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A Missense Mutation ($Trp^{86}\rightarrow Arg$) in Exon 3 of the Lipoprotein Lipase Gene: A Cause of Familial Chylomicronemia

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Summary

We have investigated a patient of English ancestry with familial chylomicronemia caused by lipoprotein lipase (LPL) deficiency. DNA sequence analysis of all exons and intron-exon boundaries of the LPL gene identified two single-base mutations, a T→C transition for codon 86 (TGG) at nucleotide 511, resulting in a $Trp^{86}\rightarrow Arg$ substitution, and a C→T transition at nucleotide 571, involving the codon CAG encoding Gln^{106} and producing $Gln^{106}\rightarrow Stop$, a mutation described by Emi et al. The functional significance of the two mutations was confirmed by in vitro expression and enzyme activity assays of the mutant LPL. Linkage analysis established that the patient is a compound heterozygote for the two mutations. The $Trp^{86}\rightarrow Arg$ mutation in exon 3 is the first natural missense mutation identified outside exons 4–6, which encompass the catalytic triad residues.

Introduction

Familial lipoprotein lipase (LPL) deficiency is a rare autosomal recessive disorder characterized by chylomicronemia, recurrent episodes of abdominal pain, acute pancreatitis, eruptive xanthomas, hepatosplenomegaly, and failure to thrive (Brunzell 1989). LPL is a glycoprotein enzyme that hydrolyzes dietary or endogenously produced triglycerides transported in the circulation by the triglyceride-rich lipoproteins, chylomicron, and very-low-density lipoproteins. The sequence of human LPL was deduced from its cDNA sequence (Wion et al. 1987) and the structure of the human LPL gene has been determined (Deeb and Peng 1989; Kirchgessner et al. 1989; Oka et al. 1990). It contains 10 exons and 9 introns and shows considerable similarity to the hepatic lipase (HL) and pancreatic lipase (PL) genes. A number of different types of mutations have been identified in the LPL gene in patients with familial LPL deficiency (reviewed by Hayden et al. 1991). To date, all the missense mutations that result in a nonfunctional LPL have been found in exons 4–6 of the LPL gene. In the present communication, we have identified a missense mutation ($Trp^{86}\rightarrow Arg$) in a compound heterozygous patient with familial LPL deficiency. This is the first exon 3 mutation that has been shown to produce a nonfunctional enzyme in a patient with this syndrome.

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Subject and Methods

Subject

The proband (individual 6 in fig. 1) is a 1-year-old male of English descent (Cheng et al. 1991). He was investigated at 11 d of age because of jaundice, eruptive xanthoma, and lipemia retinalis. The proband was diagnosed as having type I hyperlipoproteinemia. Postheparin plasma LPL and HL activity were measured in the proband and in family members by using a gum-arabic stabilized emulsion of [^{3}H]-triolein as a substrate (Goldberg et al. 1982).

PCR Amplification and Sequencing of the LPL Exons

The coding regions of exons 1–9, part of exon 10 containing the termination codon, and the flanking regions of introns were amplified from leukocyte DNA by PCR according to a method described by Ishimura-Oka et al. (in press). The PCR products were cloned

into pBluescript KS. At least six independent clones were isolated and sequenced for each exon.

Allele-specific Oligonucleotide (ASO) Hybridization

Exon 3 of the LPL gene was amplified by PCR, transferred to a nylon membrane (Amersham, Arlington Heights, IL), and hybridized to ^{32}P-end-labeled wild-type or mutant ASO (Emi et al. 1990).

Expression of Wild-Type and Mutant LPLs In Vitro

A full-length human LPL cDNA subcloned into M13 mp19 was used as a template for site-specific mutagenesis (Semenkovich et al. 1990). The following mutagenic oligonucleotides were used: 5′-ATTGTGGTGGACCGGCTGTCACGGGCT-3′ for Trp^{86}→Arg mutation and 5′-AAACTGGTGGGATAGGATGTGGCCCGG-3′ for the nonsense mutation at codon 106. Mutagenesis and expression in COS-6 cells by using the vector p91023(B) were carried out according to a method described elsewhere (Faustinella et al. 1991*a*).

Results

DNA Analysis and Family Study

The 10 exons as well as all exon-intron boundaries of the LPL gene were amplified by PCR, cloned in pBluescript KS, and sequenced. The nucleotide sequences of all exons and exon-intron boundaries were identical to those in other published data (Deeb and Peng 1989; Kirchgessner et al. 1989; Oka et al. 1990), except for exon 3, where two different nucleotide substitutions were detected in six independent clones. Three clones contained a C-to-T transition at nucleotide position 571, and the other three contained a T-to-C transition at nucleotide position 511 (fig. 2). The first mutation changes the codon CAG, encoding Gln^{106}, to TAG, a termination codon. The second mutation changes codon 86 (TGG), encoding Trp, to CGG, encoding Arg. Since only one of the two mutations was present in each individual clone, the two mutations must be present on different chromosomes, and the proband must be a compound heterozygote for two different LPL mutant alleles.

Lipid profile and LPL and HL activities of the proband and relatives are summarized in table 1. Linkage analysis was carried out by ASO hybridization for the nonsense mutation at nucleotide 571 and by *Hpa*II digestion for the missense mutation at nucleotide 511. As shown in figure 1, ASO hybridization reveals that

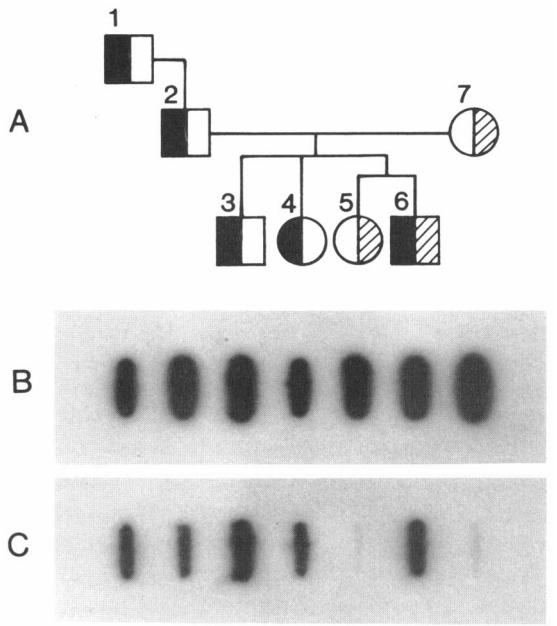

Figure 1 ASO hybridization among family members. In the pedigree (*A*), partial blackening denotes the Gln^{106}→Stop allele, and partial hatching denotes the Trp^{86}→Arg allele. Exon 3 was amplified by PCR, using the method described by Emi et al. (1990), and hybridized to the normal probe (*B*) or mutant ASO (*C*) probe.

the paternal grandfather, father, brother, sister, and proband are heterozygous for the Gln^{106}→Stop mutation. The sister and the mother carry only the wild-type sequence. Therefore, the nonsense mutation was inherited from the paternal side of the family. For the Trp^{86}→Arg mutation in exon 3, we amplified the region by PCR. Digestion, by *Hpa*II, of the PCR product from the normal allele produces two bands, of 181 bp and 46 bp. In the mutant allele, the T-to-C transition at position 511 creates an additional *Hpa*II site, and *Hpa*II digestion generates three bands, of 109 bp, 72 bp, and 46 bp. As shown in figure 3, the proband, his twin sister, and his mother are heterozygous for the Trp^{86}→Arg mutation, and the other family members have the normal allele on both chromosomes. Therefore, the mutant Arg^{86} allele was inherited from the maternal side of the family.

In Vitro Expression of Wild-Type and Mutant LPLs

To assess the functional significance of the two mutations, we studied the catalytic activities of the wild-type and mutant LPLs expressed in COS cells (table 2). Activity was easily detectable in both the medium and cellular extracts in the wild-type LPL vector-

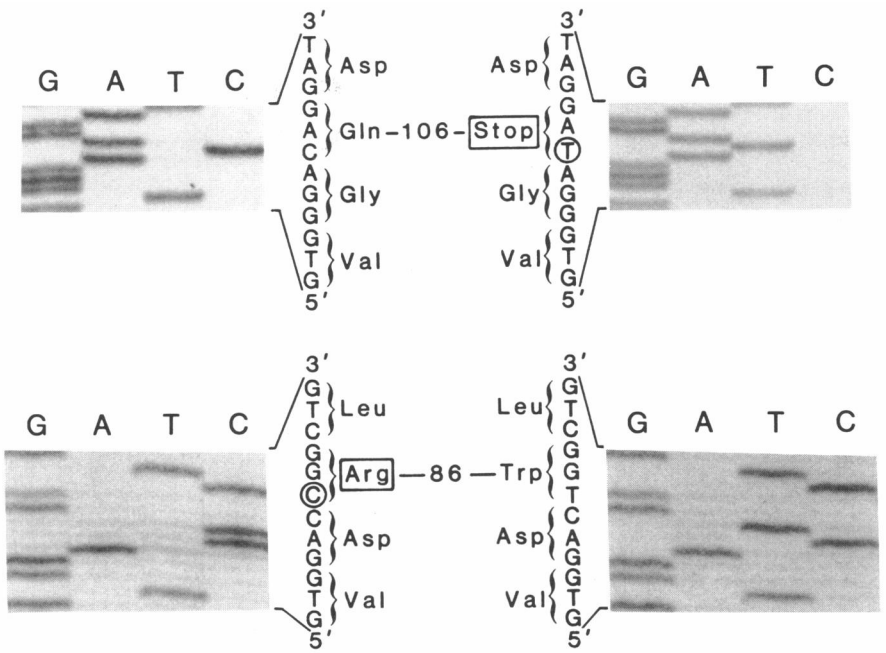

Figure 2 Sequence of cloned exon 3 fragments amplified by PCR from the proband. *Left,* Sequence of a clone containing the Trp^{86}→Arg mutation. *Right,* Sequence of a clone carrying the Gln^{106}→Stop mutation. Note that only a single mutation is present in an individual clone.

transfected cells. In contrast, it was undetectable in the mutant construct containing the Gln^{106}→Stop substitution. The new mutation described in this study, an Arg-for-Trp^{86} substitution, is associated with total loss of LPL enzyme activity. Furthermore, we used ELISA to measure the amount of immunoreactive LPL produced by the COS cells. The truncated nonsense Gln^{106}→Stop mutant transfected cells did not produce any detectable immunoreactive LPL. In contrast, the amount of the missense (Trp^{86}→Arg) mutant LPL and wild-type LPL were readily detectable. The specific activity of the missense mutant (Trp^{86}→Arg) LPL is negligible.

Discussion

We have characterized the molecular defect in a patient with familial chylomicronemia and have found that he is compound heterozygous for two mutations: (1) a nonsense (Gln^{106}→Stop) mutation and (2) a missense mutation changing a TGG codon, encoding Trp^{86}, to a CGG codon, encoding Arg. The Gln^{106}→

Table 1

Lipid Profile and LPL and HL Activities of the Proband and Relatives

Lipid	Concentration in Subject[a]						
	1	2	3	4	5	6	7
TC (mg/dl)	147	183	136	160	216	141	172
TG (mg/dl)	466	405	91	78	167	705	223
HDL (mg/dl)	55	26	29	34	38	18	28
LDL (mg/dl)	NA	NA	92	113	145	NA	NA
LPL[b] (μmol/ml/h) ...	4.1	3.5	NA	NA	2.8	.1	4.9
HL (μmol/ml/h)	10.2	11.2	NA	NA	11.1	16.0	9.9

[a] The number of the subject corresponds to that in the pedigree in fig. 1. NA = not available.
[b] The range of postheparin plasma LPL activity of normal subjects is 5–20 μmol/ml/h.

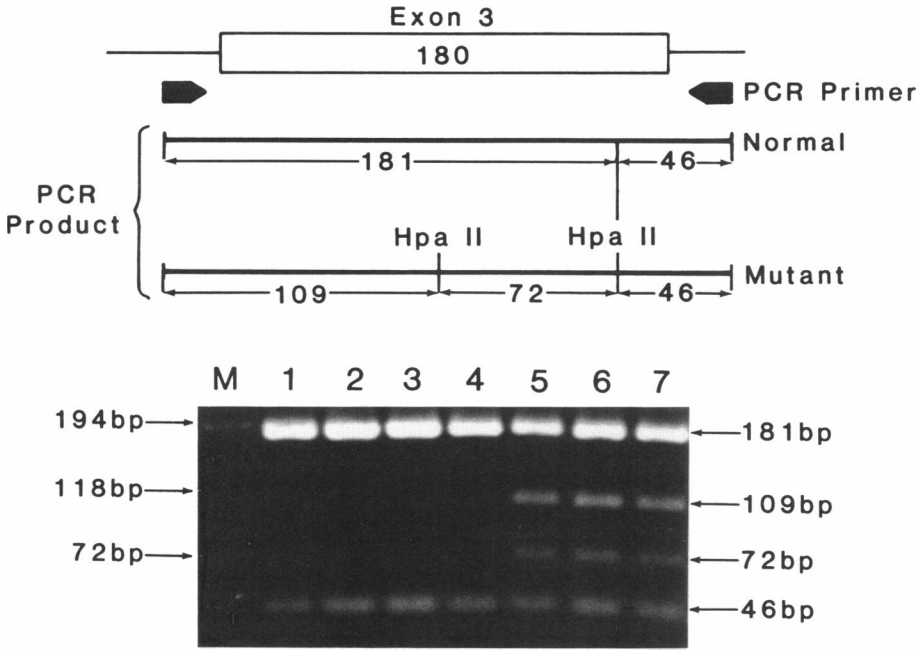

Figure 3 Detection of Trp^{86}→Arg mutation in the proband and in the relatives by restriction-enzyme digestion with *Hpa*II. A 227-bp fragment of exon 3 was enzymatically amplified by using the method described by Monsalve et al. (1990), was digested with *Hpa*II, and was electrophoresed on a 2.5% agarose gel. Lane M, Marker. Lanes 1–7, Individuals 1–7, respectively, in the pedigree in fig. 1.

Stop mutation is identical to that described by Emi et al. (1990).

The Trp^{86}→Arg missense mutation is the first mutation found in exon 3 of the LPL gene of a patient with familial chylomicronemia. All previously described missense mutations (Hayden et al. 1991) occur in exons 4–6. Not only are these highly conserved exons, they also contain the catalytic triad residues Ser^{132} (exon 4), Asp^{156} (exon 5), and His^{241} (exon 6). The identification of an exon 3 mutation and the nature of the Trp^{86}→Arg substitution causing an enzymatically inactive LPL provide plausible clues to an important role that this region of LPL plays in its catalytic function.

LPL shows high homology to PL. The three-dimensional structure of PL has recently been deduced from its crystal structure (Winkler et al. 1990). The high degree of sequence conservation between LPL and PL and the site-dependent variation in the enzymatic activities of a large number of site-specific LPL mutants (Faustinella et al. 1991*a*, 1991*b*) strongly suggest that LPL has a three-dimensional structure very similar to that of PL. An alignment of the residues that flank Trp^{86} in LPL and the corresponding residues in PL is as follows:

human LPL:	Asn	Val	Ile	Val	Val	Asp	Trp^{86}	Ser	Arg	Ala	Gln
human PL:	Asn	Cys	Ile	Cys	Val	Asp	Trp^{107}	Gly	Gly	Ser	Arg

Table 2

LPL Enzyme Activity and Immunoreactive Mass in COS Cells Transfected with Wild-Type and Mutant Expression Vectors

Expression Vector	LPL Activity (milliunits/dish)		LPL Mass (μg/dish)		Specific Activity (milliunits/μg)	
	Cell	Media	Cell	Media	Cell	Media
Wild type	214 ± 7.9	858 ± 35	6.8 ± 1.5	26 ± 1.5	31	33
Gln^{106}→Stop ...	0	0	0	0	. . .	. . .
Trp^{86}→Arg	3.6 ± 5.7	12 ± 17	3.2 ± .9	14 ± 1.9	1.1	.9

This part of the sequence is at the C-terminal end of an exon 3 sequence that is highly conserved among LPL, PL, and HL. In fact, the Val-Asp-Trp motif is conserved in the three enzymes in all species that have been sequenced (Datta et al. 1988; Semenkovich et al. 1990). By comparing LPL structure and the known PL crystal structure, we can infer that the Val and the Trp serve as a portion of the substrate-binding site by positioning the side chains of these residues within the Van der Waals distance of an acyl chain of the triglyceride (substrate). Thus, the loss of catalytic activity of the $Trp^{86}\rightarrow Arg$ mutant LPL could be the result of two independent factors. First, the introduction of a highly polar, charged guanidino group of Arg would preclude the functioning of the newly exposed surface as a nonpolar interface with the lipid substrate. The second possible effect is the formation of inappropriate salt bridges that would prevent the enzyme's segmental motion needed for the substrate to reach its catalytic center (Winkler et al. 1990).

In addition to providing information on the structure-function relationship of LPL, the identification of a new mutation in exon 3 is a patient with type I hyperlipoproteinemia indicates that mutations that are remote from the catalytic triad residues can impair enzyme function. Although functionally significant mutations appear to occur most commonly in exons 4–6, mutations in other parts of the LPL gene should be excluded both in patients with familial chylomicronemia and in those with the syndrome of familial combined hyperlipidemia. The latter syndrome is the most common form of heritable hyperlipidemia and is a major cause of premature atherosclerosis that has been linked to heterozygous LPL deficiency (Babirak et al. 1989).

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References

Babirak SP, Iverius P-H, Fujimoto WY, Brunzell JD (1989) Detection and characterization of the heterozygote state for lipoprotein lipase deficiency. Arteriosclerosis 9:326–334

Brunzell JD (1989) Familial lipoprotein lipase deficiency and other causes of the chylomicronemia syndrome. In: Scriver CR, Beaudet AL, Sly WS, Valle D (eds) The metabolic basis of inherited disease, 6th ed. McGraw-Hill, New York, pp 1165–1180

Cheng Q, Blackett PR, Wang C-S, Fesmier JO, Corder CN (1991) Use of plasma lipoprotein lipase activity without heparin injection for detection of homozygous and heterozygous lipoprotein lipase deficiency. Ann NY Acad Sci 623:410–412

Datta S, Luo C-C, Li W-H, Van Tuinen P, Ledbetter DH, Brown MA, Chen S-H, et al (1988) Human hepatic lipase: cloned cDNA sequence, restriction fragment length polymorphisms, chromosomal localization and evolutionary relationships with lipoprotein lipase and pancreatic lipase. J Biol Chem 263:1107–1110

Deeb SS, Peng R (1989) Structure of the human lipoprotein lipase gene. Biochemistry 28:4131–4135

Emi M, Hata A, Robertson M, Iverius RH, Hegele R, Lalouel JM (1990) Lipoprotein lipase deficiency resulting from a nonsense mutation in exon 3 of the lipoprotein lipase gene. Am J Hum Genet 47:107–111

Faustinella F, Chang A, Van Biervliet VP, Rosseneu M, Vinaimont N, Smith LC, Chen S-H, et al (1991*a*) Catalytic triad residue mutation ($Asp^{156}\rightarrow Gly$) causing familial lipoprotein lipase deficiency: coinheritance with a nonsense mutation ($Ser^{447}\rightarrow Ter$) in a Turkish family. J Biol Chem 266:14418–14424

Faustinella F, Smith LC, Semenkovich CF, Chan L (1991*b*) Structural and functional role of highly conserved series in human lipoprotein lipase. J Biol Chem 266:9481–9485

Goldberg IJ, Le N-A, Paterniti JR Jr, Ginsberg HN, Lindgren FT, Brown WV (1982) Lipoprotein metabolism during acute inhibition of hepatic triglyceride lipase in the cynomolgus monkey. J Clin Invest 70:1184–1192

Hayden MR, Ma Y, Brunzell JD, Henderson HE (1991) Genetic variants affecting human lipoprotein and hepatic lipases. Curr Opin Lipidol 2:104–109

Ishimura-Oka K, Semenkovich CF, Faustinella F, Goldberg IJ, Shachter N, Smith LC, Coleman T, et al. A missense ($Asp^{250}\rightarrow Asn$) mutation in the lipoprotein lipase gene in two unrelated families with familial lipoprotein lipase deficiency. J Lipid Res (in press)

Kirchgessner TG, Chuat JC, Heinzman C, Etienne J, Guilhot S, Svenson K, Ameis D, et al (1989) Organization of the human lipoprotein lipase gene and evolution of the lipase family. Proc Natl Acad Sci USA 86:9647–9651

Monsalve MV, Henderson H, Roederer G, Julien P, Deeb S, Kastelein JJP, Peritz L, et al (1990) A missense mutation at codon 188 of human lipoprotein lipase gene is a frequent cause of lipoprotein lipase deficiency in persons of different ancestries. J Clin Invest 86:728–734

Oka K, Tkalcevic GT, Nakano T, Tucker H, Ishimura-Oka K, Brown WV (1990) Structure and polymorphic map

of human lipoprotein lipase gene. Biochim Biophys Acta 1049:21–26

Semenkovich CF, Luo C-C, Nakanishi MK, Chen S-H, Smith LC, Chan L (1990) In vitro expression and site-specific mutagenesis of the cloned human lipoprotein lipase gene: potential N-linked glycosylation site asparagine 43 is important for both enzyme activity and secretion. J Biol Chem 265:5429–5433

Winkler FK, D'Arcy A, Hunziker W (1990) Structure of human pancreatic lipase. Nature 343:771–774

Wion KL, Kirchgessner TG, Lusis AJ, Schotz MC, Lawn RM (1987) Human lipoprotein lipase complementary DNA sequence. Science 235:1638–1641