Interaction Between Variant Apolipoproteins C-II and E That Affects Plasma Lipoprotein Concentrations

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The genes for apolipoprotein (apo) C-II, a cofactor for activation of lipoprotein lipase, and apo E, a ligand for receptor-mediated uptake of triglyceride-rich lipoproteins, are physically linked on chromosome 19q13.1. In a large Caribbean Caucasian family, several individuals had clinical features of the complete absence of lipoprotein lipase activity and were homozygous for a DNA frameshift mutation of apo C-II, imparting functional inactivity to the mutant protein. Plasma from heterozygous carriers of this mutation, when compared with plasma from relatives who were noncarriers, had significantly diminished capacity to activate lipoprotein lipase in vitro. We also observed in heterozygotes for this mutation a wide range of serum lipid and lipoprotein levels. When age and sex were taken into account, the presence of a single apo E allele encoding the E4 isoform occurring in individuals with a single mutant apo C-II allele was strongly associated with higher levels of cholesterol, triglycerides, very low density lipoprotein cholesterol, and non–high density lipoprotein cholesterol when compared with those of relatives who carried neither or only one variant allele. This suggests that a single genetic mutation that usually has a recessive effect on lipoprotein metabolism can have an interactive effect on lipid phenotype when it is co-inherited with a single mutation at another gene whose product affects the same metabolic pathway. (Arteriosclerosis and Thrombosis 1991;11:1303–1309)

The products of many genes individually affect lipid and lipoprotein levels.1 For example, mutations of either the low density lipoprotein receptor (LDLR) or apolipoprotein (apo) B genes cause elevations of LDL cholesterol.2–4 Other mutations of the apo B gene cause lowering of LDL cholesterol.5–7 A mutation in the cholesterol ester transfer protein (CETP) gene underlies genetically elevated levels of high density lipoprotein (HDL) cholesterol.8 Mutations of the lipoprotein lipase (LPL) gene cause chylomicronemia and underlie hyperlipoproteinemia type I.9–11

In addition to the clinical lipoprotein disorders caused by these and other monogenic defects, there is evidence that concurrent inheritance of mutations at more than one genetic locus underlies a particular phenotype. For example, homozygosity for a functionally defective apo E isoform, called E2, in combination with a second, as-yet-uncharacterized genetic or environmental factor is a necessary condition for expression of hyperlipoproteinemia type III.12 Heterozygosity for a mutant LPL gene in combination with another factor may underlie hyperlipoproteinemia types IIb and/or IV.10,13,14 In one kindred with familial hypercholesterolemia (FH) due to an LDLR defect, a second factor, unidentified but segregating as a Mendelian dominant trait, obliterated the usually deleterious phenotypic effect of the mutant LDLR gene, with a resulting neutral lipoprotein phenotype.15

Large kindreds are useful for identification of polygenic interactions that affect lipoprotein phenotypes. In unrelated subjects the phenotypic variability due to the underlying genetic heterogeneity of a putatively "monogenic" trait may mask the possible independent effect(s) on phenotype of other monogenic variables. However, within a large family in which two independent genetic variables clearly seg-
regate in a Mendelian fashion, there should be individuals with two truly monogenic variables in numbers sufficient for meaningful comparison of phenotypes between family members classified according to their genotypes at the two loci. Because of control over background genetic and environmental variables, an observed phenotypic difference between family members grouped according to their genotypes is likely related to the effects of the variant gene products, either singly or through an interaction of the products in the lipoprotein metabolic pathway.

Apo C-II is a cofactor for activation of LPL. Absence of apo C-II activity, due either to a lack of circulating apo C-II or to homozygosity for a functionally abnormal circulating apo C-II variant, results in chylomicronemia and a clinical picture indistinguishable from that for hyperlipoproteinemia type I due to genetically defective LPL.

Apo E is a structural component of very low density lipoprotein (VLDL) and a ligand for receptor-mediated uptake of triglyceride-rich lipoproteins. Apo E, apo C-II, and apo C-I form a gene cluster on chromosome 19q13.1. Apo E exists in three forms: E4, E3, and E2. The isoforms differ from one another by a single charge unit due to specific amino acid changes within the mature apo E polypeptide, in turn caused by two common DNA point mutations within exon 4 of the apo E gene. Different apo E isoforms are associated with differences in the metabolism of chylomicrons and their remnants and with variation in the levels of LDL within populations.

The current study investigated a digenic interaction in a large kindred with C-II-Toronto (CII-T), a circulating mutant apo C-II. The molecular basis for CII-T is a DNA frameshift, resulting from a single DNA base deletion that causes a change in the amino acid sequence from residue 69 onward. CII-T homozygotes have frank hyperchylomicronemia. Among CII-T heterozygotes, plasma levels of triglycerides and VLDL cholesterol vary from normal to hypertriglyceridemic (hyperlipoproteinemia type V). We have found that variation at the apo E gene can affect the biochemical phenotype in CII-T heterozygotes.

**Methods**

**Kindred C2T**

This kindred was ascertained as previously reported and is now designated as C2T. A total of more than 400 family members were identified: height, weight, and succinct medical histories were obtained in the field. A priori exclusion criteria (in decreasing order of effect on total sample size) included 1) inadequate blood sample available for all determinations (nonfasting state or small quantity), 2) unrelated spouse, and 3) homozygosity for CII-T, E4, or E2 alleles. After these exclusions, 140 subjects remained, and fasting blood samples were used for lipoprotein analyses and isoelectric focusing of apolipoproteins. One further a priori criterion for selection of subjects was matching of CII-T heterozygotes and non-CII-T control subjects according to age and gender after their identification by isoelectric focusing. Any control subject whose age was within 5 years of any CII-T heterozygote of the same gender was included in the data set. After these further exclusions, samples from 113 family members remained. Of these, 45 were CII-T heterozygotes, as defined by the presence of both normal and abnormal apo C-II isoforms or by obligate heterozygote status within the pedigree. No differences in age or body mass index were found between CII-T heterozygotes and the selected non-CII-T controls. Thus, data from these 113 subjects were analyzed.

**Biochemical Determinations**

Assays of plasma lipids and lipoproteins, electrophoretic analysis of the apolipoproteins of chylomicrons and VLDL, and assays of apo C-II activation of bovine lipoprotein lipase by plasma, with activity expressed as micromoles free fatty acid released x 10^-3 per milliliter plasma per minute. 

**Statistical Analyses**

Nonparametric analysis was performed with the Kruskal-Wallis χ² approximation test of significance of Wilcoxon rank sums with the SAS statistical package. Nonparametric tests were used because it was found that the variables chosen for analysis were not normally distributed. χ² tests were used when comparing lipoprotein phenotypes in various genotypic groups.

**Results**

**Effect of CII-T Status on Biochemical Variables**

Table 1 shows a nonparametric comparison of biochemical features of family members classified...
according to whether they were heterozygous for CII-T (CII-T+) or were homozygous for the normal apo C-II isoform (CII-T-). The groups were comparable with respect to age and sex ratio. Among the tested variables, only apo C-II activation of bovine LPL was significantly different \((p=0.0001)\), with a 30% reduction of the capacity of total plasma apo C-II to activate LPL in CII-T heterozygotes compared with normal control family members (Table 1).

There was a trend toward higher triglyceride and VLDL cholesterol levels and to lower HDL cholesterol levels in CII-T heterozygotes. This trend was also observed when individual percentiles for age and sex for these variables were compared, but these differences were not significant (data not shown).

**Effect of Apolipoprotein E Isoforms on Plasma Lipids and Lipoproteins in Carriers of Either Normal Apolipoprotein C-II or CII-T**

Tables 3 and 4 show nonparametric comparisons of biochemical features of family members classified according to apo E phenotype within a grouping based on apo C-II genotype. In CII-T heterozygotes classified according to apo E isoform phenotype (Table 3), significant between-group variation was observed in age \((p=0.05)\), total cholesterol \((p=0.006)\), triglycerides \((p=0.034)\), VLDL cholesterol \((p=0.036)\), and non-HDL cholesterol \((p=0.005)\). One CII-T heterozygote with E4/3 had type V hyperlipoproteinemia, with pancreatitis and hypertriglyceridemia almost as severe as in his relatives who were CII-T homozygotes (total cholesterol of 340 mg/dl, and triglycerides of 1,280 mg/dl with fasting chylomicronemia). When his values were excluded and the analysis repeated, the significant associations persisted (data not shown). In a similar analysis of noncarriers of CII-T (Table 4), significant variation in HDL cholesterol \((p=0.04)\) was observed.

The results of nonparametric pairwise comparisons of biochemical features of family members

### Table 2. Biochemical Characteristics of C2T Family Members Grouped by Apolipoprotein E Phenotype

<table>
<thead>
<tr>
<th>Variable</th>
<th>Apolipoprotein E phenotype</th>
<th>(E^3/2)</th>
<th>(E^3/3)</th>
<th>(E^4/3)</th>
<th>(p) (All phenotypes)</th>
<th>(p) ((E^3/3) vs. (E^4/3))</th>
</tr>
</thead>
<tbody>
<tr>
<td>(n) (M/F)</td>
<td></td>
<td>7/4</td>
<td>51/34</td>
<td>10/7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (yr)</td>
<td></td>
<td>58.3±19.9</td>
<td>40.4±15</td>
<td>46.4±16.3</td>
<td>0.005</td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m(^2))</td>
<td></td>
<td>23.9±4.4</td>
<td>25.0±4.6</td>
<td>26.2±4.5</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Chol (mg/dl)</td>
<td></td>
<td>184±37.9</td>
<td>181±32.1</td>
<td>200±44.1</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Trig (mg/dl)</td>
<td></td>
<td>138±67.1</td>
<td>125±66.1</td>
<td>341±507</td>
<td>0.025</td>
<td></td>
</tr>
<tr>
<td>VLDL chol (mg/dl)</td>
<td></td>
<td>29.2±14.0</td>
<td>22.5±14.3</td>
<td>43.9±37.6</td>
<td>0.032</td>
<td></td>
</tr>
<tr>
<td>LDL chol (mg/dl)</td>
<td></td>
<td>104±32.9</td>
<td>116±25.8</td>
<td>104±47.5</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>HDL chol (mg/dl)</td>
<td></td>
<td>51.2±16.7</td>
<td>42.7±10.9</td>
<td>38.7±11.7</td>
<td>0.08</td>
<td></td>
</tr>
<tr>
<td>CII act</td>
<td></td>
<td>35.1±9.3</td>
<td>32.6±8.7</td>
<td>36.0±8.6</td>
<td>NS</td>
<td></td>
</tr>
</tbody>
</table>

| Abbreviations are as in the footnote to Table 1. |
Lipoprotein Phenotypes in Study Subjects were found, but there was a trend toward higher levels of triglycerides and non-HDL cholesterol. Further analysis of individuals homozygous for normal apo C-II, no significant between-group differences were found, but there was a trend toward higher levels of triglycerides (p = 0.11) and body mass index (p = 0.06) in E4/3 subjects.

Between age-matched CII-T heterozygotes classified as E3/3 or E3/2, no significant between-group differences were observed (data not shown). In a similar analysis of individuals homozygous for normal apo C-II, no significant between-group differences were found, but there was a trend toward higher levels of HDL cholesterol in E3/2 heterozygotes (data not shown).

Lipoprotein Phenotypes in Study Subjects

No subject had LDL cholesterol levels that exceeded the 95th percentile for age and sex. The only lipoprotein phenotypes seen in this family were hyperlipoproteinemia types IV (triglycerides >95th percentile for age and sex), V (type IV plus chylomicronemia), and/or hypoalphalipoproteinemia (HDL cholesterol <5th percentile for age and sex). Of the 113 subjects analyzed, 23 were dyslipidemic by these definitions (eight with type IV, one with type V, 12 with hypoalphalipoproteinemia, and two with combined type IV and hypoalphalipoproteinemia). Among CII-T subjects, three of six with E4/3 were hypertriglyceridemic compared with two of 34 with E3/3 (p < 0.05).

Discussion

In the current study of an extended family, we observed significant elevation of triglycerides and total, VLDL, and non-HDL cholesterol in the subgroup who were heterozygous for both a rare circulating structural variant of apo C-II and for the E4 isoform. Each of these genetic variants has been shown to individually affect lipoprotein phenotype. Homozygosity for CII-T results in hyperlipoproteinemia type I, with complete absence of LPL activity, hyperchylomicronemia, and pancreatitis. In other populations E4 has been shown to occur significantly more frequently among individuals with hypertriglyceridermia, hyperchylomicronemia, hyperprebeta- lipoproteinemia, and pancreatitis.

In the kindred described the E3 isoform was closely linked with CII-T, effectively forming an allelic haplotype. Thus, CII-T heterozygotes had at least one E3 isoform. The second apo E isoform, present on the other chromosome 19 in CII-T heterozygotes, could thus be either E4, E3, or E2. The fortuitous linkage between CII-T and E3 permitted control over the effect of apo E variation on the lipids, as CII-T heterozygotes could at most be heterozygous for either E4 or E2. Individuals homozygous for CII-T, E4, or E2 were excluded from the analysis. Any observed interaction was thus due to the effect on phenotype of a single copy of the variant of each gene. Simple pairwise comparisons based on classification according to genotype revealed that the ability of total serum C-II to activate LPL was diminished in CII-T heterozygotes. This would be compatible with a codominant model of inheritance, in which heterozygosity for CII-T results in an approximate 50% reduction in activation of LPL by total serum C-II. This further implies that CII-T probably does not inhibit the ability of native C-II to normally activate LPL. If this were the case, heterozygosity for an inactive mutant form of CII-T that further acts to inhibit activation of LPL by total serum C-II might reduce LPL activation by much more than 50%.

Analysis of biochemical variables based on classification of subjects according to genotype at both the apo E and apo C-II genes revealed that there was significant between-group variation in triglycerides and total, VLDL, and non-HDL cholesterol. Further
Thus, in C2T members with E2 and normal apo C-II, HDL uptake of HDL. In relatives with E2 and CII-T, the variance with epidemiological data from unrelated HDL may accumulate due to impaired apo E-dependent clearance of HDL because E2 is a poor ligand for receptor-mediated uptake. 22-34-36 Levels of HDL. This might be attributed to a relative impact of E4 on LDL cholesterol levels.

Lipoprotein particles bearing E4 have been shown to be metabolized more rapidly than those bearing E3,28,34 and subjects with E4 have a lower concentration of apo E than do subjects with either E3 or E2.35 In addition, apo E isoforms are distributed unequally between lipoproteins, with E4 preferentially bound to VLDL,34-36 and E2 preferentially bound to HDL.34 E3 contains a cysteine at residue 112, which may form disulfide linkages;36 E4 has no cysteine at residue 112 and thus should always be in a functional form. E3-CII-T dimers have been observed in CII-T homozygotes and heterozygotes (P.W. Connelly et al, unpublished data). It has been suggested that apo E in vitro has the ability to inhibit LPL.37 Others have shown that the triglyceride substrate bound to apo E has a greater capacity to activate LPL and that the degree of this capacity to activate may be related to the amount of apo E bound to triglyceride.38,39 VLDL from hyperlipoproteinemia type IV subjects is relatively E poor.40 Thus, the triglyceride-rich particles in individuals with E4 may be relatively E poor. This could lead to less efficient activation of LPL or to an inefficient apo E-dependent clearance from plasma.

Simple pairwise comparisons based on classification of subjects according to apo E phenotypes revealed significant between-group variation in triglycerides and VLDL cholesterol but no differences in LDL cholesterol, a result that is somewhat at variance with epidemiological data from unrelated subjects. The observed effect overall within the family on triglycerides and on VLDL cholesterol may result from the effect of E4 on these variables in CII-T heterozygotes. Larger numbers of subjects are likely required to demonstrate a possible monogenic impact of E4 on LDL cholesterol levels.

Individuals with E2 and normal C-II had higher levels of HDL. This might be attributed to a relative lack of E-dependent clearance of HDL because E2 is a poor ligand for receptor-mediated uptake.22,34,36 Thus, in C2T members with E2 and normal apo C-II, HDL may accumulate due to impaired apo E-dependent uptake of HDL. In relatives with E2 and CII-T, the generation of HDL particles may be somewhat impaired due to a lower LPL activity. Activation of bovine LPL by total plasma C-II was significantly diminished in CII-T heterozygotes with the E3 isoform but not in CII-T heterozygotes with the E4 isoform. Previous attempts to classify subjects in this family as “CII-T heterozygotes” based on the ability of their plasma to activate bovine LPL revealed some discordance between the genotype predicted either by obligate heterozygote status or by the presence of the CII-T band and the observed activation of LPL by the subject’s plasma. Classifying subjects according to either obligate heterozygote status and/or the presence of the band and taking into account apo E isotype clarifies this discordance. CII-T subjects with E4 had “normal” ex vivo C-II activation and had high levels of triglycerides. The total level of apo C-II has been shown to be directly correlated with the triglyceride level.41 Thus, part of the heterogeneity of levels of apo C-II activation of LPL is attributed to the effect of E4.

We cannot rule out the possibility that the E4 isoform was only a linked marker in this family for another functionally relevant mutation, possibly involving the closely linked apo C-I gene. If this were the case, then the association of hyperlipidemia with the E4 isoform would still represent an interaction between two gene loci affecting phenotype.

Heterozygosity for a mutant LPL gene has been shown to be associated with elevated triglyceride levels, although not exclusively.10,14 The assessment of potentially interacting genetically determined factors, perhaps even apo E isoforms, may have further explained the phenotypic variability seen in that family. Others have observed in unrelated subjects that obligate carriers of defective LPL genes have biochemical features, such as a diminished LPL activity to LPL mass ratio that distinguished them from normal subjects.19 Some investigators have suggested that heterozygosity for defective LPL might underlie the “familial combined hyperlipidemia” (hyperlipoproteinemia type IIB) phenotype. In families in which both hyperlipoproteinemia types IIb and III are expressed, there is significant variability in lipoprotein phenotype. The severity of the perturbation of lipoprotein phenotype in such families may be due to interactions of two or more candidate genes. Mutations at some candidate genes on their own might affect the lipoprotein phenotype in a recessive manner. The paradigm observed in the C2T family may thus be generally important: a gene defect that normally has a recessive effect on lipoprotein phenotype can interact with a variant at a second gene to produce a discrete biochemical abnormality.

In summary, we have identified a digenic interaction in which the E4 isoform was associated with significantly elevated triglycerides and total, VLDL, and non-HDL cholesterol levels in heterozygotes for CII-T. Because E4 has a significant effect on CII-T heterozygotes, we hypothesize that in vivo lipolysis is suboptimal in individuals who have in-
herited both variants. Each variant on its own may not overtly compromise lipolysis, but together they can interact to overwhelm lipolytic capacity. This study exemplifies the usefulness of the family approach in the identification of interactions of a small number of genes. A silent phenotype due to one mutation can be converted into an abnormal phenotype when there is coinheritance of a second mutation at another gene. Family studies are likely essential for the ascertainment of such oligogenic interactions. In unrelated individuals in the absence of a family context, identification of oligogenic interactions that affect phenotype would be much more difficult, if not impossible.

References


**KEY WORDS** • hypertriglyceridemia • oligogenic interaction • apolipoprotein C-II-Toronto • apolipoprotein E phenotype
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