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A heterozygous mutation(the codon for Ser^{447}→a stop codon) in lipoprotein lipase contributes to a defect in lipid interface recognition in a case with type I hyperlipidemia

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Previously, we reported a case with type I hyperlipidemia due to a lipid interface recognition deficiency in lipoprotein lipase (LPL)(1). The LPL from postheparin plasma of this patient did not hydrolyze TritonX-100-triolein or very low density lipoprotein-triolein but did hydrolyze tributyrin and LysoPC-triolein substrates Sequence analysis of the proband's DNA revealed a heterozygous nucleotide change: a C→G transversion at position of 1595, resulting in changing the codon for Ser^{447} to a stop codon. Expression studies of this mutant LPLcDNA in Cos-1 cells produced and secreted considerable amounts of LPL mass in the culture media. The mutated LPL hydrolyzed much less TritonX-100-triolein than wild type LPL, whereas hydrolysis of tributyrin and LysoPC-triolein was the same with both the mutant and wild type LPL. These results suggest that this mutation might be responsible for the property of the LPL with a defect in lipid interface recognition in the type I patient we reported.

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Abbreviations: LPL, lipoprotein lipase; HL, hepatic lipase; Triton X-100-triolein, triolein emusified with Triton X-100; LysoPC-triolein, triolein emusified with lysophosphatidyl choline; PHP, postheparin plasma; PCR, polymerase chain reaction; LPL446, LPLcDNA with substitution of terminating codon for Ser^{447}; LIRS, lipid interface recognition site.

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Lipoprotein lipase(LPL) is a member of a lipase gene family that includes hepatic lipase and pancreatic lipase(2,3). Recently, numerous mutations in the LPL gene of type I hyperlipidemic patients have been reported(4-10). These reports include rearrangements of DNA(4,5) as well as single-base substitutions generating missense(6-9) and nonsense mutations(10). Among these alterations, missense mutations appear to yield important information concerning structure-function relationships in LPL molecule. The majority of these missense mutations occur within the highly conserved region encoded by exons 4, 5 and 6 of LPL that shows considerable homology to human pancreatic lipase and human hepatic lipase(2,3). By analogy with the three dimensional structure of human pancreatic lipase, many of the naturally occurring mutants of LPL are located close to the LPL catalytic triad(Ser-His-Asp). However, the problem of structure-function relationships has not been fully solved, and more detailed evaluations are necessary. As for the functional structure of LPL, the presence of a lipid interface recognition site(LIRS) and a catalytic site have been proposed(11-13). This lipase is believed to recognize the lipid interface of triglyceride-rich lipoproteins or lipid emulsion through LIRS.

Recently we reported a case of type I hyperlipidemia purportedly due to a LPL defect in the LIRS(1). In this report, we describe an alteration of the LPL gene sequence of this patient.

Methods

Subjects:

Previously we reported a case of hypertriglyceridemia presumably due to a defect in the LIRS of LPL in postheparin plasma(PHP)(1). The proband is a 14-year-old girl suffering from recurrent pancreatitis. Her maximum plasma triglyceride level was 3600mg/dl and total cholesterol level was 970mg/dl. The plasma total cholesterol and triglyceride levels of the proband's parents were within normal limits(The normal ranges of plasma total cholesterol and triglyceride levels are 130-220mg/dl and 30-130mg/dl,respectively.). However, the LPL avtivity from PHP of both the parents were approximately 1/2 of normal controls(data not shown).

Measurement of LPL activity, esterase activity and mass:

LPL activity was measured using Triton X-100-triolein and lysophosphatidyl choline(LysoPC)-triolein as substrates. Esterase activity was measured using tributyrin as substrate. LPL mass was determined by a sandwich enzyme-linked immunosorbent assay(14).

Preparation of DNA and oligonucleotide primers:

DNA was extracted from either peripheral-blood lymphocyte mononuclear cells or Epstein-Barr-virus-transformed lymphoblastoid cell lines(15,16). Oligonucleotide primers were synthesized on a model 381A synthesizer (Applied Biosystems

Inc.,Foster City,CA) using the β-cyanoethyl phosphoparamidine method, and were purified by reverse-phase chromatography cartridges(OPC.Applied Bio System,Inc.). The Polymerase Chain Reaction(PCR) primers were constructed complementary to DNA sequences flanking exons 1-9 of the LPL gene(3).

PCR amplification and direct sequencing determination of amplified DNA: Target DNA sequences were amplified(17) in a 100 μl reaction volume containing 0.5 μg of chromosomal DNA, 50 pmol of each oligonucleotide primer, 200 μM each of dATP,dCTP,dGTP and dTTP, reaction buffer(50mM KCl,10mM Tris-HCl, pH 8.4, 3mM MgCl2, 0.05% polysorbate[Tween20], 0.05% TritonX-100, 200 μg/ml of gelatin), and 2U of Taq DNA polymerase(Strategene,Inc, San Diego, CA). Amplified DNA was purified from 1% agarose gels. Sequence analysis was performed with gel-purified PCR products using the dideoxy-chain termination method(18). The obtained DNA was sequence using [α -^{35}S]dATP(600Ci/mmol; Amersham Corp.Arlington Heights) and T7 DNA polymerase(United States Biochemical Corp.Cleveland,OH). Sequencing reaction mixtures were subjected to electrophoresis on 6% acrylamide gels containing 8M urea. Dried gels were exposed to Kodak XAR film for 48-72h.

Synthesis and Enzymatic Amplification of cDNA:

Complementary DNA was synthesized from 0.5 μg of total RNA from the patient's adipose tissue by incubating the RNA for 40 min. at 37℃ in 25 μl of a reaction mixture containing 50mM Tris-HCl(pH8.3), 75mM KCl, 3mM MgCl2, 10mM dithiothreitol, 0.5mM each dATP,dCTP,dGTP and dTTP, 50 μg/ml oligonucleotide(dT), 40units of RNasin(Promega Biotek,Madison,WI), and 200units of mouse mammary leukemia virus reverse transcriptase(Bethesda Research Laboratory). The resulting cDNAs were subjected to PCR, using 50 pmol of each primer and 2.5 units of Taq DNA polymerase.

Site-directed mutagenesis and expression:

The expression vector pCDE-LPL was constructed by placing the cDNA insert downstream of SV40 promoter in the expression vector pCDE(19). The constructs, pCDE-NLPL and pCDE-PLPL, containing wild type and mutated cDNA, respectively, were characterized by DNA sequencing before transfecting into COS-1 cells. DNA transfections were performed by the DEAE-dextran method(20).

Results

Polymerase Chain Reaction(PCR) amplification and sequencing of LPL gene:

The nine coding exons of LPL were amplified and sequenced. Except for exon 9, no differences in nucleotide sequence were found in the DNA of the proband, her mother and father, and 2 control subjects. Fig.1 shows the results of direct sequence analysis for exon 9 of the LPL gene from the family members and control subjects. All family members were found to have a heterozygous point mutation in exon 9: a C→G transversion at nucleotide position 1595, resulting in changing the codon for Ser^{447} to a stop codon. This finding was verified by determining the nucleotide sequence of the complementary strand(data not shown) PHP from both the mother and father showed approximately 1/2 of the LPL catalytic activity with Triton X-100-triolein when compared with normal controls(data not shown).

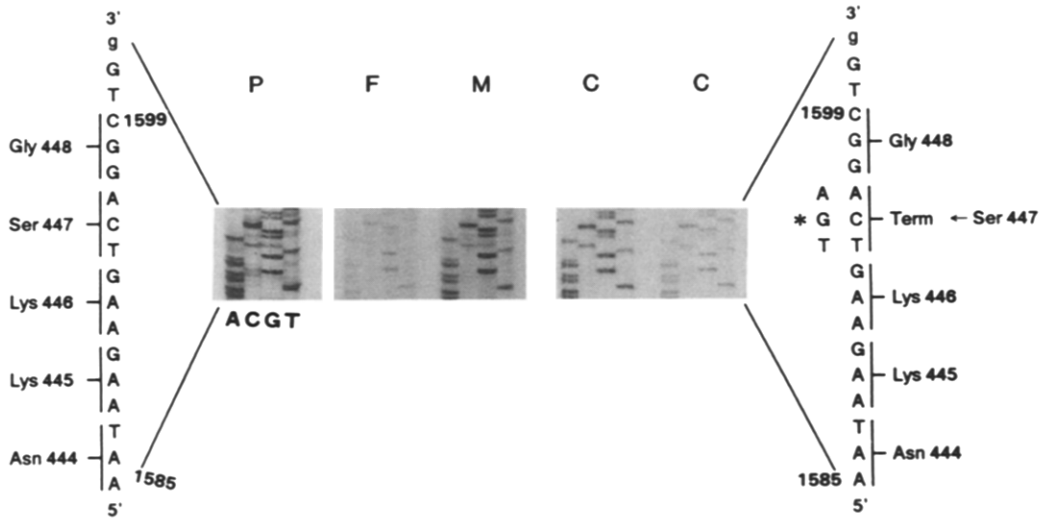

Figure 1. DNA sequence of LPL exon 9.
P,F,M,and C designate proband,father,mother and control,respectively. DNA sequence analysis was performed with oligonucleotide primers FSE9(5´-GACAGAACTGTACCTTT-3´) and RSE9(5´-TGAAGCTGCCTCCCTTAGGGTGCAAG-3´) and analyzed by electrophoresis on 6 % acrylamide/8M urea sequencing gel. The order of lanes on the corresponding autoradiograms is A,C,G,and T, showing the DNA sequence derived from oligonucleotide primer FSE9.
A heterozygous mutation (a C→G transversion)was shown at nucleotide position 1595(asterisk)in proband,father and mother.

Isolation of Adipose Tissue RNA, Synthesis and Enzymatic Amplification of LPLcDNA

The heterozygous mutation in this subject was confirmed at position 1595 by sequencing the cDNA obtained from adipose RNA. We found a C→G transversion at position 1595 in 3 out of 6 clones selected(Fig.2). This result is compatible with a heterozygous nucleotide change: a C→G transversion at position 1595 in the LPL gene.

Expression of and functional analysis of the mutant LPL(Table 1)

To assess whether the observed amino acid substitution contributes to formation of the dysfunctional LPL in this patient, we expressed LPL cDNA with a C→G transversion at 1595 (LPL-446 construct)using Cos-1 cells. The mass and catalytic function of the expressed LPL using TritonX-100-triolein, LysoPC-triolein and a water-soluble substrate, tributyrin, were investigated. The culture media of Cos-1 cells transfected with wild type LPLcDNA and LPL-446 constructs showed a considerable amount of LPL mass, indicating that a C→G transversion did not impair the expression of LPL mass.

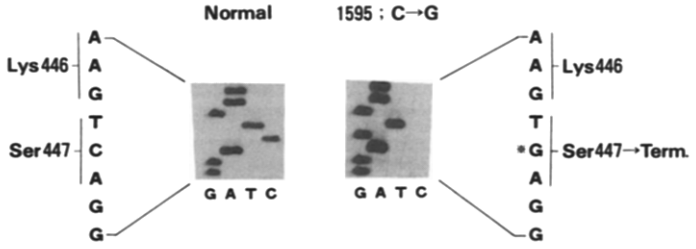

Figure 2. LPL cDNA sequence from proband's adipose tissue. Complementary DNA was synthesized from 0.5 μg of total RNA from patient adipose tissue by incubating the RNA for 40 min. at 37℃ in 25 μl of reaction mixture containing 50mM Tris-HCl(pH8.3),75mM KCl,3mM MgCl2,10mM dithiothreitol, 0.5mM each of dATP,dCTP,dGTP and dTTP, 50 μg/ml oligonucleotide(dT), 40units of RNasin (Promega Biotek,Madison,WI), and 200units of mouse mammary leukemia virus reverse transcriptase(Bethesda Research Laboratory). The cDNA was recovered by ethanol precipitation after extraction with phenol/chloroform. The cDNAs were each subjected to polymerase chain reaction on using 50 pmol of each primer and 2.5 units of Taq DNA polymerase. The reaction mixtures were denatured at 95℃ for 1min, primer annealed at 55℃ for 1min., and primer extended at 72℃ for 5min. for 30 cycles.

In order to determine the function of the expressed LPL, we checked the catalytic function using TritonX-100-triolein,LysoPC-triolein and a water soluble substrate,tributyrin(1).
The culture media of Cos-1 cells transfected with LPL-446 construct hydrolyzed much less TritonX-100-triolein substrate than the wild type LPL. On the other hand, the culture media of Cos-1 cells transfected with wild type LPL cDNA and with LPL-446 construct hydrolyzed almost equal amounts of tributyrin, indicating that the function of the catalytic site is not

Table 1

LPL mass and specific activities with various substrates in the media of Cos-1 cells transfected with wild type LPLcDNA and LPL446

	LPL mass (ng/ml)	LPL specific activity (μ moles/ μ g/h)		
		tributyrin	TritonX-100-triolein	LysoPC-triolein
Wild type	28.0	1.39±0.204	12.8±1.4	15.0±1.88
LPL 446	35.1	1.24±0.172	5.80±0.72	17.1±2.44

LPL mass values are the mean of duplicate experiments.
All other values are the mean±SD of triplicate analysis.

impaired by this mutation because tributyrin is a substrate for evaluating the function of the catalytic site(11-13).
The culture media of Cos-1 cells transfected with LPL-446 construct showed almost the same activity against Lyso-PC-triolein as those with wild type LPL. The rationale for using LysoPC-triolein as substrate in this study is that LPL from PHP of this patient did not hydrolyze Triton X-100-triolein but did hydrolyze Lyso-PC-triolein as well as normal control LPL(1).

Discussion

We have described in this study a heterozygous type of C→G transversion in exon 9 of the LPL gene from a type I hyperlipidemic patient due to the LPL with a defect in lipid interface recognition. The expressed LPL in the culture media of Cos-1 cells transfected with LPL mutant cDNA containing the C→G transversion at 1595 hydrolyzed the water-soluble substrate tributyrin and LysoPC-emulsified triolein as well as wild type LPL, in spite of the fact that it shows a lower specific activity with Triton X-100-triolein(45.3% of wild type LPL). These results indicate that the expressed LPL from Cos-1 cells transfected with LPL446 construct showed properties consistent with the LPL found in PHP from this type I patient as we previously reported(1). Thus, we concluded that the observed mutation may contribute to the lipid interface recognition defect in this subject.

Recently, Faustinella et al.(21) concluded that this mutation had no effect on the function of LPL, based on their expression study using Cos cells transfected with LPL cDNA with the Ser^{447}→Ter mutation. However, a detailed analysis of their data reveals that the LPL specific activity in the media of Cos cells transfected with Ser^{447}→Ter mutant was in fact decreased by 31%.

Few data are available concerning the clinical significance of heterozygous LPL deficiency. Recently,however, it has been reported that obligate heterozygotes for LPL deficiency show hypertriglyceridemia(22) or familial combined hyperlipidemia(23). Furthermore, a single heterozygous mutation has been found in several subjects with complete hepatic lipase deficiency (24). The mutation comprises a C→T substitution in exon 8, resulting in a substitution of Met for Thr at position 383 in hepatic lipase mature protein. That report suggested that a heterozygous mutation occuring in region near the C-terminal of

hepatic lipase mature protein might contribute to dysfunctional enzyme, resulting in complete hepatic lipase deficiency.

Our patient had no mutation in the highly conserved region of exons 3,4 and 5, but did exhibit a mutation in the C-terminal region of exon 9 of the LPL gene. This suggests that even a structural change of this terminal region must cause a great conformational change in tertiary structure, resulting in the impairment of lipid interface recognition of the LPL molecule in this patient.

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