A novel missense mutation in the C-terminal domain of lipoprotein lipase (Glu^{410}→Val) leads to enzyme inactivation and familial chylomicronemia

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Abstract Lipoprotein lipase (LPL) is a complex enzyme consisting of multiple functional domains essential for the initial hydrolysis of triglycerides present in plasma lipoproteins. Previous studies have localized the catalytic domain of LPL, responsible for the hydrolytic function of the enzyme, to the N-terminus whereas the C-terminal end may play a role in lipid and heparin binding. To date, most described missense mutations resulting in a nonfunctional LPL have been located in the N-terminal region of the enzyme. In this manuscript we describe the defect in the LPL gene of a patient with triglycerides ranging from normal to 12,000 mg/dl, low LPL mass, and no LPL activity in post-heparin plasma. Sequencing of patient PCR-amplified DNA identified two separate mutations in the C-terminal domain of LPL: an A → T transversion at nucleotide 1484 resulting in a Glu^{410}→Val substitution and a C→G mutation at position 1595 that introduces a premature stop codon at position 447. Digestion with MaeIII and MnII established that the patient is a true homozygote for both mutations. In order to investigate the functional significance of these defects, mutant enzymes containing either the Val^{410} or the Ter^{447} mutations as well as both Val^{410} and Ter^{447}, were expressed in vitro. Compared to the wild-type enzyme, LPL^{447} demonstrated a moderate reduction of specific activity using triolein (70% of normal) and tributyrin (74% of normal) substrates, while LPL^{410} had a significant (11% and 23% of normal) reduction of the normal lipase and esterase specific activities, respectively. Mutant-LPL$^{410/447}$ was virtually inactive using either triolein or tributyrin substrates establishing the functional significance of this combined defect. When analyzed by heparin-Sepharose affinity chromatography, a small fraction of LPL^{410}, like the native LPL dimer, eluted at an NaCl concentration of 1.3 M and had a normal specific activity. However, most of the LPL^{410} mass was detected in an inactive peak that, like the normal LPL-monomer, eluted at 0.8 M NaCl, indicating that the Glu^{410}→Val substitution may alter the stability of the LPL dimer. In summary, we have identified a unique mutation in the C-terminal domain of LPL that significantly affects the function of the mutant enzyme. Despite its location in the C-terminal domain of LPL, this mutation does not directly disrupt the heparin-binding properties of the mutant enzyme or its ability to interact with or hydrolyze either lipid or water-soluble substrates. Instead, the loss of enzyme activity appears to be related to a change in the monomer-dimer equilibrium of the mutant enzyme. Our studies indicate that, in addition to the proposed role in the lipid binding function, the C-terminal domain may play an important role in the formation of the active LPL dimer.—**Previato, L., O. Guardamagna, K. A. Dugi, R. Ronan, G. D. Talley, S. Santamarina-Fojo, and H. B. Brewer, Jr.** A novel missense mutation in the C-terminal domain of lipoprotein lipase (Glu^{410}→Val) leads to enzyme inactivation and familial chylomicronemia. *J. Lipid Res.* 1994. **35:** 1552–1560.

Supplementary key words apoC-II • hepatic lipase • pancreatic lipase • PCR-amplified genomic DNA

Lipoprotein lipase (LPL) is a 55-kD glycoprotein that plays a major role in the hydrolysis of triglycerides present in chylomicrons and very low density lipoproteins (VLDL) (1). LPL is produced primarily by adipose tissue, heart and skeletal muscle, macrophages, and lactating mammary gland and is bound, in its active homodimeric form, to the luminal surface of vascular endothelium (2). The importance of LPL in triglyceride hydrolysis has been established by the identification of patients with a deficiency of LPL. Affected individuals present with the familial chylomicronemia syndrome, a rare autosomal recessive disorder, characterized by accumulation of chylomicrons in fasting plasma and massive elevation of plasma triglycerides. This metabolic defect is often associated with recurrent episodes of abdominal pain or pancreatitis in infancy or childhood, hepatosplenomegaly, eruptive cutaneous xanthomas, and lipemia retinalis (2, 3).

Abbreviations: LPL, lipoprotein lipase; PCR, polymerase chain reaction; PL, pancreatic lipase; apoC-II, apolipoprotein C-II; HL, hepatic lipase.

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The cDNA and genomic organization of the LPL gene have been previously described (4–7). The high degree of primary amino acid sequence homology as well as similarity of genomic organization between LPL and hepatic (HL) and pancreatic lipase (PL) indicate a common evolutionary origin for these three enzymes (6) and suggest that the three lipases share analogous structural domains with similar secondary and tertiary structures (8, 9). Analysis of the three-dimensional crystallographic structure of human PL described by Winkler, D'Arcy, and Hunziker (10) indicates that PL contains two major structural domains. The amino-terminal region consists of an α/β structure dominated by a central parallel β-sheet and a C-terminal domain containing a β-sandwich structure formed by two β-sheets of four antiparallel strands each. By analogy to PL, LPL has also been proposed to consist of two structurally distinct regions consisting of an N-terminal and a C-terminal domain. Recent studies involving site-directed mutagenesis of LPL (11, 12) and the synthesis of chimeric lipases between LPL and HL (13, 14) have localized the catalytic domain as well as the site of apoC-II interaction to the N-terminal region of LPL. In addition, the N-terminal domain contains a loop covering the catalytic site of the enzyme that appears to be important for the interaction of LPL with its lipid substrate (15). Recent studies have indicated that the C-terminal domain of LPL may play a role in mediating heparin binding as well as the interaction of the enzyme with its lipid substrate (13, 14). However, the functional role of the C-terminal domain of LPL remains to be definitively established. Although most of the mutations identified in the LPL gene (16, 17) have been located in the N-terminal region of the enzyme, three mutations involving the C-terminal domain have been described. These defects result in the synthesis of truncated mutants LPL^{382} (18) and LPL^{447} (19) as well as LPL^{334} (20) which contains an Ala→Thr substitution at residue 334. The functional significance of LPL^{334}, however, remains to be established.

In this report we describe the molecular defect leading to a functional deficiency of LPL in a patient presenting with the familial chylomicronemia syndrome. The patient is homozygous for a unique missense mutation that results in the substitution of valine for glutamic acid at residue 410. This defect represents the first amino acid substitution described in the carboxyl-terminus of LPL that has been functionally demonstrated by in vitro studies to result in a severe loss of activity of the enzyme. Additionally, the proband is also homozygous for the previously described Ser^{447}→Ter mutation. In vitro expression of LPL^{410} and LPL^{447} results in the production of mutant enzymes with reduced specific triolein hydrolytic activity (11% and 70% of normal LPL, respectively). The combined presence of both Val^{410} and Ter^{447} mutations completely inactivates LPL, establishing the functional significance of these defects.

MATERIALS AND METHODS

Clinical data

The proband is a 3-year-old male of Egyptian descent who presented at age 2 months with acute pancreatitis and hepatosplenomegaly. Plasma lipid analysis revealed chylomicronemia with plasma triglyceride and cholesterol levels of greater than 12,000 mg/dl and 1,100 mg/dl, respectively. During the hospitalization the patient responded favorably to a blood transfusion and lipid-free parenteral nutrition. Diagnosis of LPL deficiency was established by demonstrating the absence of LPL activity assayed in post-heparin plasma in the presence of exogenous apolipoprotein C-II. Eruptive cutaneous xanthomas and lipemia retinalis have never been described and no other episodes of pancreatitis have been reported. The patient, who shows normal physical and intellectual development, is currently on a low fat diet (15% of total caloric intake) supplemented with medium-chain triglyceride oil. There is no history of consanguinity in the family.

Quantitation of post-heparin plasma HL activity and LPL activity and mass

Blood was collected in EDTA tubes before and 10 min after an intravenous bolus of heparin was administered at 60 units/kg body weight. LPL and HL lipolytic activities were assayed in triplicate using a radiolabeled triacylglycerol–phosphatidylcholine emulsion as previously reported (21). LPL and HL activities in post-heparin plasma were determined by selectively blocking LPL with 1 M NaCl. LPL mass was measured by an ELISA using the monoclonal antibody 5D2 (22), and the chicken polyclonal anti-human LPL polyclonal antibody as described previously (23).

DNA analysis

Genomic DNA was extracted from leukocytes isolated from the proband and his parents (24). The coding sequence of LPL and the intron–exon junctions were amplified by the polymerase chain reaction using intronic primers as previously reported (25, 26). The downstream primer used for the amplification of part of exon 10 annealed at base 1622–1645 of the published cDNA sequence (4). DNA analysis was performed using a combination of double-stranded DNA sequencing from pGEM7 vector (Promega Corp., Madison, WI) or direct PCR sequencing as described below. After the first standard PCR the product was gel purified in low melting point agarose and approximately 100 ng of the purified fragment was reamplified in an asymmetric polymerase chain reaction (PCR) using an unequal molar ratio (1/100) of the same primers (27). After filtration using a Centricon 100 microconcentrator (Amicon Corp., Danvers, MA) the product of the second PCR was sequenced

with the dideoxy-nucleotide chain termination method (28) using Sequenase (United States Biochemicals, Cleveland, OH) for single-stranded DNA sequencing. Restriction analysis of PCR-amplified exons 8 and 9 was performed by digestion with MaeIII (Boehringer Mannheim, Germany) and MnII (New England Biolabs, Beverly, MA). The resulting DNA fragments were analyzed on a 3% agarose minigel.

LPL cDNA expression vectors

The normal human LPL cDNA construct, designated pCMV-NL, has been described previously (29). It contains the cytomegalovirus early promoter driving the expression of a 1470 bp fragment of the LPL cDNA spanning the signal peptide through the termination codon. The mutant LPL cDNAs, pCMV-410, pCMV-447, and pCMV-410-447, were generated from the pCMV-NL construct by site-directed mutagenesis using the overlap extension method (30), and DNA sequence analysis of the constructs was performed by the dideoxynucleotide chain termination method (28). Large-scale preparation of the normal and mutant LPL constructs was performed by double equilibrium centrifugation in CsCl (31).

In vitro expression

Human embryonic kidney 293 cells (ATCC, Rockville, MD) were transfected with 40 μg plasmid DNA/100-mm dish using the calcium phosphate co-precipitation method (32). After 24 h of incubation with cells, media supplemented with 2 units/ml sodium heparin (Lipho Med, Melrose Park, IL) were harvested. Cell extract was prepared as previously described (33). Cells, washed and scraped in phosphate-buffered saline, were lysed and sonicated in 0.22 M Tris-HCl, pH 8.5, 0.25 M sucrose, 10 mg/ml BSA, 50 μg/ml heparin, 0.2% Nadeoxycholate, and 0.008% (v/v) Nonidet P40. Culture media, supplemented with glycerol at a final concentration of 30% (v/v), as well as cell extracts were aliquoted, flash-frozen, and stored at −70°C for determination of LPL mass. One aliquot was kept unfrozen and LPL activity was assayed in triplicate 2 h after harvesting as previously described (21). LPL lipolytic activity was also measured using tributyrin as a hydrosoluble substrate (34).

Heparin-Sepharose affinity chromatography

Affinity chromatography of conditioned media from cells transfected with normal and mutant LPL was performed as described by Ostlund-Lindqvist and Boberg (35). Ten ml of transfected media was applied to a 1-ml column of heparin-Sepharose CL-6B (Pharmacia-LKB Biotechnology, Uppsala, Sweden) at 15.6 ml/cm² per h. The unbound protein was eluted with 10 ml 0.4 M NaCl in 0.01 M sodium phosphate, pH 7.4, 0.1% CHAPS, and 30% glycerol. The column was then developed with a 30-ml linear gradient to 2.0 M NaCl in 0.01 M sodium phosphate, pH 7.4, 0.1% CHAPS, and 30% glycerol. One-ml fractions containing 2 USP units of sodium heparin were assayed for enzyme mass and activity. NaCl concentration of alternating fractions was monitored with a Radiometer conductivity meter at 5°-7°C. The conductivities were then related to a theoretical linear curve of NaCl concentrations of the developing and limiting buffer of the gradient. For calculation of LPL^{410} specific activity, ten 1-ml fractions eluting at 1 M to 1.5 M were pooled, concentrated using Centricon 30 (Amicon, Corp., Danvers, MA), and assayed for LPL mass and activity as indicated.

RESULTS

Table 1 summarizes the biochemical profile of fasting plasma samples obtained from the proband and his parents. While the patient observed a strict low-fat diet his triglycerides were moderately elevated, total cholesterol (C) was normal, and high density lipoprotein (HDL)-C was markedly decreased. Apolipoprotein C-II plasma levels (3.4 mg/dl) were normal as was the apparent molecular weight of apoC-II on two-dimensional electrophoresis (data not shown). The patient's apoE pheno-

TABLE 1. Biochemical profile of fasting plasma samples from the patient and his parents

	Lipids and Lipoproteins						Lipase Activity		LPL Mass	
	Total		VLDL		LDL	HDL				
Subject	TC	TG	TC	TG	TC	TC	LPL	HL	Pre	Post
	mg/dl						*nmol/ml/min*		*μg/ml*	
Proband	113	477	53	425	44	16	0	415	0	55
Father	151	125	16	72	87	43	199	171	0	450
Mother	194	172	24	125	137	33	187	616	0	622

TC, total cholesterol; TG, triglyceride; VLDL, very low density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein; LPL, lipoprotein lipase; HL, hepatic lipase.

type was E3/E3. The plasma lipid, lipoprotein, and apolipoprotein values as well as lipase activities of the father were normal, while the mother had slightly elevated triglycerides and low levels of HDL-C. The LPL concentration in the patient's post-heparin plasma (Table 1) was significantly reduced and LPL activity was undetectable. The HL activity in the proband's post-heparin plasma was within the normal range.

Analysis of the proband's LPL gene by both direct PCR sequencing as well as sequence analysis of six independent clones obtained from PCR amplification of the LPL exons revealed the presence of a transversion, A→T, at position 1484 in the eighth exon, resulting in a Glu→Val substitution at residue 410 of the mature LPL. Autoradiography of DNA sequencing gels illustrating the patient's A→T substitution as well as the normal LPL sequence is shown in **Fig. 1.** DNA sequence analysis revealed the presence of a second mutation, a C→G transversion in exon 9 at nucleotide position 1595, which introduces a premature termination codon at Ser^{447}, resulting in the synthesis of a truncated LPL missing 2 amino acid residues at the carboxyl terminus. The mutation at codon 447 of LPL has been previously described in heterozygous and homozygous normolipidemic healthy subjects (36, 37) as well as in one hypertriglyceridemic patient (19).

The A→T and C→G transversions generate new restriction sites for MaeIII and MnII, respectively, in the patient's LPL gene. **Figure 2A** illustrates the restriction pattern expected when the normal and the mutant amplified exon 8 are digested with MaeIII. Amplification of exon 8 results in the synthesis of a 233 bp product (Fig. 2A, lane 1). Digestion of the mutant exon 8 with MaeIII generates two fragments of 194 and 39 bp (Fig. 2A, lane 3), while digestion of the normal exon 8 (Fig. 2A, lane 2), which has no MaeIII sites, does not alter the size of the amplification product. MaeII digestion of PCR-amplified DNA from either parent (Fig. 2A, lanes 4 and 5) resulted in the formation of normal (233 bp) as well as abnormal (194 bp and 39 bp) fragments consistent with their status as obligate heterozygotes. The G→C transversion creates a new restriction site for MnII in the patient's gene. Fig. 2B illustrates the restriction enzyme pattern of the amplified exon 9 from the proband, family members, and a normal control after digestion with MnII. As expected, the size of amplified control DNA (Fig. 2B, lane 2) was not modified by MnII digestion. Incubation of patient amplified DNA with MnII resulted in the formation of two fragments of 125 and 53 bp in length (Fig. 2B, lane 3). The presence of all three fragments (53, 125, and 178 bp) after digestion of the parents' amplified DNA with MnII (Fig. 2B, lanes 4 and 5) is consistent with their status as obligate heterozygotes. Thus, the patient is a true homozygote for both gene defects.

Despite the loss of a negative charge resulting from the

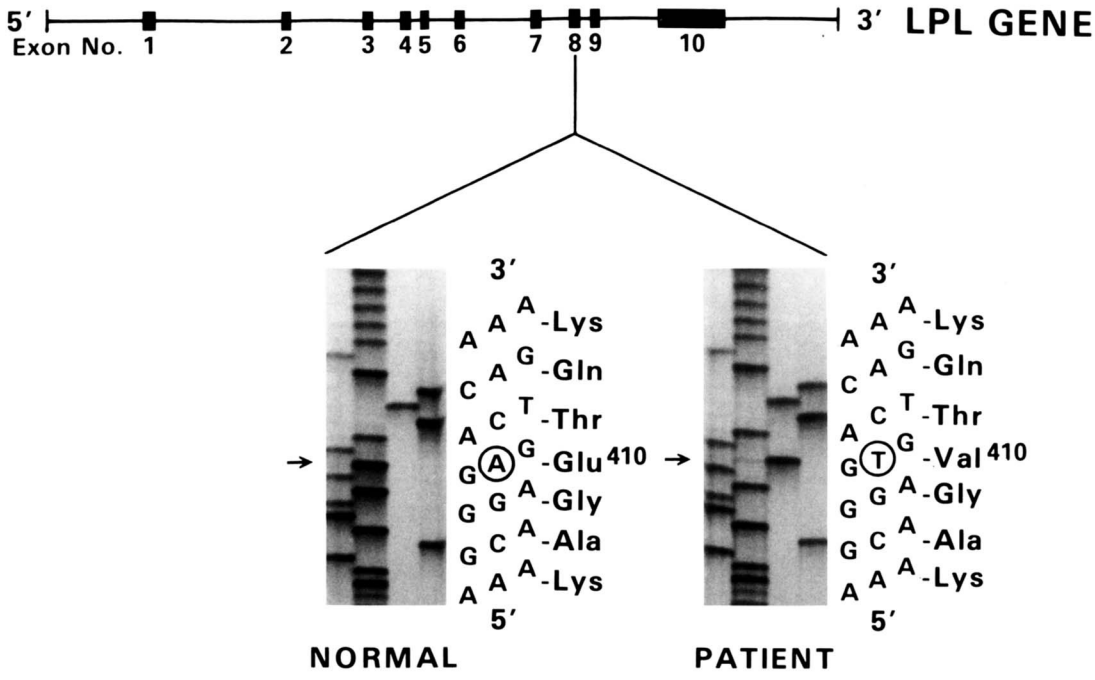

Fig. 1. Autoradiography of DNA sequencing gels illustrating the normal LPL sequence and the patient's mutation. The genomic organization of the LPL gene is illustrated on top. Autoradiography of the sequencing gels of DNA from a normal subject and the patient illustrating the A to T substitution in codon 410 of the patient's LPL gene.

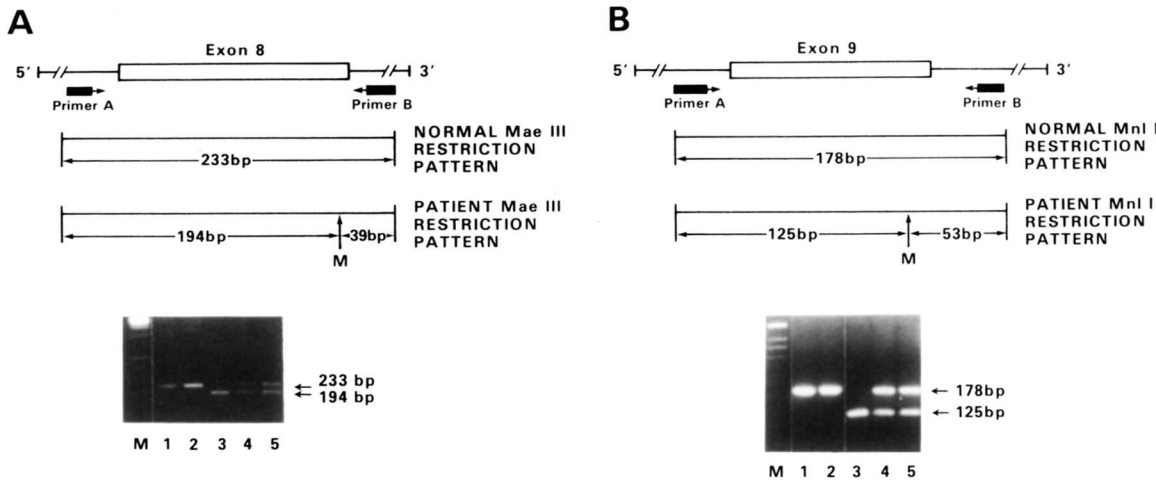

Fig. 2. Restriction enzyme analysis of the amplified exons 8 and 9 from the patient, family members, and a normal control. Panel A: Expected MaeIII restriction pattern of PCR-amplified exon 8 for normal LPL DNA and DNA containing the patient's mutation. The agarose gel on the bottom illustrates the size of undigested PCR-amplified exon 8 (lane 1), as well as PCR-amplified exon 8 from control DNA (lane 2), the patient's DNA (lane 3), and DNA from either parent (lanes 4 and 5) after digestion with MaeIII. Panel B: Expected MnII restriction pattern of PCR-amplified exon 9 for normal and patient DNA. The agarose gel illustrates undigested PCR-amplified exon 9 (lane 1) and PCR-amplified exon 9 from normal control (lane 2), patient (lane 3), and parent (lanes 4 and 5) DNA after digestion with MnII.

Val^{410} mutation, computer analysis (PC Gene, IntelliGenetics, Mountain View, CA) performed by the methods of Novotny and Auffray (38), Chou and Fasman (39), and Garnier, Osguthorpe, and Robson (40) on the mutated protein sequences to examine for changes in hydrophilicity, hydrophobicity and secondary structure did not predict any significant differences between the normal and the mutant LPLs containing one or both mutations (data not shown).

In order to test the functional significance of the Glu^{410}→Val and the Ser^{447}→Ter mutations, expression plasmids encoding for the normal and the mutant LPLs were transfected into human embryonal kidney 293 cells. The media and cellular extracts were then assayed for LPL activity and mass. LPL activity and mass were completely absent from media incubated with untransfected cells as well as cells transfected with plasmids lacking LPL cDNA (pCMV). **Table 2** summarizes the concentration of LPL present intracellularly and in the media of cells transfected with the LPL^{410}, LPL^{447}, or $LPL^{410/447}$ mutant plasmids. Significant amounts of normal as well as all three mutant LPLs were detected in the transfection media.

TABLE 2. Intracellular and media concentration of lipoprotein lipase in transfected 293 cells

Vector	Media	Intracellular	% of Total LPL in Media
	μg/plate	*μg/plate*	*%*
pCMV-NL	9111 ± 2918	1312 ± 480	87.4
pCMV-410	6166 ± 636	1893 ± 591	76.5
pCMV-447	15820 ± 3672	2329 ± 284	87.2
pCMV-410/447	9962 ± 2725	1560 ± 504	86.5

The specific activity of the expressed LPL present in the transfection media using either triolein or tributyrin substrates is summarized in **Table 3.** Transfection with the normal LPL construct, pCMV-NL, resulted in significant expression of an active enzyme with a specific activity of 263.2 nmol/μg per min using triolein as substrate. The specific activities of the expressed LPL^{410} and LPL^{447} were reduced to 11% and 70% of normal LPL, respectively. Unlike LPL^{410} and LPL^{447}, which retained some residual enzyme activity, $LPL^{410/447}$ was completely inactive, thus establishing the functional significance of the combined mutations.

Similar results were obtained when the LPL activity in the media was tested using the water-soluble lipid substrate, tributyrin (Table 3). Hydrolysis of tributyrin, a water-soluble short-chain triglyceride substrate, is independent of enzyme-lipid interaction and reflects the catalytic function of the enzyme. The mutants LPL^{410}, LPL^{447}, and $LPL^{410/447}$ exhibited a reduction in esterase specific activities (23%, 74%, and 5% of normal, respectively) that was similar to that observed when triolein was used as substrate. Thus, there was no significant difference in the ability of either mutant to hydrolyze triolein or tributyrin, suggesting that the two mutations do not preferentially disrupt the lipid-binding properties of LPL^{410} and LPL^{447}. The presence of both mutations,

TABLE 3. Mass and hydrolyzing activity of media from 293 cells transfected with normal and mutant lipoprotein lipase

Vector	Mass[a]	Triolein		Tributyrin	
		Activity[a]	Specific Activity[a]	Activity	Specific Activity
	ng/ml	*nmol/μl/min*	*nmol/μg/min*	*nmol/μl/min*	*nmol/μg/min*
pCMV-NL	473 ± 64	124.5 ± 3.88	263.2	2.13 ± 0.47	4.50
pCMV-410	406 ± 89	12.28 ± 1.35	30.2	0.42 ± 0.06	1.03
pCMV-447	805 ± 112	149.1 ± 4.08	185.2	2.67 ± 0.14	3.32
pCMV-410/447	555 ± 89	0	0	0.13 ± 0.03	0.23

LPL mass and activity in media from untransfected cells was 0 and 2.86 ± 0.35, respectively. LPL mass and activity in media of cells transfected with parent vector (pCMV) were 0 and 3.14 ± 0.52, respectively.

[a]LPL mass and activity were calculated after subtraction of background values obtained from media of untransfected cells.

however, eliminated the ability of the mutant enzyme to hydrolyze both emulsified and hydrosoluble substrates.

The effect of the Glu^{410}→Val as well as Ser^{447}→Ter mutations on the heparin binding properties of LPL was analyzed by heparin-Sepharose affinity chromatography using a linear NaCl elution gradient (**Fig. 3**). Consistent with previous studies that indicate that normal LPL elutes primarily as two separate immunoreactive peaks consisting of a functional LPL dimer and an inactive LPL monomer (41), normal expressed LPL eluted at two different peak NaCl concentrations of approximately 1.2 M and 0.7 M (bottom panel, Fig. 3). LPL activity was associated only with the 1.2 M fraction. Analysis of the mutant enzymes revealed that most of the LPL^{410} and a significant proportion of LPL^{447} eluted with the low affinity inactive 0.7 M LPL peak (top and middle panels, Fig. 3). In contrast, active LPL^{410} and LPL^{447} were detected in the 1.2 M elution fractions at a position similar to that of normal active LPL. The specific activities of these active LPL^{410} and LPL^{447} fractions were similar to that of normal LPL (0.2–0.4 nmol/ng per min).

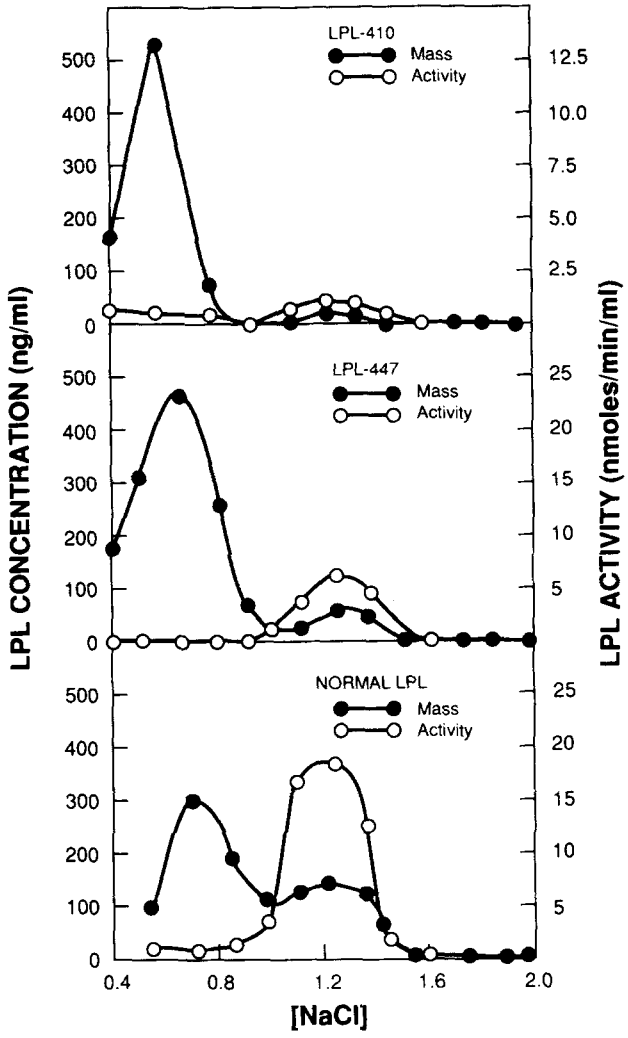

Fig. 3. Heparin-Sepharose affinity chromatography of normal LPL and mutant LPL. The elution profile of LPL^{410}, LPL^{447}, and normal LPL is illustrated. LPL mass and activity are indicated by the closed and open circles, respectively.

DISCUSSION

LPL is a complex enzyme that consists of multiple functional domains required for activity. Recent studies investigating the structure–function relationship of different LPL domains have provided new insights into LPL function (13–15, 42). Thus, the N-terminal region of LPL appears to be essential for interaction of LPL with its cofactor, apoC-II, as well as for the enzyme's catalytic function. In addition, the loop covering the catalytic site of LPL is important for the hydrolysis of lipid substrates (15). Although recent studies (13, 14) have indicated that the C-terminal domain plays a role in mediating heparin binding and is essential for the interaction of the enzyme with its lipid substrates, the functional role of the C-terminal domain of LPL remains to be elucidated.

In this report we describe a patient presenting with familial chylomicronemia due to unique mutations located in the C-terminal domain of LPL which provide new insights into a potential role of the C-terminal domain of LPL in LPL-dimer formation. DNA sequencing and restriction enzyme digestion of proband PCR-amplified genomic DNA indicate that the patient is homozygous for two mutations: an A→T transversion at nucleotide 1484 producing a Glu→Val substitution at residue 410 and a C→G transversion at nucleotide 1595 resulting in the nonsense mutation Ser^{447}→Ter. Glu^{410} is conserved in all the LPLs described from different species (mouse, bovine, guinea pig, and chicken) as well as in the human and the rat HL (9, 43). The analogous residue in human pancreatic lipase is located within the loop connecting the β-strands 5 and 6 in the C-terminus of PL corresponding to a stretch of amino acids highly conserved among all the described mammalian lipases (**Fig. 4**) (16, 43).

Residue 447, on the other hand, is not conserved in human PL indicating it may not be essential for lipase function. In fact, previous reports have described this mutation in the LPL gene of normotriglyceridemic healthy subjects that are either heterozygous or homozygous for the defect. In addition, the allelic frequency of the Ser^{447}→Ter substitution in two different normal Caucasian populations is fairly high, 8% and 17%, respectively (36, 37), suggesting that LPL^{447} may represent a normal functional LPL variant.

In order to establish the functional significance of the Glu^{410}→Val and Ser^{447}→Ter mutations, we expressed the mutant enzymes in human embryonal kidney 293 cells. Transient transfection of plasmids containing the LPL cDNAs for either the Ser^{447}→Ter or Glu^{410}→Val substitutions resulted in the synthesis of mutant enzymes with reduced but significant residual activity of 70% and 11%, respectively. The reduction of activity detected in LPL^{410} is similar to that of a previously described clinically significant mutant LPL^{172} (44) that retained 24% of normal LPL function. In contrast, $LPL^{410/447}$ mutant was completely inactive against either substrate. Thus, our expression studies indicate that the combined defects in $LPL^{410/447}$ are responsible for the patient's clinical presentation with hypertriglyceridemia and chylomicronemia.

Consistent with previous studies (36), expression of the single mutant, LPL^{447}, resulted in the synthesis of an enzyme exhibiting only a slight reduction in LPL specific activity (70% that of normal) using triolein as substrate. Our studies indicate that the specific tributyrin hydrolyzing activity of LPL^{447} was reduced to a degree similar to that observed in the specific triolein hydrolyzing activity. The mild impairment of the LPL function due to the Ser^{447}→Ter mutation, particularly in heterozygotes, does not appear sufficient to significantly modify in vivo triglyceride metabolism. Thus, the father of our proband, although a carrier for the mutant allele, is normolipidemic while the mother is only slightly hypertriglyceridemic. It is possible that other genetic or environmental factors affecting lipid metabolism are required for the expression of hyperlipidemia in these individuals (45, 46).

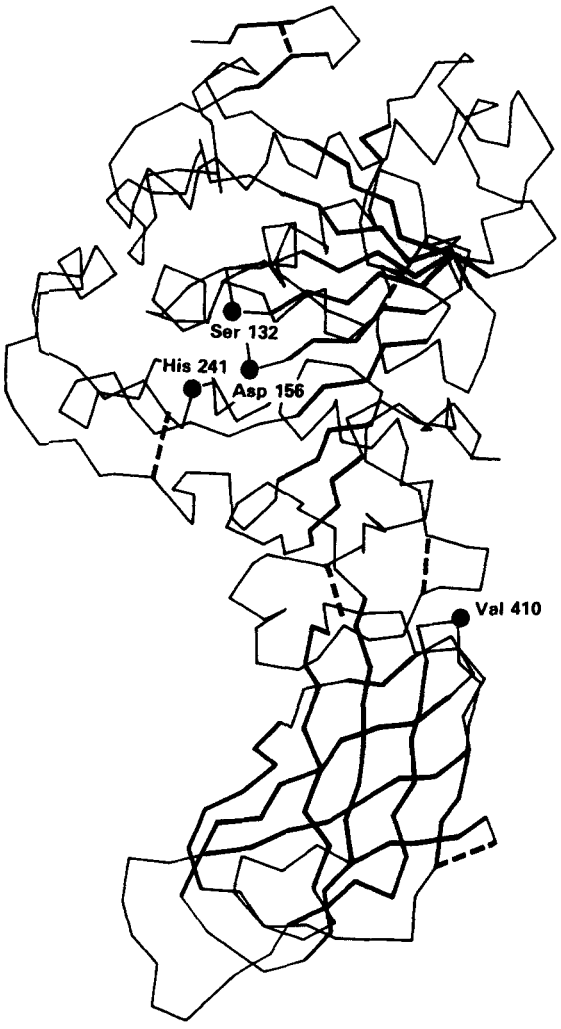

Fig. 4. Schematic representation of the α-carbon backbone of human pancreatic lipase (10). The proposed location of the amino acid residues of the catalytic triad and the amino acid substitution in LPL^{410} (Glu^{410}→Val) are indicated in the figure based on the sequence homology of pancreatic and lipoprotein lipase; (---) disulfide bonds; (●) highlighted amino acid residues.

Expression of the LPL^{410} mutant alone resulted in the production of an enzyme exhibiting a more severe loss of activity. Thus, the triolein and tributyrin specific activities were reduced to 11% and 22% that of normal, respectively. As with LPL^{447}, the substitution of Glu^{410} by Val did not appear to preferentially disrupt the lipid binding domain of LPL as the efficiency of hydrolysis of either substrate was similarly altered.

To further explore the underlying mechanism resulting in a 90% and 30% loss of activity in LPL^{410} and LPL^{447}, respectively, we analyzed their heparin binding properties

on heparin-Sepharose affinity chromatography. Previous studies have indicated that normal LPL elutes as two immunoreactive peaks at approximately 0.7 M and 1.2 M NaCl, representing the inactive LPL monomer and active dimer LPL (41), respectively. Functional LPL^{410} and LPL^{447}, with a specific activity similar to that of normal LPL, eluted at the same position as the normal active LPL, suggesting the presence of functional LPL^{410} and LPL^{447} dimers. Most of LPL^{410} as well as a significant amount of LPL^{447} eluted as an inactive enzyme at a position in the gradient similar to that of the normal LPL monomer (0.7 M NaCl). Based on these findings we speculate that the majority of LPL^{410} as well as a significant proportion of LPL^{447} were present in the monomeric, inactive state. The presence of a fully functional mutant enzyme indicates that the Glu^{410}→Val and Ser^{447}→Ter substitutions do not directly disrupt the catalytic function of the mutant enzyme. Instead, our studies indicate that the loss of activity in both mutants may result from an inability to either generate or maintain the LPL dimer, resulting in a shift of the normal LPL monomer-dimer equilibrium in favor of the inactive LPL monomer.

In contrast to the partial loss of enzyme activity observed in LPL^{410} and LPL^{447}, combined mutant $LPL^{410/447}$ was completely inactive against either triolein or tributyrin substrates. Thus, we postulate that the presence of both carboxyl-terminal mutations in $LPL^{410/447}$ may further disrupt the ability of the combined mutant enzyme to form and/or maintain a stable dimer, leading to complete enzyme inactivation. Alternatively, the combined mutation in $LPL^{410/447}$ may lead to major conformational changes in LPL structure resulting in overall disruption of LPL function. Recently, characterization of exon 5 missense mutations located in the N-terminus of LPL by Hata et al. (47) has indicated that these substitutions lead to a disruption of the LPL dimer. The present study represents the first description of a mutation in the C-terminus of LPL affecting monomer-dimer equilibrium and suggests that the C-terminal domain of LPL and specifically residue Glu^{410} may play an important role in LPL dimer formation.

In summary, we have identified a unique missense mutation located in the C-terminal domain of LPL resulting in a Glu→Val substitution at residue 410 that leads to a loss of enzymatic activity. The Glu^{410}→ Val substitution does not directly disrupt the catalytic, heparin, or lipid binding properties of the mutant enzyme but results in a shift of the LPL monomer-dimer equilibrium in favor of the formation of the LPL monomer. The simultaneous presence of the Ser^{447}→Ter mutation potentiates the effect of the first defect resulting in the synthesis of a completely inactive LPL. Our studies indicate that the C-terminal domain of LPL may play an important role in LPL dimer formation.

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