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 Ava II site, the Arg^{243} → His mutation, a Hha I site, and the Asp^{250} → Asn mutation, a Taq I 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. —**Ishimura-Oka, K., C. F. Semenkovich, F. Faustinella, I. J. Goldberg, N. Shachter, L. C. Smith, T. Coleman, W. A. Hide, W. V. Brown, K. Oka, and L. Chan.** A missense (Asp^{250} → Asn) mutation in the lipoprotein lipase gene in two unrelated families with familial lipoprotein lipase deficiency. *J. Lipid Res.* 1992. **33:** 745–754.

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^{132} -Asp^{156}-His^{241} (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. In this study, we describe the genetic abnormality in two unrelated families. Each was a compound heterozygote for two independent mutations (Gly^{188} → Glu and Asp^{250} → Asn, and Arg^{243} → His and Asp^{250} → Asn, respectively). One of the two missense mutations, Asp^{250} → Asn, was found to be common in the two families. We further demonstrate that the three different observed mutations individually produce an LPL with only 0–5% normal activity. The location and nature of the mutations provide information on the probable structural role for each of these residues.

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 and various antilipidemic agents including clofibrate and gemfibrozil. When she was seen at age 15, she was on gemfibrozil 600 mg twice daily. She denied estrogen or alcohol use and had normal thyroid functions. Her height was 163 cm and her weight 57.5 kg. Physical examination was normal except for eruptive xanthomata. At that time her mother was 42 years old and had borderline hypertension and diabetes mellitus. Her father's health was unknown because he had left the family when the patient was 6 months old. The patient has been maintained on a low fat diet but still had multiple episodes of recurrent pancreatitis including one severe episode at the age of 18 that required hospitalization. At age 19 she delivered a normal 4463-g baby boy. Her pregnancy was uncomplicated but in the 1½ years since delivery, she has had four episodes of mild pancreatitis that did not require hospitalization. At age 25, when she was seen following a mild pancreatitis, her total cholesterol was 327 mg/dl, triglyceride 2261 mg/dl, and HDL cholesterol 16 mg/dl.

Family 2. The index patient in family 2 (III-1 in Fig. 5) is a 15-year-old female of Italian origin. There was no consanguinity in the family history. The patient presented with recurrent vomiting at 3 months of age and was found to have hyperlipidemia. She was put on a low fat diet and has had no additional 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 grandparents, 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 [^{14}C]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 ^{32}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. Tkalcevic and K. Oka, unpublished results). The oligonucleotides used were: 5' primer (GAAGGATCCATTATACACATCTCCCCTG), 3' primer (GTTACTTCCTCCACTTCATTCTTCACAG). Tubes containing 1.0 μg of genomic DNA, 0.2 μM PCR primers, PCR buffer, 1.0 mM magnesium, and 0.2 mM deoxynucleotide 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 BamH1 and EcoRI sites of pBluescript vector. For each exon, at least six independent clones were isolated and sequenced by the dideoxy method with the Sequenase II DNA sequencing kit (U.S. Biomedical Corp., Cleveland, OH). DNAs from the proband and family members were amplified by PCR using primers for exon 6 and digested by the appropriate restriction enzymes (see below). All of the digested PCR products were 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 ^{32}P 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 M13mp19 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 $Gly^{188} \rightarrow Glu$, ACATTCACCAGAGAGTCCCCTGGTCGA, $Arg^{243} \rightarrow His$, TGCTCCCACGAGCACTCCATTCATCTC, $Asp^{250} \rightarrow Asn$ CATCTCTTCATCAACTCTCTGTTGAAT (the mutant bases are underlined). Mutagenesis was carried out as described by Taylor, Oh, and Eckstein (28) using an oligonucleotide-directed in vitro mutagenesis system (Amersham, Arlington Heights, IL). Mutant and wild-type LPL cDNAs were used to transfect *E. coli* TG1 cells and positive clones were identified by direct sequencing. Replicative form DNAs were isolated, digested with EcoRI, and inserted into the EcoRI site of p91023(B) (29). After transformation of DH 5α cells, positive clones were selected by cDNA hybridization. DNA was isolated, and orientation of inserts was determined by restriction mapping.

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).

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).

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

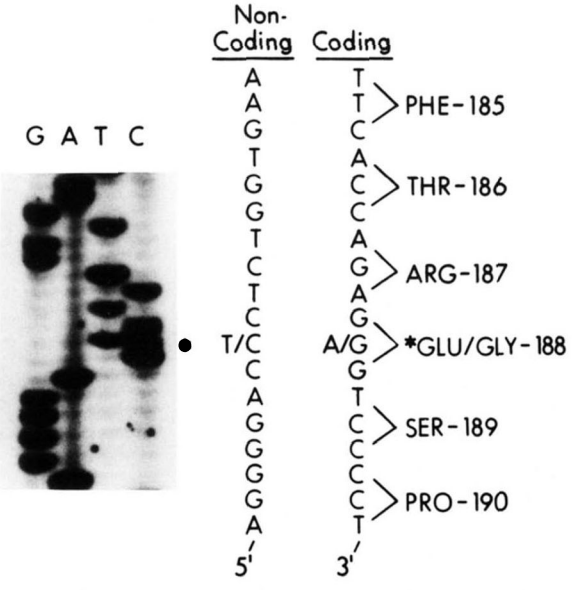

Fig. 1. Detection of the codon 188 mutation in the proband for family #1. LPL exon 5 was amplified from genomic DNA from the subject with Type I hyperlipoproteinemia in family #1 and sequenced as described in Methods. Thymine and cytosine were present at the same position (asterisk) on the noncoding strand indicating that the proband is heterozygous for a Glu for Gly substitution at codon 188.

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 II restriction site; the PCR-amplified 114 bp fragment of exon 5 described above contains only one Ava II 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 $Gly^{188} \rightarrow Glu$ 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 Asp^{250} 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 $Gly^{188} \rightarrow Glu$ mutation which was seen in the proband and her relatives, the second mutation ($Asp^{250} \rightarrow Asn$) is present only in the proband with hyperchylomicronemia but absent in her asymptomatic mother and maternal grandmother.

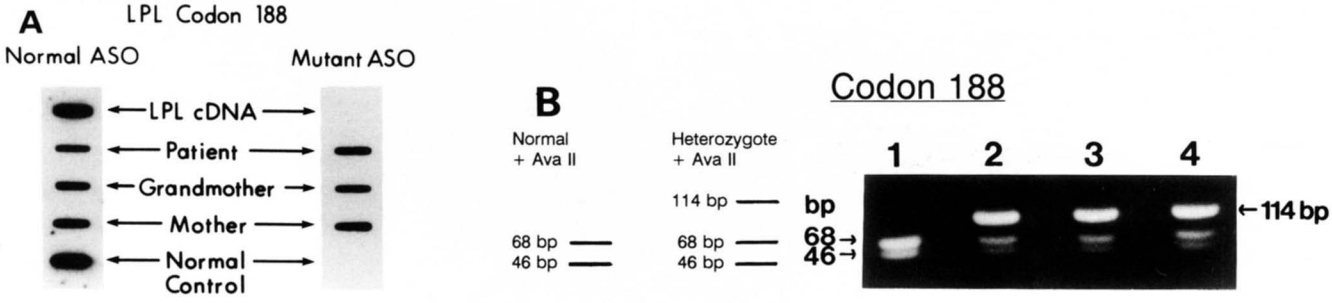

Fig. 2. A: Detection of the codon 188 mutation by ASO hybridization in family #1. A 114 bp fragment of exon 5 was amplified from the LPL cDNA, each family member, and from a normal control. PCR was performed by using the primers: 5' GAAGCCCCGAGTCGTCTTTCT and 5' AACATGCCCAACTGGTTTCTG. PCR products were slot-blotted and hybridized with either a normal or mutant ASO probe for the codon 188 mutation (Normal ASO = 5' CCAGGGGACCCTCTGGTGA; 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.

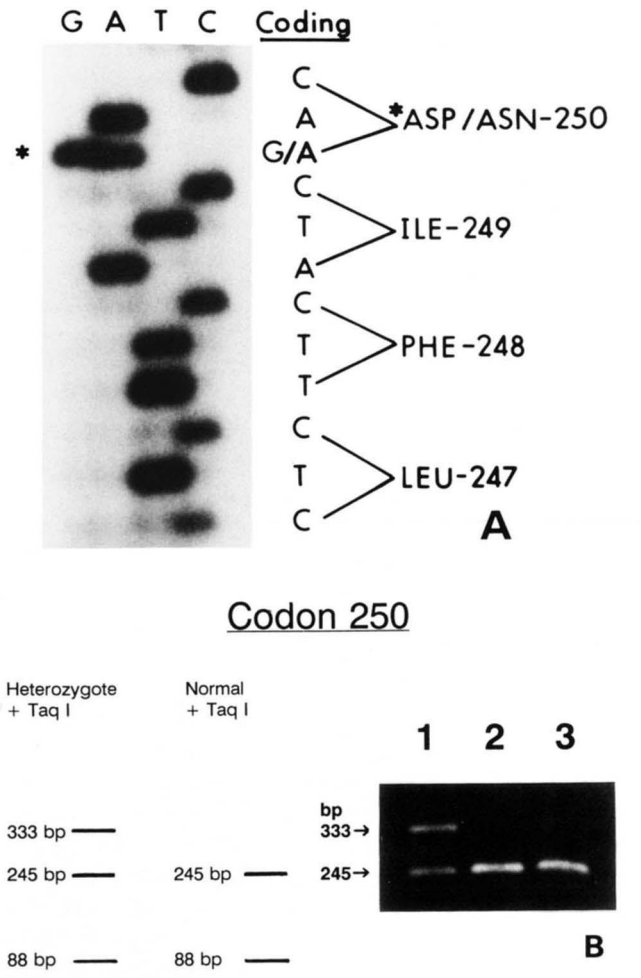

Fig. 3. A: Detection of the codon 250 mutation in the proband for family #1. LPL exon 6 was amplified from genomic DNA from the proband for family #1 and sequenced as described in Methods. Adenine and guanine are present at the same position (asterisk) on the coding strand indicating that the proband is heterozygous for an Asn for Asp substitution at codon 250. B: Confirmation of the presence of the codon 250 mutation in the proband for family #1 and its absence in clinically unaffected family members by Taq I digestion. 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 electrophoresed on a 2.0% agarose gel. An undigested 333 bp fragment confirming the presence of the codon 250 mutation is present in the proband (lane 1) but not in her relatives (lanes 2 and 3). An 88 bp band was also present in each lane but did not photograph well in this experiment.

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 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 has low postheparin LPL activity, and all family members investigated had normal HL activity.

DNA analysis. Genomic Southern hybridization following BamH1, EcoRI, Hind III, Pvu II and Sac I restriction enzyme 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 Arg^{243} 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 (GCG ↓ 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 Arg^{243} → 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 His^{243} allele was inherited from the maternal side of the family. The second mutation which produces the Asp^{250} → Asn substitution is identical to that in family 1. The mode of inheritance of this mutant allele was studied by Taq I digestion of the same 316 bp PCR product (Fig. 5, top). In this case, the normal PCR product was sensitive to Taq I producing a 230 bp and an 86 bp band whereas the mutant PCR product was resistant to the enzyme. The restriction patterns shown in Fig. 5 indicate that the proband and her two siblings, her father, and paternal grandfather were heterozygous for the mutation, whereas the other family members carried the wild-type allele on both chromosomes. Thus, the mutant Asn^{250} allele was inherited from the paternal side of the family.

TABLE 1. Lipid profile, LPL and HL activities of family 2 proband and relatives

	I-1	I-2	II-1	III-1	III-2	III-3	II-2	I-3	I-4
TC (mg/dl)	188	312	175	209	199	118	155	256	248
TG (mg/dl)	199	325	226	1520	153	858	79	123	204
HDL-C (mg/dl)	34	35	52	11	40	15	46	44	40
LDL-C (mg/dl)	114	212	78	106	128	ND	93	187	167
Postheparin LPL activity (μmol/h/ml)	4.3	5.9	NA	0.8	NA	0.6	6.8	4.1	NA
Postheparin HL activity (μmol/h/ml)	5.1	5.1	NA	4.5	NA	5.6	4.7	7.0	NA

Postheparin plasma HL activity was measured by inhibiting LPL activity with monoclonal antibody against human milk LPL and the LPL activity is expressed as the difference between total lipolytic activity and HL activity. The range of postheparin plasma LPL activity of normal subjects is 5–20 μmol/h per ml. The number of the subject corresponds to that in the pedigree in Fig. 5. Abbreviations: TC, total cholesterol; TG, triglyceride; ND, not determined; NA, not available for this study.

In vitro expression of wild-type and mutant LPLs

The proband for family 1 is a compound heterozygote for two different amino acid substitutions, $Gly^{188} \rightarrow Glu$ and $Asp^{250} \rightarrow Asn$. Affected individuals in family 2 are also compound heterozygotes for two substitutions, $Arg^{243} \rightarrow His$ and $Asp^{250} \rightarrow Asn$. 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 $Gly^{188} \rightarrow Glu$ and the $Arg^{243} \rightarrow His$ substitutions. The new mutation described in this study, which involves an Asn for Asp^{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, $Arg^{243} \rightarrow His$ and $Asp^{250} \rightarrow Asn$, produced approximately half the normal amount of LPL enzyme mass both intracellularly and in the medium, and the other mutant ($Gly^{188} \rightarrow Glu$) 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/μg and those of the two mutant LPLs, $Gly^{188} \rightarrow Glu$ and $Arg^{243} \rightarrow His$, were both 0 mU/μg, indicating that they are totally devoid of enzyme activity. The $Asp^{250} \rightarrow Asn$ LPL mutant appears to have minimal activity about 5% that of the wild-type enzyme (Table 2).

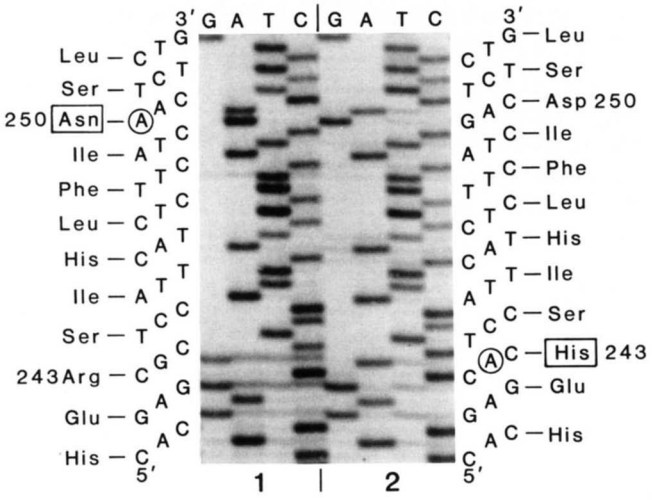

Fig. 4. Sequence of cloned Exon 6 fragments amplified by PCR in LPL-deficient patient for family #2. LPL exon 6 was amplified by PCR from genomic DNA from the proband in family #2 as described in Methods. PCR products were subcloned in pBluescript vector and sequenced by the dideoxy method. 1: Sequence of a clone containing the $Asp^{250} \rightarrow Asn$ mutation. 2: Sequence of a clone carrying the $Arg^{243} \rightarrow His$ mutation. Note that only a single mutation is present in an individual clone.

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, $Gly^{188} \rightarrow Glu$ and $Asp^{250} \rightarrow Asn$ for family 1, and $Arg^{243} \rightarrow His$ and $Asp^{250} \rightarrow Asn$ for family 2. Therefore, they share one common mutation, $Asp^{250} \rightarrow Asn$. Using in vitro expression and site-directed mutagenesis, we found that all three mutations produced essentially inactive LPLs. Two of these mutations, $Gly^{188} \rightarrow Glu$ and $Arg^{243} \rightarrow His$, have been described previously (15, 17, 20). The missense mutation at codon 188 has been found in patients with diverse ethnic

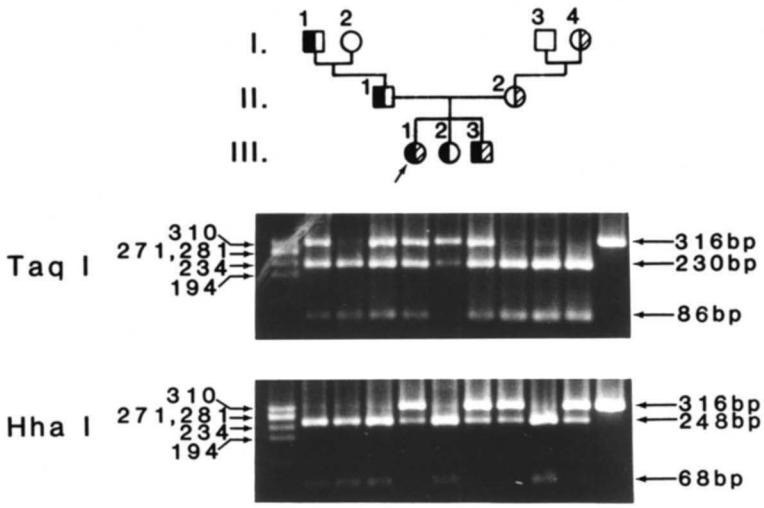

Fig. 5. Mapping of the codon 243 and 250 mutations in family 2 by restriction enzyme digestion. Exon 6 was amplified by PCR as described by Emi et al. (12). A 316 bp fragment was digested with Taq I or Hha I and electrophoresed on 2.5% agarose gel. Filled-in symbols and hatched symbols in the pedigree represent the $Asp^{250} \rightarrow Asn$ and $Arg^{243} \rightarrow His$ 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 Arg^{243} substitution has been described in a Caucasian (20) and a Japanese (11) family. The $Asp^{250} \rightarrow Asn$ 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, $Arg^{243} \rightarrow His$ and $Asp^{250} \rightarrow Asn$. 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 $Asp^{250} \rightarrow Asn$ 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 $Gly^{188} \rightarrow Glu$ mutation from her mother (Fig. 2).

The occurrence of a common mutation ($Asp^{250} \rightarrow Asn$) 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 $Asp^{250} \rightarrow Asn$ 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	Media	Cell	Media	Cell	Media
	milliunits/dish		*μg/dish*		*milliunits/μg*	
Wild-type	214 ± 7.9	858 ± 35	6.8 ± 1.5	26 ± 1.5	31	33
$Asp^{250} \rightarrow Asn$	5.4 ± 1.8	27 ± 3	2.8 ± 0.9	15 ± 2.7	1.3	1.8
$Arg^{243} \rightarrow His$	0	0	3.1 ± 1.2	16 ± 2.8	0	0
$Gly^{188} \rightarrow Glu$	0	0	1.7 ± 0.6	12 ± 2	0	0

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

```
                188                 243       250
humlpl ----FTRGSPGRSIG------HERSIHLFIDSLL-----
bovlpl ----FTRGSPGRSIG------HERSVHLFIDSLL-----
muslpl ----FTRGSPGRSIG------HERSIHLFIDSLL-----
 gplpl ----FTRGSPGRSIG------HERSIHLFIDSLL-----
cknlpl ----YTRGSPDRSIG------HERSIHLFIDSLL-----
 humhl ----FTREHMGLSVG------HERSVHLFIDSLL-----
 rabhl ----FTREHMGLSVG------HERSVHLFIDSLL-----
 rathl ----FTREHMGLSVG------HERSVHLFIDSLQ-----
```

Fig. 6. Sequence alignment of LPL and hepatic lipase of different vertebrates around the missense mutations identified in this study. HL, hepatic lipase; hum, human; bov, bovine; mus, mouse; gp, guinea pig; ckn, chicken; rab, rabbit. The residue numbers are from the human LPL sequence.

heterogeneous [for a review, see Hayden et al. (32)]. There seems to be a concentration of such mutations in exons 5 and 6 of the LPL gene, although mutations in exons 3 and 4 have also been observed. The three mutations in the two families described here occur within exons 5 (codon 188) and 6 (codons 243 and 250). Our previous study of the structural and functional roles of highly conserved serines in human lipoprotein lipase has given strong support to the view that the enzyme has a three-dimensional structure very similar to that of human pancreatic lipase (27). The crystal structure of human pancreatic lipase (8) reveals the presence of two α-helical regions encompassing residues 85–94 (α-2) and 261–275 (α-6) which could be positioned such that their respective side chains would participate in charge interactions or hydrogen bonding. The almost totally inactive $Asp^{250} \rightarrow Asn$ mutant identified in this study, together with the artificial Ser^{251} substitution mutants we previously characterized (27), indicate that Asp^{250} and Ser^{251} have a major role in the maintenance of the overall enzyme structure in this region. The Asp^{250} (corresponding to Asp^{272} in human pancreatic lipase), which is conserved in all lipases (ref. 6 and **Fig. 6**), may interact with a polar side chain in a helical region of LPL, the homolog of the α-2 helical region of pancreatic lipase. The almost complete loss of catalytic activity in the Asn^{250} variant suggests that the carboxyl group of Asp is involved in a charge interaction rather than participating in simple hydrogen bonding. The Lys^{66} of human LPL corresponds to Asn^{88} in human pancreatic lipase. The juxtaposition of these two helices would allow either Lys^{66} in LPL or Asn^{88} in pancreatic lipase to form hydrogen bonds with the ionized carboxyl group of the conserved Asp.

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 (5, 6, 32). 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 $Arg^{243} \rightarrow His$ mutation identified here [and by Dichek et al. (20)] and the $Ser^{244} \rightarrow Thr$ 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 Arg^{243} and Ser^{244} 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 $Ser^{244} \rightarrow Thr$, the $Ser^{244} \rightarrow Ala$ variant (an artificial mutant) is fully active (27), suggesting that the Thr for Ser^{244} 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 Gly^{188} 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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