Lipoprotein Lipase Gene Variation Is Associated With a Paternal History of Premature Coronary Artery Disease and Fasting and Postprandial Plasma Triglycerides

The European Atherosclerosis Research Study (EARS)

Steve E. Humphries, Viviane Nicaud, Joaquim Margalef, Laurence Tiret, Philippa J. Talmud, for the EARS

Abstract—The H-allele of the intron 8 HindIII polymorphism in the lipoprotein lipase (LPL) gene has been associated with a lower risk of myocardial infarction (MI) and plasma levels of triglycerides (TG). To test whether the HindIII site was in linkage disequilibrium with the functional variant LPL Serine447Stop (S447X), subjects from the European Atherosclerosis Research Study (EARS I) were genotyped for both polymorphic sites. This study included 515 offspring of fathers with a premature (<55 years old) MI, who were designated cases, and 930 age- and sex-matched control subjects from five different regions of Europe. Linkage disequilibrium between the two sites was very strong (>99), with only three of the four possible haplotypes identified: H+S447, H−S447, and H−X447. The frequency of the H−X447 but not of the H−S447 haplotype was significantly lower in cases than in control subjects (.090 versus .117, P<.01) suggesting a protective effect for MI, with this difference being consistent in all five regions of Europe. Compared with individuals homozygous for the H+S447 haplotype, the odds ratio of having a paternal history of premature MI for H−X447 heterozygotes (≈20% of the population) was 0.71 (95% confidence interval, 0.55 to 0.92).

In addition, there was an increase of the H−X447 haplotype frequency from north to south in control subjects (0.119 in Finland to 0.143 in the Mediterranean region, P<.01). Compared with the H+S447 haplotype, the H−X447 haplotype was associated with significantly lower concentrations of plasma TGs (5.4% lower, P<.01), with this effect being consistent over the regions of Europe. There was no significant evidence for a heterogeneity of effect between males and females or between cases and control subjects, although the effect on TG levels appeared to be the greatest in male cases (11% lower, P=.05). In a second study (EARS II), of 332 cases and 342 control subjects, postprandial clearance of TGs after a standard fat meal was examined. The H−X447 haplotype was associated with significantly lower postprandial triglyceride levels than was the H+S447 haplotype (9.4% smaller area under the curve, P<.05).

Thus, the effects on MI risk and plasma lipids associated with the H allele appeared to be mainly mediated by the X447 mutation, and although the lowering effects associated with the H−X447 haplotype on fasting and postprandial TGs are not large, they are consistent with the lowering effect observed on MI risk throughout Europe. (Arterioscler Thromb Vasc Biol. 1998;18:526-534.)

Key Words: lipoprotein lipase HindIII polymorphism ■ Ser447X ■ offspring

The European Atherosclerosis Research Study was designed to assess the involvement of a number of measurable environmental and genetic factors on the gradient of CAD risk seen across Europe and to explore predisposing factors determining the early development of disease. The study compares the young (mean age, 23 years) offspring of fathers with MI before the age of 55 years (designated as cases) with age- and sex-matched control subjects from five different regions of Europe. Differences in lipid variables were observed with plasma total and LDL cholesterol and TG levels that were higher in cases than control subjects, with the TG difference being stronger in males (0.98 versus 0.88 mmol/L, P<.005) than in females. Levels of apoB and TGs were the strongest discriminators between offspring of fathers with premature MI and control subjects. In this current article, the role of variation in the LPL gene in determining the risk of paternal MI, the north-south gradient of risk, and the case-control difference in plasma TG levels were explored.

LPL is one of the key enzymes in the metabolism of the TG-rich lipoproteins. LPL is a heparin-releasable enzyme, bound to glycosaminoglycan components of the capillary

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endothelium in adipose and muscle tissues, which hydrolyzes TG in chylomicrons and VLDL (reviewed in Reference 4). To date, >60 rare LPL gene mutations have been identified (reviewed in Reference 5), which result in a nonfunctional LPL enzyme; inheritance of two defective LPL genes leads to fasting chylomicronemia (type I hyperlipoproteinemia). A number of common sequence variants have been reported,6–9 and in particular, the intron 8 HindIII polymorphism.8 In studies of healthy individuals, the effects associated with this polymorphism have been relatively consistent with regard to levels of plasma TG and/or HDL-C, with the H-allele associated with higher values of HDL-C and lower values of plasma TG.10–12 Several studies have also reported a lower frequency of the H-allele in individuals with hypertriglyceridemia and in patients with CAD compared with normotriglyceridemic subjects.13

It has been proposed that the H-allele of the LPL HindIII polymorphism acts as a genetic marker for a functional mutation that could cause either enhanced enzyme activity or more efficient lipid binding or, if it occurred in the gene promoter, could result in increased LPL expression. This would then result in lower plasma TG and higher HDL-C concentrations in carriers of the H-allele. Searches for common sequence changes in the coding region identified three common sites that alter amino acids: D9N, N291, and S447X, all identified by single-strand conformation polymorphism and direct sequencing.9,14,15 Although carriers of either of the first two mutations tend to have elevated levels of plasma TG, lower HDL-C levels, and a greater risk of atherosclerosis,16,17 their low frequency of occurrence in the population means that they cannot explain the HindIII effect. A third polymorphism has been identified which alters the penultimate amino acid Serine447 to a stop codon (S447X) resulting in a truncation of the enzyme.9,11 and higher expression in in vitro studies.19 This polymorphism is common, with the allele frequency of the S447X mutation being ∼20% in healthy individuals.9,12,20–25 Although early studies examining the association between the S447X polymorphism and lipid levels did not give consistent results,12,20 the X447 allele occurs less frequently in patients with hypertriglyceridemia than in healthy whites and is more common in healthy control subjects compared with MI patients.22 Recent studies have demonstrated clearly that the X447 mutation is associated with higher postheparin LPL activity in patients23 and a favorable lipid profile, with lower plasma cholesterol, lower TG, and higher HDL-C levels.22–25 although in the ECTIM study, the X447 allele was not associated with the risk of MI.24 Thus, there is strong evidence to suggest that the X447 mutation is associated with a beneficial lipid profile and that it may therefore confer protection against CAD.

Therefore, the S447X polymorphism is a strong candidate that might explain the reported associations with the HindIII H-allele. The two variant sites are within 600 bp of each other in the gene, and the X447 variant is found almost exclusively on the H-allele. However, the H-allele also occurs with S447, thereby allowing a population association approach to test whether both the H–X447 and H–S447 haplotypes are associated with significant effects on the risk of MI and plasma lipids. We therefore compared the frequency of the LPL HindIII–S447X haplotypes in the subjects recruited into EARS I and examined the association between the polymorphism and levels of plasma lipid traits. To confirm these results and to explore the possible mechanisms of the effects of the polymorphisms, genotype was also determined in EARS II, wherein the effects of the polymorphisms in determining postprandial handling of plasma lipids were examined.

Methods

Subjects

EARS I has been described previously.1 In brief, male and female university students between 18 and 26 years with a paternal history of MI before the age of 55 years (cases) were recruited in 1990 from 14 university student populations from 12 European countries: Austria, Belgium, Denmark, Finland, France, Germany, Italy, Spain, Sweden, Switzerland, and the United Kingdom. Two age- and sex-matched control subjects per case were randomly selected from the same university populations. Details of lifestyle, medical history, and physiological measurements were established using standardized protocols.2,26 Venous blood was collected after a 14-hour fast. EARS II was carried out in 1993.27,28 Male students aged 18 to 28 were recruited from 14 university student populations from 11 European countries: Estonia, Finland, Belgium, Denmark, Germany, Switzerland, United Kingdom, Spain, Italy, Portugal, and Greece. Subjects were recruited and data collected using the same protocol as described above, with the exception that cases and control subjects were matched 1:1. EARS II also included an oral glucose tolerance test and an oral fat tolerance test.

Lipid and Lipoproteins Analyses

All lipid analyses were performed in Glasgow by using procedures recommended by the Lipid Research Clinic’s Manual of Operations.29 LDL-C was calculated by the Friedewald formula.30 The apoAI and B levels were measured in Lille by immunonephelometry on a Behring BNA nephelometer using Behring antisera and standards.31 Details of the lipoprotein distributions have been published elsewhere.2 Levels of LpCIII:B were measured as described.24

Oral Glucose Tolerance Test and Oral Fat Tolerance Test

Subjects were given a standard (75-g) oral glucose tolerance test.27,28 One week later they were given an oral liquid lipid load containing 42 g saturated fat, 22 g protein, 56 g carbohydrate, and 417 mg cholesterol. The formula contained 6186 kJ. Blood was drawn at baseline and at 2, 3, 4, and 6 hours afterward. Biochemical analyses were performed as described.
Isolation of DNA and Genotype Analysis

DNA was isolated using the “salting-out” procedure.\(^3\) HindIII analysis was performed as described.\(^4,5\) The cutting site was at position +477 of the intron between exons 8 and 9. Genotype was assigned on the basis of the presence (+) or absence (−) of the cutting site. The S447X polymorphism in exon 9 was identified by the introduction of a forced HindII restriction enzyme site into the PCR product. The following primers were used: forward primer 5′-CATCCATTCTTCCACAGGG-3′ and reverse primer 5′-TAGCCCGAGATGCTACACAGACT-3′ (Genosys). The reaction was carried out in the standard PCR buffer supplied by Gibco-BRL with 100 ng of each primer and a final MgCl\(_2\) concentration of 2 mmol/L, with 0.2 U of Taq polymerase per reaction in a final volume of 25 mL. PCR was performed on an MJ Research thermal cycler PTC-220 (MJ Research Inc). After an initial PCR cycle of 95°C for 5 minutes, 30 rounds of PCR followed under these conditions: 94°C for 1 minute, 55°C for 1 minute, and 72°C for 1 minute. PCR product (8 mL) was digested overnight with HindII and the buffer supplied by the manufacturer (Gibco-BRL). The introduced HindII site is absent in the S447 allele, yielding an “uncut” PCR product of 137 bp. The forced HindII site is present in the X447 allele and after HindII digestion, the fragment sizes are 114 and 23 bp. The fragments were separated by 7.5% polyacrylamide gel electrophoresis with microtiter array diagonal gel electrophoresis.\(^6\) Due to technical difficulties in isolating DNA from some samples from several centers (low yields of DNA or contamination with PCR inhibitors), genotypes were not obtained for 349 samples (111 cases, 238 control subjects). From a total of 1994 recruited subjects, 1645 were unambiguously genotyped, which represents 83% of the EARS I population.

Statistical Analysis

The recruitment centers in EARS I were grouped into five regions based on published age-standardized mortality rates, geography, and language: Finland, Great Britain, and northern, middle, and southern Europe. In EARS II, we defined four regions: Baltic (Finland and Estonia), United Kingdom, middle Europe (Denmark, Germany, Belgium, and Switzerland), and southern Europe (Portugal, Italy, Spain, and Greece). Subjects who were carriers of either the N9 or the S291 mutation were excluded from analysis. The data were analyzed using the SAS statistical software package (SAS Institute Inc). Observed numbers of each genotype were compared with those expected if the sample was in Hardy-Weinberg equilibrium by \(\chi^2\) analysis. Because of complete linkage disequilibrium between HindIII and S447, only three haplotypes existed: H+S447, H−S447, and H−X447 (with the exception of one recombinant). Haplotypes could then be assigned unambiguously, even for heterozygotes for both polymorphisms. In all analyses, the haplotype H+S447 was used as a reference, and the effects of H−S447 and H−X447 were tested by introducing two variables reflecting the number of copies (0, 1, or 2) of each of the two haplotypes carried by a given subject.

This scheme of coding implicitly hypothesizes the absence of dominance between alleles (ie, heterozygotes are strictly intermediate between the two types of homozygotes). No significant deviation from this hypothesis was observed in any analysis. Haplotype distributions were compared between cases and control subjects by logistic regression analysis adjusted for age, sex, and region. Association of haplotypes with fasting lipid levels and postprandial response was investigated by ANOVA (general linear model procedure) adjusted for age, region, sex, and case/control status. The postprandial response in EARS II was characterized by two different parameters: (1) the AUC above the fasting concentration, calculated by the trapezoidal rule, and (2) the peak value, calculated as the highest value minus the fasting value. To remove positive skewness, the distribution of fasting TG levels, AUCs, and peaks were logarithmically transformed for testing and estimating the haplotype effects. Because of this logarithmic transformation, haplotype effects on lipid traits were expressed in percent reduction rather than absolute difference. The observed value of TG in the H+S447/H+S447 haplotypes was used as a reference, and the effects estimated for the H−S447 and H−X447 haplotypes were applied to this value. The resulting value of TG in millimoles per liter in heterozygotes (H+S447/H−S447 and H+S447/H−X447) was thus estimated and plotted. The homogeneity of all genetic effects in cases and control subjects, in men and women, and by region was systematically tested by introducing the corresponding interaction term in all stages of the analysis.

Results

The characteristics of the EARS I sample have been described before in detail,\(^1,2\) and key traits are presented in Table 1, after exclusion of individuals known to be carriers for either of the common LPL missense mutations (D9N and N291S).\(^2,7\) TG levels were significantly higher in cases than control subjects, with this effect being seen in both males and females. Cases also had higher cholesterol levels than did control subjects, with this effect being seen in both males and females. Cases also had higher cholesterol levels than did control subjects, but this difference reached statistical significance in males only. Genotype was determined for the two LPL gene polymorphisms, HindIII in exon 8 (alleles designated + or −) and S447X in exon 9 (alleles designated S447 and X447). For all regions and in both cases and control subjects, the distribution of genotypes was as expected from Hardy-Weinberg proportions, and the data for the combined genotype groups are shown separately for cases and control subjects in Table 2. There are nine possible genotype groups, but from Table 2, only six of these classes were seen in cases, with only one control individual in a seventh class (genotype H+H+/S/X). From these data, it can be deduced that only

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**TABLE 1. Mean (±SE) Characteristics of Subjects From EARS I and EARS II**

<table>
<thead>
<tr>
<th>Trait</th>
<th>EARS I Females</th>
<th>EARS I Males</th>
<th>EARS II Females</th>
<th>EARS II Males</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>258</td>
<td>480</td>
<td>257</td>
<td>470</td>
</tr>
<tr>
<td>Age, y (years)</td>
<td>22.33 (0.12)</td>
<td>22.34 (0.09)</td>
<td>22.73 (0.13)</td>
<td>22.76 (0.10)</td>
</tr>
<tr>
<td>BMI, kg/m(^2)</td>
<td>21.50 (0.17)</td>
<td>21.32 (0.13)</td>
<td>22.76 (0.17)</td>
<td>22.41 (0.12)</td>
</tr>
<tr>
<td>Total C, mmol/L</td>
<td>4.62 (0.05)</td>
<td>4.51 (0.04)</td>
<td>4.57 (0.05)(^\dagger)</td>
<td>4.33 (0.04)</td>
</tr>
<tr>
<td>Total TG, mmol/L</td>
<td>0.89 (0.02)*</td>
<td>0.84 (0.02)</td>
<td>0.98 (0.02)(^\dagger)</td>
<td>0.87 (0.02)</td>
</tr>
<tr>
<td>Total HDL-C, mmol/L</td>
<td>1.56 (0.02)</td>
<td>1.58 (0.02)</td>
<td>1.26 (0.02)</td>
<td>1.30 (0.01)</td>
</tr>
</tbody>
</table>

*BMI indicates body mass index. Subjects with LPL N9 and S291 mutations were excluded. Means were adjusted for age and region. For EARS II, means of the two baseline values of TGs at the oral glucose tolerance test and oral fat tolerance test are presented. Tests were performed on log of TG values.

\(^*\)P<0.05, \(^\dagger\)P<0.001.
TABLE 2. Distribution of Cases and Control Subjects According to HindIII and S447X Combined Genotypes and Estimated Linkage Disequilibrium (D)

<table>
<thead>
<tr>
<th>HindIII/S447X</th>
<th>S/S</th>
<th>S/X</th>
<th>X/X</th>
</tr>
</thead>
<tbody>
<tr>
<td>H+/H+</td>
<td>280/450</td>
<td>0/1</td>
<td>0/0</td>
</tr>
<tr>
<td>H+/H−</td>
<td>121/241</td>
<td>67/155</td>
<td>0/0</td>
</tr>
<tr>
<td>H−/H−</td>
<td>25/45</td>
<td>18/49</td>
<td>4/9</td>
</tr>
</tbody>
</table>

For cases, $D^* = +1.00 \ (P<.001)$; for control subjects, $D^* = +0.99 \ (P<.001)$. D was not significantly different between cases and control subjects.

three common haplotypes exist: $H+S447$, $H−S447$, and $H−X447$, with the haplotype $H+X447$ being seen unambiguously in only one individual. Therefore, haplotypes could be assigned unambiguously, even for those individuals heterozygous for both polymorphisms, on the assumption that $H+X447$ is extremely rare. The frequencies of the three haplotypes among cases and control subjects in the five regions are presented in Table 3. For the $H−X447$ haplotype overall, there was a statistically significant higher frequency in the control subjects than in the cases (.117 versus .090, $P<.01$). Compared with the haplotype $H+S447$, the haplotype $H−X447$ was associated with a population-adjusted OR of paternal CAD of 0.71 (95%CI = 0.55 to 0.92). After adjustment for the matching and stratification criteria (age, sex, and region) and for the case/control difference in TGs, this OR was essentially unchanged (OR = 0.73, 95%CI = 0.56 to 0.95, $P<.02$).

In addition, as shown in Table 3, in the control subjects there was a gradient in frequency of the $H−X447$ haplotype across Europe, from Finland (.119±.024) to the south (.143±.024), and these regional differences were statistically significant ($P<.01$). Moreover, in each region the frequency of the $H−X447$ haplotype was higher in control subjects than in cases. For the $H−S447$ haplotype there was also an increasing frequency from the north to the south. Although the overall frequency was higher in control subjects than in cases (.20 versus .184; OR = 0.85, 95%CI = 0.07 to 1.04), this trend was not consistent in all regions, and neither of these effects reached statistical significance. Thus, the $H−X447$ haplotype but not the $H−S447$ haplotype is associated with CAD protection from a paternal history of MI.

To explore possible mechanisms for this protective effect, the association between LPL haplotypes and three key plasma lipid traits (total cholesterol, TG, and HDL-C) was next examined separately in males and females and by status (case and control subjects). Statistically significant effects were seen with plasma TGs only, and the data are presented in Table 4 (excluding the single individual with the genotype $H+H+/S/X$). Although there was no significant heterogeneity of genotype effects on TG between females and males and cases and control subjects, the data are presented separately. The effect on TG levels associated with LPL haplotypes was estimated on the assumptions that there are only three common haplotypes and an additive genetic model on log values of TG (ie, a multiplicative effect on TG). These estimates are presented in Table 4 and summary data presented in Fig 1. Overall the $H−X447$ haplotype was associated with a lowering effect on TG levels of 5.4% ($P=.01$) by comparison with $H+S447$. This means that heterozygotes for this haplotype had their TG values lowered by 5.4% (95%CI = −9.4% to −1.3%) and homozygotes had their TG levels lowered by 10.6% (95%CI = −18.0% to −2.5%). This haplotype effect was larger in the male control subjects than the female control subjects (−7.1%, $P<.05$, versus −4.2%, NS) and largest in male cases (−11.0%, $P=.06$). This lowering effect was seen consistently in the five regions of Europe, and there were no significant differences in the magnitude of the effect (data not shown). By contrast, for the

TABLE 3. Frequency of LPL Haplotypes Among EARS I Cases and Control Subjects in the Five Regions of Europe (N9 and S291 Excluded)

<table>
<thead>
<tr>
<th>Region</th>
<th>Status</th>
<th>n</th>
<th>$H+S447$</th>
<th>$H−S447$</th>
<th>$H−X447$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Finland</td>
<td>Case</td>
<td>78</td>
<td>.731</td>
<td>.179</td>
<td>.090</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>177</td>
<td>.700</td>
<td>.181</td>
<td>.119</td>
</tr>
<tr>
<td>G.B.</td>
<td>Case</td>
<td>51</td>
<td>.765</td>
<td>.157</td>
<td>.078</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>94</td>
<td>.750</td>
<td>.160</td>
<td>.090</td>
</tr>
<tr>
<td>North</td>
<td>Case</td>
<td>127</td>
<td>.736</td>
<td>.197</td>
<td>.067</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>264</td>
<td>.718</td>
<td>.189</td>
<td>.093</td>
</tr>
<tr>
<td>Middle</td>
<td>Case</td>
<td>127</td>
<td>.689</td>
<td>.228</td>
<td>.083</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>198</td>
<td>.657</td>
<td>.211</td>
<td>.132*</td>
</tr>
<tr>
<td>South</td>
<td>Case</td>
<td>132</td>
<td>.735</td>
<td>.140</td>
<td>.125</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>217</td>
<td>.620</td>
<td>.237†</td>
<td>.143</td>
</tr>
<tr>
<td>All</td>
<td>Case</td>
<td>515</td>
<td>.726</td>
<td>.184</td>
<td>.090</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>950</td>
<td>.683</td>
<td>.200</td>
<td>.117</td>
</tr>
</tbody>
</table>

Region difference, $P=$ NS, <.01

Case/control difference, $P=$ NS, <.01

*Test was performed by taking $H+S447$ as the reference haplotype.
†$P<.05$. †$P<.01$. 

For cases, $D^* = +1.00 \ (P<.001)$; for control subjects, $D^* = +0.99 \ (P<.001)$. D was not significantly different between cases and control subjects.

The effect on TG levels associated with LPL haplotypes was estimated on the assumptions that there are only three common haplotypes and an additive genetic model on log values of TG (ie, a multiplicative effect on TG). These estimates are presented in Table 4 and summary data presented in Fig 1. Overall the $H−X447$ haplotype was associated with a lowering effect on TG levels of 5.4% ($P=.01$) by comparison with $H+S447$. This means that heterozygotes for this haplotype had their TG values lowered by 5.4% (95%CI = −9.4% to −1.3%) and homozygotes had their TG levels lowered by 10.6% (95%CI = −18.0% to −2.5%). This haplotype effect was larger in the male control subjects than the female control subjects (−7.1%, $P<.05$, versus −4.2%, NS) and largest in male cases (−11.0%, $P=.06$). This lowering effect was seen consistently in the five regions of Europe, and there were no significant differences in the magnitude of the effect (data not shown). By contrast, for the
530  LPL Gene Variation and Premature CAD

TABLE 4. Mean (±SEM) TG Concentrations (mmol/L) According to LPL HindIII and S447X Genotypes and Haplotypes by Sex and Case/Control Status. Values Were Adjusted for Age and Region

<table>
<thead>
<tr>
<th>Haplotype Effect, Females</th>
<th>Haplotype Effect, Males</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype</td>
<td>Cases</td>
</tr>
<tr>
<td>S447</td>
<td>−4.8% (−12.1 to +3.2)</td>
</tr>
<tr>
<td>X447</td>
<td>+0.8% (−9.5 to +12.3)</td>
</tr>
</tbody>
</table>

Number in brackets indicates number of subjects. H-S447 and H-X447 haplotype effects were estimated by reference to the H+S447 haplotype: % difference (95% confidence interval). Model assumes a codominant effect on log TG. No significant heterogeneity between males and females or between cases and control subjects was observed.

†P < .05; †P < .01.

Overall H-S447 effect (adjusted for age, sex, case/control status, and region): −1.3% (−4.3 to +1.9) NS; overall H-X447 effect (adjusted for age, sex, case/control status, and region): −5.4% (−9.4 to −1.3) P = .01.

Figure 1. Association between LPL haplotypes and levels of TGs in individuals in Europe. Effects associated with the H−S447 haplotype were estimated as described in “Methods” for females and males (after adjustment for region, age, and case-control status), cases and control subjects (after adjustment for region, age, and sex), and all (after adjustment for region, age, sex, and case-control status). **P < .05; ***P < .01. Overall there was no significant evidence of heterogeneity between cases and control subjects and between males and females.

H−S447 haplotype the lowering effect in the whole group combined was much smaller than the effect of the H−X447 haplotype (overall −1.3%, 95%CI = −4.3% to +1.9%, NS); moreover, this small lowering was not seen consistently throughout the five regions. Only in male cases did the lowering effect associated with this haplotype approach statistical significance (−7.7%, P = .06).

Because of its role in plasma lipid metabolism, mutations that affect LPL activity or levels should have an effect on the clearance of TGs after a meal. To investigate this possibility, the subjects from EARS II were also genotyped for both polymorphisms. The key characteristics of these individuals (all male) are presented in Table 1 (excluding carriers for D9N and N291S) and show, as before, that cases have significantly higher cholesterol levels but that the effect on TG was smaller than in EARS I and not statistically significant. Although there were some differences in location of recruitment centers and smaller sample size, estimates of the haplotype frequencies confirmed the differences seen in EARS I, with a higher frequency of the H−X447 haplotype seen in control subjects than in cases (1.47 versus .108, OR = 0.67, 95%CI = 0.49 to 0.92, P < .05) and a north-south gradient in frequency (P < .05). For the H−S447 haplotype, results similar to those in EARS I were seen, with no differences reaching statistical significance (overall control-case frequency, .196 versus .174, OR = 0.82, 95%CI = 0.62 to 1.07).

The effect associated with the haplotypes on plasma TG levels after a standard fat meal was examined, and data were obtained for 332 cases and 342 control subjects. For the H+S447 homozygotes in both cases and control subjects, there was a rapid increase in plasma TG after the meal, peaking by 3 hours and declining to almost fasting values by 6 hours. Compared with the H+S447 haplotype, the H−X447 haplotype was associated with a significantly smaller increase in TG levels after the meal, with the difference being particularly marked at later times and statistically significant at 3 and 4 hours, even after accounting for the small differences in fasting TG levels (P < .05 for both). This smaller rise in TG levels was also seen in a 9.4% smaller estimate (95%CI = −16.7% to −1.5%, P = .02) for the AUC and a 4.1% smaller peak height (95%CI = −8.2% to +0.1%, P = .053) in heterozygotes for this haplotype than in H+S447 homozygotes. By contrast, the differences between the H−S447 haplotype and the reference H+S447 haplotype were very small and nonsignificant (AUC 0.8%, 95%CI = −6.2% to +8.4%; peak height 0.4%, 95%CI = −3.3% to +4.2%).

Overall there was no statistically significant evidence for heterogeneity of haplotype effect between cases and control subjects, but in Fig 2, these results are presented for cases and control subjects separately, which show that the lowering effect associated with the H−X447 haplotype was larger in cases than control subjects, being statistically significant...
in cases and control subjects. The larger effect in cases than in control subjects was also observed for the AUC (−14.4%, \( P < .05 \), versus −5.9%, NS) and peak height estimates (−6.7%, \( P = .05 \) versus −2.6%, NS).

In most of the EARS II subjects (n = 618), measurement at 0 and 4 hours of LpCIII:B levels was made as a surrogate measure of the levels of remnants of TG-rich lipoproteins. As expected, overall these levels were 8% higher 4 hours after the meal (\( P = .05 \)), compatible with their generation during lipolysis of chylomicrons. At 0 hours, the \( H−X447 \) haplotype was associated with levels of LpCIII:B that were 12% lower than for the \( H+S447 \) haplotype (\( P < .01 \)). At 4 hours after the meal, the \( H−X447 \) haplotype effect was of the same magnitude (−12%, \( P < .01 \)). No significant differences between the \( H+S447 \) and the \( H−S447 \) haplotypes were seen at baseline or 4 hours (not shown).

### Discussion

#### Common Variants That Might Explain the HindIII Effect

The mutation creating the HindIII variable site occurs in intron 8 of the LPL gene. Because of its location, it is unlikely to be functionally important itself but rather a marker for a functional change elsewhere in the gene. During studies to try to identify the common functional change that explains the HindIII effect, a number of relatively common mutations have been identified, such as D9N and N291S.\(^{14−16} \) In EARS I and II, carriers of the N9 and S291 mutations occur at a frequency of 2% to 4%, with carriers having elevated plasma lipid levels and, for the S291 mutation, significantly delayed postprandial clearance.\(^{27} \) However, because of their relatively low frequency of occurrence, these variants are unlikely to be the cause of the HindIII effect, and to avoid any possible confounding effect, carriers of N9 or S291 were excluded from the current analysis.

The major outcome of this study is the higher frequency of the LPL \( H−X447 \) haplotype in EARS control subjects than in cases, suggesting that this haplotype is associated with a strong protective effect on risk for MI. This finding confirms and extends the association between both the HindIII \( H \)-allele and the X447 allele and lower risk of MI that has been observed in several case-control comparisons.\(^{22−24} \) Overall, compared with those homozygous for the \( H+S447 \) haplotype, individuals heterozygous for the \( H−X447 \) haplotype (representing \( ∼20\% \) of the population) have a population-adjusted OR for paternal history of MI of 0.71, and though not measured directly, this implies that \( H−X447 \) homozygotes (representing \( ∼1\% \) of the population) would have an OR of 0.50. Unlike the usual case-control comparison study, the EARS design has the advantage of investigating the prevalence of elevated levels of measured risk factor in healthy individuals who are the offspring of cases and control subjects. Although this avoids the bias that occurs in the study of individuals with different degrees of disease, it has a reduced power to identify genetic factors, since by chance any offspring has only a 50% probability of inheriting the “high-risk” allele from the (affected) father. Thus, any significant finding, such as the lower relative risk associated with the \( H−X447 \) haplotype observed here, is likely to be an underestimate of the size of the true effect on MI associated with LPL genotype. These estimates were confirmed in the EARS II sample, and the consistency across regions of Europe and the strength of the effect puts beyond doubt that variation at the LPL locus is important in determining risk of MI in these populations.

#### North-South Gradient Across Europe

Overall in the two EARS studies, subjects from 14 different countries in Europe have been investigated, and the results demonstrate a significant north-south gradient in the frequency of LPL haplotypes, with the frequency of the protective \( H−X447 \) haplotype generally being higher in countries from southern Europe than in the north. This mirrors the gradient in CAD prevalence seen in many studies (eg, MONICA) and suggests that variation at this gene locus may be one of the genetic factors contributing to this north-south gradient. EARS I had previously shown a north-south gradient in the frequency of the apoE alleles\(^{33} \) but not for genetic variation in the apoB,\(^{34} \) Lp(a),\(^{35} \) or apoAIV alleles.\(^{36} \)

#### \( H−S447 \) Haplotype Effect

The data herein strongly suggest that the previously reported “protective” effects associated with the \( H \)-allele are explained by the effect of the X447 polymorphism and, almost without exception, that the X447 variant is found on the \( H \)-allele of the HindIII polymorphism. However, the \( H−S447 \) haplotype is present at a higher frequency in the populations studied.
than is the \(H^*\!\!-\!\!X447\) haplotype, thus allowing a population association approach to test whether the \(H\!\!-\!\!S447\) haplotype might also be associated with significant effects on plasma lipids and MI risk. Compared with the \(H\!\!-\!\!X447\) haplotype, the \(H\!\!-\!\!S447\) haplotype is associated with a much smaller and nonsignificant effect on risk of paternal history of MI. This suggests that the risk associated with these LPL genotypes is explained in large part by the effect associated with the \(X447\) and not the \(H\) polymorphism. Only in male cases is the \(H\!\!-\!\!S447\) haplotype associated with a statistically significant lowering effect on TG levels, and in this group, the effect was of similar magnitude to that seen with \(H\!\!-\!\!X447\). However, overall the lowering effect associated with this haplotype was not seen consistently throughout the regions of Europe, and the haplotype also showed no significant effect on postprandial TG levels. This suggests that any as-yet-undiscovered functionally important sequence changes on this haplotype are of limited impact on the risk of paternal MI or on TG levels in their male offspring.

Effect of the X447 Mutation on LPL Function

Several in vitro studies have examined the effect of the X447 mutation on LPL activity, and although early studies gave conflicting results (References 18, 37, and 38 and the “Discussion” in Reference 25), a recent, careful study has looked in detail at the effect of this mutation on LPL activity by expression in a COS cell transfection system.19 This study reported that the \(X447\) variant was catalytically normal and also manifested normal homodimer stability but had a 31% higher total secreted mass than the \(S447\) variant, most likely due to enhanced secretion of the monomeric form. It is still not clear precisely how the mutation has this effect, eg, whether by increasing mRNA stability or LPL protein stability, but extrapolation of these results to effects on LPL secretion from adipose tissue and muscle in vivo would suggest that carriers of the \(H\!\!-\!\!X447\) haplotype would have higher levels of LPL. Support of this notion, higher levels of postheparin LPL in \(X447\) carriers have been recently reported in a study of CAD patients from the Netherlands.25 Unfortunately, direct measures of LPL mass or activity were not available for either of the EARS studies, but the effect in the EARS II postprandial study associated with the \(X447\) variant is indicative of higher LPL activity, as evidence by lower fasting plasma TGs and greater postprandial clearance of plasma TGs and LpCIII:B particles, which were used as a surrogate measure of remnant lipoproteins.19

Mechanism of the Effect on MI Risk

The mechanisms of the protective effect on MI risk associated with the \(X447\) variant have only been partly elucidated by the biochemical analyses and metabolic investigations carried out in this study. Since it is clear that the \(S447X\) polymorphism is affecting the level and/or activity of LPL itself, the most likely mechanism for the protective effect associated with the \(X447\) allele is through an increase in the clearance of postprandial lipids and reduction in the degree of fasting and postprandial lipemia experienced by the \(X447\)-carrying individuals. Both lower plasma TGs, and the associated levels of HDL-C are likely to contribute to CAD risk,40–42 but changes in the metabolism of these two lipoprotein classes may also result in differences in the proportion of small, dense LDL, which itself has been associated with an increased risk for atherosclerosis.43 In addition, since hypertriglyceridemia is associated with hypercoagulation and impaired fibrinolysis,44 lower TG levels may also reduce thrombotic risk. However, the large protective effect associated with the \(H\!\!-\!\!X447\) haplotype can be explained only in part by the small lowering effect on plasma TGs observed in these young, healthy individuals. In males (cases and control subjects combined), the effect associated with the \(H\!\!-\!\!X447\) haplotype was a lowering of plasma TG levels by 8.1% compared with the \(H\!\!+\!\!S447\) haplotype, the size of this effect being similar to that reported by others in healthy males.6,12,45 From the postprandial data, the degree of lipemia as estimated by the AUC was 14.4% lower in cases and 5.9% lower in control subjects. However, although the effect associated with the \(H\!\!-\!\!X447\) haplotype on fasting and postprandial TG levels was consistent with the protective effect seen on the risk of MI, the low magnitude of the effects raises the possibility that other mechanisms are involved. Thus, for example, it is possible that the \(X447\) mutation may affect nonenzymatic functions of LPL, such as its bridging function, and may therefore affect postlipolytic clearance of atherogenic lipoprotein remnants. However, since the study subjects were young and all relatively healthy, it is also possible that the small effects on these lipid traits observed in these 23-year-old individuals will be magnified as the subjects become older, obese, or diabetic or start to develop more manifest signs of atherosclerosis.

One piece of evidence in support of this hypothesis is the suggestion from the findings in both EARS I and EARS II subjects that the magnitude of the \(H\!\!-\!\!X447\) haplotype effects on lowering plasma TGs are larger in the offspring of cases than in the offspring of control subjects. It is theoretically possible that the LPL haplotype inherited by the offspring of cases is genetically different from that inherited by the offspring of control subjects, with this genetic difference being reflected in the production of a functionally different LPL enzyme. However, such a case-control stratification is genetically unlikely, and there is, for example, no evidence from the homogeneous linkage disequilibrium estimates in cases and control subjects to support this. Other possibilities for the larger haplotype-associated effect on lipid traits is that the offspring of cases have either adopted a “proatherogenic lifestyle” or inherited alleles at a number of different gene loci (eg, apoE, apoB, etc) that contribute to greater genetic susceptibility. In both EARS I and EARS II, detailed information was collected about lifestyle variables such as smoking, diet, exercise, etc, and none of these measured differences were significantly different between cases and control subjects. It may be relevant that on average the cases were 1.5 cm shorter than the control subjects (\(P<.001\)),46 a finding that has been made in a number of other case-control studies. There was also a tendency for the offspring of cases to be more obese than the offspring of control subjects, but there was no statistically significant difference in body mass index among individuals of different LPL genotypes. It is therefore unlikely that body mass index could explain this different
case-control genotype association. Identification of these other genetic or lifestyle factors that interact with the LPL genotype to determine MI risk is of obvious interest.

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References


Lipoprotein Lipase Gene Variation Is Associated With a Paternal History of Premature Coronary Artery Disease and Fasting and Postprandial Plasma Triglycerides: The European Atherosclerosis Research Study (EARS)

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