Amino Acid Substitution (Ile^{194} → Thr) in Exon 5 of the Lipoprotein Lipase Gene Causes Lipoprotein Lipase Deficiency in Three Unrelated Probands

Support for a Multicentric Origin

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Abstract

Studies on the molecular biology of lipoprotein lipase (LPL) deficiency have been facilitated by the availability of LPL gene probes and the recent characterization of gene mutations underlying human LPL deficiency. Typically, missense mutations have predominated and show a preferential localization to exons 4 and 5. This distribution supports earlier studies attributing functional significance to residues encoded by these exons. We now report a further missense mutation within exon 5 of the LPL gene in three unrelated patients. Amplification of individual exons by the polymerase chain reaction and direct sequencing revealed a T → C transition at codon 194 of the LPL cDNA which results in a substitution of threonine for isoleucine at this residue. The catalytic abnormality induced by this mutation was confirmed through in vitro mutagenesis studies in COS-1 cells. Transfection with a LPL cDNA containing the codon 194 transition resulted in the synthesis and secretion of a catalytically defective protein. The Thr194 substitution was associated with two different DNA haplotypes, consistent with a multicentric origin for this mutation. (*J. Clin. Invest.* 1991. 87:2005–2011.) Key words: lipoprotein lipase • hypertriglyceridemia • molecular genetics • mutations

Introduction

Lipoprotein lipase (LPL[1]; triacylglycerolprotein acylhydrolase, EC 3.1.1.34) is a major enzyme in lipid transport and lipoprotein metabolism. It is located on the capillary endothelium where it generates free fatty acids through the hydrolysis of the triacylglyceride core of circulating chylomicrons and VLDL. This process initiates the conversion of these lipoproteins to their remnant particles and also modulates the levels and lipid composition of high density lipoproteins.

Genetic deficiencies of LPL are uncommon, occurring at a carrier frequency of 1/500 except in certain areas of Quebec where a founder effect has increased the frequency to 1/40 (1). Affected persons typically present in childhood with acute pancreatitis, lipaemia retinalis, and eruptive xanthoma, together with laboratory findings of chylomicronemia (2).

Cloning of the LPL cDNA (3) established the amino acid sequence and yielded a total of 448 residues for the mature protein. Subsequent structural studies on the LPL gene identified 10 exons spanning 30 kb (4, 5) on the short arm of chromosome 8 (6). This has facilitated a search for gene mutations underlying LPL deficiency and several have been characterized. These include, two gene rearrangements comprising a 2-kb direct tandem duplication of the major portion of exon 6 and its 3′ flanking intron (7), and a 6-kb intragenic deletion of exons 3, 4, and 5 with breakpoints in introns 2 and 5 (8). A missense mutation has been identified in exon 4 at residue 142 (Gly → Glu) (9), while four other mutations are located in exon 5 and occur at amino acid positions 157 (Pro → Arg) (Kastelein, J. J. P., T. Bruin, M. V. Monsalve et al., manuscript in preparation), 176 (Ala → Thr) (10), 188 (Gly → Glu) (11, 12), and 207 (Pro → leu) (Ma, Y., H. E. Henderson, P. Julien et al., manuscript submitted). A further single base transition has resulted in the introduction of a termination codon at position 106 in exon 3 (13). A frameshift insertion of 5 bp has also been found in exon 3, which results in the production of a truncated LPL species through the generation of a premature termination codon within exon 4 (14). The residue 188 mutation has proved to be common and accounts for ~ 25% of all the mutant alleles in a large cohort of patients of widely differing ancestries (12).

There is considerable homology between LPL of different species (15, 16). One of the longest conserved regions showing complete residue identity between man, cow, mouse, and guinea pig LPL spans residues 178–210 of human LPL and is encoded by exon 5 (17). In this manuscript we describe a further missense mutation in this region which results in the substitution of threonine for isoleucine at residue 194. In vitro mutagenesis studies confirmed that this amino acid substitution significantly impaired the catalytic activity of human LPL and was the cause of LPL deficiency. This point mutation was found on five alleles from three unrelated persons and in association with two different LPL gene haplotypes suggesting a multicentric origin. The finding of yet another mutation in exon 5 causing LPL deficiency demonstrates the structural and functional significance of residues encoded by this exon.

Methods

Subjects. The index patient (II.1) with LPL deficiency was from a South African family of Dutch and French descent (kindred 1; Fig. 1).

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1. *Abbreviations used in this paper:* FFA, free fatty acid; HL, hepatic lipase; PL, pancreatic lipase; LPL, lipoprotein lipase.

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This patient presented at the age of 4 yr with a history of hepatosplenomegaly and abdominal pain. Plasma lipid analysis revealed chylomicronemia with triglyceride levels in excess of 30 mmol/liter. LPL deficiency was previously established by demonstrating an absence of LPL activity in post heparin plasma using a radiolabeled triolein-triton ×100 emulsion. Plasma apo CII activator activity was observed to be normal as was the position and intensity of the apo CII spot on two-dimensional chromatography. Fasting lipid profiles were determined as described previously (8). Fasting blood samples were also collected for DNA analysis from 50 unrelated normolipidaemic South Africans of Dutch descent.

Lipoprotein lipase activity and mass. Blood samples were collected from all family members and controls after an overnight fast. Postheparin plasma was obtained from blood taken 10′ after an intravenous bolus of heparin administered at 60 U/kg body mass. Protease inhibitors were added to give concentrations of 10 mg/ml for leupeptin and 25 IU/ml for trasylol. Aliquots were either assayed immediately or snap frozen in liquid nitrogen and stored at −70°.

LPL activity was assayed using a radiolabeled triacylglycerol-phosphatidylcholine emulsion as previously described (18); results are expressed as nmol free fatty acid (FFA)/min/ml in post heparin plasma and nmol FFA/min/dish in the COS-1 cell medium and homogenates. LPL mass was measured by ELISA using the monoclonal antibody 5D2 in a sandwich assay which detects the homodimer form of LPL (19); results are expressed as ng/dish.

DNA analysis. Genomic DNA was isolated from leucocytes in EDTA-treated blood as described previously (20). The entire coding sequence of LPL is covered by exons 1–9 except for the last base of the termination codon which is coded by exon 10. The former exons were amplified by PCR using flanking, intronic sequence oligonucleotides and sequenced directly as previously described (12), while the first 160 bp of exon 10 were amplified using the 5′ flanking intron primer 5′-CCCTTTTTCCTGTGCTTTTTC-3′ and an exon 10 primer 5′-AGTGCTTGAGACTGTCTCCTAA-3′ complimentary to bases 1756–1776 of the published LPL cDNA sequence (3). This PCR product was cloned into pUC vectors and sequenced by standard protocols. DNA haplotypes in the patients and controls were constructed from three restriction fragment length polymorphisms (RFLP) at the LPL locus (21, 22).

LPL expression phagemids. The full length human cDNA clone used in this study, pLPL35, was a kind gift from R. Lawn (Genetech Inc., San Francisco). An LPL expression phagemid was constructed by cloning a 1.6-kb DraI/EcoR1 fragment of pLPL35 in the sense orientation into the expression vector CDM8 (23) which served as a dual function vector for both mutagenesis and expression. The 1.6-kb fragment contained the entire coding sequence of LPL flanked by 113 bp 5′ to the initiation codon and 40 bp 3′ to the termination codon.

Site-directed mutagenesis. Double-stranded CDM8-LPL DNA was purified and transfected into a dut- ung- host (BW313/p3/F′). Uracil-containing single stranded CDM8-LPL phage DNA was prepared by superinfection of the BW313/p3/F′ host with helper phage R408 (Stratagene Inc., La Jolla, CA). Mutant oligonucleotides were synthesised on an automated DNA synthesizer (model 380A; Applied Biosystems, Inc., Foster City, CA). In vitro mutagenesis was performed using the double-primer method (24). The newly synthesized heteroduplex DNA was transfected into a dut+ ung+ host (MC1061/p3) in which the uracil-containing wild-type target strand was degraded resulting in consequent suppression of the wild-type colonies. Mutant clones were identified by oligonucleotide hybridization and verified by DNA sequencing.

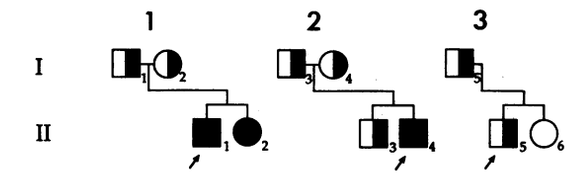
COS-1 cell transfections. Expression phagemids were introduced into the COS cells by electroporation using a Bio-Rad Laboratories (Cambridge, MA) Gene Pulser apparatus according to the manufacturer's instructions. Cells (5×10^6), harvested at ¾ confluence, were resuspended in 0.8 ml of PBS, transfected with 20 μg of each phagemid by pulsing at 270 V/500 μFD. The cells were kept on ice for 10 min and transferred to three 10-cm culture dishes containing 15 ml incubation medium supplemented with heparin at 40 μ/ml. Cells were grown for 72 h and judged to be of equivalent densities in all dishes by grid counting. The medium was removed, replaced with 6 ml fresh medium containing heparin, and the cells grown for a further 12 h. The medium from each dish was cleared of free cells by centrifugation and snap frozen (−70°) in 1-ml aliquots. Cell monolayers were washed with PBS, containing heparin (40 μg/ml), and removed from the dishes by incubation for 5 min at room temperature in PBS containing 2.5 mM EDTA. Cells from each dish were washed once in PBS, split into two, and subjected to protocols for the isolation of RNA (25) and the preparation of cell lysates for LPL mass and activity determinations (26).

Results

The proband from kindred 1 (II. 1; Fig. 1) presented with the classical clinical features of LPL deficiency and was found to have markedly reduced levels of LPL activity but normal mass in post-heparin plasma (Fig. 1). Plasma apo CII activator activity was within the normal range. Chromatography of the catalytically defective enzyme on a heparin-Sepharose column revealed that 67% of the immunoreactive material in post-heparin 0.7 plasma eluted at 1.1 M NaCl where normal LPL elutes, 30% at 0.7 M NaCl, and 2% in the void volume for a total recovery of 99%.

One of the obligate heterozygotes for the Thr 194 mutation (II 2; Fig. 1) presented with an elevated LPL mass level in pre-heparin plasma. Similar levels are sometimes encountered in pre-heparin plasma samples from normolipidemic individuals. The nature of this material is unknown and may represent a partially degraded or aggregated form of LPL.

Mutant allele sequence. Sequence analysis of exon 5 DNA from the kindred 1 proband (II 1; Fig. 1) revealed homozygosity for a T → C transition at position 836 of the published LPL cDNA sequence (3). The single base transition has occurred at the second position of the ATT codon for isoleucine at position 194 and results in the substitution of this residue by threonine (ACT) (Fig. 2). No other sequence alteration was detected in exon 5 and the base substitution was confirmed by sequencing



Plasma Triglyceride *	2.0	1.4	41.8	17.8	3.5	0.9	1.1	38.4	8.1	21.5	1.2
Total cholesterol *	5.2	6.8	7.4	5.7	5.8	5.5	4.1	8.3	8.8	7.6	5.6
LDLc *	3.5	4.6	-	-	-	-	-	-	7.2	-	4.0
HDLc *	0.8	1.6	0.3	0.4	-	-	-	-	1.1	0.4	1.0
Apo A1 **	105	134	-	-	-	-	-	-	114	-	113
Apo B **	90	98	-	-	-	-	-	-	201	-	92
LPL Activity #	138	68	0	-	-	-	-	-	110	0	92
HL Activity #	244	168	412	-	-	-	-	-	124	237	178
LPL Mass T=0′ ##	-15	244	27	-	-	-	-	-	-18	-7	-5
LPL Mass T=10′ ##	435	621	354	-	-	-	-	-	426	288	75
Incremental Mass ##	435	377	327	-	-	-	-	-	426	288	75

Figure 1. Kindreds of the three LPL-deficient patients examined in this study. Shading indicates the presence of the Ile^{194} → Thr mutation. Fasting plasma lipid levels, apolipoproteins A1 and B concentrations, LPL, HL activities, and LPL mass levels are shown. Proband II.4 (kindred) was shown to be LPL deficient in previous studies in South Africa. LDLc/HDLc, LDL/HDL cholesterol. Concentrations are given as: *mmol/liter; **μg/ml; #nmol FFA/min per ml; and ##ng/ml.

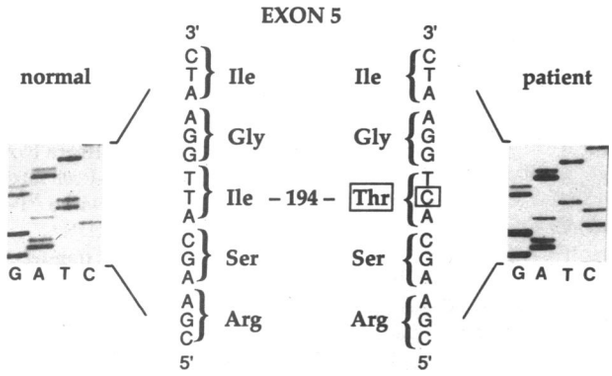


Figure 2. Nucleotide sequence of the sense strand from exon 5 of the kindred 1 proband showing the T → C transition at codon 194, which results in the substitution of threonine for isoleucine. Codon positions are taken from the published cDNA sequence (3).

the opposing strand and by repeat sequencing of exon 5 DNA from several independent PCR amplifications. The base substitution does not create or destroy a restriction enzyme site and could not be confirmed in genomic DNA by restriction fragment length analysis. The nucleotide sequence of exons 1–4 and 6–9 together with the exon donor and acceptor splice sites was determined and found to be normal. The LPL termination codon was established by amplifying and sequencing the 5′ splice site plus the first 160 bp of exon 10 and was found to be normal. RFLP analysis shows that this patient has two intact LPL alleles and it has thus been assumed that the PCR primers have amplified the individual exons from both these alleles.

Dot blot hybridization analysis. Detection of the Thr^{194} mutation was carried out using 20 ng of PCR amplified exon 5 DNA, transferred to Hybond N+ filters (Amersham Corp., Arlington Heights, IL) and probed with [γ-^{32}P] labeled oligonucleotides (17 mers) complementary to the normal or mutant sequence spanning the T → C transition. The allele specific oligonucleotides used were ASO I, 5′-TCGAAGCATTGGAATCC-3′ (normal allele); and ASO II, 5′-TCGAAGCACTGGAATCC-3′ (mutant allele). Filters were hybridized with the labeled oligomers, washed, and set up for autoradiography as previously described (14). The autoradiograph of a filter containing amplified DNA from kindred 1 is shown in Fig. 3. This result confirms the sequencing data on proband II.1 (Fig. 1), showing this individual to be a true homozygote for the mutation and confirms carrier status for the Thr^{194} mutation in both parents. By similar analysis we have probed exon 5 DNA from 50 unrelated normolipidemic control subjects and not detected this mutation.

Mutation prevalence. To determine the prevalence of the Thr^{194} mutation in patients with LPL deficiency we screened DNA from 20 unrelated affected probands of differing nationalities in whom the underlying gene mutations were unknown. Two additional probands were positive for this mutation by dot blot hybridization analysis and both were South Africans. The proband II.4 from kindred 2 (Fig. 1) was also of Dutch extraction and was unrelated to our index patient. This subject proved to be a true homozygote while the third affected individual II.5 (kindred 3) was heterozygous for this mutation (Fig. 3). To validate the presence of the 836 T → C transition in these two additional subjects, we determined the exon 5 sequence in DNA from several independent PCR amplifications and found the expected transitions. The remainder of the LPL coding sequence in these two subjects was determined by PCR amplification and sequencing as described for proband II.1 (Fig. 1) and found to be normal.

Mutagenesis. To establish whether the substitution of threonine for isoleucine at amino acid position 194 destroyed the catalytic activity of LPL and was the cause of the enzyme deficiency in our patients, we introduced the 836 T → C transition into the LPL expression phagemid using the mutagenic primer 5′-GGTCGAAGCACTGGAATCCAG-3′. The resulting phagemid LPL cDNA.T194C was used to transfect COS 1 cells. The suitability of this cell culture system to the analysis of the synthesis, secretion, and activity of LPL was determined by transfecting cells with the normal cDNA cloned into the same expression phagemid. Control cultures were also established to determine the effects on cell viability of transfection with the vector alone and to establish endogenous levels of LPL synthesis and secretion by COS-1 cells. The results obtained are depicted in Fig. 4, *a* and *b*. Measurement of LPL activity in the medium from the four cell cultures (Fig. 4 *a*) revealed almost zero endogenous lipolytic activity secreted by the transfected COS-1 cells, while the medium from the cells transfected with the normal LPL cDNA showed a substantial level of activity at 288 nmol FFA/min per dish (total medium/dish = 6 ml) and mass of 1,266 ng/dish. This latter finding contrasted sharply with the basal activity level of less than 10 nmol FAA/min per dish in the medium from the cells carrying the mutant LPL cDNA. Enzyme mass determinations, however (Fig. 4 *b*), did reveal the presence of LPL protein in this medium. These data, therefore, show that the mutant cDNA is expressed in the transfected COS-1 cells and that the secreted LPL is catalytically inactive. This observation was confirmed by a repeat transfection where similar results were obtained.

LPL mRNA levels in transfected cultures with both normal and mutant cDNAs were similar, as assessed by Northern blot analysis (Fig. 5). This finding suggests that equivalent levels of transfection and expression were obtained with both the normal and mutant LPL cDNAs.

The LPL mass level in the medium of the cells transfected with the mutant cDNA was found to be 420 ng/dish (Fig. 4 *b*),

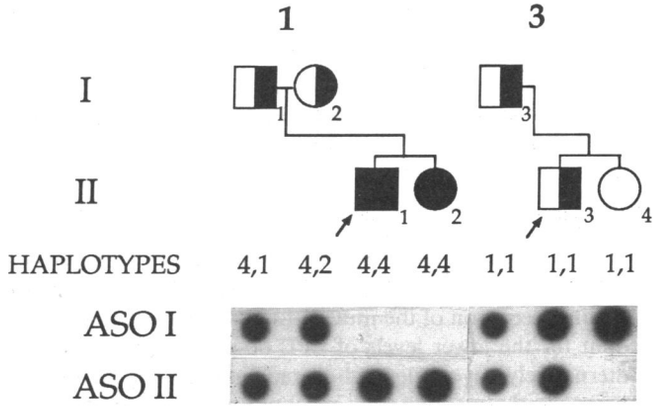


Figure 3. Autoradiograph of exon 5 dot blots from kindreds 1 and 3 when probed with ^{32}P-labeled allele-specific oligomers complimentary to the normal (*ASO I*) or mutant allele sequence (*ASO II*).

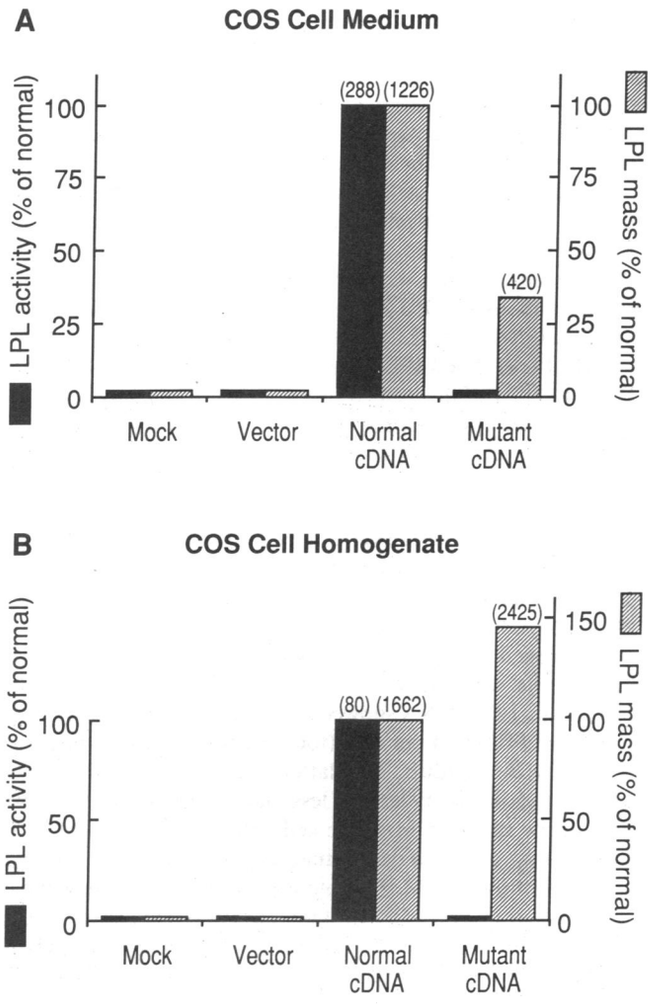


Figure 4. LPL activity and mass levels in the 12-h culture medium (*A*) and cell homogenate (*B*) from COS-1 cells transfected with the normal human LPL cDNA and the mutant cDNA. Control media are from cells subjected to a mock transfection and cells transfected with the expression phagemid alone. Results are expressed as a percentage of the levels determined in the medium and homogenate of cells transfected with the normal LPL cDNA. The actual values found are given in brackets and are nmol FFA/min per dish (total medium/dish = 6 ml) for activity and ng/dish for protein mass in both the medium and the cell homogenate.

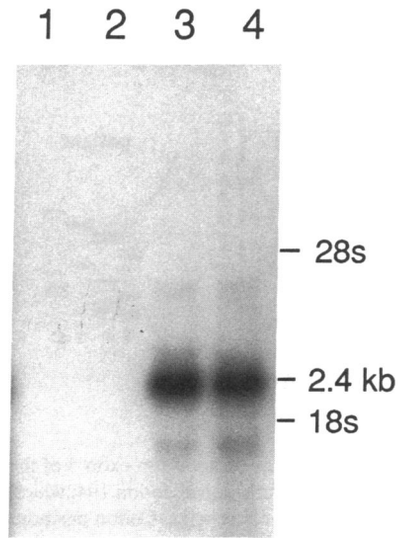


Figure 5. Autoradiograph of a Northern blot of 10 μg COS-1 cell total RNA probed with a ^{32}P-labeled LPL cDNA. Lane *1*, mock transfection; lane *2*, cells transfected with the CDM8 vector; lane *3*, cells transfected with the normal LPL cDNA; and lane *4*, cells transfected with the Thr^{194} LPL cDNA.

which is approximately one-third of the 1,226 ng/dish detected in the medium of the cells transfected with the normal cDNA. Determination of the intracellular LPL mass, however, gave a value of 2,425 ng/dish for the cells expressing the mutant cDNA, which is significantly higher (~ 45%) than the 1,662 ng/dish for the wild-type expressing cells. It is of interest that the total LPL mass (intracellular and secreted) is similar in both cell cultures (2,888 ng/dish for the wild-type cDNA vs. 2,845 ng/dish for the mutant cDNA). These data may indicate impaired secretion of the mutant LPL which may, in part, account for the lower levels of LPL observed in the medium. Alternatively, an accelerated degradation rate for the mutant enzyme in the medium may account for this difference.

RFLPs and haplotype analysis. In order to determine whether the Thr 194 mutation occurred on the same chromosomal haplotype in all the kindreds, we constructed haplotypes from three RFLPs at the LPL locus. The probands in kindreds 1 (Fig. 3) and 2 proved to be homozygous for haplotype 4, which is associated with the mutant allele in these families, while the proband in kindred 3 was homozygous for haplotype 1, which characterizes the mutant allele(s) in this kindred (Fig. 3). Haplotypes 1 and 4 differ at the HindIII and PvuII restriction sites. Kindreds 1 and 2 are unrelated but of Dutch and French descent, while the ancestry of kindred 3 is mixed, being derived from the indigenous Khoisan peoples with admixture from early Dutch, German, and French settlers. The normal alleles in these three kindreds are associated with either haplotypes 1 or 2. The use of three RFLPs in haplotype construction gives a theoretical possibility of $8[(2)^3]$ different haplotypes. The LPL gene haplotypes segregating in the general population were determined by analysis of DNA from 33 normolipidaemic unrelated South Africans of Dutch descent. The RFLP frequencies detected in this group are given in Table I and are similar to those originally published (21, 22). The haplotype distribution is given in Table II. Haplotype 2 is the most common in the South African control population and accounts for 39% of the alleles examined. The mutant alleles in our kindreds are associated with the less frequent haplotypes 4 and 1, which each account for ~ 10% of the alleles examined in this control population.

Discussion

In this manuscript we describe a T → C transition in exon 5 of the LPL gene which results in the substitution of threonine for

Table I. LPL Gene RFLP Frequencies in Normolipidemic Controls

		(+)		(−)	
Population	RFLP	*n*	Frequency	*n*	Frequency
Caucasian (Afrikaner)	HindIII	44	(0.67)	22	(0.33)
n = 66	Bam HI	22	(0.33)	44	(0.67)
	PvuII	40	(0.61)	26	(0.39)

Presence (+); absence of restriction sites (−).

Table II. LPL Gene RFLP Haplotype Frequencies in 33 Normolipidemic Afrikaners

Haplotype	HindIII	BamHI	PvuII	Alleles	%
1	+	−	−	7	10
2	+	−	+	26	39
3	−	+	+	3	5
4	−	−	+	8	12
5	+	+	−	8	12
6	−	+	−	8	12
7	+	+	+	3	5
8	−	−	−	3	5
Total				66	100

* Haplotype nomenclature according to Monsalve et al., 1990.

isoleucine at residue 194 of the mature enzyme. This transition was detected in three unrelated persons with LPL deficiency and was confirmed as the cause of their enzyme abnormality by in vitro mutagenesis studies in COS-1 cells. Transfection with an LPL cDNA incorporating this mutation resulted in the synthesis and secretion of a catalytically defective LPL protein.

This is the sixth missense mutation to be described to cause LPL deficiency (Fig. 6). All these mutations occur within a highly conserved segment, spanning residues 117–232 encoded by exons 4 and 5 and which shows significant homology with human pancreatic lipase (hPL) and human hepatic lipase (hHL) (16). The localization of the mutations to this region of the protein is unlikely to be due to an ascertainment bias through preferential sequence analysis of exon 5 in affected patients, as these mutations together account for a significant majority of the mutant alleles in a cohort of 75 LPL-deficient patients, of various ancestries, examined in this laboratory. Rather, this observation supports earlier data alluding to the structural and functional significance of the residues encoded by exons 4 and 5 (17). These residues include those comprising the proposed interfacial binding site and the active site serine at position 132 (15, 27).

The pronounced homology between the known amino acid sequences of human LPL, HL, and PL permits an alignment with few ambiguities (16, 5) and suggests that their three-dimensional structures are likely to be similar. The known three-dimensional structure of PL (27) was used to examine the possible effect of the Thr194 substitution on the structure and function of LPL. The active site of PL contains an Asp(176) . . . His(263) . . . Ser(152) triad similar to that found in trypsin-like proteases (27). Isoleucine at position 194 of LPL corresponds to phenylalanine 215 (Phe215) of PL which is located near the essential histidine of the Asp . . . His . . . Ser catalytic triad of PL. Phe215 is one of the hydrophobic residues surrounding the active site (Fig. 7) and is likely to be in hydrophobic interaction with the alkyl side chains of the triglyceride substrate. Further, the side chain of Phe215 is in van der Waals contact with Tyr114, Ala171, and Pro180 which are all conserved in LPL. An isoleucine side chain can be easily accommodated in the same environment and Ile194 can be assumed to occupy a very similar position in lipoprotein lipase.

The replacement of isoleucine by the smaller and more hydrophilic threonine in the inactive mutant could affect the enzymatic activity in various ways. The smaller size is unlikely to have a dramatic effect and indeed HL has a valine in the equivalent position. A substitution of valine for isoleucine in LPL at position 194 would not be expected to alter the catalytic activity of the enzyme. The hydrophilic hydroxyl group of threonine, however, might disrupt the hydrophobic surface that is needed for a favorable interaction with the alkyl chains of triacylglyceride substrates. Alternatively, it could lead to a change in the hydration of the active site region with effects on the catalytic mechanism and/or the interaction with substrate. Assessment of these suggested mechanisms awaits the elucidation of the three-dimensional structure of LPL.

We have clearly shown through in vitro mutagenesis studies that the Thr[194] substitution impairs the catalytic activity of LPL towards long chain triacylglycerides, but its effects on the other functional properties and molecular interactions of the mutant enzyme are unclear. The heparin binding site, however, appears to be unaffected as the mutant enzyme binds to the luminal surface of the capillary endothelium. This was revealed by the very low levels of LPL mass detected in pre-heparin plasma from proband II.1 (Fig. 1) and the substantial increase observed in the plasma after heparin infusion (Fig. 1). Further, 97% of the incremental mass in post-heparin plasma bound to a heparin-Sepharose affinity column and the major fraction of this material (67%) eluted at the same sodium chloride concentration as normal LPL.

We have not established whether the relative decrease in the concentration of the mutant LPL protein in the medium from the transfected COS cells is due to impaired secretion or accelerated degradation. However, it would appear that LPL mRNA levels are similar in both transfected cell cultures, indicating that levels of phagemid expression are similar and cannot account for the observed differences.

The association of the 836 T → C transition with two different RFLP haplotypes in our patients was unexpected. Studies of human genetic diseases have revealed that specific mutations are usually associated with only one haplotype and probably represent single ancestral events (28, 29). Less commonly however, mutations have been found to segregate with different haplotypes and are likely to be either very old or have occurred more than once on different chromosomal backgrounds (30, 31). Many of these mutations have occurred at CpG dimers, which may be hot spots for mutation and may account for the recurrent events at these sites. The haplotypes associated with the Thr[194] mutation differ at two of the three polymorphic restriction sites and it is unlikely, therefore, that these changes have recently occurred through random mutations af-

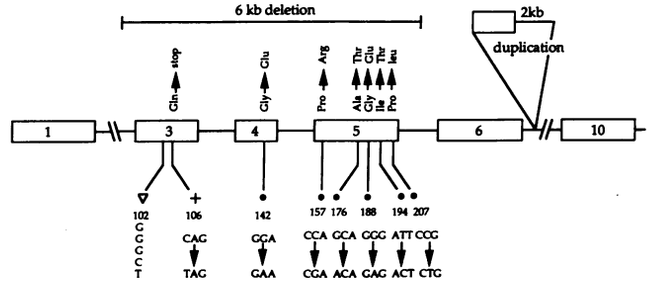


Figure 6. The identity and location of the reported mutations in the LPL gene which result in LPL deficiency. Missense mutations (●); premature stop codon (+); frameshift insertion (▽) (references are given in the introduction).

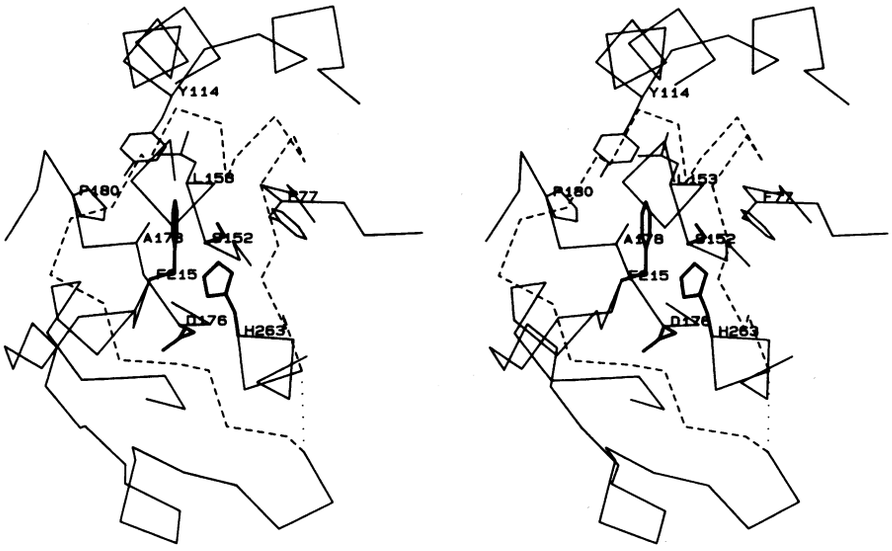


Figure 7. Stereo view of the active site region of human pancreatic lipase. Alpha carbon traces of the segments forming the active site are drawn together with the side chains of the Asp176 . . . His 263 . . . Ser152 catalytic triad (hydrogen bonds indicated by dashed lines) and the hydrophobic side chains in its neighborhood (Phe 77, Tyr 114, Ile 153, Ala 178, Pro 180, and Phe 215). Only one residue is labeled in a given segment. The corresponding residues in hLPL are Asp 156, His 241, and Ser 132 for the triad and Trp 55, Tyr 94, Ile 133, Ala 158, Pro 160, and Leu 194 for the rest. The segment between the disulphide-linked residues 237 to 261 (indicated by *dotted lines*) forms the flap and hydrophobic side chains of this segment have been omitted as their position in the open, active state is not known.

fecting these specific cutting sites. Further, the possibility that the multiplicity of haplotypes is indicative of a very old mutational event is slight, as the mutation was not found in a large cohort of LPL-deficient patients of different nationalities. These data are more consistent with the occurrence of two separate ancestral events on different chromosomal backgrounds and suggest a multicentric origin for this mutation.

Knowledge of DNA sequence alterations in the LPL gene and the synthesis of mutation-specific oligonucleotides simplifies the unequivocal diagnosis of carriers in affected families. The probands examined in this study come from extensive kindreds and it will now be possible to collect blood from large numbers of family relatives and identify carriers in significant numbers to examine the relationship between heterozygosity for LPL deficiency and lipoprotein abnormalities. In addition, an association between the heterozygous state and the presence of premature coronary atherosclerosis can now be explored (19).

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References

1. Gagne, C., L. D. Brun, P. Julien, S. Moorjani, and P. J. Lupien. 1989. Primary lipoprotein lipase activity deficiency: clinical investigation of a French Canadian population. (Can. Med. Assoc. J.) 140:405–411.

2. Brunzell, J. D. 1989. Lipoprotein lipase deficiency and other causes of the chylomicronaemia syndrome. *In* Metabolic Basis of Inherited Disease. 6th ed. C. R. Scriver, A. L. Beaudet, W. S. Sly, and D. Valle, editors. McGraw-Hill Book Co., New York. 45.

3. Wion, K. L., T. G. Kirchgessner, A. J. Lusis, M. C. Schotz, and R. M. Lawn. 1987. Human lipoprotein lipase complementary DNA sequence. *Science (Wash. DC).* 235:1638–1641.

4. Deeb, S. S., and R. Peng. 1989. Structure of the human lipoprotein lipase gene. *Biochemistry* 28:4131–4135.

5. Kirchgessner, T. G., J. C. Chuat, C. Heinzmann, J. Etienne, S. Guilhot, K. Svenson, D. Ameis, C. Pilon, L. d'Auriol, A. Andalibi, M. C. Scholz, F. Galibert, and A. J. Lusis. 1989. Organization of the human lipoprotein lipase gene family. *Proc. Natl. Acad. Sci. USA.* 86:9647–9651.

6. Sparkes, R. S., S. Zollman, I. Klisak, T. G. Kirchgessner, M. C. Komaromy, T. Mohandas, M. C. Schotz, and A. J. Lusis. 1987. Human genes involved in lipolysis of plasma lipoproteins: mapping of loci for lipoprotein lipase to 8p22 and hepatic lipase to 15q21. *Genomics.* 1:138–144.

7. Devlin, R. H., S. Deeb, J. Brunzell, and M. R. Hayden. 1990. Partial gene duplication involving exon-Alu interchange results in lipoprotein lipase deficiency. *Am. J. Hum. Genet.* 46:112–119.

8. Langlois, S., S. Deeb, J. D. Brunzell, J. J. P. Kastelein, and M. R. Hayden. 1989. A major insertion accounts for a significant proportion of mutations underlying human lipoprotein lipase deficiency. *Proc. Natl. Acad. Sci. USA.* 86:948–952.

9. Ameis, D., J. Kobayashi, R. C. Davis, O. Ben-Zeev, M. J. Malloy, J. P. Kane, G. Lee, H. Wong, R. J. Havel, and M. C. Schotz. 1991. Familial hyperchylomicronemia (Type I Hyperlipoproteinemia) due to a single missense mutation in the lipoprotein lipase gene. *J. Clin. Invest.* 87:1165–1170.

10. Beg, O. U., M. S. Meng, S. I. Skarlatos, L. Previator, J. D. Brunzell, H. B. Brewer, Jr., and S. S. Fojo. 1990. Lipoprotein lipase$_{\text{Bethesda}}$: a single amino acid substitution (Ala176 → Thr) leads to abnormal heparin binding and loss of enzymic activity. *Proc. Natl. Acad. Sci. USA.* 87:3474–3478.

11. Emi M., D. E. Wilson, P. H. Iverius, L. Wu, A. Hata, R. Hegele, R. R. Williams and J. M. Lalouel. 1990. Missense mutation (Gly → Glu^{188}) of human lipoprotein lipase imparting functional deficiency. *J. Biol. Chem.* 265:5910–5916.

12. Monsalve, M. V., H. Henderson, G. Roederer, P. Julien, S. Deeb, J. J. P. Kastelein, L. Peritz, R. Devlin, T. Bruin, M. R. V. Murthy, C. Gagne, J. Davignon, P. J. Lupien, J. D. Brunzell, and M. R. Hayden. 1990. A missense mutation at codon 188 of the human lipoprotein lipase gene is a frequent cause of lipoprotein lipase deficiency in persons of different ancestries. *J. Clin. Invest.* 86:728–734.

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

14. Henderson, H. E., R. Devlin, J. Peterson, J. D. Brunzell, and M. R. Hayden. 1990. A frameshift mutation in exon 3 of the lipoprotein lipase gene causes a premature stop codon and lipoprotein lipase deficiency. *Mol. Biol. Med.* 7:511–517.

15. Persson, B., G. Bengston-Olivecrona, S. Enerback, T. Olivecrona, and H. Jornvall. 1989. Structural features of lipoprotein lipase: lipase family relationships, binding interactions, non-equivalence of lipase cofactors, vitellogenic similarities and functional subdivision of lipoprotein lipase. *Eur. J. Biochem.* 179:39–45.

16. Datta, S., C. C. Luo, W. H. Li, P. von Turner, D. H. Ledbetter, M. A. Brown, S. H. Chen, S. W. Liu, and L. Chan. 1988. Human hepatic lipase: cloned cDNA sequence, restriction fragment length polymorphisms, chromosomal local-

ization and evolutionary relationships with lipoprotein lipase and pancreatic lipase. *J. Biol. Chem.* 263:1107–1110.

17. Semenkovich, C. F., S. H. Chen, M. Wims, C. C. Luo, W. H. Li, and L. Chan. 1989. Lipoprotein lipase and hepatic lipase mRNA tissue specific expression, developmental regulation and evolution. *J. Lipid. Res.* 30:423–431.

18. Iverius, P. H., and J. D. Brunzell. 1985. Human adipose tissue lipoprotein lipase: changes with feeding and relationship to post heparin plasma enzyme. *Am. J. Physiol.* 249:E107–E114.

19. Babirak, S. P., P. H. Iverius, W. Y. Fujimoto, and J. D. Brunzell. 1989. The detection and characterization of the heterozygous state for lipoprotein lipase deficiency. *Arteriosclerosis.* 9:326–334.

20. Ponez, M. D. Solowiejezyk, B. Harpel, Y. Morey, E. Scwartz, and S. Surrey. 1982. Construction of gene libraries from small amounts of peripheral blood: analysis of alpha-like globin genes. *Hemoglobin.* 6:27–36.

21. Fisher, K. L., G. A. Fitzgerald, and R. M. Lawn. 1987. Two polymorphisms in the human lipoprotein lipase (LPL) gene. *Nucleic Acids Res.* 15:7657.

22. Funke, H., J. Klug, and G. Assmar. 1987. HindIII RFLP in the lipoprotein lipase gene (LPL). *Nucleic Acids Res.* 15:9102.

23. Wang, X., R. Milne, Y. Marcel, and E. Rassalt. 1989. A vector that expresses secreted proteins on the cell surface. *DNA.* 8:753–758.

24. Zoller, M. J., and M. Smith. 1987. Oligonucleotide-directed mutagenesis: a simple method using two oligonucleotide primers and a single stranded DNA template. *Methods Enzymol.* 154:329–350.

25. Chomczynski, P., and N. Sacchi. 1987. Single step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal. Biochem.* 162:156–159.

26. Chait, A., P. H. Iverius, and J. D. Brunzell. 1982. Lipoprotein lipase secretion by human monocyte-derived macrophages. *J. Clin. Invest.* 69:490–493.

27. Winkler, F. K., A. D'Arcy, and W. Hunziker. 1990. Structure of human pancreatic lipase. *Nature (Lond.).* 343:771–774.

28. Dilella, A. G., J. Marvit, K. Brayton, and S. E. Woo. 1987. An amino acid substitution involved in phenylketonuria is in linkage disequilibrium with DNA haplotype 2. *Nature (Lond.).* 327:333–336.

29. Wainscoat, J. S., A. V. S. Hill, A. L. Boyce, J. Fluit, M. Hernandez, S. L. Thein, J. M. Old, J. R. Lynch, A. G. Falusi, D. J. Weatherall, and J. B. Clegg. 1986. Evolutionary relationships of human populations from an analysis of nuclear DNA polymorphisms. *Nature (Lond.).* 319:491–493.

30. Yoshioka, K., H. Furuya, H. Sasaki, M. J. M. Saraiva, P. P. Costa, and Y. Sakaki. 1989. Haplotype analysis of familial amyloidotic polyneuropathy: evidence for multiple origins of the val-met mutation most common to the disease. *Hum. Genet.* 82:9–13.

31. Wong, C., S. E. Antonarakis, S. C. Goff, S. H. Orkin, C. D. Boehm, and H. H. Kazazian, Jr. 1986. On the origin and spread of β-Thalassaemia: recurrent observation of four mutations in different ethnic groups. *Proc. Natl. Acad. Sci. USA.* 83:6529–7532.