infarction before the age of 50 years had lower apoAI levels, higher HDL-C/apoAI ratios, and lower levels of HDL-C subfractions compared with control subjects, but the levels of LDL-C and apoB did not differ significantly between the two groups.7 Conversely, Cambien et al6 report an increased concentration of apoB in adults with a parental history of myocardial infarction compared with control subjects. Finally, in first-degree relatives of patients with angiographically defined coronary artery disease, Kukita et al23 report lower levels of apoAI and HDL-C and higher levels of apoB and triglycerides than in control subjects. Several hypotheses may account for these variable associations with the different lipoprotein subfractions. First, plasma lipid and lipoprotein levels vary during different periods of life. For example, statistically significant familial correlations between HDL-C levels of parents and offspring are detected only between the ages of 15 and 20 years.24 That may explain why significant relationships with HDL-C and apoAI levels are found mainly in studies including subjects under 20 years of age.5,6,7,25 and not in studies including subjects over 20 years.3,4 This heterogeneity associated with age could be due to interactions between genetic and environmental factors that might only become apparent in adults and mask the effects of constitutional factors. Our sample, composed of adolescents whose mean age was 17.5 years, spanned a favorable period of life for finding relations with HDL subfractions and LpAI. Second, imprecise measurements of confounding factors may account for another source of variation in familial aggregation studies. Associations with LDL-C and apoB levels are strongly influenced by environmental factors and can disappear when strict matching of controls and precise measurement of covariates are performed.26 Case subjects of the present study were matched to control subjects for an important number of factors (age, gender, BMI, smoking habit, and oral contraceptive use) and differences in their plasma levels of LDL-C and apoB were not found when compared with the control group. Finally, the ascertainment of myocardial infarction diagnoses in parents is achieved through an interview in most of the studies.5,6 An excess of false-positive heart attacks could modify the power of these studies.26 In our sample, all the diagnoses of myocardial infarction were reviewed according to WHO-MONICA criteria through an inspection of all relevant medical records. This protocol allowed us to exclude at least 25% of the cases recruited through interview.

ApoAI-containing lipoprotein particle plasma levels were inversely associated with a family history of myocardial infarction: LpAI levels exhibited a strong relation but not LpAll:AI levels. In addition, when data for men and women were analyzed separately, low HDL-C

<table>
<thead>
<tr>
<th>Variable</th>
<th>Male Cases (n=41)</th>
<th>Male Controls (n=82)</th>
<th>Female Cases (n=56)</th>
<th>Female Controls (n=112)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>4.39 (0.77)</td>
<td>4.33 (0.75)</td>
<td>4.90 (1.34)</td>
<td>5.01 (0.95)</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>0.76 (0.30)</td>
<td>0.80 (0.29)</td>
<td>0.81 (0.43)</td>
<td>0.80 (0.33)</td>
</tr>
<tr>
<td>HDL-C, mmol/L</td>
<td>1.29 (0.23)*</td>
<td>1.39 (0.26)</td>
<td>1.42 (0.21)†</td>
<td>1.63 (0.31)</td>
</tr>
<tr>
<td>LDL-C, mmol/L</td>
<td>2.71 (0.72)</td>
<td>2.58 (0.67)</td>
<td>3.12 (1.26)</td>
<td>3.04 (0.88)</td>
</tr>
<tr>
<td>ApoAI, g/L</td>
<td>1.39 (0.18)</td>
<td>1.42 (0.19)</td>
<td>1.48 (0.20)†</td>
<td>1.57 (0.21)</td>
</tr>
<tr>
<td>ApoB, g/L</td>
<td>0.60 (0.17)</td>
<td>0.78 (0.18)</td>
<td>0.89 (0.31)</td>
<td>0.88 (0.22)</td>
</tr>
<tr>
<td>ApoAl/ApoB</td>
<td>1.82 (0.49)</td>
<td>1.93 (0.54)</td>
<td>1.80 (0.52)</td>
<td>1.88 (0.49)</td>
</tr>
<tr>
<td>LpAI, g/L</td>
<td>0.46 (0.09)*</td>
<td>0.51 (0.11)</td>
<td>0.53 (0.11)†</td>
<td>0.60 (0.15)</td>
</tr>
<tr>
<td>LpAll:A, g/L</td>
<td>0.75 (0.15)</td>
<td>0.75 (0.17)</td>
<td>0.77 (0.18)</td>
<td>0.77 (0.13)</td>
</tr>
<tr>
<td>LpE:B, g/L</td>
<td>0.17 (0.10)</td>
<td>0.19 (0.10)</td>
<td>0.17 (0.11)</td>
<td>0.17 (0.10)</td>
</tr>
<tr>
<td>LpCIII:B, g/L</td>
<td>0.04 (0.04)</td>
<td>0.05 (0.05)</td>
<td>0.04 (0.04)</td>
<td>0.04 (0.04)</td>
</tr>
</tbody>
</table>

HDL-C indicates high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; ApoAI, apolipoprotein AI; ApoB, apolipoprotein B; LpAI, apolipoprotein particle without apoAI; LpAll:AI, apolipoprotein particle with apoAI; LpE:B, apolipoprotein particle with apoE; and LpCIII:B, apolipoprotein particle with apoCIII. Data are mean (SD).

*P<.05.
†P<.01.
‡P<.001.
and low LpAI concentrations remained associated with parental history of CHD in both sexes. The strength of the relation was higher for LpAI in young men than in women. The plasma levels of AI-related variables were higher in young women, whereas B-related variables did not differ. The different effects of endogenous sex hormones on serum lipids and apolipoproteins19 and the higher content of total cholesterol, cholesteryl ester, and phospholipids in female LpAI particles27 may account for the different discriminative power according to gender. LpAI seemed to be more predictive of family history in men than in women. A still more informative approach would be to measure the cholesterol content of these lipoprotein particles.

In conclusion, our results indicated that adolescents with a family history of early myocardial infarction had decreased levels of HDL-C and LpAI, which may in turn increase their own CHD risk. Further studies and especially family studies are needed to distinguish between the effects of environmental and genetic factors in the occurrence of this familial aggregation.

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References

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