Apolipoprotein E Polymorphism Association
With Lipoprotein Profile in Endogenous
Hypertriglyceridemia and
Familial Hypercholesterolemia

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Apolipoprotein (apo) E polymorphism was among the first-reported genetic polymorphisms that explained part of the normal variation in plasma cholesterol concentrations in humans. The aim of this study was to assess the influence of allelic variation at the apo E gene locus on the plasma lipoprotein profile in hyperlipidemia. The lipoprotein levels of hyperlipidemic subjects of the major apo E phenotypes (E3/2, E3/3, and E4/3) were compared. One hundred eighty-two subjects with endogenous hypertriglyceridemia and 98 subjects with familial hypercholesterolemia due to a 10-kb deletion in their low density lipoprotein (LDL) receptor genes were compared with 424 normolipidemic controls from the same environmental background. LDL concentrations were lower in the E3/2 subset than in the E3/3 or E4/3 subset in the control, hypertriglyceridemic, and familial hypercholesterolemic groups. In absolute values, the magnitude of the effect was greatest in the familial hypercholesterolemic group. However, the direction and percentage change were identical in the presence or absence of the LDL receptor defect, indicating that the apo E phenotype effect is independent of LDL receptor status. Triglyceride and very low density lipoprotein (VLDL) cholesterol concentrations were higher in E3/2 than in E3/3 or E4/3 hypertriglyceridemic subjects, but this difference was not found in the familial hypercholesterolemic or control group. Thus, there seems to be a specific interaction between apo E isoforms and VLDL metabolism in hypertriglyceridemia; allelic variation at the apo E gene locus seems to be associated with specific alterations in the plasma lipoprotein profile of subjects with well-defined types of hyperlipidemia. (Arteriosclerosis and Thrombosis 1991;11:272-278)

Plasma concentrations of lipoproteins, as well as their metabolic fate, are regulated by apolipoproteins (apos) on their surfaces. Among these apos, apo E is a polymorphic protein that determines the catabolism of chylomicron remnants. Three major alleles (e2, e3, and e4) at a single gene locus determine the phenotypic expression of apo E.1 The E2 isoform differs from the most common E3 by a single amino acid substitution in position 158 (Arg→Cys) of the 299-amino acid chain and displays low-affinity binding to the low density lipoprotein (LDL) receptor in vitro.2 E4 differs from E3 by a single amino acid substitution in position 112 (Cys→Arg) and displays normal affinity for the LDL (B/E) receptor.3 In vivo, there is evidence that apo E2 carried on triglyceride-rich particles is cleared from plasma more slowly and apo E4 more rapidly than is apo E3.4 The genetic polymorphism of apo E contributes to the normal variation of plasma lipid and lipoprotein concentrations in population studies.5 This has been demonstrated in normolipidemic individuals and in various selected populations.6-17 Total and LDL cholesterol concentrations are consistently lower in apo E2-bearing subjects,6-17 whereas apo E4 is associated with increased LDL cholesterol in a variety of population samples.5,8,11,12 The apo E effect on total triglycerides, very low density lipoprotein (VLDL) cholesterol, and high density lipoprotein (HDL) cho-
lesterol concentrations has not been as clearly defined.

Previous work in hyperlipidemic subjects has shown an apparent increased frequency of the e2 and e4 alleles in hypertriglyceridemic and hypercholesterolemic populations, respectively. However, little information is available on the influence of the apo E phenotype on lipid and lipoprotein concentrations in well-defined specific types of dyslipoproteinemias. Earlier work from our laboratory suggested that apo E might modulate the phenotypic expression of and/or the susceptibility to hyperlipidemia. To test this hypothesis, we compared the effect of the apo E phenotype on plasma lipid and lipoprotein levels in three groups of subjects: one with familial hypercholesterolemia (FH), where the lipid accumulation is mainly due to reduced catabolism of LDL; another with endogenous hypertriglyceridemia (HTG), attributable to an overproduction of VLDL; and third, a control group of healthy normolipidemic individuals.

Methods

Subjects

Subjects with hyperlipidemia were selected from among new untreated patients referred to our lipid clinic. Blood samples were obtained before institution of dietary therapy. Control subjects were employees of Hydro-Quebec, a utility company in Montreal, Canada. The protocol was approved by the ethics committee of our institute.

Hypertriglyceridemia. Subjects with endogenous HTG were referred to our lipid clinic between January 1985 and December 1988. All individuals fulfilling the following criteria were included in the study: presence, after a 12-hour fast, of plasma triglyceride levels between 200 and 1,000 mg/dl in the absence of chylomicrons on paper or agarose electrophoresis and LDL cholesterol lower than 190 mg/dl. Subjects with secondary causes of hyperlipidemia, such as diabetes; thyroid, liver, or renal dysfunction; massive obesity; or chronic ethanol ingestion were excluded.

Familial hypercholesterolemia. Subjects with FH were selected on the following basis: LDL cholesterol levels above 190 mg/dl, presence of tendon xanthomas in the patient or in a first-degree relative, and presence of the French-Canadian 10-kb deletion of the LDL receptor gene detected by Southern blot analysis.

Normolipidemic controls. Normolipidemic controls belonged to a cohort of healthy subjects being investigated in our laboratory. Individuals selected for this study had plasma triglyceride levels below 150 mg/dl and total and LDL cholesterol values below 240 mg/dl and 190 mg/dl, respectively.

Any individuals receiving lipid-lowering drugs, hormone therapy, or drugs that might interfere with lipid metabolism were excluded from the study. Only subjects bearing the apo E phenotypes E3/2, E3/3, or E4/3 were selected. This selection procedure yielded 182 HTG subjects (29 women), 98 FH subjects (57 women), and 424 normolipidemic controls (138 women) for study.

Procedures

To study the effect of the apo E phenotype, patients were compared who shared one common apo E allele (e3) but who differed in the other allele (e2, e3, or e4). This approach is logical in view of the expected low numbers of E2/2 and E4/4 homozygotes.

Baseline blood samples were collected after a 12-hour fast in the absence of any lipid-lowering therapy for determination of plasma total, VLDL, LDL, and HDL cholesterol; total triglyceride; and apo B levels. Lipoproteins were separated under standard conditions by a combination of ultracentrifugation at d=1.006 g/ml and heparin/manganese precipitation of the apo B-containing lipoproteins in the d>1.006 g/ml infranatant, according to the Lipid Research Clinics protocol. Plasma total and lipoprotein cholesterol and triglycerides were measured enzymatically on an automated analyzer (Abbott Bichromatic Analyzer 100, Abbott Laboratories, Pasadena, Calif.). Total and LDL apo B were measured by electroimmunoassay in total plasma and in the d>1.006 g/ml ultracentrifugal fraction essentially by the technique of Reardon et al with some modifications. Lipoprotein electrophoresis was initially performed by the procedure of Lees and Hatch and, since September 1987, by the technique of Noble (Beckman Paragon Electrophoresis System, Beckman Instruments, Palo Alto, Calif.). The apo E phenotype was determined as previously described.

Food intake was assessed in the HTG patients by a dietary history combining an assessment of usual daily intake and a food-frequency questionnaire. Food analysis was computed from the Canadian Nutrition Files (Health and Welfare Canada, Health Protection Branch, 1988). Mean plasma levels of glucose and uric acid, as well as food consumption, were similar among apo E phenotype subgroups.

Statistical Analysis

A χ2 test was used to compare gender distribution among the various subsets. One- and two-way analyses of variance (ANOVAS) with group (control, HTG, and FH) and group plus apo E phenotype (3/2, 3/3, and 4/3) as categorical variables, respectively, were performed to assess their effects on lipid and lipoprotein levels. Significant interactions and main effects were then analyzed with multiple-comparison tests corrected according to Bonferroni (p values shown in the text, tables, and figures). Whenever it was noted that the standard deviation of a variable increased with the mean value of the variable (triglycerides, VLDL cholesterol), a logarithmic transformation of the data was done. The analysis was repeated on the log-transformed data.
TABLE 1. Plasma Lipid, Lipoprotein, and Apolipoprotein B Concentrations in Control, Hypertriglyceridemic, and Familial Hypercholesterolemic Subjects

<table>
<thead>
<tr>
<th>Variable</th>
<th>Controls (n=424)</th>
<th>HTG (n=182)</th>
<th>FH (n=98)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (F/M)</td>
<td>138/286</td>
<td>29/153</td>
<td>57/41</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>37.5±7</td>
<td>44.2±4*‡</td>
<td>38.4±11†‡</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.6±2.5</td>
<td>27.3±3.7*</td>
<td>24.1±3.9‡</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>179.9±27</td>
<td>237.6±41.8‡</td>
<td>372.9±52§</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>76.4±29.9</td>
<td>420.4±178.5‡</td>
<td>148.8±80*§</td>
</tr>
<tr>
<td>VLDL cholesterol (mg/dl)</td>
<td>19.9±7.9</td>
<td>85.1±38.8‡</td>
<td>39.9±25.3*§</td>
</tr>
<tr>
<td>LDL cholesterol (mg/dl)</td>
<td>114.9±25</td>
<td>121.9±33.4</td>
<td></td>
</tr>
<tr>
<td>HDL cholesterol (mg/dl)</td>
<td>49.9±10</td>
<td>30.6±8§</td>
<td>36.0±9*†</td>
</tr>
<tr>
<td>VLDL apo B (mg/dl)</td>
<td>8.9±6</td>
<td>72.8±36</td>
<td>...</td>
</tr>
<tr>
<td>LDL apo B (mg/dl)</td>
<td>98.9±21</td>
<td>122.1±30</td>
<td>...</td>
</tr>
</tbody>
</table>

HTG, hypertriglyceridemia; FH, familial hypercholesterolemia; BMI, body mass index; VLDL, very low density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein; apo, apolipoprotein.

One-way analysis of variance (group as independent factor) followed by multiple-comparison test corrected according to Bonferroni (p values): Significantly different from controls (p<0.005, §p<0.0005; ||p<0.05); significantly different from HTG (p<0.005, $p<0.0005).

Results

Comparison Among Groups

Gender distribution was unbalanced among the three groups (control, HTG, FH; Table 1). There were 32% women in the control group and 16% in the HTG and 58% in the FH groups (p<0.001). Preliminary ANOVA indicated significant differences in age (p<0.0001) and body mass index (BMI) (p<0.0001) among groups. Multiple-comparison tests corrected according to Bonferroni confirmed that HTG subjects were significantly older than both controls (p<0.005) and FH patients (p<0.005). Their BMIs were also higher than those of controls (p<0.005) or FH patients (p<0.005).

Mean values for plasma lipid and lipoprotein cholesterol concentrations are presented in Table 1 for the three groups. One-way ANOVA showed a statistically significant group effect for cholesterol, triglycerides, VLDL cholesterol, LDL cholesterol, HDL cholesterol, VLDL apo B, and LDL apo B (p<0.0001). As expected, HTG subjects had higher levels of triglycerides and VLDL cholesterol than did controls, while FH subjects had higher levels of cholesterol and LDL cholesterol than other groups. Triglyceride levels in FH subjects were about twice the values found in controls but were lower than those in the HTG subjects. HDL cholesterol was lower in HTG and FH individuals than in controls, but was higher in the FH than in the HTG group. All these differences reached statistical significance, as specified in Table 1.

Apolipoprotein E Phenotypes and Group Interaction

Table 2 shows the age, BMI, and lipid and lipoprotein concentrations of control and hyperlipidemic subjects as a function of apo E phenotype. Gender distribution was again unbalanced among the three groups but was equally distributed by apo E phenotype (E3/2, E3/3, E4/3) within groups, with the exception of the FH group. There were more women in the FH E3/3 subgroup (p<0.01). However, there was no significant interaction between gender and apo E phenotype within each group for all plasma lipid variables that were studied.

Two-way ANOVA (group, apo E phenotype) showed a significant interaction for the following variables: total cholesterol (p<0.001), triglycerides (p<0.02), and VLDL cholesterol (p<0.0001), suggesting different effects of apo E isoforms in each group. Results of the ANOVA on LDL cholesterol levels also revealed a significant main effect (p<0.0001) for apo E phenotype but without significant interaction (p>0.18), indicating a similar effect of apo E isoforms on LDL cholesterol levels within groups (Figure 1). There were no statistically significant effects or interactions for HDL cholesterol and the VLDL cholesterol/triglyceride ratio.

Multiple comparisons corrected according to Bonferroni were then performed to identify the effect of apo E isoforms within groups. Results are summarized in Table 2. LDL cholesterol was significantly lower in E3/2 subjects than in E3/3 subjects in the control, HTG, and FH groups. Triglycerides and VLDL cholesterol were higher for the E3/2 HTG subjects but not for control or FH subjects. In the FH group, the triglyceride and VLDL cholesterol levels were intermediate between those of controls and HTG subjects. As in the HTG group, a trend to higher values for triglycerides and VLDL cholesterol in the apo E3/2 group was noted. Interestingly, total cholesterol was significantly lower in the FH E4/3 subjects than in the FH E3/3 group (p<0.05), a finding consistent with observations in coronary artery disease subjects.10,32

Since LDL cholesterol levels were inversely correlated with total triglycerides (r=-0.44, p<0.0001) and VLDL cholesterol (r=-0.36, p<0.0001) in the HTG group, the lower LDL cholesterol in the apo E3/2 HTG subjects might be related to their higher triglyceride and VLDL cholesterol levels. Consequently, the influence of the apo E phenotype on LDL cholesterol concentrations in the HTG group was tested with triglyceride or VLDL cholesterol concentration as a covariable. The significant main effect for apo E phenotype on LDL cholesterol concentration persisted after adjustment (p<0.003), further suggesting that this effect was independent of triglyceride and VLDL cholesterol levels.
The VLDL cholesterol/triglyceride ratio was not statistically different among apo E phenotypes. However, when the frequency distribution of the apo E phenotypes was determined at different ratios of VLDL cholesterol/triglycerides (Figure 2), there was a progressive increase in the proportion of apo E3/2 subjects with increasing ratio values ($>0.0005$).

Finally, ANOVA was repeated on the log-transformed data as well as with quantitative variables adjusted for age and BMI using a stepwise regression analysis. These analyses yielded the same results as above.

### Discussion

The results of the present study indicate that lipid and lipoprotein concentration variability within dyslipoproteinemic groups (FH and endogenous HTG) is related to apo E phenotype. This suggests that apo E isoforms specifically modulate the lipoprotein profile expression in each of these hyperlipidemia classes.

The apo E polymorphism was among the first reported to contribute to the normal variation of cholesterol levels in population samples. Subjects carrying the $e2$ allele have lower cholesterol concentrations, whereas the effect of $e4$ in raising cholesterol levels is less pronounced and may depend on associated factors. Similarly, we have observed a significant effect of the $e2$ allele but not of the $e4$ allele in our well-defined dyslipidemic groups (Table 2). It is possible that the specific factors necessary for the expression of a putative $e4$ effect are not present in our sample of hyperlipidemic subjects, therefore explaining the absence of an $e4$ allele effect.
Population studies in normolipidemic individuals have clearly shown that LDL cholesterol concentrations are modulated by the apo E phenotype. We have extended these earlier observations to hyperlipidemic subjects (Figure 2). The e2 allele was found to be associated with lower LDL cholesterol concentrations in control, FH, and HTG subjects, strongly indicating that the effect of apo E2 is independent of the LDL B/E receptor status and of the type of hyperlipidemia. Moreover, the influence of allelic variation at the apo E gene locus on LDL cholesterol concentrations persisted after adjustment for triglyceride levels in HTG. Thus, at the same level of plasma triglycerides, subjects bearing the e2 allele tend to have lower concentrations of LDL cholesterol than do subjects with the e3 or e4 allele in HTG.

The mechanisms whereby apo E2, which is not normally present in LDL, is associated with reduced plasma LDL concentrations remain speculative. Chylomicrons and large VLDL, whose clearance is regulated by apo E, have been postulated as possible effectors. The conjunction of a reduced cholesterol absorption in e2 subjects and defective apo E2 remnant uptake may decrease the delivery of cholesterol to the liver. This may result in the upregulation of liver LDL receptors and accelerated fractional catabolic rates of plasma LDL in apo E2 subjects. Apo E2 may also be less efficient than the other isoforms in promoting VLDL transformation to LDL. Finally, in vitro studies have demonstrated that apo E2 dimyristoylphosphatidylcholine complexes are poorer competitors for the LDL receptor than are those with apo E3 and E4. Extended to remnant metabolism, this might result in lower plasma LDL cholesterol levels in E3/2 subjects.

Unlike LDL, VLDL cholesterol concentrations are modulated by the apo E phenotype only in HTG. Earlier population studies in normolipidemic subjects did not demonstrate consistent effects of apo E on triglyceride and VLDL concentrations. Triglyceride levels were either higher in E3/2 subjects or lower, when compared with those of E3/3 or E4/3 subjects, suggesting no consistent effect in normolipidemic populations. Our findings in control subjects are in agreement with these observations. Conversely, in HTG but not in control or FH subjects, apo E2 is clearly associated with higher triglyceride and VLDL cholesterol concentrations. This suggests that apo E2 may alter VLDL metabolism only in the presence of VLDL overpro-
duction and/or defective catabolism, consistent with the pathophysiology of type III hyperlipidemia.33

There is evidence indicating that impaired VLDL catabolism in E3/2 HTG subjects may increase VLDL concentrations. First, defective lipolysis of apo E2–rich particles was found in vitro.40,41 Second, human metabolic studies have shown delayed plasma clearance of apo E2–containing lipoproteins.3 Third, VLDL from HTG subjects but not from normolipidemic subjects bind to the LDL B/E receptor via apo E in vitro.42 Our observations are consistent with these apparent differences (interaction) in metabolism of normal and hypertriglyceridemic VLDL (and apo E isoforms).

Elevated levels of VLDL in subjects with HTG may also result from the accumulation of triglyceride-rich lipoprotein remnants of both intestinal and hepatic origin.43,44 Apo E2 could exacerbate this potentially atherogenic effect. Experiments with orally administered fat loads have demonstrated that HTG subjects have a lower rate of clearance of chylomicron remnants than do normotriglyceridemic subjects.45 The same defect is found in normolipidemic E3/2 subjects relative to normolipidemic E3/3 and E4/3 subjects.46,47 In agreement with these observations, we found more subjects with the E3/2 phenotype as the VLDL cholesterol/triglyceride ratio increased, consistent with chylomicron remnant accumulation (Figure 2). Alternatively, cholesterol enrichment of apo E3/2 HTG VLDL might be explained by the longer exposure of VLDL-containing apo E2 to lecithin–cholesterol acyltransferase and plasma cholesterol ester transfer protein activity.

Lower levels of total cholesterol are also found in apo E3/2 controls, but differences reach statistical significance only in FH. In both groups, the effect is accounted for by the reduction in LDL cholesterol. Conversely, the lower concentrations of LDL cholesterol in apo E3/2 HTG subjects were balanced by higher values of VLDL cholesterol, with no resultant effect on total cholesterol concentrations in HTG. The impact of this last plasma lipoprotein pattern on cardiovascular risk is not predictable, since one might expect increased atherogenicity because of remnant accumulation, but reduced risk because of lower LDL cholesterol concentrations.

In summary, we have shown that variation in apo E phenotype, reflecting allelic variation at the apo E gene locus, is associated with significant alterations of plasma lipid and lipoprotein concentrations in well-characterized subjects with two widely differing forms of dyslipoproteinemia. The apo E2 isoform is associated with low plasma LDL cholesterol irrespective of the type of dyslipidemia and LDL receptor status. By contrast, it is associated with elevated VLDL cholesterol only in the presence of VLDL overproduction and/or defective catabolism.

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