A missense (Asp$^{250}$→Asn) mutation in the lipoprotein lipase gene in two unrelated families with familial lipoprotein lipase deficiency

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Abstract We have identified the molecular basis for familial lipoprotein lipase (LPL) deficiency in two unrelated families with the syndrome of familial hyperchylomicronemia. All 10 exons of the LPL gene were amplified from the two probands' genomic DNA by polymerase chain reaction. In family 1 of French descent, direct sequencing of the amplification products revealed that the patient was heterozygous for two missense mutations, Gly$^{188}$→Glu (in exon 5) and Asp$^{250}$→Asn (in exon 6). In family 2 of Italian descent, sequencing of multiple amplification products cloned in plasmids indicated that the patient was a compound heterozygote harboring two mutations, Arg$^{243}$→His and Asp$^{250}$→Asn, both in exon 6. Studies using polymerase chain reaction, restriction enzyme digestion (the Gly$^{188}$→Glu mutation disrupts an AvaII site, the Arg$^{243}$→His mutation, a HhaI site, and the Asp$^{250}$→Asn mutation, a TaqI site), and allele-specific oligonucleotide hybridization confirmed that the patients were indeed compound heterozygous for the respective mutations. LPL constructs carrying the three mutations were expressed individually in Cos cells. All three mutant LPLs were synthesized and secreted efficiently; one (Asp$^{250}$→Asn) had minimal (<5%) catalytic activity and the other two were totally inactive. The three mutations occurred in highly conserved regions of the LPL gene. The fact that the newly identified Asp$^{250}$→Asn mutation produced an almost totally inactive LPL and the location of this residue with respect to the three-dimensional structure of the highly homologous human pancreatic lipase suggest that Asp$^{250}$ may be involved in a charge interaction with an α-helix in the amino terminal region of LPL.

The occurrence of this mutation in two unrelated families of different ancestries (French and Italian) indicates either two independent mutational events affecting unrelated individuals or a common shared ancestral allele. Screening for the Asp$^{250}$→Asn mutation should be included in future genetic epidemiology studies on LPL deficiency and familial combined hyperlipidemia.


Supplementary key words compound heterozygote • familial hyperchylomicronemia

Lipoprotein lipase (LPL) is a crucial enzyme in the metabolism of the triglyceride-rich lipoproteins, chylomicrons, and very low density lipoproteins. An inherited deficiency of LPL results in Type I hyperlipoproteinemia which is characterized by hyperchylomicronemia, recurrent abdominal pain, hepatosplenomegaly, and failure to thrive (1).

The structure of the human LPL gene has been published recently (2–4). The LPL gene is part of a supergene family of lipases that also includes hepatic triglyceride lipase (HL) and pancreatic lipase (3, 5, 6). In addition, there is also limited sequence similarity between LPL and the Drosophila yolk proteins (vitellogenins) (3, 6). The high homology among the three lipases suggests that they have similar mechanism of action.

The human LPL gene contains 10 exons and 9 introns. Certain regions of the LPL gene are much better conserved than others. The strictly conserved catalytic triad

Abbreviations: LPL, lipoprotein lipase; HL, hepatic lipase; PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism; ASO, allele-specific oligonucleotide; bp, basepair(s); HDL, high density lipoprotein.

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residues, Ser<sup>152</sup> - Asp<sup>156</sup> - His<sup>241</sup> (7, 8), span 3 different exons (nos. 4, 5, and 6). To date, a number of natural mutations in the LPL gene have been identified in patients with familial hyperchylomicronemia. These include one insertion (9), one duplication (10), one deletion (9), three nonsense mutations (11-13), one frameshift mutation (14), and at least nine missense mutations (11, 15-24). The location and nature of the missense mutations provide interesting information about the structure-function relationship of LPL. Some, but not all, of the identified missense mutations have been proven to impair LPL function by direct testing of the mutant enzyme produced in vitro.

**MATERIALS AND METHODS**

**Subjects**

Family 1. The patient (D. T.) in this family is a 21-year-old female of French descent who first presented at 6 months of age when she had undergone an operation for removal of a 2-cm “fat lump” in her leg. At age 6, she had her first episode of pancreatitis. At age 11, she had a total cholesterol of 277 mg/dl, triglyceride of 1929 mg/dl, and HDL cholesterol of 15 mg/dl. A recurrent attack occurred at age 13, at which time she was started on a low fat diet but still had multiple episodes of recurrent vomiting or pancreatitis. She had normal growth and was seen by one of us (W. V. B.) at the age of 7 when she was diagnosed to have LPL deficiency and Type I hyperlipoproteinemia. She was noted to have lipemia retinalis and splenomegaly, but no eruptive xanthoma or hepatomegaly. Her grand-parents, parents, and siblings were also investigated. A brother (III-3 in Fig. 5) was also found to have Type I hyperlipoproteinemia and has been maintained on a low fat diet.

Measurement of lipoprotein lipase and hepatic lipase activities

Heparin (60 U/kg) was administered intravenously after 12-h fast. Venous blood was obtained before and 15 min after heparin injection. LPL and HL activities were quantitated in triplicate as previously described (25) using [14C]triolein as substrate.

**DNA isolation and Southern blotting**

Genomic DNA was prepared from the buffy coat of whole blood cells of LPL-deficient probands and their family members. For Southern blotting, 5-µg aliquots of genomic DNA were digested with 5-10 units of enzyme per µg DNA under conditions recommended by the supplier. Samples were partially evaporated, electrophoresed on 0.7% agarose gels followed by partial depurination with 0.2 N HCl, rinsed with water, and denatured with 0.2 N NaOH-0.6 M NaCl. DNA was transferred to nylon membranes under alkaline conditions (0.4 N NaOH). The membranes were neutralized, baked at 80°C under vacuum for 2 h, pre-hybridized, and then hybridized with a <sup>32</sup>P-labeled human LPL cDNA (26) under standard conditions.

**PCR amplification and sequencing of the LPL gene**

For the proband of family 1, exons 1-9 were amplified by polymerase chain reaction (PCR) using the oligonucleotide primers described by Monsalve et al. (17). Exon 10 was amplified using primers based on LPL genomic sequence (G. T. Tkalec and K. Oka, unpublished results). The oligonucleotides used were: 5' primer (GAAGAGCTCCATTTACACATCCTCCCCCTG), 3' primer (GTTACCTGCTTCCACTACATTTACACAG). Tubes containing 1.0 µg of genomic DNA, 0.2 µM PCR primers, PCR buffer, 1.0 mM magnesium, and 0.2 mM deoxy-nucleotide triphosphates were heated at 95°C for 5 min followed by addition of 2.5 units Taq polymerase and cycling in a Perkin-Elmer DNA thermocycler as follows: denaturation at 94°C for 1 min, annealing at 50-60°C for
1 min, and extension at 72°C for 1 min for 35 cycles. For exons 1-6, double-stranded PCR products of the predicted size were gel-purified then subjected to asymmetric PCR essentially as described by Monsalve et al. (17). For exons 7-10, PCR products were subcloned into the replicative form of M13 and single-stranded DNA was sequenced using an Applied Biosystems Model 340A Automated DNA Sequencer. With rare exceptions, both strands of each exon were sequenced in their entirety to clarify ambiguities.

For family 2, exons 1-9 were amplified by PCR using the oligonucleotides described by Emi et al. (12), and exon 10 using primers from LPL exon 10 and intron 9 sequences as for family 1, and the PCR products were then subcloned at the BamHI and EcoRI sites of pBluescript vector. For each exon, at least six independent clones were isolated and sequenced by the dideoxy method with the oligonucleotides described by Emi et al. (12), and exon sequences as for family 1, and the PCR products were then electrophoresed and analyzed by ethidium bromide staining of 2.5% agarose gel.

**Allele-specific oligonucleotide (ASO) hybridization**

Regions of interest were amplified by PCR as described above, extracted once with phenol-chloroform, denatured with NaOH, and applied to nylon filters using a slot-blot apparatus (Schleicher and Schuell, Keene, NH). Filters were hybridized with either a 32P end-labeled normal or mutant ASO essentially as described by Emi et al. (15).

**Human LPL cDNA expression vector**

The human LPL cDNA spanning nucleotides 320 to 1466 was subcloned into M13mpl9 and used as a template for site-specific mutagenesis as described previously (26, 27). Oligonucleotides were synthesized on an Applied Biosystems Inc. 380A DNA Synthesizer. The sequences for the mutagenic oligonucleotides are: for Gly188 the wild type LPL cDNA, were sequenced. Replicative form DNAs were isolated, digested with the following restriction enzymes: Stu I, Hind III, EcoRI, Pst I, Nco I, Xba I, and Pvu I. After Southern blotting and hybridization with the LPL cDNA as described in Methods, no major rearrangements of the LPL gene were detected (data not shown).

For the proband of family 1, exons 1 through 9, representing the translated region of the LPL cDNA, as well as exon 10, corresponding to the relatively long 3' untranslated region of the LPL cDNA, were sequenced. Each intron-exon junction was sequenced and found to be normal. The proband of family 1 was found to be a compound heterozygote for LPL deficiency. The first mutation is shown in Fig. 1. Both cytosine and thymine are seen at the asterisk (only a cytosine should be present) on

**RESULTS**

**Family 1**

To exclude the presence of major structural defects of the LPL gene in family 1, genomic DNA from the proband, her relatives, and a normal control subject was digested with the following restriction enzymes: Stu I, Hind III, EcoRI, Pst I, Nco I, Xba I, and Pvu II. After Southern blotting and hybridization with the LPL cDNA as described in Methods, no major rearrangements of the LPL gene were detected (data not shown).

Transfection of mammalian cells

Transfection was performed as previously reported (26, 27) with some modification. Cos 6 cells were transfected with normal or mutant LPL sequences in p91023(B) using the DEAE dextran method (30). After 48-72 h, culture media and cells were collected to measure LPL activity and mass in culture media or cell extracts as reported previously (27).

![Fig. 1. Detection of the codon 188 mutation in the proband for family #1. LPL exon 5 was amplified from genomic DNA from the subject with Type I hyperlipoproteinemia in family #1 and sequenced as described in Methods. Thymine and cytosine were present at the same position (asterisk) on the noncoding strand indicating that the proband is heterozygous for a Glu for Gly substitution at codon 188.](image-url)
the noncoding strand of the PCR product for exon 5. This finding was confirmed by sequencing the coding strand (not shown) which revealed both an adenine and guanine at the same position. The proband is thus heterozygous for a missense mutation at codon 188, resulting in a Glu for Gly substitution, identical to the mutation described by Emi et al. (15).

To confirm the presence of the codon 188 mutation in the proband, ASO hybridization studies were performed. A 114 bp region of exon 5 that includes codon 188 was amplified from the LPL cDNA as well as from genomic DNA from a normal control, the proband, and the proband’s mother and grandmother. PCR products were slot-blotted and subjected to ASO hybridization using a normal ASO complementary to the wild-type coding sequence and an ASO complementary to the codon 188 mutation coding sequence. As shown in Fig. 2A, DNA from the patient hybridized with both the normal and mutant ASO, confirming that she is heterozygous for the codon 188 mutation. The same pattern was seen in both of the proband’s asymptomatic relatives, consistent with the fact that they are also heterozygous for the defective allele. The presence of the heterozygous codon 188 mutation was confirmed by sequencing in each of the family members (not shown). Neither the LPL cDNA nor DNA from a normal control hybridized with the mutant ASO. The intensity of the normal ASO signal for the LPL cDNA and the control subject was twice that for the family members, consistent with the presence of two copies of the normal allele in the cDNA and the control but only one copy in the family members.

The codon 188 mutation disrupts an Ava I1 restriction site; the PCR-amplified 114 bp fragment of exon 5 described above contains only one Ava I1 site. As an independent confirmation that each of the members of family 1 is heterozygous for the codon 188 mutation, the 114 bp region of exon 5 containing the codon 188 mutation was amplified, digested with Ava II, and subjected to agarose gel electrophoresis followed by staining with ethidium bromide. As shown in Fig. 2B, two bands, 68 and 46 bp in length, are seen after Ava II digestion of DNA from the normal subject (lane 1), consistent with the presence of an intact Ava II site on both chromosomes. However, these bands as well as the undigested 114 bp fragment are seen in DNA from the proband and her mother and maternal grandmother (lanes 2–4), consistent with the presence of an intact Ava II site on one chromosome and a disrupted site on the other, indicating that all three family members are heterozygous for the Gly188 → Glu mutation.

The second missense mutation in the proband for family 1 is shown in Fig. 3A. Both guanine and adenine are present at the asterisk (only a guanine should be present) on the coding strand of the PCR product for exon 6. Both cytosine and thymine were present at the same position on the noncoding strand (not shown), confirming the authenticity of the identified mutation. This mutation changes the codon CAG, encoding Asp250 to CAA, encoding Asn.

The codon 250 mutation disrupts a Taq I site in exon 6. The primers used to amplify exon 6 yield a 333 bp fragment (including part of the intron sequence). Digestion with Taq I should produce two fragments, 245 and 88 bp in length. Exon 6 DNA from each of the family members was amplified, digested with Taq I at 65°C for 2 h, and subjected to agarose gel electrophoresis and ethidium bromide staining. As shown in Fig. 3B, an undigested 333 bp exon 6 band is present in DNA from the proband (lane 1) but not in DNA from her relatives (lanes 2 and 3). Therefore, unlike the Gly188 → Glu mutation which was seen in the proband and her relatives, the second mutation (Asp250 → Asn) is present only in the proband with hyperchylomicronemia but absent in her asymptomatic mother and maternal grandmother.

![Fig. 2](image-url)

**Fig. 2.** A: Detection of the codon 188 mutation by ASO hybridization in family #1. A 114 bp fragment of exon 5 was amplified from the LPL cDNA, each family member, and from a normal control. PCR was performed by using the primers: 5' GAAGCCCCGAGTCGTCTTTCT and 5' AACATGCCCAACTGGTCTG. PCR products were slot-blotted and hybridized with either a normal or mutant ASO probe for the codon 188 mutation (Normal ASO = 5' CCAGGGGACCCTCCTGGTGA; Mutant ASO = 5' CCAGGGGACTCTCTGGTGA). B: Confirmation of the presence of the codon 188 mutation in the members of family #1 by Ava II digestion. A 114 bp fragment of exon 5 was PCR-amplified, digested with Ava II, and electrophoresed on a 2.0% agarose gel. Lane 1, normal control subject; lane 2, proband for family #1; lane 3, proband's maternal grandmother; lane 4, proband's mother.
Fig. 3. A: Detection of the codon 250 mutation in the proband for family #1. LPL exon 6 was amplified from genomic DNA from the proband for family #1 and sequenced as described in Methods. Adenine and guanine are present at the same position (asterisk) on the coding strand indicating that the proband is heterozygous for an Asn for Asp substitution at codon 250. B: Confirmation of the presence of the codon 250 mutation. A 333 bp fragment of genomic DNA containing exon 6 was amplified for the proband and for her relatives. The PCR products were digested with Taq I and electroforesed on a 2.0% agarose gel. An undigested 333 bp fragment of the normal sequence. The Hha I digestion of DNAs from family members did not show any major insertions, deletions, or rearrangements of the LPL gene (data not shown). To determine the complete sequence of the coding regions of the LPL gene, 10 exons as well as all exon—intron boundaries were amplified enzymatically by PCR and cloned in pBluescript vector for sequence analysis. Except for exon 6, nucleotide sequences of all the exons, and exon—intron boundaries were identical to the published data (2-4). For exon 6, six independent clones were isolated and DNA sequence analysis revealed two different mutations. Three clones contained a G → A transition at nucleotide position 983, and the other three clones carried a G → A transition at nucleotide position 1003 (Fig. 4). The first mutation changes the codon CGC encoding Arg983 to CAC encoding a His at this position, and is identical to the mutation described by Dichek et al. (20). The second mutation changes codon 250 from GAC, encoding Asp, to AAC, encoding an Asn residue at this position. Since only one of the two mutations was present in each individual clone, the proband must be a compound heterozygote for two different LPL mutant alleles.

The first (G → A) mutation affecting codon 243 disrupts a Hha I restriction enzyme recognition sequence (GGC ↓ C) at position 981 → 984. We determined the restriction digestion pattern of this region of the LPL gene in the proband and her family members (Fig. 5, bottom). When exon 6 was amplified by PCR using the flanking intron primers [described by Emi et al. (12)], a 316 bp DNA band was produced. Digestion using Hha I would produce two bands of 248 bp and 68 bp, respectively, in the normal sequence. The Hha I restriction patterns of the family members reveal that the proband, her affected brother, her mother, and maternal grandmother each had three bands of 316 bp, 248 bp, and 68 bp, respectively, indicating that they were heterozygous for the Arg983 → His mutation. In contrast, the other family members shown in Fig. 5 each had two bands, indicating that they had the normal alleles on both chromosomes. Therefore, the mutant His983 allele was inherited from the maternal side of the family. The second mutation which produces the Asp250 → Asn in LPL gene causing LPL deficiency

**Family 2**

Lipoprotein profile, LPL and HL activities. The plasma lipids, LPL and HL activities of the patient and the family are shown in Table 1. Elevated fasting triglycerides and very low HDL-C levels were found in the proband (III-1) and her younger brother (III-3). The lipid and lipoprotein values in this kindred were consistent with the presence of familial combined hyperlipidemia since different phenotypes, including elevated LDL-C, were detected in the family. The proband and her brother have low postheparin LPL activity, and all family members investigated had normal HL activity.
In vitro expression of wild-type and mutant LPLs

The proband for family 1 is a compound heterozygote for two different amino acid substitutions, Gly\textsubscript{188} \rightarrow Glu and Asp\textsubscript{250} \rightarrow Asn. Affected individuals in family 2 are also compound heterozygotes for two substitutions, Arg\textsubscript{243} \rightarrow His and Asp\textsubscript{250} \rightarrow Asn. There are, therefore, three mutations in these two families that potentially could produce an inactive LPL. We tested the functional significance of these mutations by expressing the wild-type enzyme and each of the mutant LPLs in Cos cells in vitro and assaying for their enzymatic activities.

The expression vector p91023(B) (29) had previously been used for expressing a wide variety of proteins including human LPL (23, 26, 27). The results of the transfection experiment are shown in Table 2. There was no measurable LPL activity in mock-transfected Cos cells. It was easily detectable in both the medium and cellular extract in the wild-type LPL vector-transfected cells. In contrast, LPL enzyme activity was undetectable in the two mutant constructs containing the Gly\textsubscript{188} \rightarrow Glu and the Arg\textsubscript{243} \rightarrow His substitutions. The new mutation described in this study, which involves an Asn for Asp\textsubscript{250} substitution, is associated with loss of over 95% of LPL enzyme activity (Table 2). To ensure that the absence of enzyme activity was not caused by the failure of production of the mutant LPLs, we measured the amount of immunoreactive LPL produced by the Cos cells in vitro by an ELISA (Table 2). It is evident that compared to the wild-type construct, the two mutant constructs, Arg\textsubscript{243} \rightarrow His and Asp\textsubscript{250} \rightarrow Asn, produced approximately half the normal amount of LPL enzyme mass both intracellularly and in the medium, and the other mutant (Gly\textsubscript{188} \rightarrow Glu) LPL was produced at approximately one-fourth the wild-type level intracellularly and half the wild-type level in the medium. Therefore, all mutant enzymes were synthesized and there was no evidence for impairment of secretion. The specific activity of the wild-type LPL produced in vitro was 31-33 mU/\mu g and those of the two mutant LPLs, Gly\textsubscript{188} \rightarrow Glu and Arg\textsubscript{243} \rightarrow His, were both 0 mU/\mu g, indicating that they are totally devoid of enzyme activity. The Asp\textsubscript{250} \rightarrow Asn LPL mutant appears to have minimal activity about 5% that of the wild-type enzyme (Table 2).

### DISCUSSION

In this study, we have characterized the molecular basis for familial hyperchylomicronemia in two unrelated families. Both were found to be compound heterozygous for two missense mutations, Gly\textsubscript{188} \rightarrow Glu and Asp\textsubscript{250} \rightarrow Asn for family 1, and Arg\textsubscript{243} \rightarrow His and Asp\textsubscript{250} \rightarrow Asn for family 2. Therefore, they share one common mutation, Asp\textsubscript{250} \rightarrow Asn. Using in vitro expression and site-directed mutagenesis, we found that all three mutations produced essentially inactive LPLs. Two of these mutations, Gly\textsubscript{188} \rightarrow Glu and Arg\textsubscript{243} \rightarrow His, have been described previously (15, 17, 20). The missense mutation at codon 188 has been found in patients with diverse ethnic
Fig. 5. Mapping of the codon 243 and 250 mutations in family 2 by restriction enzyme digestion. Exon 6 was amplified by PCR as described by Emi et al. (12). A 316 bp fragment was digested with Taq I or Hha I and electrohoresed on 2.5% agarose gel. Filled-in symbols and hatched symbols in the pedigree represent the Asp2’0-, Asn and Arg24’+His allele, respectively. Undigested exon 6 PCR product is shown in the first lane on the right, and DNA size markers are in the first lane on the left.

ancestries including those of French, Canadian, British, Polish, Dutch, German, and Asian Indian descent (15, 17). The second missense mutation causing a His for Arg243 substitution has been described in a Caucasian (20) and a Japanese (11) family. The Asp250 → Asn mutation common to the two families described in this communication has not been reported previously.

Of the two families studied, a more complete analysis of multiple family members was possible only with family 2. The data presented in Fig. 4 and 5 show definitively that the proband and her affected brother were compound heterozygous for the two mutations, Arg243 → His and Asp250 → Asn. Not only were the defective alleles inherited from different sides of the family, but the cloning and sequencing experiments established that the two mutations were on different clones and therefore on separate chromosomes. The sequence data for family 1 did not allow the assignment of the two mutations to different or the same chromosomes. However, the fact that the Asp250 → Asn mutation was detected only in the proband but not in her relatives (Fig. 3) indicates that this allele was inherited from the father who was not available for testing. It is evident that she inherited the other defective allele containing the Gly188 → Glu mutation from her mother (Fig. 2).

The occurrence of a common mutation (Asp250 → Asn) among two unrelated families of different ethnic origin is interesting. Whether the mutation arose de novo in the ancestors of the two families as independent events or was inherited from a common ancestral allele can be inferred by large scale screening of LPL-deficient patients for this mutation and haplotype analysis among Caucasians in the U.S. and Europe. The Asp250 → Asn substitution should be included in the evaluation not only of patients with familial hyperchylomicronemia but also those with familial combined hyperlipidemia. Heterozygous LPL deficiency appears to be an important underlying cause of familial combined hyperlipidemia which is commonly associated with atherosclerosis (31).

Missense mutations causing LPL deficiency are highly TABLE 2. LPL enzyme activity and immunoreactive mass in Cos cells transfected with wild-type and mutant expression vectors

| Expression Vector | LPL Activity | | LPL Mass | | Specific Activity |
|-------------------|--------------|-----------------|-----------------|-----------------|
|                   | Cell         | Cell            | Media           | Media           | Cell            |
| Wild-type         | 214 ± 7.9    | 858 ± 35        | 6.8 ± 1.5       | 26 ± 1.5        | 31              |
| Asp250 → Asn      | 5.4 ± 1.8    | 27 ± 3          | 2.8 ± 0.9       | 15 ± 2.7        | 1.3             |
| Arg243 → His      | 0            | 0               | 3.1 ± 1.2       | 16 ± 2.8        | 0               |
| Gly188 → Glu      | 0            | 0               | 1.7 ± 0.6       | 12 ± 2          | 0               |

The data presented represent the results of five different transfection experiments. Values are reported as means ± SD.

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The almost totally inactive AspZ5O (a-6) sequence in Am250 variant suggests that the carboxyl group of the residue at this position is involved in a charge interaction rather than participating in simple hydrogen bonding. The Ly@ and SerZ5' substitution mutants we previously characterized may interact with a polar side chain in a helical region of LPL, the homolog of the pancreatic lipase. The juxtaposition of these two helices would allow hydrogen bonds with the ionized carboxyl group of the Asp residue at the homologous position in native hepatic lipase. Glu is the natural residue at the homologous position in native hepatic lipase and obviously does not impair enzyme activity. These observations underscore the complexity of the structure-function relationship of LPL and hepatic lipase.

In analyzing various natural missense mutations resulting in inactive LPLs, different authors have attempted to infer the structure-function relationships of the observed amino acid substitutions, and have generally concluded that such missense mutations affect residues that are crucial to LPL function. Such conclusions are supported by the fact that many of these mutations, including the three described here (Fig. 6), occur in highly conserved regions of the LPL gene. However, in most reports, the simplistic interpretation is based on the examination of a single amino acid substitution at each natural mutation site. For example, the Arg2^243 $\rightarrow$ His mutation identified here [and by Dichek et al. (20)] and the Ser2^44 $\rightarrow$ Thr mutation affecting the neighboring Ser residue (19) have been described as the underlying defects for familial hyperchylomicronemia in three different families. Both mutant enzymes were found to be inactive in vitro, suggesting that Arg2^243 and Ser2^44 are crucial to LPL function. However, additional site-specific mutagenesis experiments indicate that such a straightforward interpretation may not be appropriate. For example, compared to the totally inactive Ser2^44 $\rightarrow$ Thr, the Ser2^44 $\rightarrow$ Ala variant (an artificial mutant) is fully active (27), suggesting that the Thr for Ser2^44 substitution could have inactivated the LPL because of its more bulky side chain, an effect not reproduced by substitution with the smaller Ala residue. Furthermore, although the inactive enzyme resulting from a Glu for Gly1^48 substitution could be interpreted to result from the bulkiness or the negative charge of Glu that causes a perturbation of LPL structure, Glu is the natural residue at the homologous position in native hepatic lipase and obviously does not impair enzyme activity. These observations underscore the complexity of the structure-function relationship of LPL and hepatic lipase. A clearer picture of the structural basis of LPL function must await the determination of its three-dimensional structure when crystals of the enzyme are obtained.

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