Association of Lipoprotein Lipase Gene Polymorphisms With Coronary Artery Disease

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OBJECTIVES The purpose of this study was to test whether the HindIII (+) and PvuII (−) or (+) restriction enzyme–defined alleles are associated with angiographic coronary artery disease (CAD).

BACKGROUND Lipoprotein lipase (LPL) plays a central role in lipid metabolism, hydrolyzing triglyceride in chylomicrons and very low density lipoproteins. Polymorphic variants of the LPL gene are common and might affect risk of CAD.

METHODS Blood was drawn from 725 patients undergoing coronary angiography. Leukocyte deoxyribonucleic acid segments containing the genomic sites were amplified by the polymerase chain reaction and digested, and polymorphisms were identified after electrophoresis in 1.5% agarose gel.

RESULTS In no-CAD control subjects (n = 168), HindIII (−) and (+) allelic frequencies were 28.6% and 71.4%, and (−) and (+) alleles were carried by 44.0% and 86.9% of subjects, respectively. Control PvuII (−) and (+) allelic frequencies were 41.7% and 58.3%, and (−) and (+) alleles were carried by 64.3% and 81.0%, respectively. In CAD patients (>60% stenosis; n = 483), HindIII (+) allelic carriage was increased (93.8% of patients, odds ratio [OR] = 2.28, confidence interval [CI] 1.27 to 4.00). Also, PvuII (−) allelic carriage tended to be more frequent in CAD patients (OR = 1.33, CI 0.92 to 1.93). Adjusted for six CAD risk factors and the other polymorphism, HindIII (+) carriage was associated with an OR = 2.86, CI 1.50 to 5.42, p = 0.0014, and PvuII (−) carriage, OR = 1.42, CI 0.95 to 2.12, p = 0.09. The two polymorphisms were in strong linkage disequilibrium, and a haplotype association was suggested.

CONCLUSIONS The common LPL polymorphic allele, HindIII (+), is moderately associated with CAD, and the PvuII (−) allele is modestly associated (trend). Genetic variants of LPL deserve further evaluation as risk factors for CAD. (J Am Coll Cardiol 1999;33:1013–20) © 1999 by the American College of Cardiology

Atherosclerotic cardiovascular disease, predominantly coronary artery disease (CAD), is the single greatest cause of mortality in the United States, being responsible for about one third of all deaths annually (1). Coronary artery disease shows strong familial aggregation, especially when it presents at an early age and when many relatives are affected (2). Dyslipidemias form a well known risk factor for CAD; however, only a small percentage of CAD patients have recognized monogenetic disease due to dysfunctional mutations in lipoproteins or lipoprotein–related genes (e.g., receptors, catabolic enzymes) (2). Thus, as much as one half of risk remains unexplained (2,3) and is believed to be due to the interaction of multifactorial inheritance and environmental factors (e.g., diet, exercise, tobacco) (2). In this regard, common variants of genes central to lipid metabolism that are associated with modest changes in protein function might be important contributors to risk at a population level, perhaps in association with other specific environmental and genetic factors (2,4).

The lipoprotein lipase (LPL) gene represents an excellent candidate to explain portions of the genetically determined risk of atherosclerosis (5). Mature LPL is a 448 amino acid glycoprotein with chromosomal location 8p22 (6,7). It is one of about 15 genetically mapped, lipid-related proteins known to be capable of contributing to CAD risk (2). Lipoprotein lipase, strategically anchored to vascular endothelium, plays a central role in lipid metabolism. Lipoprotein lipase–catalyzed hydrolysis of triglycerides constitutes the rate-limiting step in the removal of triglyceride-rich lipoproteins such as chylomicrons and very low density lipoproteins (VLDLs) from the circulation (8,9). Lipoprotein lipase is multifunctional, recently having been shown to

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serve as a ligand for low density lipoprotein (LDL) receptor–related protein and to influence the hepatic secretion and uptake of VLDL and LDL cholesterol (10). Rare LPL mutations are known that cause marked dyslipidemias (e.g., familial LPL deficiency with chylomicronemia [8,11]), at least some of which are known to be associated with premature atherosclerosis (11). However, we were interested in exploring whether common variants of LPL (i.e., allele frequency ≥0.20) might contribute importantly to risk for CAD at a population level through subtle changes in LPL function associated with milder clinical phenotypes.

Several polymorphic variants of the LPL gene have been identified in recent years and evaluated for their effects on plasma lipids and atherosclerosis risk (11–24). Those defined by the HindIII and PvuII restriction fragment length polymorphism sites, located on introns 8 and 6, respectively, of the LPL gene, are common and may be associated with subtle alterations in plasma lipids. The HindIII (+) allele or the HindIII (+/+ ) genotype has been reported to be associated with an atherogenic profile (elevated triglycerides and/or decreased high density lipoprotein [HDL] cholesterol), and the HindIII and PvuII polymorphisms have been variably reported to associate with CAD (12,14,16,17,19,20,22). However, the studies often have been small and the results inconsistent. Polymorphisms in genes related to CAD pathophysiology often have required multiple and large studies to clearly define attributable genetic risk (4,25–27).

Given the importance of LPL as a candidate gene for CAD risk, we prospectively evaluated a large, independent, well defined, ethnically relatively homogeneous and angiographically controlled study population to determine whether the HindIII and PvuII polymorphisms were associated with angiographically defined CAD.

### Abbreviations and Acronyms

- CAD = coronary artery disease
- Cl = confidence intervals
- DNA = deoxyribonucleic acid
- HDL = high density lipoprotein
- LDL = low density lipoprotein
- LPL = lipoprotein lipase
- MI = myocardial infarction
- OR = odds ratio
- VLDL = very low density lipoprotein

### METHODS

#### Study hypotheses

We tested whether: 1) carriage of the more common HindIII (+) variant allele would be CAD-associated (13,16,17,19,20,22,23), and 2) carriage of either the PvuII (+) (more common) or PvuII (−) (less common) alleles (12,16,19–22) would be disease-associated in patients whose coronary status was defined by angiography.

### Patient and control populations

Study patients and control subjects consisted of consecutive consenting subjects presenting for coronary angiography at LDS Hospital because of either symptoms relating to suspected CAD or unrelated conditions requiring angiographic evaluation (e.g., valvular disease, cardiomyopathy). Included subjects were of unrestricted age and gender who gave written informed consent for a blood draw at the time of angiography for deoxyribonucleic acid (DNA) extraction to be used in studies approved by the hospital’s institutional review board. Subjects were residents of Utah, southwestern Idaho or southeastern Wyoming, a population that is ethnically primarily of northern European (Anglo-Scandinavian) descent, and which has previously been shown to be genetically representative of North American Caucasians (28).

Key demographic characteristics of subjects were recorded on computerized angiographic data forms, including age, gender and past history of myocardial infarction (MI) (4). Assessment of CAD was made by review of angiograms by the patient’s cardiologist, who was uninformed as to LPL genotypes. Results were entered into the computer database in a format modified after the Coronary Artery Surgery Study protocol (4,25,29,30). Patients were designated as having significant CAD if they had >60% stenosis of at least one coronary artery or major branch and no CAD if <10% stenosis was present. Of the 483 CAD patients, 172 presented for angiography with a history of MI (acute or recent onset MI in 109), 176 with unstable angina, 141 with other chest pain syndromes, 72 with valvular heart disease and 1 with cardiomyopathy, singly or in combination. Of the 168 control subjects with normal coronary arteries, 108 presented for angiography with chest pain syndromes without CAD, 45 with valvular disease and 6 with cardiomyopathy, singly or in combination, or other indications. Patients with minor CAD (10% to 60% stenosis; n = 74) were designated as having an “indeterminate” CAD status and were not included in the comparative analyses.

### Deoxyribonucleic acid extraction

Approximately 20 to 30 ml of blood was withdrawn by venipuncture at the time of coronary angiography and collected in ethylenediaminetetraacetic acid. The leukocyte buffer coat was separated by centrifugation and genomic DNA prepared using a standard phenol extraction, isopropanol precipitation method as previously described (25,26).

### Genotyping

Genotyping was performed by polymerase chain reaction amplification of the polymorphic regions found in introns eight and six followed by digestion of these amplified fragments with HindIII and PvuII restriction endonucleases, respectively.

The region in intron 8 containing the HindIII polymorphism was amplified using the following primer pair (16): LPL–H1 5′–TGA AGC TCA AAT GGA AGA GT–3′, LPL–H2 5′–TCA AAG CAA ATG ACT ATG AAA–3′. The amplification protocol consisted of a denaturing segment (94°C, 1 min), an annealing segment (53°C, 45 s) and an
common genetic factors associated with even smaller odds ratios (≈1.5) for multifactorial diseases such as CAD also may be of interest. Thus, a sample size was calculated that would provide adequate power to determine an odds ratio of 1.5 to 2.0 for an association with CAD. For LPL \( \text{HindIII} \) (+) allelic carriage, occurring in ≈80% of control subjects, a sample size of 260 to 683 subjects per group would be required; for the \( \text{PvuII} \) (−) polymorphic allele, with a ≈60% carriage rate in control subjects, the required size is 152 to 421 subjects per group, assuming a power of 80% at a two-sided alpha level of 0.05. We assembled and studied a population of ≈725 subjects; 67% of the population sampled had severe CAD and 27% had a history of MI.

A chi-square analysis was used to evaluate the allelic and genotypic frequencies that were calculated from the observed genotypic counts and to assess Hardy–Weinberg expectations. The same methodology was applied to comparisons between allelic and genotypic frequencies. Associations were determined as odds ratios (ORs) and 95% confidence intervals (CIs) as previously described (32). The odds of carrying a specific allele is defined as the frequency of subjects in whom it occurs divided by the frequency of subjects in whom it does not occur. The odds ratio for CAD is the odds of allelic carriage in the diseased [CAD] group divided by the odds in the undiseased [no-CAD] group.

Exploratory analyses were prospectively planned in the stratified subgroups of interest presented in Figure 2. Multivariate logistic regression was used to determine adjusted ORs for the genetic markers, conditioned on the major CAD risk factors (SPSS 6.1, Chicago, Illinois). Linkage disequilibrium, the tendency of a specific combination of linked loci to occur on the same chromosomal homologue more frequently than explained by chance, was assessed by estimating the observed frequencies of specific haplotypes, that is, the possible combinations of alleles at the \( \text{HindIII} \) and \( \text{PvuII} \) loci, using the linkage utility program EH and comparing it with the expected frequencies using the likelihood test statistic.

RESULTS

Characteristics of the patient groups. A total of 725 angiographically assessed subjects were studied. Their age averaged 64 years (range 17 to 89); 196 (27%) had a history of MI, and 486 (67%) had significant CAD. Their key identifying characteristics are summarized by patient subgroup in Table 1. Characteristics of the CAD and no-CAD control groups were approximately comparable, except that those with CAD were somewhat older (by 4 years) and more frequently male (79% vs. 53%) and diabetic (18% vs. 12%), compared with no-CAD control subjects.

Genotypic and allelic frequencies in the control group. Genotypic and allelic frequencies in the study groups are shown in Table 2. The \( \text{HindIII} \) (−) and (+) allelic frequencies in the pool of no-CAD angiographic control subjects (n = 168) were 28.6% and 71.4%, respectively, and the \( \text{HindIII} \) (−) and (+) alleles were carried by 44.0% and
86.9%, respectively, of these subjects. The PvuII (−) and (+) allelic frequencies in no-CAD control subjects were 41.7% and 58.3%, and the PvuII (−) and (+) alleles were carried by 64.3% and 81.0% of subjects, respectively. Genotypic distributions in the control groups conformed with Hardy–Weinberg expectations.

HindIII polymorphism and CAD. Among CAD patients, HindIII (−) allelic frequency was 26.3% (OR = 0.89, CI 0.73 to 1.09, p = 0.42), and HindIII (+) frequency was 73.7% (OR = 1.12, CI 0.92 to 1.37). The distribution of genotypes differed between cases and control subjects (p = 0.006).

Carriage of the HindIII (+) variant allele was observed in 93.8% of CAD patients, corresponding to an odds ratio of 2.28, CI 1.27 to 4.00, p = 0.005, compared with no-CAD control subjects. Homozygous (+/+ genotype carriage was associated with an odds ratio for CAD of 2.02, CI 1.11 to 3.70, p = 0.02, compared with the homozygous (−/−) genotype (Table 3).

PvuII polymorphism and CAD. Among CAD patients, PvuII (−) allelic frequency was 46.2% (OR 1.20, CI 0.93 to 1.54, p = 0.15, vs. control subjects). Overall, genotypic distributions did not differ significantly from control subjects. However, carriage of the PvuII (−) variant allele, present in 70.6% of patients, was associated with a modest odds ratio trend for CAD: OR = 1.33, CI 0.92 to 1.93, p = 0.05.

Table 1. Characteristics of CAD Patients and Control Subjects

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>CAD Patients</th>
<th>CAD Controls</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>483</td>
<td>168</td>
<td>—</td>
</tr>
<tr>
<td>Age (yr) (mean ± SD)</td>
<td>65 ± 10</td>
<td>61 ± 12</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Range</td>
<td>37 to 89</td>
<td>17 to 84</td>
<td></td>
</tr>
<tr>
<td>Gender (% male)</td>
<td>79</td>
<td>53</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>49</td>
<td>45</td>
<td>0.4</td>
</tr>
<tr>
<td>Systolic BP (mm Hg)</td>
<td>139 ± 24</td>
<td>140 ± 22</td>
<td>0.5</td>
</tr>
<tr>
<td>Diastolic BP (mm Hg)</td>
<td>73 ± 10</td>
<td>75 ± 13</td>
<td>0.08</td>
</tr>
<tr>
<td>Smoker (%)</td>
<td>24</td>
<td>21</td>
<td>0.4</td>
</tr>
<tr>
<td>Diabetes (%)</td>
<td>18</td>
<td>12</td>
<td>0.05</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>207 ± 46</td>
<td>203 ± 60</td>
<td>0.6</td>
</tr>
<tr>
<td>n</td>
<td>368</td>
<td>91</td>
<td></td>
</tr>
<tr>
<td>Cholesterol ≥220 mg/dl (%)</td>
<td>35</td>
<td>35</td>
<td>0.9</td>
</tr>
</tbody>
</table>

BP = blood pressure; CAD = coronary artery disease.
Table 2. Genotypic Distributions and Allelic Frequencies of HindIII and PvuII Polymorphisms Among CAD Patients and Control Subjects

<table>
<thead>
<tr>
<th>Group</th>
<th>Genotype (−/−), % (n)</th>
<th>Genotype (−/+), % (n)</th>
<th>Genotype (+/+), % (n)</th>
<th>Allele (−), % (fraction)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HindIII polymorphism</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CAD (n = 483)</td>
<td>6.2 (30)</td>
<td>40.2 (194)</td>
<td>53.6 (259)</td>
<td>26.3 (254/966)</td>
</tr>
<tr>
<td>No CAD (n = 168)</td>
<td>13.1 (22)</td>
<td>31.0 (52)</td>
<td>56.0 (94)</td>
<td>28.6 (96/336)</td>
</tr>
<tr>
<td>PvuII polymorphism</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CAD (n = 483)</td>
<td>21.7 (105)</td>
<td>48.9 (236)</td>
<td>29.4 (142)</td>
<td>46.2 (446/966)</td>
</tr>
<tr>
<td>No CAD (n = 168)</td>
<td>19.0 (32)</td>
<td>45.2 (76)</td>
<td>35.7 (60)</td>
<td>41.7 (140/336)</td>
</tr>
</tbody>
</table>

For 2 × 3 table of genotype by disease, p = 0.006 for HindIII, p = 0.31 for PvuII.
CAD = coronary artery disease.

Carriage of the homozygous (−/−) compared with the (+/+ ) genotype was associated with an odds ratio for CAD of 1.39 (CI 0.84 to 2.28) (Table 3).

Associations between polymorphisms and CAD in prespecified subgroups. Lipoprotein lipase polymorphisms might affect risk primarily in certain subgroups (e.g., in women vs. men or in younger vs. older patients). For both HindIII (+) and PvuII (−) carriage, there was no significant heterogeneity for the associations with CAD among subgroups. Although some variations in the point estimate of the ORs between stratified subgroups were observed, the CIs were broad, and the power was low for these comparisons (Fig. 2).

Association between HindIII and PvuII polymorphisms. A contingency table relating HindIII and PvuII polymorphisms is presented in Table 4. Observed and expected numbers of cases are shown. The null hypothesis, that the genotypes were independent, was rejected by chi-square testing (chi-square = 95, p < 0.0001), demonstrating strong linkage disequilibrium between the HindIII and PvuII polymorphisms, an observation also noted by others (12,20,22).

Estimated haplotype frequencies by disease. Table 5 presents the estimated frequencies of haplotypes that best account for the observed genotypic associations, both overall and by CAD status. In CAD patients, the estimated frequency for the HindIII (+)/PvuII (−) (H+P−) haplotype is nominally greater than for control subjects, whereas for the other haplotypes, estimated frequencies are less than (H+P+, H−P+) or similar to (H−P−) control subjects.

Adjusted odds ratio estimates using conditional logistic regression. For angiographic CAD, multivariate logistic regression (simultaneously considering age, gender, cholesterol, hypertension, diabetes, smoking status, presence of HindIII (+) allele or presence of PvuII (−) allele) selected male gender (OR = 4.5), age (OR = 1.05 per year older), HindIII (+) allelic carriage (OR = 2.86, CI 1.50 to 5.42, p = 0.0014), diabetes (OR = 1.92) and (trend) PvuII (−) allelic carriage (OR = 1.42, CI 0.95 to 2.12, p = 0.09) as independent associates (Table 6). Hence, the positive associations with CAD and HindIII (+) allelic carriage and PvuII (−) allelic carriage were maintained or amplified after adjustment for known, measured risk factors (compare with Table 3).

DISCUSSION

Summary of study results. In a relatively large, prospectively studied, angiographically defined population, we found associations with CAD for common LPL polymorphisms that were of moderate strength for carriage of the HindIII (+) allele (homozygotic or homozygotic genotype) and of modest strength (trend only) for the PvuII (−) allele. Adjusted simultaneously for six CAD risk factors, the associations persisted and were strengthened (HindIII [+] adjusted OR = 2.9; PvuII [−] adjusted OR = 1.4).

Linkage disequilibrium of polymorphisms. A strong linkage disequilibrium, also reported by others, was found between the HindIII and PvuII variant polymorphisms (12,13,15–17,20,22). Linkage disequilibrium, the tendency of a specific combination of alleles at two (or more) linked loci to occur on the same chromosomal homologue more frequently than explained by chance, is determined by the proximity of the genetic loci, the rate of spontaneous mutation and the generational time since a mutation of interest occurred on the ancestral chromosome. Paradoxically, the direction of the association with CAD differed for the two polymor-
phisms, with the more common (+) allele being associated with increased risk for \textit{Hin}III but decreased risk (trend) for the more common \textit{Pvu}II (+) allele. One explanation for this paradox, consistent with our data (Table 6), is a third, linked, disease-causing mutation, located on \textit{Hin}III (+)/\textit{Pvu}II (−) haplotypic chromosomes, which originated on a \textit{Hin}III (+) chromosome before the mutation causing the \textit{Pvu}II site variation, as discussed below (22).

\textbf{Comparison with literature reports.} \textit{Associations of polymorphisms with altered plasma lipids and lipoproteins.} A number of previous studies have defined associations of the \textit{Hin}III and \textit{Pvu}II polymorphisms with plasma lipids and lipoproteins. The \textit{Hin}III (+) allele or \textit{Hin}III (+/) genotype has been found to be associated with elevated triglycerides and/or decreased HDL cholesterol in several (13,16,17,19,20,22,23) although not all (15,21) studies. Gerdes et al. found a negative gene-dosage effect of the \textit{Hin}III (+) allele on HDL (but not total) cholesterol (22), and the large ECTIM study also reported lower HDL cholesterol in association with \textit{Hin}III (+) (20).

In contrast, the \textit{Pvu}II polymorphism has not been associated with consistent changes in specific lipids or lipoprotein concentrations, and hence its associated risk often has been attributed to other or more subtle effects (13,15–17,19–22).

\textit{Associations of polymorphisms with altered risk of atherosclerosis.} Earlier, mostly small studies have not adequately defined associations between LPL polymorphisms and CAD. Several (14,19,20,22) but not all (12,16,21) studies have suggested that \textit{Hin}III (+) allele or \textit{Hin}III (+/) genotype to be associated with measures of ischemic heart disease, whereas associations with \textit{Pvu}II polymorphisms have been inconsistent or absent (12,16,19–21). Thorn et al. found increased \textit{Hin}III (+) allelic or \textit{Hin}III (+/) genotypic frequencies in patients with coronary atherosclerosis (14), whereas Peacock et al. observed no differences in either \textit{Hin}III or \textit{Pvu}II allelic frequencies in young MI survivors (16). Ukkola et al. found reductions in various measures of ischemic heart disease in non-insulin-dependent diabetics who were \textit{Pvu}II (+/) but increases in those who were \textit{Hin}III (−/) (21). In contrast, Gerdes et al. found the \textit{Hin}III (+/) genotype to be positively associated with a family history of premature ischemic heart disease in 40-year old Danish men (22). Similarly, Mattu et al. found an association between the \textit{Hin}III (+) allele (but not the \textit{Pvu}II [+] allele) and CAD (19). Also, the ECTIM case–control study (20) reported an increased odds ratio (2.1) for MI with \textit{Hin}III (+/+) compared with \textit{Hin}III (−/) genotypes. Wang et al. (12) found an association between extent but not occurrence of CAD and \textit{Pvu}II (\textit{Pvu}II [+/+]) but not \textit{Hin}III polymorphisms.

Our study adds substantially to previous evidence for an association between the \textit{Hin}III (+) allele and CAD in terms of both size and the certainty of diagnosis (angiographic). Our minor (if any) association between \textit{Pvu}II (−) carriage and CAD differs from two reports (12,20) but is consistent with three others (16,19,21). These differences are likely explained by differences in trial size and design, ethnic/genetic makeup, disease marker, and chance (given the relatively modest degrees of association, especially for \textit{Pvu}II). In addition, though these two genetic polymorphisms are linked to each other, it is possible that they function primarily as markers for another linked, etiologically important genomic locus (see next section).

\textbf{Pathophysiologic rationale for study findings.} The catabolism of chylomicrons and VLDL by LPL results in the formation of chylomicron remnants depleted in triglyceride but enriched in cholesterol esters (a result of cholesteryl ester transfer protein) and intermediate density lipoproteins; intermediate density lipoproteins are further metabolized to LDL (8,22,23). A further function of LPL is to bind and

### Table 4. Assocations Between \textit{Hin}III and \textit{Pvu}II Polymorphisms: Observed (Expected) Genotypic Frequencies

<table>
<thead>
<tr>
<th>Genotype</th>
<th>\textit{Hin}III (−/−)</th>
<th>\textit{Hin}III (−/)</th>
<th>\textit{Hin}III (+/)</th>
<th>Totals</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{Pvu}II (−/−)</td>
<td>33 (10.8)</td>
<td>64 (53.3)</td>
<td>46 (78.9)</td>
<td>143</td>
</tr>
<tr>
<td>\textit{Pvu}II (−/)</td>
<td>16 (27.5)</td>
<td>151 (135.2)</td>
<td>196 (200.3)</td>
<td>363</td>
</tr>
<tr>
<td>\textit{Pvu}II (+/)</td>
<td>6 (16.6)</td>
<td>55 (81.6)</td>
<td>158 (120.8)</td>
<td>219</td>
</tr>
<tr>
<td>Totals</td>
<td>55</td>
<td>270</td>
<td>400</td>
<td>725</td>
</tr>
</tbody>
</table>

Data are presented as observed numbers of cases and expected numbers of cases (in parentheses) for each cell. By chi-square testing, the null hypothesis of independence of genotypes is rejected, chi-square = 94.8, \( p < 0.0001 \).

### Table 5. Estimated Haplotypic Frequencies With Association

<table>
<thead>
<tr>
<th>Group</th>
<th>\textup{H} + \textup{P}+</th>
<th>\textup{H} + \textup{P}−</th>
<th>\textup{H} − \textup{P}+</th>
<th>\textup{H} − \textup{P}−</th>
<th>\textup{D}</th>
<th>\textup{LTS}</th>
<th>\textbf{p Value}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
<td>0.476</td>
<td>0.262</td>
<td>0.077</td>
<td>0.186</td>
<td>0.068</td>
<td>78.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CAD+</td>
<td>0.465</td>
<td>0.272</td>
<td>0.074</td>
<td>0.189</td>
<td>0.068</td>
<td>48.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CAD−</td>
<td>0.490</td>
<td>0.224</td>
<td>0.093</td>
<td>0.192</td>
<td>0.073</td>
<td>25.9</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Estimated frequencies calculated using the linkage utility program EH assuming association. CAD = coronary artery disease; \textit{H} = \textit{Hin}III; LTS = Likelihood Test Statistic; \textit{P} = \textit{Pvu}II.
and found that the associations persisted and, indeed, were well known CAD risk factors, including age and gender, populations (12–23). Also, we adjusted our odds ratios for similarity in the LPL polymorphic allelic frequencies in our ple. However, if present, it appears to be small, given the angiography, compared with a free-living population sam-

According to prospective studies. Disease and control groups differed in some baseline variables, as expected. However, when differences were accounted for by conditional multivariate logistic regression, the relative risk associated with the polymorphisms was maintained or augmented.

Conclusions. In a moderately large, prospectively studied, angiographically defined U.S. population of European ex-

strengthened. At least, the study is clinically relevant for the group of subjects who present for angiographic evaluation because of a suspicion of CAD.

Mistyping is a theoretical concern and was dealt with by retyping ≈10% of our samples, with identical results. Also, polymorphic allelic frequencies are similar to those reported in other white populations (12–23).

Our study was cross-sectional, raising the possibility of changes in prevalence of LPL polymorphisms among cases and control subjects due to differential survival rates for CAD patients presenting for angiography based on LPL genotype status. This seems unlikely, but can be addressed by prospective studies. Disease and control groups differed in some baseline variables, as expected. However, when differences were accounted for by conditional multivariate logistic regression, the relative risk associated with the polymorphisms was maintained or augmented.

Study strengths and limitations. This study has the advantage of being of relatively large size and angiographically controlled. The possibility exists of inadvertent genetic selection bias appearing in patients selected for coronary angiography, compared with a free-living population sam-

However, if present, it appears to be small, given the similarity in the LPL polymorphic allelic frequencies in our angiographic study and in several other studies from healthy populations (12–23). Also, we adjusted our odds ratios for well known CAD risk factors, including age and gender, and found that the associations persisted and, indeed, were travel with chylomicron remnants to the liver, where LPL enhances clearance of chylomicron remnants via LDL receptor-related protein (33). In the liver, triglyceride-enriched particles (including HDL and LDL) also are catabolized by another, related hydrolytic enzyme, hepatic lipase (22).

Given its complex and central role, dysfunctional LPL could affect lipoprotein metabolism in a number of ways, although the precise mechanism of enhanced atherogenesis is unclear. A change in LPL's lipolytic or clearance functions for triglyceride-rich lipoproteins and their remnants has been postulated, resulting in a more atherogenic lipid phenotype in fasting or postprandial states (22,33–35). Because both HindIII and PvuII polymorphisms involve introns (ie., 8, 6) in the LPL gene on chromosome 8 (15), protein structure should not be affected; however, their operation as risk markers could be explained through linkage disequilibrium with one (or more) etiologic genetic locus in close proximity.

Gerdes et al. postulated LPL variants to be associated with altered triglyceride metabolism, with HDL cholesterol alterations serving as an integrative marker of triglyceride metabolism (22). They interpreted their findings to suggest that the responsible variant was located on HindIII (+)/PvuII (−) haplotype chromosomes and had originated on a HindIII (+) chromosome before the mutation causing the PvuII site variation (22). Similarly, we interpret our data to be consistent with a genetic variant of LPL or a closely linked, disease-causing gene that is associated with HindIII (+)/PvuII (−) haplotypes (cf. Table 6) and believe that this hypothesis, along with other possible explanations, warrants investigation.

### Table 6. Multivariate Conditional Logistic Regression Model for CAD

<table>
<thead>
<tr>
<th>Variable</th>
<th>Beta</th>
<th>SE</th>
<th>Wald</th>
<th>df</th>
<th>Significance</th>
<th>R</th>
<th>Exp (B) (OR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (male)</td>
<td>1.503</td>
<td>0.210</td>
<td>51.50</td>
<td>1</td>
<td>&lt;0.001</td>
<td>0.258</td>
<td>4.50</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>0.0468</td>
<td>0.0090</td>
<td>27.17</td>
<td>1</td>
<td>&lt;0.001</td>
<td>0.184</td>
<td>1.048</td>
</tr>
<tr>
<td>H(+) carriage</td>
<td>0.1050</td>
<td>0.328</td>
<td>10.27</td>
<td>1</td>
<td>0.0014</td>
<td>0.106</td>
<td>2.86</td>
</tr>
<tr>
<td>Diabetes</td>
<td>0.651</td>
<td>0.283</td>
<td>5.26</td>
<td>1</td>
<td>0.0218</td>
<td>0.0663</td>
<td>1.92</td>
</tr>
<tr>
<td>P(−) carriage</td>
<td>0.348</td>
<td>0.205</td>
<td>2.87</td>
<td>1</td>
<td>0.090</td>
<td>0.0343</td>
<td>1.42</td>
</tr>
<tr>
<td>Constant</td>
<td>−3.133</td>
<td>0.635</td>
<td>20.33</td>
<td>1</td>
<td>0.001</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

Modeling used backward conditional logistic regression (SPSS 6.1 for Power Macintosh), initially entering age (in years), and dichotomous status (yes/no) for diabetes; hypertension; male gender; H(+) carriage 0.348 0.205 2.87 1 0.090 0.0343 1.42.

**REFERENCES**


