Severe Hyperlipidemia in Apolipoprotein E2 Homozygotes Due to a Combined Effect of Hyperinsulinemia and an SstI Polymorphism


Abstract—More than 90% of patients with type III hyperlipoproteinemia are homozygous carriers of the apolipoprotein (apo) E*2 allele. The great majority of these apoE2(Arg158→Cys) homozygotes in the general population, however, are normolipidemic. Apparently, expression of the hyperlipidemic state requires additional genetic and/or environmental factors, suggesting a multifactorial etiology. To elucidate these additional risk factors, we analyzed normolipidemic and hyperlipidemic apoE2 homozygotes. Hyperinsulinemia was observed in 27 of 49 apoE2 homozygotes and associated with elevated lipid levels: hyperinsulinemic apoE2 homozygotes had type III hyperlipoproteinemia 6 times more often than apoE2 homozygotes with normal insulin levels (odds ratio 6.2, P=0.02). We screened the normolipidemic and hyperlipidemic apoE2 homozygotes for common variants in candidate genes involved in lipolysis—the APOA1-C3-A4 gene cluster, lipoprotein lipase, and hepatic lipase—and analyzed for associations with the expression of hyperlipidemia. In the hyperinsulinemic group, the 7 carriers of the SstI polymorphism (S2) in the APOC3 gene displayed severely elevated VLDL cholesterol (Pinsulin by SstI<0.001) and VLDL triglyceride (Pinsulin by SstI<0.01) and low levels of HDL (Pinsulin by SstI<0.02). In the normoinsulinemic group, no such relation of the SstI polymorphism with hyperlipidemia was observed. These data provide the first evidence for a combined effect of hyperinsulinemia and the SstI polymorphism on the expression of hyperlipidemia in apoE2 homozygotes. (Arterioscler Thromb Vasc Biol. 1999;19:2722-2729.)

Key Words: apolipoprotein E ∙ hyperlipoproteinemia type III ∙ hyperinsulinemia ∙ xanthomas

Atherosclerosis has a complex etiology in which hyperlipidemia accelerates the deposition of lipids in the artery wall.1,2 One of the inherited lipid disorders, type III hyperlipoproteinemia (HLP), is characterized by dysfunctional apolipoprotein (apo) E and by increased serum concentrations of both cholesterol and triglyceride.3 Biochemically, the disorder is characterized by the presence of β-VLDL particles, which are cholesterol-enriched chylo micron and VLDL remnants. More than 90% of the patients with type III HLP are homozygous for a specific isoform of apoE: apoE2(Arg158→Cys).4 Compared with the other common isoforms, apoE3 and E4, apoE2 has <2% binding activity for hepatic lipoprotein receptors,5,6 which renders apoE2 homozygotes susceptible to accumulation of circulating remnant lipoprotein particles. However, the majority of apoE2 homozygotes in the general population are normolipidemic or even hypolipidemic.7,8 The latter is due to low plasma LDL levels as a result of (1) compensatory upregulated expression of LDL receptors on the surface of hepatocytes to maintain a normal intrahepatocellular cholesterol concentration and (2) a delayed conversion of IDL into LDL in plasma.9 The expression of overt hyperlipidemia in apoE2 homozygotes occurs despite compensatory mechanisms, when additional genetic and/or environmental factors result in large amounts of circulating remnants.4

For the majority of the apoE2 homozygotes with type III HLP, the additional factors causing hyperlipidemia are not known. It has been reported that uncontrolled diabetes mellitus10,11 and hypothyroidism12 occasionally contribute to the expression of type III HLP in apoE2 homozygotes. Obesity associates with type III HLP, which suggests an influence of insulin resistance.13,14 Even though insulin resistance is a frequent disorder of metabolism that is consistently associated with increased levels of triglyceride-rich lipoproteins, its contribution to hyperlipidemia in apoE2 homozygotes is unknown.15 Common variants of other proteins involved in lipolytic conversion, such as lipoprotein lipase (LPL), hepatic lipase (HL), and apoC3, may contribute to the accumulation of triglyceride-rich particles in the circulation and lead to the expression of type III HLP in apoE2 homozygotes. This hypothesis is supported by findings of others showing that (1) LPL gene mutations aggregate in hyperlipidemic apoE2 carriers,16 and several LPL gene mutations associate with
dyslipidemia17–20; (2) HL activity is much less stimulated by apoE2 than by apoE321; (3) apoC3 inhibits LPL22 and reduces the hepatic uptake of triglyceride-rich remnant particles23,24; (4) in mice, apoC3 was shown to decrease the lipolysis at the cell surfaces25; and (5) in various human populations, the SsrI polymorphism (S2) of the APOC3 gene associates with hypertriglyceridemia and coronary artery disease.26–30 The SsrI polymorphism is located in the 3′ noncoding region of the APOC3 gene and does not lead to a change in the amino acid sequence of the protein. Probably, the SsrI polymorphism is in linkage disequilibrium with hitherto unknown mutation(s) causing hypertriglyceridemia.24,28,31–33

The availability for research of both normolipidemic and hyperlipidemic apoE2 homozygotes enabled us to investigate additional risk factors required for the overt expression of type III HLP. Here, we report the association of (1) fasting insulin levels and (2) common polymorphisms in 3 candidate genes—LPL, HL, and the APOA1-C3-A4 gene cluster—with the expression of type III HLP in apoE2 homozygotes.

Methods

ApoE2(Arg158→Cys) Homozygotes

ApoE phenotypes were determined according to a standard procedure34 and were confirmed by APOE genotyping as previously described by others.35 A total of 49 homozygous carriers of apoE2(Arg158→Cys) were identified. Ten unrelated carriers were detected during a population-based study among 2018 randomly selected 35-year-old men36 and were analyzed in 1985 at age 35 years and again in 1995 (present study) at age 45 years. Between July 1988 and December 1990, 895 probands, who were unrelated up to the third degree, with inherited HLP were referred to our Lipid Clinic. Among them, a total of 25 probands were identified with homozygosity for apoE2. Routine measurement of the lipids by general practitioners yielded 13 of these 25 patients; a further 6 were identified after having onset of symptoms of coronary artery disease; another 6 patients were identified by lipidologists/internists of other hospitals in our region, and those patients were referred to our university outpatient Lipid Clinic for diagnostic reasons (ultracentrifugation and apoE genotyping).

Eight more men >34 years old and 6 women >38 years old were found by screening of 5 families of Lipid Clinic patients and 3 families of apoE2 homozygotes detected during the population-based study.

This combination of selection methods allowed us to include normolipoproteinemic and hyperlipoproteinemic apoE2 homozygotes in the study. The diagnosis of type III HLP among our apoE2 homozygotes was based on a ratio of VLDL cholesterol to serum triglyceride >0.689. In apoE2 homozygotes, high ratios reflect the presence of β-VLDL, which is indicative for the expression of type III HLP.5

With the exception of the sampling of 8 normolipidemic 35-year-old men in 1985, all blood samples were obtained after an overnight fast. The insulin concentration was determined by a radioimmunoassay (Ins-Ria-100, Medgenix) in which the antibody cross-reacts with proinsulin (40%) but not with C-peptide. Fasting insulin levels measured with a similar radioimmunoassay were shown to correlate consistently with insulin resistance in normoglycemic subjects.37 The other methods of the clinical chemical analyses have been described previously.38 Informed consent was given by each participant, and the study was approved by the Ethics Committee of our hospital.

Molecular Analyses of Candidate Genes

Identification of the mutations in the LPL and HL genes was performed with polymerase chain reaction followed by specific restriction digestion as previously described by others: LPL(Asp9→Asn),17 LPL(Asn291→Ser),19 LPL(Ser447→Ter),39 and HL(Val73→Met).40 Carriers of the SsrI polymorphism in exon 4 of the APOC3 gene were detected by PCR and restriction enzyme analysis: homozygosity for the wild-type allele was designated S1S1, and heterozygosity for the polymorphic variant at the restriction site was designated S1S2.24 The positions −482 and −455 of the promoter region of the APOC3 gene were screened for 2 frequently occurring point mutations.41 Two MapI sites at positions −75 and +83 in the APOA1 gene,42 which is located downstream of the SsrI polymorphism, were screened to perform haplotyping.

Statistical Analyses

Fisher exact tests were applied to compare allele frequencies between groups. The frequencies and the probabilities of allele-specific haplotypes were estimated by the expectation maximization resampling method and maximum-likelihood statistics.63 All lipid and lipoprotein values were presented as mean±SEM. Analyses were adjusted for age, sex, and fasting glucose concentration with both multiple linear regression and 2-way ANCOVA. The findings of the regression analyses were identical to those of 2-way ANCOVA. Statistical significance was assessed at the 5% level of probability. Statistical analyses of serum triglycerides and VLDL triglycerides were performed after logarithmic transformation.

Results

General Characteristics

The total of 49 apoE2 homozygotes comprised 32 men and 17 women. Their median ages were 44 years (range 27 to 83 years) and again in 1995 (present study) at age 45 years. Between July 1988 and December 1990, 895 probands, who were unrelated up to the third degree, with inherited HLP were referred to our Lipid Clinic. Among them, a total of 25 probands were identified with homozygosity for apoE2. Routine measurement of the lipids by general practitioners yielded 13 of these 25 patients; a further 6 were identified after having onset of symptoms of coronary artery disease; another 6 patients were identified by lipidologists/internists of other hospitals in our region, and those patients were referred to our university outpatient Lipid Clinic for diagnostic reasons (ultracentrifugation and apoE genotyping).

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Influence of Hyperinsulinemia

Among the 49 apoE2 homozygotes, fasting insulin levels varied from 22 to 258 pmol/L, with a median of 100 pmol/L. Persons with fasting insulin values ≥100 pmol/L were assigned to the high-insulin group and were compared with the remaining persons, who had values <100 pmol/L. In our general population, a fasting insulin concentration ≤90 pmol/L is considered normal for both women and men. The results were similar when this value was used as a cutoff point (data not shown). The 15 men and 7 women in the high-insulin group were older than the 17 men and 11 women in the low-insulin group (54±2 versus 43±2 years, P=0.001). The mean body mass index was 26.1±0.6 kg/m² in the high-insulin group and 25.1±0.9 kg/m² in the low-insulin group (P=0.4). The mean fasting glucose concentrations of the high- and low-insulin groups were 5.19±0.13 and 5.02±0.12 mmol/L (P=0.1), respectively, and the corresponding mean fasting insulin levels were 145±8 and 51±5 pmol/L. The high-insulin group had significantly higher mean levels of total cholesterol, triglycerides, LDL and IDL cholesterol, VLDL cholesterol, and VLDL triglycerides and a lower mean HDL cholesterol than the other group (Table 2).

The contribution of high insulin levels to the expression of type III HLP in apoE2 homozygotes was analyzed adjusted for age, sex, fasting glucose concentration, body mass index, and cigarette smoking. These covariables did not contribute to type III HLP (data not shown). The persons with insulin levels ≥100 pmol/L had a ratio of VLDL cholesterol to triglyceride >0.689 six times more often (odds ratio 6.2, 95% CI 1.3 to 28.7, P=0.02).

LPL Gene and HL Gene

All individuals were screened for frequently occurring mutations in the LPL and HL genes: the LPL(Asp9→Asn) mutation was not detected, but 3 heterozygous carriers of the LPL(Asn291→Ser) mutation, 2 carriers of the LPL(Ser447→Ter) mutation, and 6 carriers of the HL(Val73→Met) mutation were identified (Table 3).

### Table 1. Characteristics of ApoE2 Homozygotes With and Without Palmar or Tuberous Xanthomas

<table>
<thead>
<tr>
<th></th>
<th>Xanthomas (n=12)</th>
<th>No Xanthomas (n=37)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male, n (%)</td>
<td>8 (67±14)</td>
<td>24 (65±8)</td>
<td>0.9</td>
</tr>
<tr>
<td>Age, y</td>
<td>48±3</td>
<td>49±2</td>
<td>0.7</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>25.9±1.1</td>
<td>25.6±0.6</td>
<td>0.8</td>
</tr>
<tr>
<td>VLDL cholesterol to triglycerides &gt;0.689, n (%)</td>
<td>11 (92±8)</td>
<td>17 (46±8)</td>
<td>0.005</td>
</tr>
<tr>
<td>VLDL cholesterol, mmol/L</td>
<td>8.26±1.49</td>
<td>3.77±0.80</td>
<td>0.009</td>
</tr>
<tr>
<td>VLDL triglyceride, mmol/L</td>
<td>6.73±1.59</td>
<td>3.76±1.00</td>
<td>0.005*</td>
</tr>
<tr>
<td>Fasting insulin, pmol/L</td>
<td>131±17</td>
<td>93±9</td>
<td>0.05</td>
</tr>
<tr>
<td>Sst I polymorphism: S1S2, n (%)</td>
<td>4 (33±14)</td>
<td>11 (30±8)</td>
<td>0.8</td>
</tr>
<tr>
<td>Lipase gene mutation, n (%)</td>
<td>4 (33±14)</td>
<td>6 (16±6)</td>
<td>0.2</td>
</tr>
<tr>
<td>Coronary artery disease,† n (%)</td>
<td>2 (17±11)</td>
<td>7 (19±7)</td>
<td>0.9</td>
</tr>
</tbody>
</table>

Values are concentration or percentage±SEM. All blood samples were obtained after an overnight fast.

*Statistical analysis after logarithmic transformation.
†Two patients had coronary bypasses, 1 underwent rescue percutaneous transluminal coronary angioplasty, 4 had myocardial infarction (ECG and creatine kinase-MB monitoring during the acute phase), and 2 had angina pectoris assessed with ECG exercise testing.

### Table 2. Effect of Fasting Insulin Levels on Lipid Profile in ApoE2 Homozygotes

<table>
<thead>
<tr>
<th></th>
<th>Fasting Insulin &lt;100 pmol/L (n=22)</th>
<th>Fasting Insulin ≥100 pmol/L (n=27)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>6.69±0.51</td>
<td>10.64±0.96</td>
<td>0.001</td>
</tr>
<tr>
<td>Triglyceride, mmol/L</td>
<td>2.83±0.35</td>
<td>7.88±1.61</td>
<td>0.001†</td>
</tr>
<tr>
<td>LDL+IDL cholesterol, mmol/L</td>
<td>2.58±0.19</td>
<td>3.35±0.17</td>
<td>0.002</td>
</tr>
<tr>
<td>VLDL cholesterol, mmol/L</td>
<td>2.30±0.39</td>
<td>6.96±1.19</td>
<td>0.001</td>
</tr>
<tr>
<td>VLDL triglyceride, mmol/L</td>
<td>2.10±0.30</td>
<td>6.44±1.45</td>
<td>0.001†</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L</td>
<td>1.44±0.09</td>
<td>0.93±0.04</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Values are concentration±SEM. All blood samples were obtained after an overnight fast. A total of 14 apoE2 homozygotes were identified through family studies. In addition to the 4 first-degree relatives in both groups, the high- and low-fasting-insulin groups consisted of 23 and 18 unrelated persons, respectively.

*ANOVA with adjustment for age, sex, and fasting glucose concentration.
†Statistical analysis after logarithmic transformation.
The carriers of the specific lipase gene mutations were not related. Severe hyperlipidemia was observed only in hyperinsulinemic patients. Hyperlipidemia among carriers of the LPL(Asn291→Ser) and the HL(Val73→Met) mutation was not unequivocally related to hyperinsulinemia or the SstI polymorphic site (S1S2). The mean fasting insulin concentration of 9 LPL(Asn291→Ser) and HL(Val73→Met) carriers was 81±18 pmol/L, and that of 40 persons without such a lipase mutation was 108±9 pmol/L (P=0.2). The LPL(Asn291→Ser) and the HL(Val73→Met) mutations were expected to associate with hyperlipidemia; however, these lipase mutations were equally distributed among normolipidemic and hyperlipidemic apoE2 homozygotes: 4 (17.8%) and 5 (20±8%) carriers of a lipase gene mutation, respectively (P=0.8). The mean serum concentrations of VLDL cholesterol and triglycerides did not differ between 9 persons with and 40 without the said lipase gene mutations either in the total group or in both insulin subgroups (data not shown). Adjustment for age, sex, and fasting glucose did not change these results. Although the LPL(Ser447→Ter) mutation was expected to associate with a higher lipid profile, 1 of 2 carriers was hyperinsulinemic. The mean HDL cholesterol level of the 10 heterozygous lipase gene carriers was 1.46±0.17 mmol/L, compared with 1.08±0.06 mmol/L in the 39 persons without lipase gene mutations (P=0.01).

### Influence of APOA1-C3-A4 Gene Cluster

A total of 15 heterozygous carriers of the SstI polymorphism (S1S2) in exon 4 of the APOC3 gene were identified. We did not observe any effect of entering relatives into the study: 2 pairs of sisters were carriers of the polymorphic SstI site of the APOC3 gene, of which 1 proband was a hyperlipidemic patient whose sister was normolipidemic (family 142 in Figure 2). The other pair was normolipidemic (family 144 in Figure 2), and all other carriers of the SstI polymorphism were unrelated. The persons with an S2 allele tended to have higher concentrations of VLDL cholesterol than those with S1S1 (6.93±2.04 versus 3.96±0.56 mmol/L, P=0.06). Separate analyses in the high- and low-fasting-insulin groups showed that this trend is based on an effect of the S2 allele in hyperinsulinemic patients. In the low-fasting-insulin group, no significant effect of the SstI polymorphism was observed (Table 4). In the high-fasting-insulin group, however, the 7 unrelated subjects with S1S2 had a much more deleterious lipid profile than the 20 persons with S1S1. The mean insulin level was 172±14 pmol/L in the 7 S2 allele carriers and 136±9 pmol/L in the remaining 20 persons (P=0.05). The highly significant insulin by SstI interaction terms (I polymorphism by Ser) shown in Table 4 indicate a combined effect of hyperinsulinemia and the polymorphism on VLDL metabolism.

Because the SstI polymorphism is located in the 3′ non-coding region of the apoC3 gene, it could be argued that the APOC3 effect is not caused by the SstI polymorphism but rather is due to a closely linked functional mutation. The recently described common genetic variants in the insulin/phorbol ester response element at positions −482 and −455 of the promoter region of APOC3 could be candidates.41 We subsequently screened all individuals for these 2 point mutations and performed haplotype analysis. In both insulin groups, significant linkage disequilibrium was observed between the SstI polymorphism (S2) and the sequence variations in the promoter region (allele P2, P=0.001, Table 5). Therefore, we performed subgroup analyses with the 3 alleles of the promoter polymorphism instead of the SstI polymorphism. However, no differences were detected in lipid levels between carriers of the 3 alleles P1, P2, and P3 (data not shown). This indicates that the effect of the SstI polymorphism or a closely linked mutation is contained within a P2S2 haplotype.

### Ascertainment Through Family Studies

The 8 family trees that were studied to ascertain additional apoE2 homozygotes are shown in Figure 2. Analyses of these kindreds were inconclusive with regard to the expression of type III HLP: hyperinsulinemia, the SstI polymorphism (S1S2), and the HL(Val73→Met) mutation occurred with and without hyperlipidemia. Nonetheless, these family trees illustrate that additional familial factors are needed to express type III HLP: apoE2 homozygotes ascertained through 3 probands from a population-based study (families 126, 129, and 145) did not express HLP, whereas >50% of such

### Table 3. Ten ApoE2 Homozygotes With Lipase Gene Mutations

<table>
<thead>
<tr>
<th>Subject No.</th>
<th>Sex</th>
<th>Age, y</th>
<th>Fasting insulin, pmol/L</th>
<th>SstI</th>
<th>LPL(Asn291→Ser)</th>
<th>LPL(Ser447→Ter)</th>
<th>HL(Val73→Met)</th>
<th>VLDL Cholesterol, mmol/L</th>
<th>Triglyceride, mmol/L</th>
<th>HDL Cholesterol, mmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>FD77</td>
<td>F</td>
<td>57</td>
<td>144</td>
<td>S1S1</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>9.56</td>
<td>6.63</td>
<td>1.24</td>
</tr>
<tr>
<td>FD786</td>
<td>M</td>
<td>50</td>
<td>129</td>
<td>S1S1</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>5.11</td>
<td>3.57</td>
<td>1.08</td>
</tr>
<tr>
<td>FD1114</td>
<td>M</td>
<td>27</td>
<td>172</td>
<td>S1S2</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>17.68</td>
<td>18.86</td>
<td>0.64</td>
</tr>
<tr>
<td>FD1524</td>
<td>M</td>
<td>45</td>
<td>57</td>
<td>S1S2</td>
<td>+</td>
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<td>−</td>
<td>3.46</td>
<td>2.48</td>
<td>1.19</td>
</tr>
<tr>
<td>FD1538</td>
<td>M</td>
<td>38</td>
<td>43</td>
<td>S1S1</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>0.84</td>
<td>1.25</td>
<td>2.33</td>
</tr>
<tr>
<td>FD1669†</td>
<td>F</td>
<td>38</td>
<td>86</td>
<td>S1S2</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>0.79</td>
<td>1.37</td>
<td>1.58</td>
</tr>
<tr>
<td>FD1799</td>
<td>M</td>
<td>43</td>
<td>29</td>
<td>S1S2</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>0.76</td>
<td>0.82</td>
<td>1.36</td>
</tr>
<tr>
<td>FD1801†</td>
<td>M</td>
<td>44</td>
<td>22</td>
<td>S1S1</td>
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<td>+</td>
<td>−</td>
<td>1.21</td>
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<td>3.56</td>
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<td>25</td>
<td>43</td>
<td>S1S2</td>
<td></td>
<td>−</td>
<td>+</td>
<td>0.59</td>
<td>1.13</td>
<td>2.32</td>
</tr>
</tbody>
</table>

The carriers of the specific lipase gene mutations were not related. The LPL(Asn9→Asn) mutation was not detected among our apoE2 homozygotes.

†FD1801 is the proband of family 129 and FD1538 is his brother (Figure 2).†FD1669 is a member of family 144 (Figure 2).
relatives of the hyperlipidemic probands had type III HLP (56%, 95% CI 21% to 86%).

Discussion

The present study was initiated to identify additional factors that contribute to type III HLP in apoE2 homozygotes. Type III HLP in apoE2 homozygotes is an autosomal recessive disorder with a reduced penetrance and variable expression. In cross-sectional analyses, late onset of hyperlipidemia could explain the observation of such incomplete expression. With regard to apoE2 homozygotes, it is often stated that in men, hyperlipidemia manifests in the third decade of life and in women, after menopause. Our follow-up of 10 male apoE2 homozygotes, however, suggests that essentially no change could be observed between the lipid profiles obtained at age 35 years and those obtained at age 45 years (Figure 1). Thus, at least during this period of their life, in which most inherited lipid disorders are assumed to become manifest in men, age was not the factor leading to the expression of type III HLP. Although the sample was small, the findings suggest that we did not introduce an important bias by studying the expression of type III HLP cross-sectionally. A population-based study to investigate the expression of type III HLP is unfeasible, because the frequency of apoE2 homozygotes in the general population is low (0.5%), and only a small percentage is hyperlipidemic (~2%). Therefore, we recruited apoE2 homozygotes from our Lipid Clinic. Additional apoE2 homozygotes were ascertained by screening of families. In contrast to the normolipidemic men from the follow-up sample, inclusion of family members in the normolipidemic group may have involved differences in exposure time to additional familial risk factors. This could have diminished the contrast between the hyperlipidemic and the normolipidemic groups; however, we included only male relatives >34 years old and mostly postmenopausal women. The kindreds show a clear difference between the families that were ascertained through normolipidemic and hyperlipidemic probands. This confirms that additional familial factors are required for the expression of type III HLP. However, more families need to be studied to analyze the specific interacting factors in such kindreds properly.

Hyperinsulinemia is an independent risk factor for atherosclerosis and ischemic heart disease and associates with increased levels of triglyceride-rich lipoproteins, mainly VLDL. Hyperinsulinemia stimulates the production and secretion of VLDL, and subsequently the clearance capacity for these particles may become overwhelmed in apoE2 homozygotes. In humans, short-term infusion of long-chain triglycerides in turn enhances hyperinsulinemia by substrate competition; however, chronic endogenous hypertriglycerideremia is unlikely to produce hyperinsulinemia. In fact, hypertriglycerideremic transgenic mice carrying the human APOC3 gene were neither hyperinsulinemic nor insulin resistant. In our apoE2 homozygotes, hyperinsulinemia was associated with high ratios of VLDL cholesterol to serum triglycerides, which reflects the presence of β-VLDL, and is indicative for the expression of type III HLP. Furthermore, the hyperinsulinemic apoE2 homozygotes also had low levels
of HDL, which may further increase susceptibility for coronary artery disease. These effects of hyperinsulinemia on the expression of type III HLP and HDL levels were analyzed irrespective of the cause of the high insulin levels.

Heterozygosity for mutations in genes that are involved in the lipolytic conversion of lipoproteins could also contribute to accumulation of VLDL, as has been shown for the LPL(Asn291→Ser) mutation.18,19 Although such mutations do not always lead to metabolic disorders, hyperlipidemia may develop when other factors stress the biochemical pathway.19 However, we did not detect a contribution to hyperlipidemia in our apoE2 homozygotes of the LPL(Asn291→3) mutation. Our data suggest that apoE2 homozygotes with lipase gene mutations may have a more favorable lipid profile because of relatively high levels of HDL cholesterol. Such an advantage has been described previously in carriers of the LPL(Ser447→Ter) mutation20,22 and in carriers of a promoter polymorphism (G→C→A) of the HL gene. The heterozygous and homozygous carriers of the latter mutation were shown to have high levels of HDL3 cholesterol.53 Our heterozygous carriers of HL gene mutations probably have a more favorable lipid profile as a result of a decreased activity of HL.53–59 Recently, variation in the HL gene was shown to associate with HL activity and with HDL triglycerides but not with VLDL triglycerides.60 In agreement with the latter study, we also did not observe an influence on VLDL triglyceride levels. Zhang et al60 observed a contribution of the LPL(Asn291→Ser) sequence variation to type III HLP: the mutation was observed in 4 of 17 apoE2 homozygotes with type III HLP, which was significantly more than the 2 carriers in 230 Dutch control subjects.61 In the present study, 2 carriers of the LPL(Asn291→Ser) mutation were observed among 25 patients with type III HLP. The allele frequency (4.0%) in patients with type III HLP in the present study did not differ significantly from the allele frequency (11.8%) in apoE2 homozygotes of the study by Zhang et al and the allele frequency (0.7%) of the Dutch control subjects. Moreover, we also detected an apoE2 homozygote with the LPL(Asn291→Ser) sequence variation but without hyperlipidemia. Nevertheless, meaningful conclusions about this mutation cannot be drawn because of the small numbers in the 2 studies, and our findings do not exclude a contribution of other mutations in lipase genes.

It has been shown previously that the presence of an SstI restriction site in exon 4 of the APOC3 gene is associated with hypertriglyceridemia.26–30 Therefore, we screened our study population for this polymorphism. Although the S2 allele itself was not associated with hypertriglyceridemia in our total study group, our data show a strong interaction between the polymorphism and hyperinsulinemia: we observed expression of severe type III HLP in hyperinsulinemic apoE2 homozygotes who had the SstI restriction site (S2). An increased production of VLDL due to insulin resistance may lead to hypertriglyceridemia when remnant metabolism fails to compensate. A decreased remnant clearance in apoE2 homozygotes, who have an increased production of apoC3,
may then result in severe type III HLP. This susceptibility to accumulation of remnant particles in apoE2 homozygotes may prove to be a sensitive model for identifying additional genetic factors that are generally contributory to hyperlipidemia. In addition to endogenous hypertriglyceridemia without apoE2 homozygosity, these “second factors” may also contribute to the atherogenic lipid profile of familial combined hyperlipidemia, as suggested by the low levels of HDL in the hyperinsulinemic S2 carriers. We probably need to know the effect of such additional factors in the first place to allow analyses of the main causes of familial combined hyperlipidemia.51–63 Directly identifying additional factors in patients with endogenous hypertriglyceridemia or familial combined hyperlipidemia is much more complex than in apoE2 homozygotes because of the lack of normolipidemic controls within the affected individuals.

Dammernan et al24 found a susceptibility to hypertriglyceridemia among carriers of the SsrI polymorphism (S2). Previously, we also observed this relation with severe hypertriglyceridemia.30 The study by Dammernan et al was probably performed in a hypertriglyceridemic population that, in addition to 11 hyperlipidemic apoE2 homozygotes, consisted of a large number of patients with endogenous hypertriglyceridemia. This effect of the polymorphic site in patients with severe hypertriglyceridemia may also be based on an interaction with hyperinsulinemia. In the present study, the SsrI polymorphism was associated with hypertriglyceridemia exclusively when patients had hyperinsulinemia. Notably, the carriers of this polymorphic site, who had low levels of fasting insulin, tended to have even lower triglyceride concentrations than the apoE2 homozygotes with the wild-type variant (Table 4).

Because the SsrI polymorphism is located in the 3' non-coding region of the APOC3 gene and thus does not lead to a change in the amino acid sequence of the protein, the possibility cannot be excluded that the effect found for the SsrI polymorphism is due to another hitherto unknown mutation in the APOC3 gene or in another gene near the restriction site. Extended haplotyping and subgroup analysis with 2MspI polymorphisms in the APOA1 gene, downstream, did not identify haplotypes associated with a higher risk of expression of type III HLP (data not shown).42

The combined effect of hyperinsulinemia and an APOC3 haplotype may suggest that insulin influences the expression of apoC3. Recently, 2 common variations of an insulin/phorbol ester response element of the promoter region of the APOC3 gene were described. In vitro studies showed increased expression of apoC3 for both promoter variants due to a defective response to insulin at the DNA level.41 In transgenic mice, such increased expression of apoC3 causes hypertriglyceridemia.64 In the present study, the SsrI polymorphism was found almost exclusively in combination with the presence of the 2 promoter variants. Such a strong linkage disequilibrium between the SsrI polymorphism and the 2 promoter variants is in agreement with the findings of others.24 It could therefore be argued that the observed effect of the SsrI polymorphism is due to 1 or both promoter variants. However, we observed no effect of the 3 promoter alleles on the lipid levels. This can be explained by the fact that although the S2 allele was strictly associated with the P2 allele in the hyperinsulinemic group, an even higher number of P2 alleles were found to be linked with the S1 allele (Table 5).

This is in agreement with observations in the ARIC population33 and in Italian school children.32

In conclusion, the combination of hyperinsulinemia and the presence of the SsrI polymorphism in the APOC3 gene results in the expression of severe type III HLP in apoE2 homozygotes. This finding enables a screening strategy to identify those apoE2 homozygotes at risk for developing type III HLP and subsequently premature coronary artery disease.

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**References**

Hyperlipidemia in ApoE2 Homozygotes


Severe Hyperlipidemia in Apolipoprotein E2 Homozygotes Due to a Combined Effect of Hyperinsulinemia and an SstI Polymorphism


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