The LPL Gene in Individuals With Familial Combined Hyperlipidemia and Decreased LPL Activity

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Abstract Familial combined hyperlipidemia (FCHL) is an oligogenic disorder, with family members having elevated apolipoprotein B-100 levels and either elevated plasma cholesterol or triglyceride levels or both. Obligate heterozygous parents of children with lipoprotein lipase (LPL) deficiency express a mild FCHL phenotype. Of patients with FCHL, 36% have diminished postheparin LPL activity and mass values that are comparable with those of obligate heterozygotes for LPL deficiency. It is hypothesized that heterozygosity for mutations in the LPL gene could contribute to FCHL in this subset of patients. Single-strand conformation polymorphism (SSCP) analysis, direct DNA sequencing, and Southern blot analysis were used to examine exons 1 through 9 and exon-intron junctions of the LPL gene in 20 patients with FCHL and low LPL activity and mass. One subject had a substitution (GAC→AAC) in exon 2, changing Asp to Asn. Two subjects had a previously undescribed "silent" substitution (GTG→GTA) in exon 3 at Val. Three patients had a premature termination at codon 447 in exon 9 resulting in truncation of the mature protein by two amino acids. In addition to SSCP analysis, exons 4, 5, and 6, where almost all mutations in LPL-deficient patients have been found, were sequenced and no additional mutations were found. Southern blot analysis of the LPL gene revealed one subject with heterozygous loss of an EcoRI site but without an abnormality in Stu I restriction fragments; this mutation is therefore unlikely to be functionally significant. The substitutions identified at codons 9 and 447 have previously been found not to affect lipolytic activity when expressed in vitro. In summary, the findings suggest that mutations in the coding sequence of the LPL gene are an infrequent cause of FCHL. Other mechanisms that regulate plasma LPL activity remain to be investigated in the pathogenesis of FCHL.

Keywords lipoprotein lipase • single-strand conformation polymorphism • familial combined hyperlipidemia • triglycerides • cholesterol

Several lines of evidence support a role for abnormalities in the LPL gene or its regulation in some individuals with FCHL. Some investigators have found restriction fragment length polymorphisms and polymorphic microsatellites in the LPL gene to be associated with plasma lipoprotein levels. Previous work from our laboratory has supported the concept that one type of FCHL is due to heterozygosity for LPL deficiency. Babirak et al examined six families in which the relatives were determined by measurement of postheparin LPL activity and mass. Mild hyperlipidemia, elevated apo B levels, and reduced HDL levels, findings consistent with FCHL, were associated with heterozygosity for LPL deficiency in these families. To examine the frequency of low LPL activity in FCHL patients, Babirak et al selected 56 individuals with FCHL and measured their postheparin LPL activity and mass. Twenty of 56 subjects (36%) had reduced LPL activity and mass values in the same range as did obligate heterozygotes for LPL deficiency. Wilson et al also found similar evidence for variable hyperlipidemia (predominantly hypertriglyceridemia) in heterozygous carriers of an LPL mutation in a large pedigree. These findings led to the hypothesis that mutations in the LPL gene could be the basis for reduced LPL in this subset of FCHL patients. This report describes the results of screening the LPL gene in the aforementioned 20 subjects with FCHL and reduced LPL activity and mass.
Methods

Informed consent was obtained according to procedures approved by the Human Subjects Review Committee of the University of Washington. The lipoprotein profile and LPL activity and mass for all 20 subjects in this study have been reported in detail.\(^\text{17}\) Mean postheparin LPL activity and mass values were 102 nmol/min per milliliter (normal, 220 nmol/min per milliliter) and 86 ng/mL (normal, 196 ng/mL), respectively. Mean plasma lipid, lipoprotein, and apolipoprotein data for the 20 subjects were TG 327 mg/dL, cholesterol 268 mg/dL, VLDL cholesterol (C) 73 mg/dL, LDL-C 160 mg/dL, HDL-C 36 mg/dL, and apo B 149 mg/dL.

Genomic DNA was extracted from frozen white blood cells according to published methods.\(^\text{24}\) Polymerase chain reaction (PCR) and single-strand conformation length polymorphism (SSCP) analyses were performed as described in Reina et al.\(^\text{25}\) When available, known mutants (exons 2, 3, 4, 5, 6, and 9) were processed with the subjects’ samples. \(^\text{32P}\)-labeled dCTP exonic fragments were electrophoresed on 5% nondenaturing polyacrylamide gels containing 10% glycerol. Gels were run at approximately 40 W for 4 to 5 hours with and without a fan so that each sample was run at two temperatures (28°C to 30°C and 38°C to 40°C) to increase the probability of detecting sequence variants. Gels were dried and exposed to X-MAT film (Amersham) for 36 to 60 hours at -70°C. Autoradiograms were read and variants sequenced.

The same primers for SSCP were also used to produce unlabeled PCR products for direct sequencing. PCR products for exons 4, 5, and 6 were made for each subject, purified by electrophoresis in low-melting-point agarose (Seaplaque),\(^\text{26}\) and sequenced by the chain-termination method using \(^\text{35S}\)-dATP.\(^\text{27}\)

Restriction digests with EcoRI and Stu I with 5 μg genomic DNA were run in 15% agarose gels, and Southern analysis was performed as described in Langlois et al.\(^\text{28}\)

Results

SSCP analysis of exons 1 through 9 and exon-intron junctions identified sequence variants in exons 2, 3, and 9. No variants were found in exons 1, 4, 5, 6, 7, or 8. One of 20 subjects was heterozygous for an exon 2 substitu-

Discussion

In this study we examined the coding region and exon-intron junctions of the LPL gene in 20 well-characterized FCHL subjects with reduced plasma postheparin LPL activity and mass. Although six variants...
were found in these 20 subjects, it is unlikely that these variants alone reduce LPL activity. Aside from the new ‘‘silent’’ polymorphism at Val$^{10}$ in exon 3, previous reports have examined the other variants. The substitution that changes Asp$^7$ to Asn in exon 2 has normal activity when expressed in vitro and has normal affinity to heparin Sepharose.29 The nonsense mutation at codon 447 of exon 9, which shortens the mature protein by two amino acids to 446 residues, has been reported.30 Expression of this variant in COS cells produced LPL mass and activity comparable with that of wild type. Faustinella et al30 found a high population frequency (16%) and an asymptomatic homozygote with the codon 447 premature termination in a group of 224 normal Caucasians. Our finding of a 15% frequency of the premature termination in exon 9 is consistent with these observations. Taken together, the data argue against the concept that these variants account for the observed decrease in LPL activity. The possibility exists that nonlipolytic functions of the enzyme, such as heparin binding or remnant lipoprotein catabolism, could be affected by a coding sequence variant that secondarily reduces plasma LPL activity and mass but is not detected in an in vitro expression system that measures activity only.

SSCP has excellent sensitivity and specificity when used on the human LPL gene and many other genes, but some mutations could have been missed. Of the mutations reported from our laboratory,25 all were detected in the aforementioned SSCP system. Direct DNA sequencing of LPL exons 4, 5, and 6 confirmed that SSCP screening of the FCHL subjects had not missed any mutations in these exons. Given that the LPL mutations that have been detected in LPL-deficient patients cluster in these exons, particularly exon 5, mutations in the LPL structural gene were effectively shown not to be the cause of reduced LPL activity in our sample. The extra band on Southern analysis for one individual is most likely due to heterozygous loss of an EcoRI site in an intron. This individual had no variants on SSCP analysis of exons 1 through 9, and it is known that no EcoRI sites are present in the human LPL cDNA. Recently Chaut et al31 have reported genomic sequence for introns 6 through 9 and have located EcoRI sites in introns 6, 7, and 9. The intron 7 EcoRI site is located within an Alu repeat, which may provide a mechanism for this observation.

The observations that obligate heterozygotes for LPL deficiency exhibit mild features of FCHL,24 and that 36% of FCHL subjects have diminished levels of LPL activity and mass remain to be explained. Given that the estimated frequency of LPL heterozygotes in the population is 1 in 500 individuals, it is plausible to consider LPL heterozygosity as a mechanism for the reduced LPL activity found in 1 of 3 individuals with FCHL, a disorder estimated to affect 1% to 2% of the population, but this hypothesis has not been supported. Several alternative mechanisms for LPL reduction in this subset of FCHL patients may be responsible and can be tested. Mutations in cis-regulatory elements located 5’ and 3’ from the LPL gene may produce diminished LPL gene expression, thereby accounting for the observed phenotype. Several regions upstream from the transcription start site for the LPL gene are reported to be important in determining transcriptional activity in transient transfection experiments with 3T3-L1 adipocytes.32 Molecular scanning of these sites by SSCP can examine this possibility. Linkage analysis of FCHL families with reduced LPL activity is a complementary approach. Polymorphic LPL markers are available for linkage studies33,34 and have been used in a sib-pair linkage study.19 Evidence for linkage of these markers to FCHL would lead to further examination of LPL gene regulatory elements. Negative results of linkage analysis to markers at the LPL gene would support the alternative hypothesis that trans-acting factors or other mechanisms might be involved.

The complex pathway from posttranscriptional processing, translation, glycosylation, secretion, and finally attachment of LPL to endothelial heparan sulfate proteoglycans provides a number of sites that could be defective and ultimately cause diminished postheparin plasma LPL activity and mass. LPL binding to heparan sulfate proteoglycans or to the proposed LPL binding protein35,36 are potential sites where abnormalities in LPL binding could reduce LPL activity in plasma. Plasma constituents such as apo-CII, apo-CIII, and apo-AIV may affect postheparin plasma LPL activity. Additionally, a plasma inhibitor of LPL activity has been described in one family37 but is unlikely to explain the partial reduction in LPL activity seen in these FCHL subjects.

Finally, reduced LPL activity and mass may reflect an adaptation to changes in the transport of free fatty acids in plasma rather than a primary defect in LPL regulation. Thus, an abnormality in the adipose-acylation stimulation protein pathway that affects triglyceride transport could secondarily affect plasma LPL activity.40 In conclusion, mutations in the LPL gene coding sequence are unlikely to be the cause of reduced LPL activity in this subset of FCHL, and other mechanisms will need to be examined.
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