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

**Riadh Jemaa,* Frédéric Fumeron,[1,]* Odette Poirier,† Laure Lecerf,† Alun Evans,§ Dominique Arveiler,** 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 SC7,† 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 U325 and MONICA Project-Lille,†† Institut Pasteur, 59019 Lille, France; INSERM U326 and MONICA Project-Haute-Garonne,§§ 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, $Ser^{447}$→Ter) and the newly described mutation $Asn^{291}$→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 $Ter^{447}$ 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 LpCIII: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 $Asn^{291}$→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 $Ser^{447}$→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 coronarography 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 $Asn^{291}$→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.—**Jemaa, R., F. Fumeron, O. Poirier, L. Lecerf, A. Evans, D. Arveiler, G. Luc, J-P. Cambou, J-M. Bard, J-C. Fruchart, M. Apfelbaum, F. Cambien, and L. Tiret.** Lipoprotein lipase gene polymorphisms: associations with myocardial infarction and lipoprotein levels, the ECTIM study. *J. Lipid Res.* 1995. **36:** 2141-2146.

**Supplementary key words** RFLP • $Asn^{291}$→Ser substitution • $Ser^{447}$→Ter substitution • coronary heart disease • plasma triglycerides • VLDL • HDL • apolipoproteins

Lipoprotein lipase (LPL) is essential for the clearance of triglyceride-rich lipoproteins from circulation through its role in the hydrolysis of triglycerides in chylomicrons and very low density lipoproteins (VLDL) (1). LPL also promotes the exchange of lipids between VLDL and high density lipoproteins (HDL) (2). Because of its key role in lipoprotein metabolism, LPL is likely to have an important influence in the development of atherosclerosis.

The LPL gene is located on chromosome 8p22, spanning about 35 kb and containing 10 exons (3). Several restriction fragment length polymorphisms (RFLP) have been identified at the LPL locus and possible associations of these RFLPs with lipid levels and coronary heart disease (CHD) have been investigated. The most consistent associations are those reported between the H+ allele (presence of the restriction site) of the HindIII RFLP (intron 8) and increased triglyceride levels (4–6), hypertriglyceridemia (7), and coronary atherosclerosis (6, 8). Although less consistently, the P+ allele of the PvuII RFLP (intron 6) has also been found associated with higher triglyceride (7) and lower HDL-cholesterol (6) levels. The first common mutation described in a

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.

[1]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.



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coding sequence was the $Ser^{447}$→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, $Asn^{291}$→Ser (10), has been found in hypertriglyceridemic patients of French Canadian descent (11).

Despite growing evidence that the LPL gene is involved in the predisposition to dyslipoproteinemia, its impact on CHD is less clearly established as these studies have not always been concordant. 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 underwent angiography but not with coronary disease per se (6).

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 $Ser^{447}$→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 Témoin sur l'Infarctus du Myocarde) (12). The LPL polymorphisms examined were HindIII, PvuII, $Ser^{447}$→Ter, and $Asn^{291}$→Ser.

## METHODS

### Study populations

The populations who took part in the ECTIM study have been described in detail (12). Cases were recruited from the MONICA registers in Belfast (Northern Ireland), Lille (northern France), Strasbourg (eastern France), and Toulouse (southwestern France). Patients aged 25–64 years who survived an MI (MONICA category I) were recruited at least 3 months and at most 9 months after the event. Age-matched controls were obtained from the electoral rolls in France and from the lists of general practitioners held by the Central Services Agency in Northern Ireland. Measurements of fasting plasma lipid, lipoprotein, and apolipoprotein concentrations were carried out as previously described (12).

A coronary angiography was available for 93% of the French cases but for only 20% of the cases from Belfast. In French cases only, a coronary score was defined as the number of coronary arteries with more than 50% occlusion (range 0–3).

### DNA analysis

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

Exon 6: 5′ ATCTTGGTGTCTCTTTTTTACC 3′),
5′ TTATTTACAACAGTC TCC AG 3′
Exon 9: 5′ TGTTCTACATGGCATATTCAC 3′,
5′ TCAGGATGCCCAGTCAGCTT 3′
ASO291 frequent: 5′ TGAGATCAATAAAGTCA 3′,
ASO291 rare: 5′ TGAGATCAGTAAAGTCA 3′,
ASO447 frequent: 5′ TAAGAAGTCAGGCTGGT 3′,
ASO447 rare: 5′ TAAGAAGTGAGGCTGGT 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 disequilibria 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 observed 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 between 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 distributions were in accordance with Hardy-Weinberg expectations. 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 $Ser^{447}$→Ter mutation was in nearly complete dise-



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TABLE 1. Lipid levels (adjusted mean, 95% CI) according to LPL genotypes in control subjects

| Genotype | Triglycerides[a] | ApoC-III[a] | HDL-Cholesterol |
|---|---|---|---|
| | *g/l* | *mg/dl* | *g/l* |
| LPL HindIII | | | |
| H-H- (n = 75) | 1.32 [1.19–1.47] | 2.46 [2.24–2.71] | 0.542 [0.513–0.571] |
| H+H- (n = 313) | 1.33 [1.26–1.40] | 2.56 [2.45–2.69] | 0.523 [0.507–0.539] |
| H+H+ (n = 337) | 1.39 [1.32–1.46] | 2.76 [2.64–2.89] | 0.508 [0.494–0.522] |
| Test[b]; | NS | $P < 0.01$ | $P < 0.05$ |
| LPL PvuII | | | |
| P-P- (n = 187) | 1.27 [1.19–1.35] | 2.58 [2.42–2.74] | 0.515 [0.495–0.535] |
| P+P- (n = 351) | 1.40 [1.34–1.47] | 2.60 [2.49–2.72] | 0.520 [0.506–0.534] |
| P+P+ (n = 186) | 1.36 [1.27–1.46] | 2.79 [2.62–2.96] | 0.519 [0.499–0.539] |
| Test[b] | NS | NS | NS |
| LPL $Ser^{447}$→Ter | | | |
| Ser–Ser (n = 556) | 1.39 [1.34–1.45] | 2.72 [2.63–2.82] | 0.513 [0.501–0.525] |
| Ser–Ter (n = 152) | 1.26 [1.17–1.36] | 2.40 [2.25–2.57] | 0.538 [0.516–0.560] |
| Ter–Ter (n = 13) | 1.11 [0.86–1.43] | 2.18 [1.74–2.74] | 0.510 [0.438–0.583] |
| Test[b] | $P < 0.01$ | $P < 0.001$ | NS |
| LPL $Asn^{291}$→Ser | | | |
| Asn–Asn (n = 684) | 1.36 [1.31–1.41] | 2.65 [2.56–2.73] | 0.517 [0.507–0.527] |
| Ser–+[c] (n = 39) | 1.40 [1.20–1.63] | 2.65 [2.32–3.04] | 0.517 [0.475–0.559] |
| Test[b] | NS | NS | NS |

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

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

### Associations with lipid related parameters

In the control populations, the most significant associations were observed between the $Ser^{447}$→Ter mutation and several triglyceride-related traits (**Table 1**). The $Ter^{447}$ allele had a lowering effect on triglycerides ($P < 0.01$, $R^2 = 1\%$) and apoC-III levels ($P < 0.001$, $R^2 = 1.9\%$), and these effects were consistently observed in the four populations (**Fig. 1**). The $Ter^{447}$ allele was also associated with lower VLDL-cholesterol ($P < 0.05$, $R^2 = 0.7\%$), LpE:B ($P < 0.01$, $R^2 = 1\%$) and LpCIII:B ($P < 0.05$, $R^2 = 0.9\%$) levels, and higher apoA-I levels ($P < 0.05$, $R^2 = 0.7\%$). With respect to HindIII polymorphism, the H- allele was associated with lower apoC-III ($P < 0.01$, $R^2 = 1\%$) and higher HDL-cholesterol ($P < 0.05$, $R^2 = 0.7\%$) levels (Table 1). However, the H- effect on apoC-III level seemed mediated mainly by its linkage disequilibrium with the $Ter^{447}$ allele, since after controlling for the $Ser^{447}$→Ter polymorphism, the association was no longer significant. The PvuII and $Asn^{291}$→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-

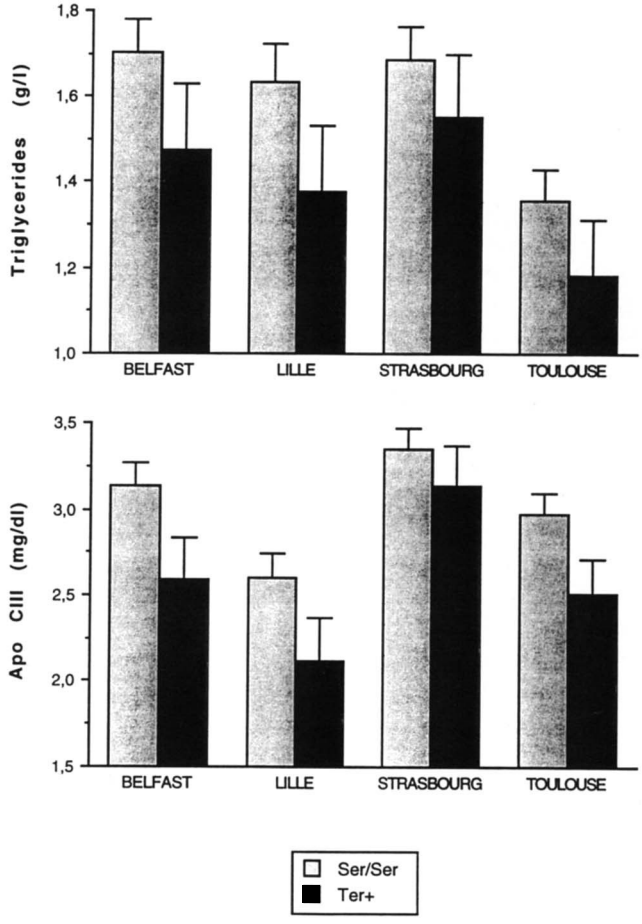


**Fig. 1.** Plasma triglycerides and apoC-III levels in the four ECTIM control populations according to LPL $Ser^{447}$→Ter genotype (Ter–Ter homozygotes are pooled with heterozygotes because of small sample size in each population).



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TABLE 2. Distribution of LPL genotypes in cases and controls in the four ECTIM populations

| | LPL HindIII | | | | | LPL PvuII | | | | | LPL $Ser^{447}$→Ter | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | H-H- | H-H+ | H+H+ | *f(H+)* | Test[a] | P-P- | P-P+ | P+P+ | *f(P+)* | Test | Ser-Ser | Ser-Ter | Ter-Ter | *f(ter)* | Test |
| Belfast | | | | | | | | | | | | | | | |
| Cases | 12 | 85 | 104 | 0.73 | NS | 40 | 94 | 67 | 0.57 | NS | 164 | 36 | 1 | 0.09 | NS |
| Controls | 15 | 76 | 89 | 0.71 | | 40 | 96 | 44 | 0.51 | | 145 | 35 | 1 | 0.10 | |
| Lille | | | | | | | | | | | | | | | |
| Cases | 5 | 25 | 36 | 0.73 | NS | 15 | 38 | 13 | 0.48 | NS | 76 | 18 | 2 | 0.11 | NS |
| Controls | 14 | 58 | 75 | 0.71 | | 48 | 62 | 37 | 0.46 | | 113 | 28 | 7 | 0.14 | |
| Strasbourg | | | | | | | | | | | | | | | |
| Cases | 12 | 90 | 97 | 0.71 | NS | 43 | 94 | 62 | 0.55 | NS | 167 | 38 | 0 | 0.09 | NS |
| Controls | 18 | 80 | 97 | 0.70 | | 47 | 90 | 58 | 0.53 | | 150 | 40 | 4 | 0.12 | |
| Toulouse | | | | | | | | | | | | | | | |
| Cases | 9 | 58 | 81 | 0.74 | < 0.001 | 30 | 76 | 42 | 0.54 | NS | 118 | 26 | 3 | 0.11 | NS |
| Controls | 29 | 100 | 82 | 0.63 | | 52 | 109 | 49 | 0.49 | | 155 | 51 | 1 | 0.13 | |
| Odds ratios [95% CI][b] | | H+H+/H-H-: 2.05 [1.32–3.20][c] | | | | P+P+/P-P-: 1.36 [0.99–1.87] | | | | | Ter-Ter/Ser-Ser: 0.52 [0.19–1.42] | | | | |
| | | H+H-/H-H-: 1.74 [1.11–2.72][d] | | | | P+P-/P-P-: 1.20 [0.90–1.60] | | | | | Ter-Ser/Ser-Ser: 0.83 [0.63–1.10] | | | | |

[a]Difference of allele frequencies.
[b]Odds ratios adjusted for population, age, body mass index, alcohol, and cigarette consumption.
[c]$P < 0.01$; [d]$P < 0.05$; test of heterogeneity between populations for HindIII: $P = 0.3$.

primarily reflected a lower frequency in the Toulouse population (**Table 2**). No such geographical variation was observed for the P+ allele (Table 2) or for the $Ter^{447}$ and the $Ser^{291}$ alleles whose frequencies were estimated in the combined control populations as 0.123 ± 0.009 and 0.027 ± 0.004, respectively. The HindIII genotype distributions significantly differed between cases and controls. By reference to the H-H- genotype, the ORs associated with H+H+ and H+H- were estimated as 2.05 ($P < 0.01$) and 1.74 ($P < 0.05$), respectively (Table 2). The case-control difference was larger in Toulouse than in the three other populations, although the test of heterogeneity among populations was not significant ($P = 0.3$). When excluding Toulouse, the ORs were 1.71 [1.00–2.90] ($P < 0.05$) and 1.64 [0.96–2.81] ($P = 0.07$) respectively. Adjustment on apoC-III level did not modify the ORs, whereas adjustment on HDL-cholesterol slightly decreased the strength of association (ORs = 1.81 and 1.60, respectively). To a lesser extent, the P+ allele was also associated with a higher risk of MI, although nonsignificantly (Table 2). The model assuming codominance well fitted to the data ($P > 0.8$) and the OR associated with the presence of the allele P+ was estimated as 1.17 [0.99–1.37] ($P = 0.06$). In studies conducted in France, an increase of the coronary score was observed with the P+ allele (**Table 3**). The mean score varied from 1.41 in P-P- individuals to 1.64 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 $Ser^{291}$ allele (1.58 vs. 1.90, $P = 0.06$). The $Ter^{447}$ 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 $Ter^{447}$ allele was estimated as 0.81 [0.63–1.03] ($P = 0.09$). The frequency of the $Ser^{291}$ allele did not differ between cases and controls (data not shown).

## DISCUSSION

This study 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 $Ser^{447}$→Ter substitution on

TABLE 3. Coronary score in cases according to PvuII and LPL $Asn^{291}$→Ser genotypes

| | |
|---|---|
| LPL PvuII | |
| P-P- (n = 82) | 1.41 (0.09) |
| P+P- (n = 190) | 1.57 (0.06) |
| P+P+ (n = 108) | 1.65 (0.08) |
| Test[a] | $P < 0.05$ |
| LPL $Asn^{291}$ → Ser | |
| Asn-Asn (n = 387) | 1.58 (0.04) |
| Ser-+[b] (n = 25) | 1.90 (0.16) |
| Test[a] | NS ($P = 0.06$) |

[a]Test of allele effect adjusted for age, population, body mass index, alcohol, and cigarette consumption.
[b]Ser-Ser + Asn-Ser genotypes.



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plasma concentrations of apoC-III and LpCIII:B and LpE:B particles. The lower levels of these particles in carriers of the $Ter^{447}$ 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 $Stop^{447}$ codon had a normal lipolytic activity (4, 16). It has been proposed from the observation of a case with type I hyperlipidemia that the functional defect could be due to an impaired lipid-binding ability (17).

Concerning the $Asn^{291}$→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 $Ser^{447}$→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 $Ser^{447}$→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 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 $HDL_2$-cholesterol 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.*

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