DNA Variants at the LPL Gene Locus Associate With Angiographically Defined Severity of Atherosclerosis and Serum Lipoprotein Levels in a Welsh Population

Raj K. Mattu, Edward W.A. Needham, Ruth Morgan, Alan Rees, Allan K. Hackshaw, Joseph Stocks, Peter C. Elwood, David J. Galton

Abstract

Coronary artery disease (CAD) patients (n=235), comprising minimal (CAD*; n=124) and severe (CAD++; n=111) CAD, were recruited on the basis of their angiographic scores. Male control subjects (n=123) were selected randomly from the Caerphilly Heart Study cohort. Subjects were genotyped for the Ser*'-Ter mutation and HindIII/Pvu II restriction fragment length polymorphisms of the lipoprotein lipase gene and investigated for associations with severity and development of CAD and lipid and lipoprotein levels. The Ser*'-Ter mutation showed no significant associations with CAD or dyslipidemia but was related to favorable lipid and lipoprotein profiles. The H2H2 genotype (P<.05) and CAD or dyslipidemia but was related to favorable lipid and lipoprotein levels. The H2H2 genotype (P<.05) and CAD or dyslipidemia but was related to favorable lipid and lipoprotein levels. The H2 allele had most significant associations with raised apolipoprotein B levels compared with other biochemical parameters. Our data suggest that the H2 allele may be a marker for an etiologic mutation for dyslipidemia and the severity and development of atherosclerosis; this is not the Ser*'-Ter mutation. (Arterioscler Thromb. 1994;14:1090-1097.)

Key Words

- LPL
- Ser*'-Ter mutation
- apo B
- CAD
- dyslipidemia

Familial clustering of coronary artery disease (CAD) has long been recognized1-2 and confirmed by many studies, implicating a genetic predisposition.3,4 In most subjects the genes conferring susceptibility to CAD have yet to be identified, but they have been elucidated in a small number of cases, eg, mutations of the low-density lipoprotein (LDL) receptor gene that determine the onset of premature CAD in familial hypercholesterolemia.5 Numerous large studies have established the association of lipid and lipoprotein abnormalities with an increased risk for the development of CAD.6-9 Between 50% and 80% of myocardial infarction survivors have some form of dyslipoproteinemia,10 and one of the most common types is hypertriglyceridemia accompanied by low high-density lipoprotein (HDL) levels.11,12

Human lipoprotein lipase (LPL) plays a role in the determination of the plasma lipid and lipoprotein profile; it is a rate-limiting enzyme in the clearance of triglyceride-rich lipoproteins from the circulation.13,14 Other known influences of the enzyme include apolipoprotein (apo) and phospholipid exchange between very-low-density lipoprotein (VLDL) and HDL,15 thereby affecting HDL conversion to HDL2 and LDL generation derived from VLDL clearance.16,17

LPL is a glycoprotein synthesized in parenchymal cells (primarily adipose tissue and muscle) from which it is secreted and anchors to the vascular endothelium bound to glycosaminoglycans.18,19 It appears to be active as a noncovalently linked homodimer that loses activity progressively and rapidly on dissociation.20-22 Activation of LPL requires apoC-II23 at the lipid interface, an obligatory cofactor present in chylomicrons, VLDL, and HDL.

The human LPL gene has been cloned and localized to chromosome 8p22 and spans approximately 30 kb.24,25 The first 9 of its 10 exons code for a protein containing 475 amino acids, including a 27-amino acid signal peptide that is cleaved posttranslationally to yield mature LPL with a molecular weight of approximately 60 000 D.26-28

Extensive research has been directed at genetic variants in dyslipidemia to elucidate the genes predisposing to CAD.10,29 Restriction fragment length polymorphisms (RFLPs) have been widely used as markers to determine associations of DNA sequence variations in and around such candidate genes with diseases or biochemical traits.30-32 The RFLPs reported in the LPL gene have shown the following significant associations:
the HindIII RFLP (intron 8) with primary hypertriglyceridermia,33,34 hypercholesterolemia,35 HDL levels,36 and premature CAD36; and the Pvu II RFLP (intron 6) with primary hypertriglyceridermia.35 These findings suggest that a relatively common mutation at the LPL gene locus, in linkage disequilibrium with these RFLPs, may be predisposing to dyslipidemia and also conferring increased risk for the development of CAD.

Recently, the first common mutation within an exon of the LPL gene was discovered just 635 bp downstream from the HindIII RFLP (intron 8), involving a Ser477-Ter mutation.36 The Ser477-Ter mutation is a consequence of a C-G transversion at nucleotide 1595 in exon 9, converting the serine 447 codon (TCA) to a premature termination codon (TGA). This would be anticipated to yield a truncated enzyme lacking the two carboxy terminal amino acids (Ser-Gly).

In this study we have investigated the Ser477-Ter mutation to determine whether it influences the development or severity of CAD. In view of its proximity to the RFLPs, we analyzed whether the RFLPs are markers for the Ser477-Ter mutation, thereby accounting for the previously reported RFLP associations. We have also examined for possible effects of the mutation on plasma lipid and lipoprotein levels and in addition any associations with the RFLPs.

Methods

Subjects

White subjects were recruited over 18 months from 1371 consecutive patients undergoing coronary angiography during investigation of chest pain at the Department of Cardiology, University of Wales College of Medicine, Cardiff. The extent of coronary atherosclerosis was defined by the modified Brandt scoring system37 using a scale of 0 to 15. Patients were selected for the study having scores of less than 4.5 or greater than 8.5, constituting groups with minimal (CAD−, n=124) and severe (CAD+, n=111) atherosclerosis, respectively. Only patients with a fasting plasma glucose less than 6.5 mmol/L were recruited. After recruitment of angiographic subjects, a male population-derived control group was collected from the same region of South Wales(n=123); this involved the random selection of subjects from the Caerphilly Prospective Heart Disease Study, details of which are described elsewhere.38 No angiography was performed on the population control group because of ethical considerations. This precluded us from obtaining the ideal negative control group for comparisons with CAD∗ subjects, as we are unable to perform angiograms on healthy individuals to determine an asymptomatic, angiographically defined disease-free group. However, a population control subgroup (n=92), comprising asymptomatic and clinically disease-free subjects, was analyzed after exclusion of subjects with a diagnosis of CAD (namely, World Health Organization criteria for myocardial infarction or on angiography). Although the CAD∗ group does not represent disease-free or healthy subjects, it provides a “control” group for the CAD∗ patients to analyze the severity of CAD. No angiographic patients had received any hypolipidemic drugs or dietary advice.

Lipid Measurements

Lipid measurements were undertaken on serum separated within 6 hours of venesection from clotted venous samples collected after a 12-hour fast. Cholesterol39 and triglyceride40 levels were measured using fully enzymatic methods (Boehringer Mannheim). HDL cholesterol was measured after phosphotungstate/magnesium precipitation41 and apoA-I and apoB by immunonephelometry (Beckman Immunochemistry Ana-
TABLE 1. Biochemical Characteristics of Patients With CAD, Patients Without CAD, and Control Subjects

<table>
<thead>
<tr>
<th></th>
<th>Men</th>
<th>P</th>
<th>Women</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CAD+ (n=90)</td>
<td>CAD- (n=97)</td>
<td>Controls* (n=92)</td>
<td>P</td>
</tr>
<tr>
<td>Age, y</td>
<td>55.1±7.5</td>
<td>53.4±10.1</td>
<td>57.7±4.5</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>26.9±2.9</td>
<td>26.1±3.3</td>
<td>26.1±3.6</td>
<td>NS</td>
</tr>
<tr>
<td>Cholesterol, mmol/L</td>
<td>6.39±0.14</td>
<td>5.97±0.13</td>
<td>5.78±0.11</td>
<td>.002</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>2.08±1.05</td>
<td>1.75±1.05</td>
<td>1.71±1.04</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>HDL, mmol/L</td>
<td>97.5±2.9</td>
<td>98.9±2.8</td>
<td>95.3±2.3</td>
<td>.12</td>
</tr>
<tr>
<td>ApoA-I, mg/dL</td>
<td>130.0±3.4</td>
<td>141.9±3.3</td>
<td>122.2±2.7</td>
<td>.001</td>
</tr>
<tr>
<td>ApoB, mg/dL</td>
<td>97.5±2.9</td>
<td>88.9±2.8</td>
<td>95.3±2.3</td>
<td>.08</td>
</tr>
<tr>
<td>LDL-C, mmol/L</td>
<td>4.69±0.13</td>
<td>4.28±0.13</td>
<td>3.89±0.10</td>
<td>.0001</td>
</tr>
<tr>
<td>Lp(a), mg/dL†</td>
<td>357</td>
<td>134</td>
<td>99.5</td>
<td>.0001</td>
</tr>
</tbody>
</table>

*Unadjusted median values and range.

CAD indicates coronary artery disease; CAD+, with CAD; CAD-, without CAD; BMI, body mass index; HDL, high-density lipoprotein; Apo, apolipoprotein; LDL-C, low-density lipoprotein cholesterol; and Lp(a), lipoprotein(a).

Linkage disequilibrium was assessed using the ASSOC 2.2 computing program at the Human Genome Resource Centre.

Results

Epidemiologic data show that biochemical traits and the natural history of CAD differ between the sexes; therefore, male and female patients were analyzed separately. Furthermore, our population-derived control group consisted entirely of men, and significant differences were apparent in age and body mass index between the sexes in our angiographic patients.

Biochemical Characteristics and CAD

Table 1 summarizes the adjusted biochemical characteristics for the study groups for men and women. Clinical details have been described elsewhere. As expected, the mean levels of cholesterol, triglycerides, LDL-C, and Lp(a) were significantly and progressively higher between the men in the control, CAD-, and CAD+ groups, respectively. HDL levels followed a similar significant inverse trend. The women did not exhibit the same significant trends regarding triglyceride and HDL levels, probably reflecting the smaller numbers of women recruited into the study.

Genotyping

The map in the Figure represents the region of exons 8 and 9 of the LPL gene showing the positions of the HindIII RFLP and the Ser*7-Ter mutation and the methods used to analyze the genotypes. The C-G transition at nucleotide 1595 does not create or abolish a restriction site; by using a modified 3' amplimer, an additional mutation (G—>T) is introduced at nucleotide 1598 into the amplification product. Thus, a novel HindIII restriction site is created solely in the presence of the G allele (Ser*7-Ter mutation), as the enzyme has the recognition sequence GA(N)TC.

LPL Genotypes in Control and Angiographic Subjects

The genotype distributions of the Ser*7-Ter mutation and RFLPs examined at the LPL gene locus were in Hardy-Weinberg equilibrium and are shown in Table 2 for the male subjects. Comparison between the CAD+ and CAD- groups did not show any significant differences in genotype or allelic distributions in female patients (data not shown).

Schematic diagram shows lipoprotein lipase gene. Shown is the region between exons 8 and 9 with the HindIII restriction fragment length polymorphism and Ser*7-Ter mutation. PCR indicates polymerase chain reaction.
TABLE 2. Genotypes for RFLP Alleles and Ser^-Ter Mutation at the Lipoprotein Lipase Gene Locus In Male Control Subjects and Subjects With Angiographically Defined Severe (CAD) or Minimal (CAD') Atherosclerosis

<table>
<thead>
<tr>
<th>No. of Subjects Represented by Genotype (%)</th>
<th>Allelic Frequencies</th>
</tr>
</thead>
<tbody>
<tr>
<td>H1H1</td>
<td>H1H2</td>
</tr>
<tr>
<td>Control subjects</td>
<td>6</td>
</tr>
<tr>
<td>(5)</td>
<td>(37)</td>
</tr>
<tr>
<td>Control subjects*</td>
<td>3</td>
</tr>
<tr>
<td>(3)</td>
<td>(41)</td>
</tr>
<tr>
<td>CAD-</td>
<td>10</td>
</tr>
<tr>
<td>(10)</td>
<td>(49)</td>
</tr>
<tr>
<td>CAD+</td>
<td>6</td>
</tr>
<tr>
<td>(7)</td>
<td>(38)</td>
</tr>
</tbody>
</table>

RFLP indicates restriction fragment length polymorphism; CAD, coronary artery disease.

†Excludes subjects with established clinical diagnosis of CAD.
††HindIII genotypes and alleles between CAD- and control subjects, x^2 = 4.13, P < .05.
§HindIII alleles in CAD- vs CAD+ by a 2 x 2 contingency table, * x^2 = 6.24 (P < .05) and 5.45 (P < .05), respectively.

**Ser^-Ter Mutation**

Allelic frequencies in pooled male subjects (data not shown) for the Ser^-Ter mutation were 0.09 (G allele) and 0.91 (C allele). No significant differences in distribution of genotypes or alleles were observed between the CAD+, CAD-, and control groups.

**Pvu II RFLP**

Allelic frequencies in the control group were 0.45 (P1) and 0.55 (P2). Comparison of the genotype and allelic frequencies between the control, CAD-, and CAD+ groups showed no significant differences in distribution.

**HindIII RFLP**

There was a significantly higher frequency of the H2H2 genotype among subjects with CAD- versus CAD- (P < .05). Examination of allelic frequencies showed a significant association of the H2 allele with the severity of CAD (CAD- versus CAD+, P = .05). Similarly, significant differences were observed between the CAD- patients and control subjects for both genotypic and allelic distributions.

**LPL Genotypes and Biochemical Traits**

Comparisons of lipid, lipoprotein, and apo levels based on genotypes at the LPL gene locus among the control group are presented in Table 3.

**Ser^-Ter Mutation**

Comparisons among the control group of subjects possessing at least one Ser^-Ter mutation (CG or GG) with those without did not show any significant differences in biochemical traits. However, the homozygotes with the Ser^-Ter mutation (n = 3) had significantly higher HDL levels (mean, 1.69 mmol/L) than either the heterozygotes or wild-type homozygotes (mean, 0.87 mmol/L) (P < .002) when all the male data were pooled, but interpretations should remain cautious because of the small number of homozygous GG patients.

**Pvu II RFLP**

Control subjects with the genotype P2P2 had significantly lower HDL levels than control subjects with alternative genotypes for this RFLP (P < .03). Conversely, these individuals showed a trend toward higher LDL-C levels, which failed to reach similar statistical significance (P < .054).

**HindIII RFLP**

Comparisons among the control group showed significantly higher cholesterol (P < .021), LDL-C (P < .01), and apo B (P < .005) levels in subjects with the genotype H2H2 compared with H1H1 or H1H2. Pooled data involving all male subjects are represented in Table 4 and show stronger associations. This probably reflects the larger numbers of individuals being analyzed. The strongest association with the HindIII RFLP was again found with apo B (P = .0002). In the pooled group the H2 allele was also associated with significantly higher triglyceride levels (P < .04).

**Haplotype Associations**

Unequivocal haplotypes were constructed using data from all subjects having heterozygosity at no more than one site out of the three studied. The Ser^-Ter mutation was never observed on the same chromosome as the alleles of the RFLPs containing the restriction sites P2 (Pvu II) and H2 (HindIII). The pooled haplotype distributions for the male subjects showed no significant differences between the CAD+, CAD-, and control groups. However, a significant association of haplotypes was observed with biochemical traits on comparisons within these groups (data not shown).

**P1H,C Haplotype**

On comparisons among control subjects, this haplotype was associated with significantly lower cholesterol...
TABLE 3. Mean Biochemical Values ± SEM (Adjusted for Age and BMI) for Genotypes at the Lipoprotein Lipase Gene Locus in the Male Population Control Subjects (n = 123)

<table>
<thead>
<tr>
<th></th>
<th>Pvu II</th>
<th>HindIII</th>
<th>Ser^47-Ter</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P1P1</td>
<td>P1P2</td>
<td>P2P2</td>
</tr>
<tr>
<td>Cholesterol, mmol/L</td>
<td>5.63±0.20</td>
<td>5.70±0.12</td>
<td>5.98±0.16</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>1.58±1.09</td>
<td>1.81±1.05</td>
<td>1.63±1.07</td>
</tr>
<tr>
<td>HDL, mmol/L</td>
<td>1.13±0.05</td>
<td>1.00±0.03</td>
<td>0.98±0.04†</td>
</tr>
<tr>
<td>ApoA-I, mg/dL</td>
<td>123.7±3.9</td>
<td>121.7±2.4</td>
<td>121.2±3.1</td>
</tr>
<tr>
<td>ApoB, mg/dL</td>
<td>92.9±4.6</td>
<td>94.4±2.7</td>
<td>98.2±3.6</td>
</tr>
<tr>
<td>LDL-C, mmol/L</td>
<td>3.70±0.18</td>
<td>3.80±0.114</td>
<td>4.19±0.15§</td>
</tr>
<tr>
<td>Lp(a), mg/dL</td>
<td>97</td>
<td>86</td>
<td>143</td>
</tr>
</tbody>
</table>

(BMI indicates body mass index; HDL, high-density lipoprotein; Apo, apolipoprotein; LDL-C, low-density lipoprotein cholesterol; and Lp(a), lipoprotein(a).
*Genotypes H2H2 vs H1H2 vs H1H1, P = .021.
†Genotypes P2P2 vs P1P2 vs P1P1, P < .03.
‡Genotypes H2H2 vs H1H2 vs H1H1, P < .005.
§Genotypes P2P2 vs P1P2 vs P1P1, P < .05.
¶Genotypes H2H2 vs H1H2 vs H1H1, P = .01.
#Unadjusted median values and range.

levels (P < .006) and LDL-C levels (P < .002). There was also a trend toward lower apoB levels in subjects with this haplotype among the control group (P = .063).

P1H1C Haplotype

Comparison among the control group showed that subjects with this haplotype had significantly lower HDL levels (P = .05).

P1H2C Haplotype

Comparison among subjects with CAD revealed significantly lower cholesterol levels (P < .007) and apoB levels (P < .013) with this haplotype.

P,H,G Haplotype

Comparisons among subjects with CAD showed significantly higher HDL levels with this haplotype (P = .0001). These individuals also showed a trend toward lower triglyceride levels (P = .084).

Discussion

The Ser^47-Ter mutation is the first report of a common, coding sequence mutation at the LPL gene locus. The functional significance of the carboxy terminal region of LPL remains uncertain. It has been postulated to function in the interaction of LPL with lipid substrates. In vitro LPL activity studies suggest that the...
Ser^Ter mutation appears to influence hydrolysis of substrates dependent on their nature.46-50 Although LPL-deficient states give rise to type I hyperlipidemia, this is not usually associated with a predisposition to CAD. However, the obligate heterozygote state has recently been implicated in familial combined hyperlipidemia, in which there is a predisposition to premature and severe CAD.52 Our data suggest that the Ser^Ter mutation conveys no risk for the development or severity of CAD and has little influence on dyslipoproteinemia.

The Ser^Ter mutation is in significant linkage disequilibrium with the RFLPs Pvu II (P < .000002) and HindIII (P < .000001); similar allelic associations are noted between the RFLPs Pvu II and HindIII (P < .000008). These observations are consistent with previous reports of linkage disequilibrium between these sites in Caucasian33,45,51 and Japanese53 subjects, linking the PI, H1, and Ser^Ter (G) alleles. Consequently, we were not surprised to find no significant associations of the Ser^Ter mutation with the severity or development of CAD, consistent with our observations regarding allelic linkage and the previously reported association of the HindIII, H2 allele with CAD.54 Interestingly, we observed significant associations of the H2H2 genotype and the H2 allele with the severity of CAD (CAD⁺ versus CAD⁻) but no differences between the angiographic groups (alone or pooled) versus random population-derived control subjects. This may partly reflect the heterogeneous nature of the control group, which was composed of approximately 23% of subjects known to have CAD and a further 11% having possible CAD, based on clinical histories. This may have affected the power of our study to detect any asymmetric genotype distribution between the CAD and control groups. However, comparison of the control subgroup (which excludes subjects with known CAD) with CAD⁺ showed a significant difference in genotype and allelic distribution for the HindIII RFLP. This was observed despite the presence of subjects with possible CAD within this subgroup. We are unable to explain the absence of a similar genetic difference on comparison with CAD⁺.

Our observations among the control and pooled subjects support previous associations of the H2H2 genotype with significantly elevated total cholesterol, LDL-C, and triglyceride levels. In addition, we find that the strongest associations are with apoB levels. This association reflects a significant increase in levels of apoB-containing lipoproteins in subjects with the H2H2 genotype. LPL has a pivotal role in lipoprotein metabolism, affecting HDL, VLDL, and chylomicron clearance. This may influence the generation of other apoB-containing lipoproteins by LPL. Our data suggest that in apoB-containing lipoproteins in a subject's pool, we would anticipate an association between the lipids and this genotype, which is similar to our findings with CAD⁺.

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