Catalytic Triad Residue Mutation (Asp<sup>156</sup> → Gly) Causing Familial Lipoprotein Lipase Deficiency

CO-INHERITANCE WITH A NONSENSE MUTATION (Ser<sup>447</sup> → Ter) IN A TURKISH FAMILY*

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We studied the molecular basis of familial Type I hyperlipoproteinemia in two brothers of Turkish descent who had normal plasma apolipoprotein C-II levels and undetectable plasma post-heparin lipoprotein lipase (LPL) activity. We cloned the cDNAs of LPL mRNA from adipose tissue biopsies obtained from these individuals by the polymerase chain reaction and directional cloning into M13 vectors. Direct sequencing of pools of >2000 cDNA clones indicates that their LPL mRNA contains two mutations: a nonsense mutation changing codon 156 from GAU to GGU predicting an Asp<sup>156</sup> → Gly substitution and a nonsense mutation changing the codon for Ser<sup>447</sup> from UCA to UGA, a stop codon, predicting a truncated LPL protein that contains 446 instead of 448 amino acid residues. Both patients were homozygous for both mutations. Analysis of genomic DNAs of the patients and their family members by the polymerase chain reaction, restriction enzyme digestion (the GAT → GG/T mutation abolishes a TaqI restriction site), and allele-specific oligonucleotide hybridization confirms that the patients were homozygous for these mutations at the chromosomal level, and the clinically unaffected parents and siblings were true obligate heterozygotes for both mutations.

In order to examine the functional significance of the mutations in this family, we expressed wild type and mutant LPLs in vitro using a eukaryotic expression vector. Five types of LPL proteins were produced in COS cells by transient transfection: (i) wild type LPL, (ii) Asp<sup>156</sup> → Gly mutant, (iii) Ser<sup>447</sup> → Ter mutant, (iv) Gly<sup>446</sup> → Ter mutant, and (v) Asp<sup>156</sup> → Gly/Ser<sup>447</sup> → Ter double mutant. Both LPL immunoreactive mass and enzyme activity were determined in the culture media and intracellularly. Immunoreactive LPLs were produced in all cases. The mutant LPLs, Asp<sup>156</sup> → Gly and Asp<sup>156</sup> → Gly/Ser<sup>447</sup> → Ter, were devoid of enzyme activity, indicating that the Asp<sup>156</sup> → Gly mutation is the underlying defect for the LPL deficiency in the two patients. The two mutant LPLs missing a single residue (Gly<sup>446</sup>) or a dipeptide (Ser<sup>447</sup> → Gly<sup>446</sup>) from its carboxyl terminus had normal enzyme activity. Thus, despite its conservation among all mammalian LPLs examined to date, the carboxyl terminus of LPL is not essential for enzyme activity. We further screened 224 unrelated normal Caucasians for the Ser<sup>447</sup> → Ter mutation and found 36 individuals who were heterozygous and one individual who was homozygous for this mutation, indicating that it is a sequence polymorphism of no functional significance.

Human LPL shows high homology to hepatic triglyceride lipase and pancreatic lipase. The crystal structure of human pancreatic lipase (Winkler, F. K., D’Arcy, A., and Hunziker, W. [1990] Nature 343, 771–774) reveals that the enzyme contains an Asp-His-Ser catalytic triad which is chemically analogous to that in the serine proteases. By sequence alignment to pancreatic lipase, the homologous catalytic triad in human LPL corresponds to Asp<sup>156</sup>-His<sup>241</sup>-Ser<sup>332</sup>. The totally inactive Asp<sup>156</sup> → Gly LPL mutant described in this study is the first naturally occurring mutant reported that involves a catalytic triad residue among the lipases and serine proteases. It gives strong support for the essential role of Asp<sup>156</sup> in enzyme catalysis.

Lipoprotein lipase (LPL, EC 3.1.1.34) is an endothelial enzyme responsible for the hydrolysis of the triglyceride component of circulating chylomicrons and very low density lipoproteins. An inherited deficiency of this enzyme produces Type I hyperlipoproteinemia, which is characterized by recurrent abdominal pain, hepatosplenomegaly, lipemia retinalis, eruptive xanthomatosis, and lactescent serum in the postabsorptive state caused by the hyperchylomicronemia (Brunzell, 1989).

The structure of human LPL has been deduced recently from its cDNA sequence (Wion et al. [1987]). Sequence comparisons indicate that LPL shows high homology to hepatic triglyceride lipase and pancreatic lipase (Kirchgessner et al., 1989; Datta et al., 1988). At the genomic level, these three genes also show considerable similarity in structure. Human hepatic triglyceride lipase contains nine exons and eight introns, all of the latter occurring within the coding region of the gene (Cai et al., 1989). Human LPL contains eight introns at similar locations and an additional intron interrupting the translation termination codon (Deeb and Peng, 1989; Kirchgessner et al., 1989). Dog pancreatic lipase has 13 exons and 12 introns, conserving essentially all the homologous exons in the hepatic lipase gene and having some of them split by

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‡ The abbreviations used are: LPL, lipoprotein lipase; apo, apolipoprotein; PCR, polymerase chain reaction; bp, base pair(s); kb, kilobase(s); ELISA, enzyme-linked immunosorbent assay.
additional introns (Mickel et al., 1989). The similarity in function, primary sequence, and genomic organization of the three genes suggests a common evolutionary origin (Kirchgasser et al., 1989; Datta et al., 1988; Sementovich et al., 1989a), and it is likely that the three enzymes display analogous secondary and tertiary structure and structure-function relationships. The crystal structure of human pancreatic lipase was recently published by Winkler et al. (1990). The structural data indicate that Ser\(^{152}\) in pancreatic lipase is the nucleophilic residue essential for catalysis. This essential Ser residue forms part of an Asp-His-Ser triad which is chemically analogous to that found in all serine proteases (Winkler et al., 1990; Blow, 1990). Based on sequence alignment between LPL and pancreatic lipase, the homologous catalytic triad in human LPL corresponds to Asp\(^{156}\)-His\(^{347}\)-Ser\(^{372}\).

To date, at least four Type I hyperlipoproteinemic human mutant LPLs have been reported to have resulted from single amino acid substitutions that produce functionally inactive LPLs (Ameis et al., 1991; Beg et al., 1990; Emi et al., 1990b; Monsaule et al., 1990; Hata et al., 1990a). An additional Type I patient was a compound heterozygote for two other missense mutations (Dichek et al., 1991). None of these mutations involves the catalytic triad residues. In this communication, we present our studies on a family with LPL deficiency in which we found two mutations: a missense mutation resulting in an Asp\(^{156}\} \rightarrow \text{Gly substitution and a nonsense mutation that produces a truncated LPL missing a dipeptide at its carboxyl}


terminus. Functional analysis of wild type and mutant human LPLs expressed in transfected cells in vitro indicates that the carboxyl-terminal dipeptide is nonessential for activity, whereas the Asp\(^{156}\} \rightarrow \text{Gly substitution produces an inactive LPL, supporting the critical role of Asp}\(^{156}\) in the catalytic triad.
Human LPL cDNA Expression Vector—A cDNA containing 1786 bases extending from nucleotides -320 to 1466 (counting the first base of the translation initiation codon as nucleotide +1) was subcloned into M13mp19 and used as a template for site-specific mutagenesis. It contains 320 nucleotides in the 5'-untranslated region, the entire coding region, and 38 nucleotides in the 3'-untranslated region and is bounded by an artificial HindIII site in the 5' end and a natural EcoRI site in the 3' end.

Oligonucleotides (Table I) synthesized on an Applied Biosystems Inc. 380A DNA synthesizer were 5'-phosphorylated and annealed with single-stranded template. Mutagenesis was carried out as described by Taylor et al. (1985) using an oligonucleotide-directed in vitro mutagenesis kit (Amersham). Mutant and wild type LPL cDNAs were used to transfect Escherichia 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) (Wong et al., 1985). After transformation of E. coli DH 5α cells, positive clones were selected by cDNA hybridization. DNA was isolated, and orientation of inserts was determined by restriction mapping.

In Vitro Expression and LPL Enzyme Assay—COS M-6 cells (0.25-1.0 x 10⁶) were plated on 100-mm diameter tissue culture dishes and transfected with 20 μg of plasmid DNA/dish by the technique of Seldin and Leibach (1986) with the addition of a 3-h incubation with chloroquine after MeSO shock. In all experiments, collecting media contained sodium heparin (40 μg/ml). In each experiment, a parallel dish was subjected to the transfection protocol without plasmid DNA (COS cells only condition). For LPL enzyme assay experiments, after 48-72 h, aliquots of culture media were collected. Cells were washed in phosphate-buffered saline, scraped into 1 ml of 50 mM NH₄/NH₄Cl (pH 8.1) containing heparin, and sonicated. Media and cell extracts were then flash-frozen in a dry ice/ethanol bath and stored at -70°C. Just before determination of LPL activity or protein mass, samples were thawed in ice water, made 0.2% in sodium deoxycholate, and rotated at room temperature for 5 min. Cell extracts and media were assayed for LPL enzyme activity as previously described (Semenkovich et al., 1989b). LPL activity is expressed in milliunits (1 milliunit = 1 nmol of fatty acid released per min). In experiments determining enhancement of LPL activity by apoC-II, media from transfected COS cells were assayed in the presence of solvent (3 M guanidine HCl diluted to a final concentration of 12 mM) or of purified human apoC-II (kindly provided by Dr. Henry Pownall, Baylor College of Medicine) in the same solvent. As a control, bovine milk LPL was purified as previously described (Voyta et al., 1985) and assayed under the same conditions.

Quantitation of LPL Protein Mass—LPL mass was determined by a standard enzyme-linked immunosorbent assay using purified bovine milk LPL as standard. The assay utilized a monoclonal antibody raised against purified bovine milk LPL (monoclonal antibody 40) as described by Voyta et al. (1985).

RESULTS

Lipoprotein Profiles and Post-heparin LPL of Patients and Family.—The plasma lipids and apolipoprotein levels of the affected family are shown in Fig. 1. Elevated fasting triglyceride was present in both patients and their father. Also of note was that the father and one sibling had elevated lipoprotein(a). The father also had higher apoC-I 1 and apoC-I 11 levels (Table I). The older patient had easily detectable apoC-I 1 levels. Therefore, they did not predict a truncated LPL shortened at its carboxyl terminus.

Sequence Analysis of LPL cDNAs Cloned from Patients’ Adipose Tissue mRNA—In order to determine the molecular basis of the LPL defect in the two patients, we cloned their LPL cDNAs from adipose tissue mRNA. We amplified the cDNA by PCR and subcloned the PCR products into M13 vectors mp18 and mp19 by directional cloning utilizing an artificial HindIII restriction site in the 5' end and a natural EcoRI site in the 3’ end. About 2,000-4,000 independent clones were pooled and sequenced by the dideoxy chain termination technique (Sanger et al., 1977). A total of 1502 nucleotides were amplified, subcloned, and sequenced. It included 33 nucleotides in the 5'-untranslated region, the entire coding region, and 41 nucleotides in the 3'-untranslated region of the mRNA. The sequence completely matched the normal human LPL cDNA sequence published by Wion et al., 1987, except for two bases: a point mutation, an A to G transition, affecting the second base of codon 156 changing it from GAT to GGT which would result in an Asp¹⁵⁶ -> Gly substitution (Fig. 2A), and a nonsense mutation changing the second base of the codon TCA for Ser¹¹² from C to G producing a premature stop codon (Fig. 2B) that would predict a truncated LPL shortened at its carboxyl terminus by 2 amino acid residues. Both mutations were homozygous by this analysis since sequencing of >2000 pooled cDNA clones yielded only the mutant bands on the sequencing gel (Fig. 2). Identical results were obtained in the two patients who were therefore homozygous for both of these mutations.

Analysis of Genomic DNAs of the Patients, Family Members, and Unrelated Individuals—Leucocyte DNAs isolated from the two patients and available family members were analyzed by PCR, restriction enzyme digestion, and allele-specific oligonucleotide hybridization. The sequences flanking the two mutation sites were amplified by PCR. The 5'-PCR product contained 115 bp including 90 bp in exon 5 and 25 bp in intron 4. The 3'-PCR product contained 105 bp of exon 9. Only single bands of PCR products of the predicted size were identified on agarose gel electrophoresis for both regions. The exon 5 mutation results in an A -> G substitution in a tetranucleotide TCGA which is a recognition sequence for the restriction enzyme TaqI. Digestion of the PCR product with this enzyme produces a 29-bp and an 86-bp product in the wild type sequence. The mutant sequence is not recognized by TaqI and remains undigested (Fig. 3A). The PCR-TaqI digestion patterns show that the products from the two pa-
an Asp$^{156}$ → Gly missense mutation and a Ser$^{447}$ → Ter nonsense mutation, one or both mutations could be deleterious to LPL function and be the underlying defect of the hyperchylomicronemia syndrome. We investigated this issue by expressing the wild type and mutant LPL DNA constructs in transfected COS cells in vitro and analyzed the cells and media for LPL activity. The expression vector p91023(B) (Wong et al., 1986) had previously been used for expressing a wide variety of proteins including LPL (Semenkovich et al., 1990). The results of the transfection experiment are shown in Table II. There was no measurable LPL activity in mock-transfected COS cells. LPL activity was easily detectable in COS cells transfected with wild type LPL construct and the Ser$^{447}$ → Ter mutant. ApoC-II-stimulated the LPL activity approximately 5-fold in both cases (data not shown). In both cases, approximately three-fourths of the activity was secreted into the media, and one-fourth was detected intracellularly. In contrast, LPL activity was totally undetectable in the cells transfected with the Asp$^{156}$ → Gly mutant construct, whether in the media or intracellularly. This observation suggests that the Asp$^{156}$ → Gly mutation produces an inactive LPL protein and is the cause of the Type I hyperlipoproteinemia syndrome in the two patients. The Ser$^{447}$ → Ter mutant, which produces a truncated LPL protein missing its carboxyl-terminal dipeptide Ser-Gly, appears to be actively active. We also expressed a mutant construct containing a Gly$^{448}$ → Ter mutation. This mutant LPL with a single residue, Gly, missing from its carboxyl terminus was found to be fully active (Table II). Therefore, the carboxyl-terminal dipeptide appears unimportant for LPL enzyme activity. Finally, since the two patients were homozygous for both the missense (Asp$^{156}$ → Gly) and the nonsense (Ser$^{447}$ → Ter) mutations, we expressed a mutant LPL construct containing both mutations. As expected, this double mutant was completely inactive (Table II).

Although the transfection experiments were consistent with Asp$^{156}$ → Gly being the underlying defect in the LPL deficiency syndrome in this family, we had to exclude the possibility that the absence of LPL activity in the Asp$^{156}$ → Gly mutant-transfected cells was caused by a failure of production of the LPL enzyme in the COS cells transfected with the mutant constructs. We therefore simultaneously measured LPL immunoreactive mass by an ELISA and LPL enzyme activity in additional transfection experiments. The results of these experiments are also shown in Table II. It is evident that LPL activities and mass, and therefore LPL specific activities, were very similar among wild type, Ser$^{447}$ → Ter, and Gly$^{448}$ → Ter mutant-transfected COS cells. In contrast, despite the presence of immunoreactive LPL at a concentration approximately 50–60% that in wild type cells, we did not detect any LPL enzyme activity either in the cell or media of

![Fig. 2. Sequence of cloned cDNAs from adipose tissue mRNA in the Type I hyperlipoproteinemic patients and a normal subject showing the Asp$^{156}$ → Gly mutation (A) and the Ser$^{447}$ → Ter mutation (B). Each sequence was determined on >2000 pooled cDNA clones. The mutated and corresponding normal bands are highlighted by asterisks.](image)

![Fig. 3. Analysis of genomic DNA for the Asp$^{156}$ → Gly mutation by TaqI enzyme digestion of PCR-amplified genomic DNAs. A, map of exon 5 and positions of PCR primers and predicted sizes of TaqI digestion products. The open bars represent the exons, and the line connecting exons 4 and 5 represents intron 4. B, agarose gel electrophoresis of TaqI digestion products. Lanes 1 and 2, normal controls; 3, father; 4, mother; 5, clinically normal sister; 6, patient C. U.; 7, patient C. M. S, standard DNA size markers.](image)

![Fig. 4. Analyses of genomic DNA for the Ser$^{447}$ → Ter mutation by PCR and allele-specific oligonucleotide hybridization. The exon 9 region spanning the Ser$^{447}$ → Ter mutation was amplified by PCR, and the products were applied onto duplicate nitrocellulose papers by a slot-blot apparatus. The filters were hybridized to normal oligonucleotide (top strip) and mutant oligonucleotide (bottom strip) as described under “Materials and Methods.”](image)
the cultures transfected with the Asp^{356} → Gly mutant or the double mutant containing the Asp^{356} → Gly and Ser^{447} → Ter mutations. Therefore, the Asp^{356} → Gly mutation produces an inactive LPL and is the cause of the hyperchylomicronemia syndrome in the two patients.

Prevalence of Asp^{356} → Gly and Ser^{447} → Ter Mutations—Since the Ser^{447} → Ter mutation is associated with normal enzyme activity, we screened clinically asymptomatic unrelated Caucasians for the mutation by PCR amplification and allele-specific oligonucleotide hybridization. Among 224 Caucasians screened, we found that there were 36 heterozygous individuals and 1 individual who was homozygous for this mutation. Therefore, the allele frequency for the nonsense mutation is 8%. As expected, in this clinically unaffected population, the Asp^{356} → Gly mutation was not present in any of the 224 individuals that we tested.

**DISCUSSION**

In this study, we have characterized two mutations in the lipoprotein lipase gene in a Turkish family with classic Type I hyperlipoproteinemia. The nucleotide sequence was determined on the cloned cDNAs of LPL mRNA isolated from the patients' adipose tissues. Previous reports have relied on sequence determinations on cDNAs from either adipose mRNA or PCR-amplified DNAs from genomic sequences. Because of the low but definite error rate of PCR, especially when the target sequence is present in low concentrations in the starting material, e.g. genomic DNA, investigators have had to perform either direct PCR sequencing which is often poorly reproducible, or they have resorted to sequencing multiple clones to ensure accuracy. We have circumvented this problem by pooling a large number of cDNA clones and determining their sequence simultaneously. The results were confirmed at the genomic level by PCR, restriction digestion for the exon 5 mutation, and allele-specific oligonucleotide hybridization for both the exon 5 and exon 9 mutations. We believe that the approach used represents a more direct way of analyzing PCR products without having to unnecessarily perform multiple sequencing experiments.

The genetic basis of familial LPL deficiency is highly heterogeneous. To date, at least 12 different mutations have been reported to be associated with LPL deficiency (Table III). There is one nonsense mutation (the Ser^{447} → Ter mutation characterized in this study is not included because it does not impair LPL enzyme activity), one 2-kb duplication, one 6-kb deletion, one 5-bp insertion, and one probable splice site mutation. Seven missense mutations have been reported.

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**TABLE II**

LPL enzyme activity and immunoreactive mass in COS cells transfected with wild type and mutant expression vectors.

| Mutations | Location | Authors |
|-----------|----------|---------|
| I. Missense mutations | | |
| Gly^{342} → Glu | Exon 4 | Ameis et al., 1991 |
| Asp^{370} → Gly | Exon 5 | Present study |
| Ala^{378} → Thr | Exon 5 | Beg et al., 1990 |
| Gly^{336} → Glu | Exon 5 | Emi et al., 1990b; Monsalve et al., 1990 |
| Ile^{394} → Thr | Exon 5 | Dichek et al., 1991 |
| Arg^{353} → His | Exon 6 | Dichek et al., 1991 |
| Ser^{447} → Thr | Exon 6 | Hata et al., 1990a |
| II. Other mutations | | |
| 2-kb duplication | Mainly exon 6 | Devlin et al., 1990 |
| 6-kb deletion | Exons 3, 4, and 5 | Langlois et al., 1990 |
| Splice site transition* | Intron 2 | Hata et al., 1990a |
| Gln^{396} → Ter | Exon 3 | Emi et al., 1990a |
| 5-bp insertion | Exon 3 | Henderson et al., 1990 |

*In the family reported here, this mutation is co-inherited with a Ser^{447} → Ter mutation. The latter produces a normally functioning LPL and is therefore not listed.

*The two mutations Ser^{447} → Thr and splice site transition are co-inherited in the family studied by Hata et al. (1990). The Ser^{447} → Thr mutant is inactive. The functional significance of the splice site G → A transition has not been tested.

*Although the functional significance of this mutation was not tested by Emi et al. (1990a) in vitro, its location predicts that it will be inactive since the catalytic triad is completely missing in the truncated LPL, if the truncated LPL is produced at all.

*This mutation comprises a 6-bp insertion at the site of a single base pair deletion, a net insertion of 5 bp at amino acid positions 102 to 103 causing a translational frameshift generating 44 amino acid residues of random sequence and a premature stop codon within exon 4 (Henderson et al., 1990).
One of the missense mutations, Gly^{488} → Glu, was found to be a relatively common defect which accounts for approximately 25% of the mutant alleles among LPL-deficient individuals of widely different ancestries (Monsalve et al., 1990). The other mutations appear to be highly heterogeneous and independently inherited. Of the seven missense mutations, four affect residues in exon 5. Exon 5 is one of the best conserved exons among LPLs of different species (Datta et al., 1988; Semenovich et al. 1989a) and among different lipases (Datta et al., 1988; Deeb and Peng, 1989; Cai et al., 1989). Since enzymatically inactive mutant LPLs are uncovered because the affected individuals present with LPL deficiency and hyperchylomicronemia, the clustering of missense mutations within exon 5 in these Type I hyperlipoproteinemic patients is consistent with exon 5 playing a crucial role in enzyme structure and function.

The family described here is of Turkish descent. It is interesting that two apparently independent mutations are co-inherited in this family: a missense mutation at residue 156 (Asp → Gly) and a nonsense mutation at residue 447 (Ser → Ter) producing a truncated LPL protein that contains 446 instead of 448 amino acid residues. It is not clear which mutation came first. Since the Ser^{447} → Ter mutation produces no apparent effect on the catalytic activity of LPL, we speculate that it might have preceded the much more deleterious Asp^{156} → Gly mutation. Analysis of 224 unrelated Caucasians indicates that indeed the Ser^{447} → Ter mutation represents a sequence polymorphism that has an allele frequency of 8%. After we completed our study, we became aware of a study by Hata et al. (1990) who reported this mutation came first. Since the Ser^{448} amino acid residue faces the carboxyl terminus of a lipase, and pancreatic lipase shows a highly conserved gene sequence (Monsalve et al., 1988; Deeb and Peng, 1989; Cai et al., 1989). In both lipases, there is an Asp-His-Ser trypsin-like catalytic triad with an active serine. The triglyceride lipase from Mucor mitcheri and pancreatic lipase shows a highly conserved gene sequence (Chomczynski et al., 1987) as well as primary sequence (Datta et al., 1989; Kirchgessner et al., 1989) as well as primary sequence (Datta et al., 1988; Deeb and Peng, 1989; Cai et al., 1989). Since enzymatically inactive mutant LPLs are uncovered because the affected individuals present with LPL deficiency and hyperchylomicronemia, the clustering of missense mutations within exon 5 in these Type I hyperlipoproteinemic patients is consistent with exon 5 playing a crucial role in enzyme structure and function.

Comparison of the structures of LPL, hepatic triglyceride lipase, and pancreatic lipase shows a highly conserved gene organization (Cai et al., 1989; Kirchgessner et al., 1989) as well as primary sequence (Datta et al. 1988) among these lipases. Eight of ten cysteine residues of LPL are highly conserved among the lipases; all 10 cysteines in bovine LPL exist in disulfide linkage (Yang et al., 1989). The three-dimensional structure of human pancreatic lipase has been established by x-ray crystallography (Winkler et al., 1990). The triglyceride lipase from Mucor mitcheri has also been crystallized to provide the three-dimensional structure at 1.9 Å resolution (Brady et al., 1990). In both lipases, there is an Asp-His-Ser trypsin-like catalytic triad with an active serine. These amino acids form a constellation that is stereochemically very similar to that in serine proteases (Blow, 1990). The essential triad residues, and presumably the topology, are conserved in all known sequences of serine proteases and lipases. At the active site of every serine protease, there is a serine which is within hydrogen-bonding distance of a ring nitrogen of histidine, invariably N1 in chemical nomenclature. The other ring nitrogen is hydrogen-bonded to the carboxylate group of an aspartate, which is usually buried. Although the conformation of the amino acids in the active site differs, in all charge relay systems, the N2 of histidine faces the carboxylate which is always from an aspartate residue. The structural similarity does not extend beyond the position of the hydrogen bond acceptors and donors. The essential role of aspartate in trypsin catalysis was demonstrated unequivocally by Craik et al. (1987) who engineered an Asp^{156} → Asn mutant in rat trypsin, reducing catalysis by about 3 orders of magnitude.

The Asp^{156} → Gly mutation in familial LPL deficiency described in this study is the first natural mutation involving 1 of the catalytic triad residues in a lipase (or protease). The resulting loss of catalytic activity strongly supports the inference that Asp^{156} functions as a proton donor in a charge relay system in human LPL as in the case of Asp^{182} in rat trypsin (Craik et al., 1987).

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