Lipoprotein lipase gene polymorphisms: associations with myocardial infarction and lipoprotein levels, the ECTIM study

Rhadih Jemaa,* Frédéric Fumeron,† Odette Poirier,† Laure Lecerf,† Alun Evans,§ Dominique Arveiller,** Gerald Luc,‖ † Jean-Pierre Cambou,§§ Jean-Marie Bard,‖ † Jean-Charles Fruchart,‖ † Marian Apfelbaum,* François Cambien,†,*** and Laurence Tiret***

INSERM U286,* Faculté de Médecine Xavier Bichat, 75018 Paris, France; INSERM U750,† 75005 Paris, France; MONICA Project-Belfast,§ The Queen University of Belfast, Belfast BT12 6BJ, Northern Ireland, UK; MONICA Project-Bas-Rhin,** 67085 Strasbourg, France; SERLIA-INSERM U925 and MONICA Project-Lille,‖ † Institut Pasteur, 59019 Lille, France; INSERM U926 and MONICA Project-Hauts-de-France,§§ CHU Purpan, 31059 Toulouse, France; and INSERM U258,*** Hôpital Broussais, 75674 Paris, France

Abstract Several lipoprotein lipase (LPL) gene polymorphisms have been found associated with fasting lipid levels, but their impact on coronary heart disease (CHD) is less clearly established. We investigated associations of LPL polymorphisms (HindIII, PvuII, Ser447→Ter) and the newly described mutation Asn291→Ser with the risk of myocardial infarction (MI), severity of atherosclerosis, and fasting plasma lipoprotein concentrations in the ECTIM study (614 patients and 733 controls). The Ter477 allele had a lowering effect on triglycerides (P < 0.01), VLDL-cholesterol (P < 0.05), apoC-III (P < 0.001), LpE:B (P < 0.01), and LpCII:B (P < 0.05), and a raising effect on apoA-I levels (P < 0.05). The H- allele of the HindIII polymorphism was associated with lower apoC-III (P < 0.01) and higher HDL-cholesterol (P < 0.05) levels. The PvuII and Asn291→Ser polymorphisms did not exhibit any significant association with the biochemical traits examined. The HindIII genotype distributions differed between cases and controls, the odds ratios for MI associated with H+H+ and H+H- genotypes being 2.05 (P < 0.01) and 1.74 (P < 0.05) by reference to H-H-. The lack of association between Ser447→Ter and MI suggested that this mutation was unlikely to be the cause of the association found with HindIII. In some cases, the severity of atherosclerosis assessed by coronaryography increased with the presence of P+ allele (coronary scores: 1.41, 1.57, and 1.64 in P-P-, P-P+, and P+P+ individuals respectively, P < 0.05). A similar trend on the coronary score was observed with the presence of the Asn291→Ser mutation (1.58 vs. 1.90, P = 0.06).

Our results suggest that the LPL gene is involved in the determination of lipoprotein profiles, the predisposition to CHD, and the severity of atherosclerosis.

Abbreviations: LPL, lipoprotein lipase; MI, myocardial infarction; CHD, coronary heart disease; ECTIM, Étude Cas-Témoins de l’Infarctus du Myocarde; VLDL, very low density lipoprotein; HDL, high density lipoprotein; RFLP, restriction fragment length polymorphism; ASO, allele specific oligonucleotide; PCR, polymerase chain reaction; OR, odds ratio.

To whom correspondence should be addressed at: INSERM U286, Faculté de Médecine Xavier Bichat, BP 416, 16 rue Henri Huchard, 75870 Paris Cedex 18, France.

Supplementary key words RFLP • Asn291→Ser substitution • Ser447→Ter substitution • coronary heart disease • plasma triglycerides • VLDL • HDL • apolipoproteins
coding sequence was the Ser$^{447}\rightarrow$Ter mutation (exon 9)
due to a C-G transversion that results in a premature
termination codon. This mutation, which is in strong
linkage disequilibrium with H- and P- alleles, was
associated with a lower risk of primary hypertriglyceridemia
(9), but not with lipid profile (4) or CHD (6). Recently,
a newly described mutation in exon 6, Asn$^{291}\rightarrow$Ser (10),
has been found in hypertriglyceridemic patients of
French Canadian descent (11).

Despite growing evidence that the LPL gene is in-
volved in the predisposition to dyslipoproteinemia, its
impact on CHD is less clearly established. These discrepancies
could be due to the small number of subjects included
and therefore to a lack of power. Moreover, in relation to
CHD, only a few studies have been published. One
found an association (8), another no association (4). In
a third study, the HindIII polymorphism was associated
with the severity of atherosclerosis in patients who under-
went angiography but not with coronary disease per-
se (5).

Furthermore, several questions remain open about
the underlying biological mechanisms, in particular
about a possible mediation of the effects of HindIII and
PvuII by the Ser$^{447}\rightarrow$Ter mutation. We investigated
possible associations of LPL polymorphisms with the
risk of MI and several biochemical parameters related
to the metabolism of triglyceride-rich particles and HDL
in the ECTIM study (Etude Cas Témoins sur l’Infarctus
du Myocarde) (12). The LPL polymorphisms examined
were HindIII, PvuII, Ser$^{447}\rightarrow$Ter, and Asn$^{291}\rightarrow$Ser.

DNA analysis
Genotypes for the HindIII and PvuII polymorphisms
were determined by PCR using primers and amplifica-
tion conditions as described by Mattu et al. (6). The two
substitution polymorphisms, Asn$^{291}\rightarrow$Ser (exon 6) and
Ser$^{447}\rightarrow$Ter (exon 9), were studied by PCR amplification
followed by allele specific oligonucleotide (ASO) hy-
bridization of PCR products (13). The sequences of
these oligonucleotides were as follows:

Exon 6: 5’ ATCTGGGTGTCCTTTTTTACC 3’,
5’ TTATTACACAGTGCCAGTCC 3’
Exon 9: 5’ TGTTCTACATGGGATATTCC 3’,
5’ TACCGATGCCCAGTCCAGTCT 3’
AS0291 frequent: 5’ TGGAGTTCAATAAGTCA 3’,
AS0291 rare: 5’ TGGAGGCAGTTAACGTC 3’,
AS0447 frequent: 5’ TAAGAGTGAGGCTGAGT 3’,
AS0447 rare: 5’ TAAGACGTAGGCTGAGT 3’

Statistical analysis
Controls with CHD were excluded from all analyses.
Hardy-Weinberg equilibrium was tested in control
populations using a $\chi^2$ test. Pairwise linkage disequi-
lbria were estimated using log-linear model analysis (14)
and the extent of disequilibrium was expressed in terms
of D/Dmax (15). The association of lipid traits with LPL
polymorphisms was tested in control subjects by analysis
of variance controlling for age, population, body mass
index, alcohol, and cigarette consumption. A model
assuming additive allele effects was fitted to the data. As
no significant deviation from this hypothesis was ob-
served for any trait, the additive model was adopted in
subsequent analyses. The contribution of the different
polymorphisms to the variability of lipid traits was given
by the $R^2$. Triglycerides, VLDL, LpE-B, LpCIII-B, and
apoC-III values were log-transformed to remove positive
skewness. Comparison of genotype distributions be-
tween cases and controls was performed by logistic
regression analysis controlling for the same covariates
as above and adjusted odds ratios (OR) for MI were
derived from the logistic equation. A model assuming
codominance was also fitted to the data by coding the
genotype as an ordinal variable (0, 1, 2).

RESULTS

Linkage disequilibrium coefficients
In each control population, the genotype distribu-
tions were in accordance with Hardy-Weinberg expec-
tations. As previously described, HindIII and PvuII RFLPs
were in strong linkage disequilibrium (D/Dmax = 0.51,
P < 10$^{-4}$). H- and P- alleles being preferentially associated.
The Ser$^{447}\rightarrow$Ter mutation was in nearly complete dise-
TABLE 1. Lipid levels (adjusted mean, 95% CI) according to LPL genotypes in control subjects

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Triglycerides&lt;sup&gt;a&lt;/sup&gt;</th>
<th>ApoC-III&lt;sup&gt;b&lt;/sup&gt;</th>
<th>HDL-Cholesterol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>g/l [95% CI]</td>
<td>g/l [95% CI]</td>
<td>g/l [95% CI]</td>
</tr>
<tr>
<td>LPL HindIII</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H-H- (n = 75)</td>
<td>1.32 [1.19-1.47]</td>
<td>2.46 [2.24-2.71]</td>
<td>0.542 [0.513-0.571]</td>
</tr>
<tr>
<td>H+H- (n = 313)</td>
<td>1.33 [1.26-1.40]</td>
<td>2.56 [2.45-2.69]</td>
<td>0.523 [0.507-0.539]</td>
</tr>
<tr>
<td>H+H+ (n = 337)</td>
<td>1.39 [1.32-1.46]</td>
<td>2.76 [2.64-2.89]</td>
<td>0.508 [0.494-0.522]</td>
</tr>
<tr>
<td>Test&lt;sup&gt;c&lt;/sup&gt;</td>
<td>NS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LPL PvuII</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P-P- (n = 187)</td>
<td>1.27 [1.19-1.35]</td>
<td>2.58 [2.42-2.74]</td>
<td>0.515 [0.495-0.535]</td>
</tr>
<tr>
<td>P+P- (n = 351)</td>
<td>1.40 [1.34-1.47]</td>
<td>2.60 [2.49-2.72]</td>
<td>0.520 [0.506-0.534]</td>
</tr>
<tr>
<td>P+P+ (n = 186)</td>
<td>1.36 [1.32-1.46]</td>
<td>2.79 [2.62-2.96]</td>
<td>0.519 [0.499-0.539]</td>
</tr>
<tr>
<td>Test&lt;sup&gt;c&lt;/sup&gt;</td>
<td>NS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LPL Ser&lt;sup&gt;291&lt;/sup&gt;+Ter</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ser-Ser (n = 556)</td>
<td>1.39 [1.34-1.45]</td>
<td>2.72 [2.63-2.88]</td>
<td>0.513 [0.501-0.525]</td>
</tr>
<tr>
<td>Ser-Ter (n = 152)</td>
<td>1.26 [1.17-1.36]</td>
<td>2.40 [2.25-2.57]</td>
<td>0.538 [0.516-0.560]</td>
</tr>
<tr>
<td>Ter-Ter (n = 13)</td>
<td>1.11 [0.86-1.43]</td>
<td>2.18 [1.74-2.74]</td>
<td>0.510 [0.438-0.583]</td>
</tr>
<tr>
<td>Test&lt;sup&gt;c&lt;/sup&gt;</td>
<td>P &lt; 0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LPL Asn&lt;sup&gt;291&lt;/sup&gt;+Ser</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asn-Asn (n = 684)</td>
<td>1.36 [1.31-1.41]</td>
<td>2.65 [2.56-2.73]</td>
<td>0.517 [0.507-0.527]</td>
</tr>
<tr>
<td>Ser-&lt;sup&gt;c&lt;/sup&gt; (n = 39)</td>
<td>1.40 [1.20-1.63]</td>
<td>2.65 [2.32-3.04]</td>
<td>0.517 [0.475-0.559]</td>
</tr>
<tr>
<td>Test&lt;sup&gt;c&lt;/sup&gt;</td>
<td>NS</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>Antilog values.
<sup>b</sup>Test of allele effect (assuming an additive model) adjusted for age, population, body mass index, alcohol, and cigarette consumption.
<sup>c</sup>Ser-Ser + Asn-Ser genotypes.

equilibrium with HindIII (D/Dmax = 0.97, P < 10<sup>-4</sup>) and PvuII (D/Dmax = 0.94, P < 10<sup>-4</sup>), the rare allele Ter<sup>447</sup> being almost always carried by H- and P-. The Asn<sup>291</sup>+Ser mutation exhibited only a weak association with PvuII (P < 0.05), the rare allele Ser<sup>291</sup> being preferentially carried by the P+ allele.

**Associations with lipid related parameters**

In the control populations, the most significant associations were observed between the Ser<sup>447</sup>+Ter mutation and several triglyceride-related traits (Table 1). The Ter<sup>447</sup> allele had a lowering effect on triglycerides (P < 0.01, R<sup>2</sup> = 1%) and apoC-III levels (P < 0.001, R<sup>2</sup> = 1.9%), and these effects were consistently observed in the four populations (Fig. 1). The Ter<sup>447</sup> allele was also associated with lower VLDL-cholesterol (P < 0.05, R<sup>2</sup> = 0.7%), LpE:B (P < 0.01, R<sup>2</sup> = 1%) and LpCIII:B (P < 0.05, R<sup>2</sup> = 0.9%) levels, and higher apoA-I levels (P < 0.05, R<sup>2</sup> = 0.7%). With respect to HindIII polymorphism, the H- allele was associated with lower apoC-III (P < 0.01, R<sup>2</sup> = 1%) and higher HDL-cholesterol (P < 0.05, R<sup>2</sup> = 0.7%) levels (Table 1). However, the H- effect on apoC-III level seemed mediated mainly by its linkage disequilibrium with the Ter<sup>447</sup> allele, since after controlling for the Ser<sup>447</sup>+Ter polymorphism, the association was no longer significant. The PvuII and Asn<sup>291</sup>+Ser polymorphisms did not exhibit any significant association with the various biochemical traits examined (Table 1).

**Associations with CHD**

The H+ allele frequency significantly varied among control populations (P < 0.05), but this variation primar-
distribution of LPL genotypes in cases and controls in the four ECIM populations

<table>
<thead>
<tr>
<th>LPL HindIII</th>
<th>LPL PvunII</th>
<th>LPL Ser447→Ter</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Testa</td>
<td>Test</td>
</tr>
<tr>
<td></td>
<td>H+H+</td>
<td>H+H-</td>
</tr>
<tr>
<td>Belfast</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cases</td>
<td>12</td>
<td>85</td>
</tr>
<tr>
<td>Controls</td>
<td>15</td>
<td>76</td>
</tr>
<tr>
<td>Lille</td>
<td>15</td>
<td>76</td>
</tr>
<tr>
<td>Controls</td>
<td>14</td>
<td>75</td>
</tr>
<tr>
<td>Strasbourg</td>
<td>12</td>
<td>90</td>
</tr>
<tr>
<td>Controls</td>
<td>18</td>
<td>80</td>
</tr>
<tr>
<td>Toulouse</td>
<td>9</td>
<td>58</td>
</tr>
<tr>
<td>Controls</td>
<td>20</td>
<td>100</td>
</tr>
<tr>
<td>Odds ratios [95% CI]a</td>
<td>H+H+/H+H: 2.05 [1.32–3.20]</td>
<td>P+P+/P-P: 1.36 [0.99–1.87]</td>
</tr>
<tr>
<td></td>
<td>H+H-H+H: 1.74 [1.11–2.72]</td>
<td>P+P+/P-P: 1.20 [0.90–1.60]</td>
</tr>
</tbody>
</table>

aData of allele frequencies.

bOdds ratios adjusted for population, age, body mass index, alcohol, and cigarette consumption.

P < 0.01; P < 0.05; test of heterogeneity between populations for HindIII: P = 0.3.

The frequency of the Ser291 allele was estimated as 0.81 [0.63–1.03] (P = 0.09). The frequency of the Ser291 allele did not differ between cases and controls (data not shown).

**DISCUSSION**

This study is based on large population samples suggested that genetic variation at the LPL locus was associated with fasting plasma lipid and lipoprotein profiles. Despite the fact that several traits were studied, no statistical correction for multiple tests was performed because the search for possible associations was limited to triglyceride-related traits and HDL, on the basis of the known role of LPL. However, some caution is necessary when interpreting the significance of these results because all the traits investigated were not independent.

The associations observed with triglyceride and HDL-cholesterol levels, although rather weak, were in accordance with those generally reported (4–7). More interesting were the effects of the Ser447→Ter substitution on the trimetabolic trait profile. The Ser447→Ter substitution was associated with a significant increase of the coronary score in both cases and controls (Table 3). The mean score varied from 1.41 in P-P-individuals to 1.65 in P+P-individuals, with heterozygotes P+P having an intermediate score of 1.57 (test for linear trend, P < 0.05). A similar trend on the coronary score was observed with the presence of the Ser291 allele (1.58 vs. 1.90, P = 0.06). The Ter447 variant was less frequent in cases than in controls (Table 2), but this result did not reach statistical significance. Assuming a codominant model (P > 0.6 for the fit of the model), the OR associated with the presence of the Ser447 allele was estimated as 0.81 [0.63–1.03] (P = 0.09). The frequency of the Ser291 allele did not differ between cases and controls (data not shown).

**Table 3.** Coronary score in cases according to PvunII and LPL Asn329→Ser genotypes

<table>
<thead>
<tr>
<th>LPL PvunII</th>
<th>LPL Asn329→Ser</th>
</tr>
</thead>
<tbody>
<tr>
<td>P-P (n = 82)</td>
<td>Asn→Asn (n = 387)</td>
</tr>
<tr>
<td>P-P (n = 120)</td>
<td>Ser→Ser (n = 25)</td>
</tr>
<tr>
<td>P-P (n = 108)</td>
<td>Testa</td>
</tr>
<tr>
<td>1.41 (0.09)</td>
<td>1.58 (0.04)</td>
</tr>
<tr>
<td>1.57 (0.06)</td>
<td>1.90 (0.16)</td>
</tr>
<tr>
<td>1.65 (0.08)</td>
<td>NS (P &gt; 0.06)</td>
</tr>
</tbody>
</table>

aData of allele effect adjusted for age, population, body mass index, alcohol, and cigarette consumption.

aSer→Ser + Asn→Ser genotypes.
plasma concentrations of apoC-III and LpCIII:B and LpE:B particles. The lower levels of these particles in carriers of the Ter447 allele suggest that their clearance is enhanced in presence of this mutation, although the precise mechanisms are not yet elucidated. In particular, it has been shown that the truncated LPL protein created by the premature Stop447 codon had a normal lipolytic activity (4, 16). It has been proposed from the observation of a case with type I hyperlipidemia that the carriers of the Te#47 allele suggest that their clearance is enhanced in presence of this mutation, although the rate of LpE:B particles. The lower levels of these particles in carriers out of 121 (4.1%) hypertriglyceremic subjects and no carrier among 150 normotriglyceremic subjects of French Canadian descent. This LPL variant is associated with catalytic deficiency (10). An interaction of this variant with the apoE genotype was suggested to explain the hypertriglyceridemic effect (11). Such an interaction between apoE and LPL genotypes was not observed in ECTIM. It should be noted that the frequency of mutation carriers in the French Canadian hypertriglyceridemic patients was similar to that observed in our control population. The absence of carriers in normotriglyceridemic individuals is surprising and might be explained by a founder effect or by sampling bias due to small sample size.

Although the Ser447→Ter mutation had the largest effects on lipid traits, this polymorphism had no significant effects on lipid-related parameters were found in our study. This result is in contrast to a recent work (11) which found 5 mutation carriers out of 121 (4.1%) hypertriglyceremic subjects and no carrier among 150 normotriglyceridemic subjects of the French Canadian descent. This LPL variant is associated with catalytic deficiency (10). An interaction of this variant with the apoE genotype was suggested to explain the hypertriglyceridemic effect (11). Such an interaction between apoE and LPL genotypes was not observed in ECTIM. It should be noted that the frequency of mutation carriers in the French Canadian hypertriglyceridemic patients was similar to that observed in our control population. The absence of carriers in normotriglyceridemic individuals is surprising and might be explained by a founder effect or by sampling bias due to small sample size.

Concerning the Asn291→Ser mutation, no case-control difference and no significant effects on lipid-related parameters were found in our study. This result is in contrast to a recent work (11) which found 5 mutation carriers out of 121 (4.1%) hypertriglyceridemic subjects and no carrier among 150 normotriglyceridemic subjects of French Canadian descent. This LPL variant is associated with catalytic deficiency (10). An interaction of this variant with the apoE genotype was suggested to explain the hypertriglyceridemic effect (11). Such an interaction between apoE and LPL genotypes was not observed in ECTIM. It should be noted that the frequency of mutation carriers in the French Canadian hypertriglyceridemic patients was similar to that observed in our control population. The absence of carriers in normotriglyceridemic individuals is surprising and might be explained by a founder effect or by sampling bias due to small sample size.

Although the Ser447→Ter mutation had the largest effects on lipid traits, this polymorphism had no significant impact on the risk of MI. Conversely, the H+ allele was found to be associated with an increased risk of MI, especially in the Toulouse population. This association was apparently not mediated by large effects on fasting lipid and lipoprotein concentrations. The HindIII polymorphism is probably a neutral marker in linkage disequilibrium with one or several functional sites. Our results indicated that the Ser447→Ter mutation was unlikely to be one of these functional mutations, suggesting that other pathways are probably involved.

The precise mechanisms whereby the LPL gene could act on the disease process are still unclear. One hypothesis could be that unidentified functional sites in linkage disequilibrium with HindIII influence lipid levels in the postprandial rather than in the fasting state. Delayed postprandial triglyceridemia has been shown to be an important determinant of atherosclerosis (18) and CHD risk (19). Given the central role played by LPL in the catabolism of triglyceride-rich particles, the LPL gene is a strong candidate for the regulation of postprandial lipemia.

Other phenotypes not measured in the present study might also be relevant to consider, such as HDL2-choles-terol or LDL size, as these phenotypes have been shown to be modified in heterozygous states of primary LPL deficiency (20), and are known as cardiovascular risk factors (21, 22). The HindIII RFLP could be linked to functional mutations affecting these parameters through changes in LPL activity.

Another hypothesis could be the existence of LPL defects acting at a local level, for example, atherosclerotic lesions, which would not be reflected by circulating blood lipid levels. This hypothesis would be supported by the observation of an association between the PvuII polymorphism and severity of coronary lesions, despite no significant effect on fasting lipid parameters. This association between P+ and coronary score is described here for the first time. A recent observation yielded an association between severity of atherosclerosis and H+ allele but not P+ (6). The severity in that study was defined only in binary terms: severe/not severe. Thus the "severe" group in that study could be similar to the ECTIM case group, and the result they described could be comparable to our finding of an association between H+ and MI.

In conclusion, these results suggest that genetic variation at the LPL locus is involved in the determination of lipid and lipoprotein profiles and the predisposition to CHD. Further studies are needed to elucidate the underlying biological pathways and to identify the functional variants.

We thank Dr. J. M. Lalouel for providing ASO techniques and Dr. D. J. Galton for providing PCR-digestion techniques. The ECTIM study is supported by grants from Bristol-Myers Squibb, Sandoz, the British Heart Foundation, and by the Institut National de la Santé et de la Recherche Médicale and the Institut Pasteur in Lille.

Manuscript received 16 March 1995 and in revised form 22 May 1995.

REFERENCES


---

Jemaa et al.  LPL gene polymorphisms and myocardial infarction  2145


