Apo E Allele Frequency in Primary Endogenous Hypertriglyceridemia (Type IV) with and without Hyperapobetalipoproteinemia

Suzanne Lussier-Cacan, Daniel Bouthillier, and Jean Davignon

Apolipoprotein E polymorphism is responsible for the existence in the population of six apo E phenotypes determined by three alleles acting at a single gene locus. We have previously reported an enrichment in the e2 allele and the E2-bearing phenotypes in an unselected sample of subjects with primary hyperlipidemia consisting mainly of endogenous hypertriglyceridemia (Type IV). A study was carried out on 214 Type IV hypertriglyceridemic subjects to determine whether there was the same distribution in subjects with hyperapobetalipoproteinemia as in those without. The study showed that the relative enrichment in the e2 allele was associated only with Type IV subjects without hyperapobetalipoproteinemia. Since hyperapobetalipoproteinemia is a presumed marker for familial combined hyperlipidemia (FCHL), this finding may provide further evidence that FCHL and familial hypertriglyceridemia, both associated with a Type IV lipoprotein pattern, are truly separate disease entities.

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There are three major isoforms of apolipoprotein E (apo E), a protein constituent of plasma very low density lipoprotein (VLDL): E2, E3, and E4, which are under the control of three independent alleles (c2, c3, c4) acting at a single gene locus. As a result, six major apo E phenotypes occur in the population: three homozygotes, E4/4, E3/3 (the most frequent), and E2/2, and three heterozygotes, E4/3, E3/2 and E4/2.1 The phenotype E2/2 is associated with Type III hyperlipidemia.2,3 While a higher prevalence of apo E4 has been reported in Type V hyperlipoproteinemia.4 In the course of our own studies on the polymorphism of apo E, we noted that the c2 allele predisposes to higher triglyceride levels and lower low density lipoprotein (LDL) cholesterol concentrations, whereas the c4 allele is associated with higher cholesterol and LDL cholesterol levels.5–7 Of particular significance was the observation that the c4 allele raises LDL cholesterol in normolipidemic individuals.6,7 Two independent studies8,9 have reported an increased frequency of the allele c4 in “hypercholesterolemia” defined as cholesterol levels above 280 mg/dL or 260 mg/dL and normal triglycerides. We further reported6 an enrichment in the apo E2-bearing phenotypes in an unselected hyperlipidemic population consisting mainly (76%) of individuals with the Type IV lipoprotein phenotype (endogenous hypertriglyceridemia, hyperprebetalipoproteinemia). Endogenous hypertriglyceridemia represents a group of heterogeneous disease entities with different etiologies and a varied degree of associated risk for atherosclerosis.10 Two major hereditary disease entities harboring a Type IV phenotype may be considered, one with an associated elevation of LDL-apo B levels and believed to represent familial combined hyperlipidemia (FCHL) which is a multiple lipoprotein-phenotype disease; the other, familial hypertriglyceridemia (FHTG), in which LDL-apo B levels are normal.11–13 In this report, we examine the apo E allele frequency distribution of Type IV hypertriglyceridemic individuals to see whether the enrichment in the c2 allele we previously observed is found equally in patients with and without hyperapobetalipoproteinemia.

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Methods

Subjects

Subjects with endogenous hypertriglyceridemia were selected from among the new untreated patients referred to our lipid clinic between November 1982 and November 1984. Informed consent was obtained and the study protocol was approved by the institutional Ethics Committee. Included in the study were 214 individuals fulfilling the following criteria: presence after a 12-hour fast of triglyceride levels between 200 and 1000 mg/dl, a prebetalipoprotein on paper electrophoresis, VLDL cholesterol >35 mg/dl and LDL-C <190 mg/dl, absence of chylomicrons and β-VLDL in the fasting state, and absence of secondary causes of hyperlipidemia.

Type IV patients were then separated into two groups according to their LDL-apo B levels. Group 1 had LDL-apo B ≥125 mg/dl (above the 95th percentile of our control population) and Group 2, ≤104 mg/dl (below the 90th percentile of our controls). Group 1 was designated as having hyperapo beta-lipoproteinemia according to the criteria defined by Sniderman et al.14 (LDL-C <200 mg/dl, LDL-apo B >2 SD above normal mean). Patients with intermediate levels (n = 49, LDL-apo B >104 <125 mg/dl) were excluded from the study.

Normolipidemic controls were 102 civil servants from Ottawa, Canada, who were participating in a health survey program.6,7 Their cholesterol and triglyceride levels were below 240 mg/dl and 150 mg/dl respectively.

The unselected hyperlipidemic sample (102 consecutive patients referred to our lipid clinic) which led to our original observations8 was included in this study for comparison.

Procedures

In the morning after the patients had fasted for 12 to 14 hours, blood was collected from an antecubital vein into Vacutainer tubes (Becton-Dickinson) containing Na2EDTA (1 mg/ml). Sodium azide (0.02%) was added to the separated plasma. Cholesterol15 and triglycerides16 were measured enzymatically on an automated analyzer (ABA-100 bichromatic analyzer, Abbott Laboratories, Pasadena, California). The plasma lipoprotein profile was examined by paper electrophoresis according to the method of Lees and Hatch.17 Lipoproteins were separated for determination of VLDL, LDL, and HDL cholesterol by ultracentrifugation at a density = 1.006 g/ml and heparin-manganese precipitation.18

Total and LDL-apo B concentrations were measured by electroimmunoassay (EIA) on total plasma and on the d >1.006 ultracentrifugal fraction, essentially by the technique of Reardon et al.18 VLDL-apo B was obtained by subtracting the LDL-apo B from the total plasma value. An LDL fraction isolated between densities 1.030 and 1.050 g/ml was used for standardization with the addition of lipoprotein-deficient serum (d =1.21 g/ml) as suggested by Roseneu et al.19 In each assay, a commercial serum of known apo B concentration (Behringwerke, Marburg, West Germany) was used as a reference standard. The apo E phenotype was obtained by isoelectric focusing of the soluble apoproteins of VLDL on a single polyacrylamide gel.5

Results

Mean values (± sd) for plasma lipid, lipoprotein cholesterol, and apo B levels are given in Table 1 for normal controls and for the different groups of subjects with hyperlipidemia. The two groups of Type IV individuals (Group 1 with and Group 2 without hyperapo B) were different from the point of view of total triglyceride and cholesterol levels, Group 1 having higher cholesterol, and Group 2, higher triglycerides. The cholesterol difference was accounted for by LDL cholesterol, which, in Group 1, was considerably higher than in Group 2 (+73%). The difference in LDL-apo B levels was of a similar magnitude. There

| Table 1. Plasma Lipids, Lipoproteins, and Apolipoprotein B in Normolipidemic and Hyperlipidemic Subjects |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                                  | Normal lipids   | Unselected       | Hyperlipidemias | Type IV hyperlipidemias* |
| Number (males)                  | 102 (55)        | 102 (70)         | 93 (65)         | 72 (57)         | 72 (47)         | 38 (30)         |
| Age (yrs, mean ± sd)           | 36.1 ± 8.5      | 45.9 ± 12.5      | 47.0 ± 12.1     | 43.7 ± 13.6     | 49.4 ± 12.2     | 45.6 ± 13.0     |
| Total cholesterol (mg/dl)      | 174 ± 29        | 262 ± 83         | 267 ± 37†       | 212 ± 36†       | 258 ± 31†       | 195 ± 28†       |
| Triglycerides (mg/dl)          | 74 ± 30         | 506 ± 548        | 384 ± 176†      | 501 ± 235†      | 306 ± 79        | 309 ± 89        |
| VLDL-cholesterol (mg/dl)       | 24 ± 13         | 94 ± 24          | 82 ± 36         | 94 ± 41         | 68 ± 21         | 65 ± 21         |
| VLDL-apo B (mg/dl)             | 12 ± 10         | ND†              | 68 ± 38         | 82 ± 43         | 54 ± 23         | 51 ± 24         |
| LDL-cholesterol (mg/dl)        | 103 ± 27        | 132 ± 65         | 152 ± 20†       | 88 ± 22†        | 157 ± 18†       | 98 ± 22†        |
| LDL-apo B (mg/dl)              | 83 ± 21         | ND               | 144 ± 16†       | 82 ± 14†        | 146 ± 15†       | 85 ± 15†        |
| HDL-cholesterol (mg/dl)        | 47 ± 12         | 33 ± 10          | 32 ± 9          | 29 ± 9          | 33 ± 6          | 33 ± 9          |

*Groups 1 and 1a = LDL-apo B ≥125; Groups 2 and 2a = LDL-apo B ≤104. Subjects with triglycerides >500 mg/dl were excluded from Groups 1a and 2a.
†Groups 1 vs 2 and 1a vs 2a were significantly different at p < 0.001.
‡Not determined.
Table 2. Apo E Phenotype and Allele Distribution In Normolipidemic and Hyperlipidemic Subjects

<table>
<thead>
<tr>
<th>Phenotypes</th>
<th>Normal subjects</th>
<th>Unselected hyperlipidemics</th>
<th>Type IV hyperlipidemias*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apo E-4/4</td>
<td>4 (3.9)</td>
<td>2 (2.1)</td>
<td>2 (5.3)</td>
</tr>
<tr>
<td>Apo E-3/3</td>
<td>63 (61.7)</td>
<td>53 (57.0)</td>
<td>36 (50.0)</td>
</tr>
<tr>
<td>Apo E-2/2</td>
<td>2 (2.0)</td>
<td>3 (2.9)</td>
<td>42 (58.3)</td>
</tr>
<tr>
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<td>21 (20.6)</td>
<td>21 (22.6)</td>
<td>10 (13.9)</td>
</tr>
<tr>
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<td>18 (25.0)</td>
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<td>6 (6.5)</td>
<td>4 (5.5)</td>
</tr>
<tr>
<td>Total n (%)</td>
<td>102 (100.0)</td>
<td>93 (100.0)</td>
<td>72 (100.0)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Alleles</th>
<th></th>
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<tbody>
<tr>
<td>e4</td>
<td>0.152</td>
<td>0.167</td>
<td>0.153</td>
</tr>
<tr>
<td>e3</td>
<td>0.770</td>
<td>0.742</td>
<td>0.694</td>
</tr>
<tr>
<td>e2</td>
<td>0.078†</td>
<td>0.091</td>
<td>0.153†</td>
</tr>
</tbody>
</table>

*Groups 1 and 1a = LDL-apo B >125; Groups 2 and 2a = LDL-apo B <104.
†Group 2 vs normals = p < 0.04; Group 2a vs Group 1a and normals = p < 0.05.

was more fluctuation in VLDL cholesterol within both groups, but HDL cholesterol levels were not significantly different. Thus, we have two heterogeneous samples that nonetheless meet the same basic criteria for Type IV hyperlipidemia.

Another major difference between these two Type IV groups was noted in the apo E phenotype distribution (Table 2). There was enrichment in the e2 allele at the expense of the e3 allele in Group 2 (Group 2 vs normals, χ² = 11.39, p < 0.04) as was previously observed in our unselected hyperlipidemics, and this was evident at all triglyceride levels (not shown). Conversely, the allele distribution of Group 1 was the same as in normal individuals. Because Group 2 included more individuals with marked hypertriglyceridemia (>500 mg/dl) than Group 1, we decided to look only at individuals whose triglyceride levels were between 200 and 500 mg/dl. These subgroups are identified as Groups 1a and 2a in the tables. The two reduced samples had identical triglycerides and this was evident at all triglyceride levels (not shown). Conversely, the allele distribution of Group 1 was the same as in normal individuals. Since the receptor-binding defect of apo E2 has been demonstrated in hypo- and normolipidemic, as well as in hyperlipidemic E2/2 subjects, it is surmized that one or more additional factors are necessary for hyperlipidemia to develop in this phenotype. Indeed, one of these factors may be another hyperlipidemia gene as suggested by Utermann et al.

The presence of the e2 allele, even in heterozygotes, seems to predispose subjects to hypertriglyceridemia. This was first reported by Utermann et al., who noted that individuals with the apo E-ND phenotype (one e2 allele) had triglyceride levels intermediate between that of normals and of e2 homozygotes. We previously found a higher prevalence of the e2 allele in a hyperlipidemic population that consisted mostly of hypertriglyceridemic Type IV subjects. Consistent with these observations is the possibility that we inherit a predisposition to hypertriglyceridemia that is revealed when the system is overloaded by ecological or hormonal stresses (excessive caloric or alcohol intake, use of oral contraceptives, pregnancy, or hypothyroidism) or other genetic factors (diabetes, increased VLDL production, or decreased VLDL catabolism).

In this study, the primary nature of the hyperlipidemia is assumed on the basis that all secondary causes have been excluded by a thorough clinical and laboratory evaluation at the initial visits. Although a familial history was obtained in all subjects, no systematic study of first-degree relatives was carried out and the presence of occasional cases of nongenetic hyperlipidemia cannot be excluded. Our results show that the e2 allele appears to harbor Type III dysbetalipoproteinemia, as having Type III dysbetalipoproteinemia harbor the E2/2 phenotype, it has been reported that most E2/2 subjects are normolipidemic. Since the receptor-binding defect of apo E2 has been demonstrated in hypo- and normolipidemic, as well as in hyperlipidemic E2/2 subjects, it is surmized that one or more additional factors are necessary for hyperlipidemia to develop in this phenotype. Indeed, one of these factors may be another hyperlipidemia gene as suggested by Utermann et al.

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mine whether the former group represents only familial hypertriglyceridemia (FHTG) and the second only familial combined hyperlipidemia (FCHL), two disorders that have specific abnormalities. Indeed, the major metabolic defect ascribed to FHTG is an increased hepatic synthesis of triglycerides with the accumulation of triglyceride-rich VLDL in plasma, resulting from an apparent saturation of catabolic processes. In FCHL, there is increased synthesis of apo B with an overproduction of both VLDL- and LDL- apo B.

Structural abnormalities of VLDL may also influence its catabolism and determine the amount of density <1.019 g/ml apo B that proceeds to LDL. As has been demonstrated recently, large VLDL particles in hypertriglyceridemia are not a good substrate for LDL formation and appear to be removed by an alternative pathway. Concurrently, reduced amounts of abnormal LDL are formed. In our study, VLDL with apo E2 represent another structural abnormality resulting in impaired LDL formation. It appears that the additional stress imposed by poorly catabolized E2-bearing lipoproteins in subjects with the e2 allele is more likely to produce hyperlipidemia in FHTG. Apo E may also play a significant role in FCHL by allowing the increased production of VLDL to proceed through the pathway to LDL resulting in higher LDL-cholesterol and -apo B levels.

Further, we were able to confirm the findings of Sniderman et al. that a substantial proportion of hypertriglyceridemic subjects also have elevated LDL- apo B levels. The higher incidence of atherosclerotic disease they reported in this group emphasizes the importance of measuring apo B levels in hypertriglyceridemia.

Finally, as we have recently reported, the apo E locus accounts for more than 15% of the genetic variability of plasma cholesterol in a "normal" population. Additional studies are warranted to determine the impact of apo E polymorphism on the expression of various forms of dyslipidemia.

Acknowledgments

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Index Terms: apolipoprotein E polymorphism • apo E allele • endogenous hypertriglyceridemia • hyperapobetalipoproteinemia

Correction

The Editors of Arteriosclerosis regret the error in this abstract, which appeared on page 550a of the September/October issue (Arteriosclerosis 5:550a, 1985) and reprint it in its correct form.

Hepatic Uptake of LDL in Rats Induced by Lactosaminated Fab Antibodies
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Proteins modified by conjugation with lactose residues often exhibit increased hepatic uptake mediated by the galactose or asialoglycoprotein receptor. As reagents to promote plasma clearance and hepatic uptake, we prepared lactosaminated Fab fragments of sheep IgG specific for human LDL. Fab fragments of anti-LDL IgG retained their affinity after derivatization. Rats were injected first with 125I-LDL and then after 10 minutes with 1 mg of either lactosaminated Fab (lac-Fab) or Fab. Ten minutes later the radioactivity in the serum of rats treated with lac-Fab was reduced to 47.7% (±3.4%) of the injected 125I-LDL, with an increase of hepatic localization from 3% to 30%. After 60 minutes 78.5% (±5.6%) of 125I-LDL was cleared from the circulation. In the rats treated with Fab or saline, 85% of 125I-LDL remained in the circulation with only 7% present in the liver. These processes were completely inhibited by simultaneous injection of asialofetuin.

These findings suggest that lac-Fab is an effective means to induce the catabolism of LDL by the liver.
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