A Common Mutation in the Lipoprotein Lipase Gene (N291S) Alters the Lipoprotein Phenotype and Risk for Cardiovascular Disease in Patients With Familial Hypercholesterolemia

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Background—Recently, a mutation in the lipoprotein lipase (LPL) gene (N291S) has been reported in 2% to 5% of individuals in western populations and is associated with increased triglyceride (TG) and reduced HDL cholesterol (HDLc) concentrations.

Methods and Results—Here we report a significant alteration in biochemical and clinical phenotype in subjects with familial hypercholesterolemia (FH) who are heterozygous for this N291S LPL mutation. Sixty-four FH heterozygotes carrying the N291S mutation had significantly a higher TG level (P<.004), a higher ratio of total cholesterol to HDLc (P=.001), and lower HDLc concentrations (P=.002) compared with 175 FH heterozygotes without this LPL mutation. Moreover, the N291S mutation conferred a significantly greater risk for developing cardiovascular disease in FH heterozygotes compared with FH heterozygotes without this LPL mutation (odds ratio, 3.875; P=.006).

Conclusions—These data provide evidence that a common LPL variant (N291S) significantly influences the biochemical phenotype and risk for cardiovascular disease in patients with FH. (Circulation. 1998;97:729-735.)

Key Words: genetics • lipoproteins • atherosclerosis

Lipoprotein lipase is a multifunctional protein, playing a major role in the hydrolysis of TG-rich lipoproteins. Over 60 mutations in the LPL gene have been described that result in a functionally defective catalytic protein and that occur with a frequency of ≈1 in 500 persons. Recently, a more common serine for asparagine substitution at residue 291 (N291S) in exon 6 of the LPL gene has been described that is observed with high frequency, ranging from 2% to 5% in different populations. This N291S mutation is associated with partial reduction in LPL activity, both in vivo and in vitro, and has been associated with reduced levels of HDLc and elevated TGs in some populations.

Despite its association with dyslipoproteinemia, the association between this common LPL mutation and premature CVD is still controversial. We hypothesized that such an association might become particularly apparent if this N291S mutation were studied in a population at increased risk for premature atherosclerosis. Animal models particularly susceptible to atherosclerosis, such as mice with targeted disruption of the apolipoprotein E gene or the gene for the LDL receptor, have been effectively used to monitor the effects of changes in other genes. For example, mice lacking functional LDL receptors (LDLR−/−) have a predisposition to significant hypercholesterolemia and premature atherogenesis. When LDLR−/− mice are crossed with mice overexpressing LPL, a significant attenuation of the biochemical and clinical phenotype is observed.

FH is a common autosomal dominant disorder of lipoprotein metabolism, affecting ≈1 in 500 people in the western world. Defects in the gene coding for the LDL receptor result in disturbed clearance of LDL cholesterol because of impaired hepatic LDL receptor function. In subjects with heterozygous FH, concentrations of plasma cholesterol are generally increased two to three times, and cholesterol accumulates in extrahepatic sites, resulting in the typical characteristics of FH, including tendon xanthomata and premature CVD. Un-treated, ≈75% of men and 40% of women with heterozygous FH will undergo a coronary event before 60 years of age.

Recently, we reported that the N291S mutation in the LPL gene was associated with reduced HDLc concentrations in FH families, and it was suggested that LPL mutations may underlie some cases of low HDLc concentrations observed in...
patients with FH.\textsuperscript{13—15} What remains unanswered, however, is whether this common N291S LPL mutation influences lipoprotein phenotype and risk for CVD in a large cohort of FH patients. This study was therefore undertaken to assess whether this common LPL mutation might be associated with a change in the risk for CVD in patients with heterozygous FH.

**Methods**

**Study Population**

A total of 1045 patients with FH, all residing in the Netherlands, were enrolled in this study. The diagnosis of heterozygous FH was based on either the presence of a documented LDL receptor mutation and/or the following criteria: LDL cholesterol levels above the 95th percentile for sex and age, the presence of typical tendon xanthomas in the patient or in a first-degree relative, or a pediatric relative with an LDL cholesterol level above the 95th percentile for sex and age. Secondary causes of hypercholesterolemia, including renal and hepatic disease, alcohol abuse, diabetes mellitus, and hypothyroidism, were excluded in all subjects. In addition, all biochemical and clinical parameters were assessed at presenting lipid clinic visits with subjects off lipid-lowering medication for at least 3 months. No subjects were first-degree relatives.

Cases were defined as all FH heterozygotes who were positive for the N291S mutation and who did not carry the common D9N mutation in the LPL gene. From the remaining FH cohort who did not carry the N291S or the D9N mutations in the LPL gene, a control group of 175 FH patients was selected for comparison. These groups were selected in a case-control manner by selecting, from the cohort of 1045 available FH heterozygotes, control subjects for each FH case matched for age, sex, and body mass but blinded for all other factors. Of the 64 FH heterozygotes without the N291S mutation, 46 (26.3%) had a defined LDL receptor mutation. Of 175 FH heterozygote control subjects without the N291S mutation, 46 (26.3%) had a defined LDL receptor mutation (data not shown).

**DNA Analysis**

Genomic DNA was extracted from leukocytes as previously described.\textsuperscript{16} The LPL N291S mutation was assessed in 1045 FH heterozygotes by polymerase chain reaction amplification by use of a mismatch primer and followed by digestion with RS41 as described previously.\textsuperscript{17} All cases and control subjects were also analyzed for the D9N mutation in the LPL gene by methods described previously.\textsuperscript{18} Subjects were excluded from the study if they carried the D9N LPL mutation.

**Biochemical Analysis**

Total plasma cholesterol was determined by an enzymatic colorimetric procedure by use of cholesterol esterase.\textsuperscript{19} HDL cholesterol was determined after precipitation of apo-B–containing lipoproteins.\textsuperscript{19} TGs were quantified by an enzymatic colorimetric procedure by use of lipase, glycerol-3-phosphate oxidase, and glucose-6-phosphate dehydrogenase.\textsuperscript{21} LDL cholesterol was calculated by use of the Friedewald formula, which is valid up to a TG level of 8 mmol/L.\textsuperscript{22,23}

**Cardiovascular Disease**

CVD was considered to be present if subjects met one of the following criteria: (1) if subjects had undergone a myocardial infarction, proven by ECG abnormalities and enzyme changes; (2) if the patient had suffered an ischemic stroke; (3) if a diagnosis of clinically documented angina pectoris had been made; (4) if a history of intermittent claudication was present; or (5) if an intervention by either coronary bypass surgery or balloon angioplasty had been performed.

**Statistical Analysis**

FH heterozygotes with and without the N291S mutation were compared. Results are reported by use of untransformed and unadjusted variables. Because both groups were matched for age, sex, and BMI, standard t tests were used to compare means of the variables studied. TGs were log transformed before analysis, but untransformed levels are reported.

χ² analysis was used to compare the frequency of cases and control subjects in TG, HDL, and BMI tertiles. For comparison of the prevalence of CVD in both groups, the Fisher's exact test was used. Logistic regression analysis was performed to assess the impact of the N291S mutation on lipid abnormalities and cardiovascular risk.

All statistical analyses were performed by use of the SYSTAT statistical package (UBC) except the logistic regression models, which were determined by use of the SPSS package (UBC). A value of P<.05 was used to declare statistical significance.

**Results**

**Frequency of the N291S Mutation**

Of the 1045 FH heterozygotes screened, 68 subjects (32 men and 36 women; 6.5%) were carriers of the N291S LPL mutation. This frequency is not significantly different from previous estimates for the frequency of this mutation in the Dutch general population.\textsuperscript{5} Four of these subjects who also carried the common D9N LPL mutation were excluded from the case cohort for lipid, lipoprotein, and cardiovascular assessment.

**Baseline Demographics, Anthropometry, and Risk Factor Assessment**

Baseline characteristics of FH heterozygotes with and without the N291S mutation are shown in Table 1. These groups were matched for age, BMI, and sex ratio. There also were no significant differences in blood pressure, smoking, alcohol intake, and plasma glucose levels. Of the 64 FH heterozygotes with the N291S mutation, 22 (34.4%) had a defined LDL receptor mutation. Of 175 FH heterozygote control subjects without the N291S mutation, 46 (26.3%) had a defined LDL receptor mutation (data not shown).

**Lipoprotein Assessment**

Both groups of patients were similarly characterized by elevated total and LDL cholesterol concentrations as expected for patients with FH (Table 2). FH heterozygotes with the LPL N291S mutation had more marked dyslipoproteinemia, characterized by higher TG levels (P=.004) and lower HDL levels (P=.002) compared with FH heterozygotes without the N291S mutation. In addition, significantly higher TC concentrations (P=.02) and ratios of TC to HDL (P<.001) were observed in FH heterozygotes with the N291S mutation.
TABLE 1. Baseline Characteristics of FH Patients With and Without the LPL N291S Mutation

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>With N291S Mutation n=64</th>
<th>Without N291S Mutation n=175</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>64.9±16.5 (64.4-75.0)</td>
<td>75.0±15.0 (61.6-66.4)</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>23.58±3.64 (13.54-31.77)</td>
<td>23.08±3.29 (13.85-30.10)</td>
</tr>
<tr>
<td>M/F</td>
<td>64/30/34</td>
<td>175/84/91</td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>129.14±16.25 (129.86±18.21)</td>
<td>129.86±18.21</td>
</tr>
<tr>
<td>DBP, mm Hg</td>
<td>79.92±9.34 (80.11±12.43)</td>
<td></td>
</tr>
<tr>
<td>Smoking, cigarettes/d</td>
<td>5.11±8.26</td>
<td>5.66±9.13</td>
</tr>
<tr>
<td>Alcohol, units/d</td>
<td>1.51±1.89</td>
<td>1.37±1.35</td>
</tr>
<tr>
<td>Glucose, mmol/L</td>
<td>5.08±0.85</td>
<td>4.87±0.56</td>
</tr>
</tbody>
</table>

SBP indicates systolic blood pressure; DBP, diastolic blood pressure. Values are given as mean±SD, with ranges given in parentheses for age and BMI. Baseline characteristics did not differ statistically.

TABLE 2. Lipid Levels in FH Patients With and Without the LPL N291S Mutation

<table>
<thead>
<tr>
<th>Lipid</th>
<th>With Mutation N291S n=64</th>
<th>Without Mutation N291S n=175</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC, mmol/L</td>
<td>8.96±1.16 (4.05-13.60)</td>
<td>8.42±1.60 (5.18-14.4)</td>
<td>.02</td>
</tr>
<tr>
<td>LDL cholesterol, mmol/L</td>
<td>6.70±1.55</td>
<td>6.45±1.63</td>
<td>NS</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L</td>
<td>1.15±0.36 (0.40-2.47)</td>
<td>1.32±0.37 (0.65-2.77)</td>
<td>.002</td>
</tr>
<tr>
<td>TG, mmol/L</td>
<td>1.83±1.08 (0.43-5.60)</td>
<td>1.41±0.64 (0.29-3.52)</td>
<td>.004</td>
</tr>
<tr>
<td>TC/HDLC</td>
<td>8.63±3.83 (2.83-29.25)</td>
<td>6.91±2.48 (3.25-16.84)</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

TG values were log transformed before analysis. All values are given as mean±TG, with ranges given in parentheses.
addition, in this BMI tertile, significantly lower HDLC levels were observed in FH patients with the LPL mutation (1.08±0.32 mmol/L) compared with FH control subjects (1.29±0.38 mmol/L; P=.02; Fig 2B). In the middle BMI tertile, subjects with FH and the N291S mutation also had lower HDLC (1.12±0.29 mmol/L versus 1.37±0.41 mmol/L; P=.02) and higher TG levels (2.02±0.91 versus 1.42±0.55 mmol/L; P=.009) compared with FH control subjects. No significant difference between FH cases and control subjects was observed in the lowest BMI tertile with respect to either TG or HDLC concentration. These results suggest a significant interaction between BMI and this LPL mutation, with the phenotypic effects of this mutation clearly unmasked in patients with high BMIs.

Prevalence of CVD

The prevalence of CVD was almost two times greater in FH heterozygotes with the N291S mutation compared with FH heterozygotes without this mutation. Of the 64 FH cases, 19 had a history of CVD compared with 21 of 175 FH control subjects (P=.006; Table 4).

Although the prevalence of CVD was significantly higher in FH cases with the N291S mutation, no significant difference in age of onset of CVD between FH cases and FH control subjects was observed. FH patients with the N291S mutation had a mean age of onset of CVD of 49.0±12.8 years compared with 44.9±8.8 years in FH control subjects (P=.3; data not shown).

**Table 3. OR for Association Between the N291S Mutation and the Low HDL/High TG Phenotype**

<table>
<thead>
<tr>
<th>Prevalence, (%)</th>
<th>N291S</th>
<th>OR</th>
<th>P</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>HDLC &lt;0.91 mmol/L</td>
<td>36 (15)</td>
<td>2.49</td>
<td>.02</td>
<td>1.15-5.41</td>
</tr>
<tr>
<td>TG &gt;2.82 mmol/L</td>
<td>17 (7.1)</td>
<td>3.813</td>
<td>.02</td>
<td>1.25-11.63</td>
</tr>
<tr>
<td>HDLC &lt;0.91 and TG &gt;2.82 mmol/L</td>
<td>6 (2.5)</td>
<td>6.28</td>
<td>.09</td>
<td>0.71-55.51</td>
</tr>
</tbody>
</table>

Adjusted for smoking, sex, age, alcohol intake, BMI, and systolic and diastolic blood pressures.

**Table 4. Prevalence of and OR for CVD in FH Patients With and Without the N291S Mutation**

<table>
<thead>
<tr>
<th>Prevalence of CVD, (%)</th>
<th>OR</th>
<th>P</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>With N291S mutation</td>
<td>19/64 (29.69)</td>
<td>3.88</td>
<td>.006</td>
</tr>
<tr>
<td>Without N291S mutation</td>
<td>21/175 (12.0)</td>
<td>1.47</td>
<td>.3</td>
</tr>
</tbody>
</table>

CVD was defined as myocardial infarction, definite angina pectoris, documented stroke, or surgery for peripheral arterial disease. Adjusted for smoking, sex; age; alcohol intake; BMI; systolic and diastolic blood pressures; and LDL cholesterol, HDLc, and TG concentrations.
Furthermore, no difference in the type of CVD was observed between FH subjects with and without the N291S mutation. Of the 19 FH heterozygotes with the LPL mutation, 15 had coronary artery disease, 2 had cerebrovascular disease, and 2 had peripheral vascular disease. Of the 21 FH control subjects (without the N291S mutation) with CVD, 15 had coronary artery disease, 4 had cerebrovascular disease, and 2 had peripheral vascular disease. In addition, no difference in the number of coronary events was observed between FH subjects with and without the N291S mutation (data not shown).

Here, we demonstrate that the N291S LPL mutation, which is expressed in FH heterozygotes who carry this LPL mutation, significantly increased risk of developing CVD in patients with FH (OR, 3.89; \( P = 0.003; 95\% \text{ CI}, 1.59 \text{ to } 9.51 \)). The addition of HDLC, TG, and LDL-C to the model had very little effect on these results (OR, 3.88; \( P = 0.006; 95\% \text{ CI}, 1.47 \text{ to } 10.22 \); Table 4). In this model, the N291S LPL mutation was the most significant predictor of CVD status, followed by age (OR, 1.11; \( P < 0.001; 95\% \text{ CI}, 1.06 \text{ to } 1.17 \)), sex (OR, 0.23; \( P = 0.009; 95\% \text{ CI}, 0.08 \text{ to } 0.69 \)), and BMI (OR, 1.29; \( P = 0.02; 95\% \text{ CI}, 1.05 \text{ to } 1.59 \); data not shown).

**Discussion**

We have studied a cohort of FH heterozygotes to assess the effect of a common LPL mutation (N291S) on both lipoprotein phenotype and risk for atherosclerosis. Despite its previously reported association with dyslipoproteinemia, the association between this mutation and an increased incidence of atherosclerotic vascular disease remains controversial. To assess this possible association, we have studied a group of individuals already at high risk for CVD to determine whether this mutation altered the risk for developing atherosclerosis in this cohort.

Here, we demonstrate that the N291S LPL mutation, detected in 6.5% of 1045 FH heterozygotes studied, not only had a significant deleterious effect on lipoprotein phenotype but also significantly increased cardiovascular risk in subjects with this LPL mutation.

Individuals with heterozygous FH manifest with elevated TC and LDL cholesterol concentrations and have a significantly increased predisposition to premature CVD.\(^{10}\) It has been reported, however, that significant variability does exist with respect to the biochemical and clinical phenotype in FH.\(^{24,25}\) Apart from the elevated LDL cholesterol concentrations observed in FH, numerous studies of FH heterozygotes have observed a trend toward low HDLC concentrations.\(^{13-15,26}\) Low HDLC and high TG concentrations also have been shown to correlate positively with increased frequency of myocardial infarction in men with FH.\(^{27}\) Recently, we reported in FH families that mutations in the LPL gene can result in reduced HDLC concentrations in individuals carrying mutations in both the LDL receptor and LPL genes.\(^{22}\) Here, we confirm and extend these findings by illustrating the effect of the common N291S mutation in a large cohort of FH heterozygotes.

Double FH/LPL heterozygotes presented with significantly lower HDLC, higher TG, and higher TC concentrations and higher TC/HDL ratios compared with FH heterozygotes without the N291S LPL mutation. This mutation predisposed to the combined low HDLC/high TG phenotype as illustrated by logistic regression analysis. Furthermore, FH heterozygotes with this LPL mutation were enriched in the lowest HDL and highest TG tertiles in this FH cohort.

The influence of the N291S mutation on lipid levels was most apparent when acting in concert with increased BMI. Significant differences in TG and HDLC were apparent only between FH N291S carriers and FH control subjects in the middle and upper tertiles of body mass. These data support the observed association with dyslipoproteinemia, the association between this mutation and increased body mass.\(^{6,8}\) This finding may have clinical and therapeutic implications and could provide support specifically for encouraging weight loss, particularly in FH heterozygotes who carry this LPL mutation.

The overall incidence of CVD in this study population was in keeping with the reported expected incidence for FH heterozygotes of this age.\(^{16}\) Of 239 FH heterozygotes, 40 (16.7%) with a mean age of 38.7 ± 15.8 years had evidence of CVD. In addition to the effect of this mutation on lipoprotein phenotype, we were able to demonstrate a significant positive association between this N291S mutation on cardiovascular risk in FH heterozygotes. Logistic regression analysis determined that in this cohort, the effect of the N291S mutation in increasing the risk for developing CVD was greater than any other risk factors added to the model (including smoking, age, sex, alcohol intake, BMI, and systolic and diastolic blood pressures). The effect of the N291S mutation on CVD risk also was in part independent of its effect on lipid levels, as illustrated in Table 4. This finding may be related in part to the roles of LPL outside its lipolytic function. Altered proteoglycan binding or lipid particle uptake at the level of the vessel wall might account for this finding. We must also point out that only a single fasting lipid determination, as was undertaken in this study, may underestimate the long-term effect of this LPL mutation on lipoprotein metabolism. Serial lipid measurements in N291S carriers before they go on a lipid-lowering diet with subsequent weight loss may reveal a stronger association between this mutation, lipid levels, and CVD risk.

Clearly, other factors may also influence the lipoprotein phenotype and risk for atherosclerosis in patients with FH. Dietary differences may account for an altered phenotype in FH, as we have recently illustrated by comparing the phenotypes of Chinese FH heterozygotes with the same LDL receptor mutations residing in either China or Canada (S.N. Pimstone, X-M Sun, C. du Souich, J.J. Frohlich, M.R. Hayden, A.K. Soutar, unpublished data, 1997). Furthermore, additional genetic factors, Lp(a) concentrations, and the underlying LDL receptor mutation might also influence the FH phenotype. Although we were not able to directly assess all of these in this study, these factors are unlikely to account for these results. Patients with many different functional LDL receptor mutations were included in the FH cohorts with and without the N291S mutations. Furthermore, there were no differences in apo E genotype frequencies between the groups (data not shown). Within the cohort of 19 N291S subjects...
with CVD, all apo E genotypes apart from apo E2/E2 were represented. Lp(a) concentrations were not available from this cohort, but data with respect to both apo E genotype and Lp(a) concentrations factors predictably altering coronary risk in FH have been contradictory.20–23 Furthermore, dietary differences are unlikely to have had a significant influence on coronary risk in this population. All of these subjects were reported to be following a low fat/low cholesterol diet monitored by the lipid clinics at which they were being followed.

The influence of this common LPL mutation on CVD risk must, at least in part, be explained by the effect of the N291S mutation on LPL function. This mutation decreases LPL catalytic activity both in vivo and in vitro,33–34 resulting in impaired catabolism of chylomicrons and VLDL particles and in hypertriglyceridemia.3 Resulting TG elevations in carriers of the N291S mutation contribute to a reduction in LDL particle size.35 Small, dense LDL in association with impaired postprandial TG clearance observed in carriers of the N291S mutation34 may in part be responsible for increasing atherogenic risk in FH heterozygotes who carry this LPL defect.

Here, we report for the first time that a large cohort of FH heterozygous subjects also carrying the N291S LPL mutation have significantly lower HDL levels, higher TG levels, and higher ratios of TC to HDL and are at increased risk for cardiovascular disease compared with FH heterozygotes without this mutation. Because the LPL mutation segregates independently of the mutation in the LDL receptor gene, LPL mutations may account in part for the variability of TG and HDL levels observed in FH patients and FH families and furthermore may contribute to the variability in the clinical course observed in heterozygous FH. These data illustrate that this common LPL variant is an independent risk factor for atherosclerotic disease in patients heterozygous for FH and suggest that this common LPL mutation may influence risk for CVD in the general population.

Acknowledgments

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References


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