Molecular basis of familial chylomicronemia: mutations in the lipoprotein lipase and apolipoprotein C-II genes

Manuel Reina,[1] John D. Brunzell, and Samir S. Deeb[2]

Department of Medicine, University of Washington, Seattle, WA 98195

Abstract The molecular basis of familial chylomicronemia (type I hyperlipoproteinemia), a rare autosomal recessive trait, was investigated in six unrelated individuals (five of Spanish descent and one of Northern European extraction). DNA amplification by polymerase chain reaction (PCR) followed by single strand conformation polymorphism (SSCP) analysis allowed rapid identification of the underlying mutations. Six different mutant alleles (three of which are previously undescribed) of the gene encoding lipoprotein lipase (LPL) were discovered in the five LPL-deficient patients. These included an 11 bp deletion in exon 2, and five missense mutations: Trp 86 Arg (exon 3), His 136 Arg (exon 4), Gly 188 Glu (exon 5), Ile 194 Thr (exon 5), and Ile 205 Ser (exon 5). The Trp 86 Arg mutation is the only known missense mutation in exon 3. The other missense mutations lie in the highly conserved "central homology region" in close proximity with the catalytic site of LPL. These and other previously reported missense mutations provide insight into structure/function relationships in the lipase family. The missense mutations point to the important role of particular highly conserved helices and beta-strands in proper folding of the LPL molecule, and of certain connecting loops in the catalytic process. A nonsense mutation (Arg 19 Term) in the gene encoding apolipoprotein C-II (apoC-II), the cofactor of LPL, was found to underlie chylomicronemia in the sixth patient who had normal LPL but was apoC-II-deficient.—**Reina, M., J. D. Brunzell, and S. S. Deeb.** Molecular basis of familial chylomicronemia: mutations in the lipoprotein lipase and apolipoprotein C-II genes. *J. Lipid Res.* 1992. **33:** 1823–1832.

Supplementary key words polymerase chain reaction • lipoprotein lipase • apoC-II • single strand conformation polymorphism

Lipoprotein lipase (LPL) plays a key role in the metabolism of lipoproteins by catalyzing the hydrolysis of triacylglycerols of very low density lipoproteins (VLDL) and chylomicrons, thereby delivering free fatty acids to various tissues. LPL is synthesized in parenchymal cells, including adipose and skeletal muscle, and is secreted and transported to the luminal surface of vascular endothelium where catalysis occurs. Active LPL is a homodimer that is bound to heparan sulfate chains on the surface of endothelial cells (1, 2). It requires apolipoprotein C-II, which is a component of substrate lipoprotein particles, as a cofactor (for review see 3). LPL has several functional domains which include a signal peptide; sites for binding to the substrate, to apoC-II, to heparin sulfate, and to another LPL subunit; and finally a catalytic site.

The cDNA sequence (4) and the genomic structure (5, 6) of the human LPL have been determined. The coding sequence is 1425 bp in length and translates into a mature protein of 448 residues preceded by a signal peptide of 27 amino acids. The LPL gene, located on the short arm of chromosome 8 (7), is composed of 10 exons spanning some 30 kb. Exon 10 encodes the relatively long (1.95 kb) untranslated 3' end of the mRNA.

LPL deficiency is a relatively rare autosomal recessive disorder occurring at a frequency of about one in a million in most parts of the world. The clinical syndrome, referred to as type I hyperlipoproteinemia (familial chylomicronemia), is characterized by massive hypertriglyceridemia, abdominal pain, pancreatitis, eruptive xanthomas, and hepatosplenomegaly, and is usually associated with LPL deficiency. Less commonly, deficiency in apolipoprotein C-II, the cofactor of LPL, has also been observed to be associated with the above syndrome (8, 9). Diagnosis is confirmed by measurement of enzymatic activity of LPL (triacylglyceroprotein acylhydrolase; E.C.3.1.1.34) in postheparin plasma or in adipose tissue (for review see 10). Heterozygotes who have one-half normal levels of LPL activity sometimes have mild hyperlipidemia (11, 12).

A number of mutations in the LPL gene were previously shown to underlie familial chylomicronemia. Two major

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Abbreviations: HTGL, hepatic triglyceride lipase; LPL, lipoprotein lipase; VLDL, very low density lipoprotein; PCR, polymerase chain reaction; FFA, free fatty acids; SSCP, single strand conformation polymorphism.

[1]On leave from the Department of Biochemistry and Physiology, University of Barcelona, Spain.

[2]To whom correspondence should be addressed at: Division of Medical Genetics, RG-25, Department of Medicine, University of Washington, Seattle, WA 98195.

gene rearrangements in a single LPL-deficient subject, one a deletion and the other a tandem duplication, together led to undetectable levels of LPL activity and mass in plasma (13, 14). A nonsense mutation (at amino acid residue 106 in exon 3) resulting in LPL deficiency was identified in a patient of German-Polish ancestry (15). A missense mutation (Gly 188 Glu in exon 5) was found in LPL-deficient probands of different ancestries, and apparently due to a founder effect, became frequent among French Canadians (16–18). Other missense mutants in the LPL gene have been noted: Gly142Glu (19), Pro207Leu (20, 21), Pro157Arg (22), Ala176Thr (23), Ile194Thr (24, 25), Asp156Asn/Gly, Cys216Ser (26), and Arg243His (25).

Examination of the DNA of subjects with severe fasting chylomicronemia will identify mutant alleles of either the LPL or apoC-II genes. The position and nature of amino acid substitutions resulting from these and other previously reported mutations will reveal likely structure function/relationships. In the present report, among six unrelated individuals with familial chylomicronemia, five were found to be deficient in LPL and the sixth was deficient in apoC-II. Six mutant alleles of the LPL gene were found in the LPL-deficient probands and one nonsense mutant allele of the apoC-II gene was found in the apoC-II deficient patient.

Patients

Three of the probands with type I hyperlipoproteinemia, J. D., A. Q., and E. G. S. (Table 1) under the care of Drs. M. A. Lasuncion and E. Herrera at Hospital Ramon y Cajal, Madrid, Spain, a fourth, E. V., a patient of Dr. J. Amat, Hospital de Sant Joan de Deu, Barcelona, Spain, and a fifth, G. P., a 19-year-old female from the Seattle area, were found to have deficiency in LPL activity in postheparin plasma. There was no known history of consanguinity in their families through the generation of grandparents of the probands.

J. D. was hospitalized at age 25 with hepatosplenomegaly and massive hypertriglyceridemia. A. Q., a 5-year-old male was noted shortly after birth to have hypertriglyceridemia that responded to a very low fat diet. E. G. S., a 10-year-old male, was noted at birth to have marked hypertriglyceridemia. E. V., a 16-year-old female, was first hospitalized at 4 years of age with pancreatitis and massive hypertriglyceridemia. She was again hospitalized at age 15 with severe pancreatitis and massive hypertriglyceridemia. Her triglyceride levels were reduced from 2,900 to 1,400 mg/dl after she was placed on a very low fat diet. J. C., a 6-year-old female, was diagnosed shortly after birth to have plasma triglyceride levels of 1,000 mg/dl, which were reduced to 500 mg/dl after she was placed on a fat-free diet. She had normal levels of postheparin plasma LPL, but was shown to be deficient in apolipoprotein C-II, the cofactor of LPL.

Informed consent was obtained from the subjects, and all procedures were subjected to prior approval by the University's Human Subjects Review Committee.

MATERIALS AND METHODS

Blood collection and intravenous heparin bolus

After an overnight fast 20 ml of blood was collected in EDTA (1 mg/ml). Ten minutes after a heparin bolus of 60 units/kg body weight, 10 ml blood was collected in EDTA tubes for measurement of lipase activities. Plasma was separated and stored at −70°C. The cell pellet was stored also at −70°C and was used for white cell DNA isolation.

Lipoprotein lipase and hepatic triglyceride lipase (HTGL) activities

Total lipolytic activity was measured using tri-[1-^{14}C]oleoylglycerol (Amersham, Arlington Heights, IL) and lecithin emulsion as a substrate for 60 min at 37°C (27). The liberated free fatty acids (FFA) were extracted and counted. Activity is expressed as nanomoles FFA released/min per ml of plasma. To determine HTGL and LPL, LPL was selectively blocked by the use of a specific monoclonal antibody (12). This monoclonal antibody binds and completely blocks activity of human, bovine, and rabbit LPL (12). HTGL activity was determined as the lipolytic activity remaining after incubation with this antibody. LPL activity was then calculated by subtracting HTGL activity from total postheparin lipolytic activity. On each assay occasion, bovine milk lipase and human postheparin plasma standards were included to correct for interassay variation.

A functional assay for the presence of apolipoprotein C-II (modified from 28) involved the activation of partially purified bovine milk LPL by the patient's serum. This lipolytic assay was performed on two control subjects and the six patients with severe chylomicronemia.

LPL mass ELISA

LPL mass was measured by a sandwich enzyme-linked immunosorbent assay (ELISA) using the monoclonal antibody 5D2 as previously described (12). Detection of mass by this assay indicates the presence of the naturally occurring dimers of this enzyme.

DNA isolation and Southern blot analysis

The DNA was isolated from frozen white blood cells using an Applied Biosystems Model 3490 DNA extractor (Applied Biosystems, Foster City, CA), according to the manufacturer's instructions. Southern blot analysis of genomic DNA after digestion with either PvuII or StuI and probing with probe HLPL26 of LPL cDNA was as described (12).

Sequence analysis by single strand conformation polymorphism (SSCP)

Detection of mutant alleles of the LPL gene was accomplished by PCR amplification of all coding exons using the primers and conditions described previously (17) and genomic DNA as template. Radiolabeling of the PCR products and separation of single strands on nondenaturing polyacrylamide gels were performed essentially as described (29–31). The PCR reaction mixture contained, in a volume of 10 μl: reaction buffer (Perkin-Elmer-Cetus, Norwalk, CT) 100 ng of genomic DNA, 5 pmol of each primer, 0.25 units of enzyme, 10 μCi of α-^{32}P-dCTP (3,000 Ci/mM), and 0.625 pmol of each of the four deoxytriphosphates. An aliquot (2 μl) of the PCR reaction product was diluted 50-fold with 0.1% sodium dodecyl sulfate–10 mM EDTA. Three μl of the diluted DNA was then added to an equal volume of loading dye mixture (95% formamide–20 mM EDTA–0.05% bromophenol blue–0.05% xylene cyanol), heat-denatured for 3 min in a boiling water bath, fast-cooled on ice, and loaded (1 μl) onto a 5% nondenaturing polyacrylamide gel containing 10% glycerol. Electrophoresis (40 W for approximately 5 h) in 90 mM Tris-borate–2 mM EDTA (TBE) was performed at room temperature (gel plate temperature of 27–30°C) using a fan for cooling. The gel was transferred to Whatman 3 MM paper, dried, and autoradiographed at −70°C with an intensifying screen for 12–24 h.

PCR amplification and direct sequencing

Genomic DNA was amplified enzymatically (32) on a Thermal Cycler (Perkin-Elmer-Cetus). The reaction mixture contained, in a total volume of 10 μl reaction buffer (Perkin-Elmer-Cetus), 5 pmol of each primer (see Table 1), 2 pmol of each of the four deoxytrinucleotides, 100 ng of genomic DNA, and 0.25 units of Taq polymerase (Perkin-Elmer-Cetus). Oligonucleotides were synthesized on an Applied Biosystems 380 DNA Synthesizer (Applied Biosystems Inc.). The sequence of oligonucleotide primers for the nine coding exons of LPL, and the conditions for amplification were previously described (17). An apoC-II genomic fragment (864 bp) extending from the 3' end of intron 1 to 41 bp downstream of the translation termination codon was PCR-amplified using the following oligonucleotide primers:

5' AGTCAGCCTGCCACATGACACCC 3'
5' GGGACTCTCCCCTTGTCCACTGAT 3'

Thirty-five cycles of 1 min at 94°C, 1 min at 68°C, and 1 min at 72°C were used. To obtain ssDNA templates for sequencing, 1 μl of the dsDNA PCR product was used as template in a second amplification reaction containing 60 pmol of one of the primers, 0.6 pmol of the second primer, buffer, 20 pmol of each of the four deoxytrinucleotides, and 2.5 units of Taq polymerase, in a total volume of 100 μl. The single-stranded PCR product was purified and concentrated by filtration through a Centricon 100 filter (Amicon Corp. Danvers, MA) according to the protocol supplied by the manufacturer. Sequencing was performed using the dideoxy chain termination method (33) using [α-^{32}P]-dCTP (3,000 Ci/mmol; New England Nuclear, Boston, MA) and the Sequenase DNA sequencing kit (U.S. Biochemical Corp., Cleveland, OH). The sequencing primers for LPL were those used for PCR amplification and those for apoC-II included the internal primers:

5' CCTGCTGCAGCCCCACG 3'
5' ACCATCTGTGCTTTCTCC 3'
5' TGGGTCCTGGATGCACT 3'
5' GCTTGGAGCTCATTCCCCTCCC 3'

Both strands of each DNA fragment were sequenced.

RESULTS

LPL activity and mass in pre- and post-heparin plasma

LPL and hepatic triglyceride lipase (HTGL) activities were measured in postheparin plasma and LPL mass was measured in pre- and postheparin plasma samples from each proband. In addition, each patient's plasma was assayed for the ability of apoC-II, the cofactor of LPL, to activate purified bovine milk LPL. The results are shown in **Table 1.** All probands had normal plasma apoC-II activity except J. C. who had <10% of normal. Of the LPL-deficient patients, only J. D. showed a substantial increase in detectable (using the monoclonal antibody 5D2) LPL mass after intravenous heparin. As 5D2 detects homodimer complexes of LPL, it is possible that the inactive proteins encoded by the other missense mutant alleles do not form stable dimers.

Analysis of the LPL gene

The LPL gene of each LPL-deficient patient was examined for major structural rearrangements by Southern blot analysis after digestion of genomic DNA with either PvuII or StuI. No abnormal size restriction fragments resulting from these two enzymes were detected (data not shown), indicating that no major (>100 bp) rearrangements in the LPL gene were responsible for the deficiencies.

Detection of LPL mutant alleles by PCR/SSCP analysis

Exons 1–9 of the LPL gene were individually amplified by PCR and subjected to SSCP analysis in order to determine the location of DNA sequence variants. Subjects A. Q., E. V., and J. D. were found to be homozygous for a DNA sequence variant in exon 5; subjects E. V. and J. D. were also heterozygous for variants in exon 8; E. G. S. was heterozy-

TABLE 1. Triglyceride levels, lipase activity, and LPL mass in plasma of LPL-deficient subjects

Subject	TG	Activity[a]		LPL Mass		ApoC-II Activity[b]	Mutation
		HTGL	LPL	Pre	Post		
	mg/dl			*ng/ml*			
E.V.	2,900	142	0	0	0	11.0	$Ile^{205} \rightarrow Ser$
J.D.	2,500	158	0	0	292	11.3	$Ile^{194} \rightarrow Thr$
A.Q.	5,000	188	0	21	31	12.6	$Gly^{188} \rightarrow Glu$
E.G.S.	4,260	177	1	40	42	16.6	$Gly^{188} \rightarrow Glu$/11 bp deletion
G.P.	1,800	89	6	42	77	21.6	$Trp^{86} \rightarrow Arg/His^{136} \rightarrow Arg$
J.C.	>1,000	286	183	18	374	0.42	$Arg^{4} \rightarrow Term$(apoC-II)
NL_1		337	389	0	290	19.7	
NL_2		48	142	8	182	22.4	

The 95% confidence interval for LPL activity is 104–336 nmol free fatty acids (FFA)/min · ml (28) and is similar for males and females. The control range of hepatic triglyceride lipase activity (HTGL) for males and preadolescent females is 102–236 nmol FFA/min · ml (n = 17 normal adult males). The control range of HTGL activity for adult females is 44–167 nmol FFA/min · ml (n = 17 normal adult females).

[a]Values given as nmol FFA released/min · ml plasma.

[b]Values given as nmol/min · ml.

gous for exons 2, 5, and 8 variants; G. P. was heterozygous for variants in exons 3 and 4. Autoradiographs of SSCP gels used to separate single strands of amplified exons 4 and 5 of the LPL-deficient as well as normal subjects is shown in **Fig. 1.** All coding exons of the LPL gene of deficient subjects were then sequenced in order to determine the precise mutational sites and verify the results of analysis by SSCP.

Sequence of mutant alleles

Nucleotide sequence of both strands of the nine coding exons and the exon-intron junctions of the LPL genes of each LPL-deficient proband was determined by direct sequencing of PCR-amplified ssDNA using genomic DNA as template. The following variants in the coding sequence of the LPL gene were discovered. The variant in exon 8 of J. D., E. V., and E. G. S. was shown to be due to a silent polymorphism (C→A) in the third letter of codon 388. This polymorphism, which neither creates nor abolishes a restriction site, is a potentially useful marker for the LPL gene.

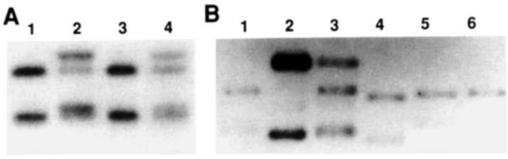

Fig. 1. Detection of LPL mutations by SSCP. Autoradiograph of single stranded DNA generated by PCR amplification, denaturation, and separation on a 4.5% nondenaturing acrylamide gel as described under Methods. A: DNA bands corresponding to exon 4 of a normal subject (lane 1), proband G.P. (lane 2), and her father (lane 4) who are heterozygous for the His136Arg mutation, and her noncarrier sister (lane 3). B: Fragments corresponding to exon 5 of two normal control subjects (lanes 1 and 6), subject A.Q. who was found to be homozygous for the residue 188 (lane 2), subject E.G.S., heterozygous for the residue 188 mutation (lane 3), subject J. D., homozygous for the residue 194 mutation (lane 4), subject E. V., homozygous for the residue 205 mutation (lane 5).

Subject E. V. was found to be homozygous for a T→G transversion in exon 5 that led to the substitution of a Ser for Ile at position 205 of the mature protein (**Fig. 2A**). Sequencing of exon 5 of two other family members, the father and a brother (both normal with respect to chylomicronemia), showed the former to be a carrier and the latter a noncarrier for the residue 205 mutation.

J. D. was shown to be homozygous for a T→C transition also in exon 5, resulting in the substitution of a Thr for Ile at position 194. The parents were found by sequence analysis to be heterozygotes, while an affected sister was shown to be homozygous for the same mutation.

A. Q. was homozygous for the previously described C→T transition leading to the substitution of a Glu for Gly at position 188 (16, 17).

E. G. S. was found to be a compound heterozygote. One of his alleles had an 11 bp deletion in exon 2 (Fig. 2C) and the other had the same residue 188 mutation found in proband A. Q. The 11 bp deletion is predicted to shift the reading frame of the protein, to introduce a termination codon (UGA) at residue 23, and lead to formation of a truncated mature protein composed of only 19 residues.

Proband G. P. was found to be a compound heterozygote for a C→T transition in exon 3 resulting in the substitution of Arg for Trp at position 86 (Fig. 2B), and for an A→G transition in exon 4 resulting in the substitution of Arg for His at position 136 of the mature protein (Fig. 2D). SSCP analysis showed G. P.'s unaffected mother to be heterozygous for the His 136 Arg, her unaffected father to be heterozygous for the Trp 86 Arg mutation, and her unaffected sister to be homozygous for the normal allele

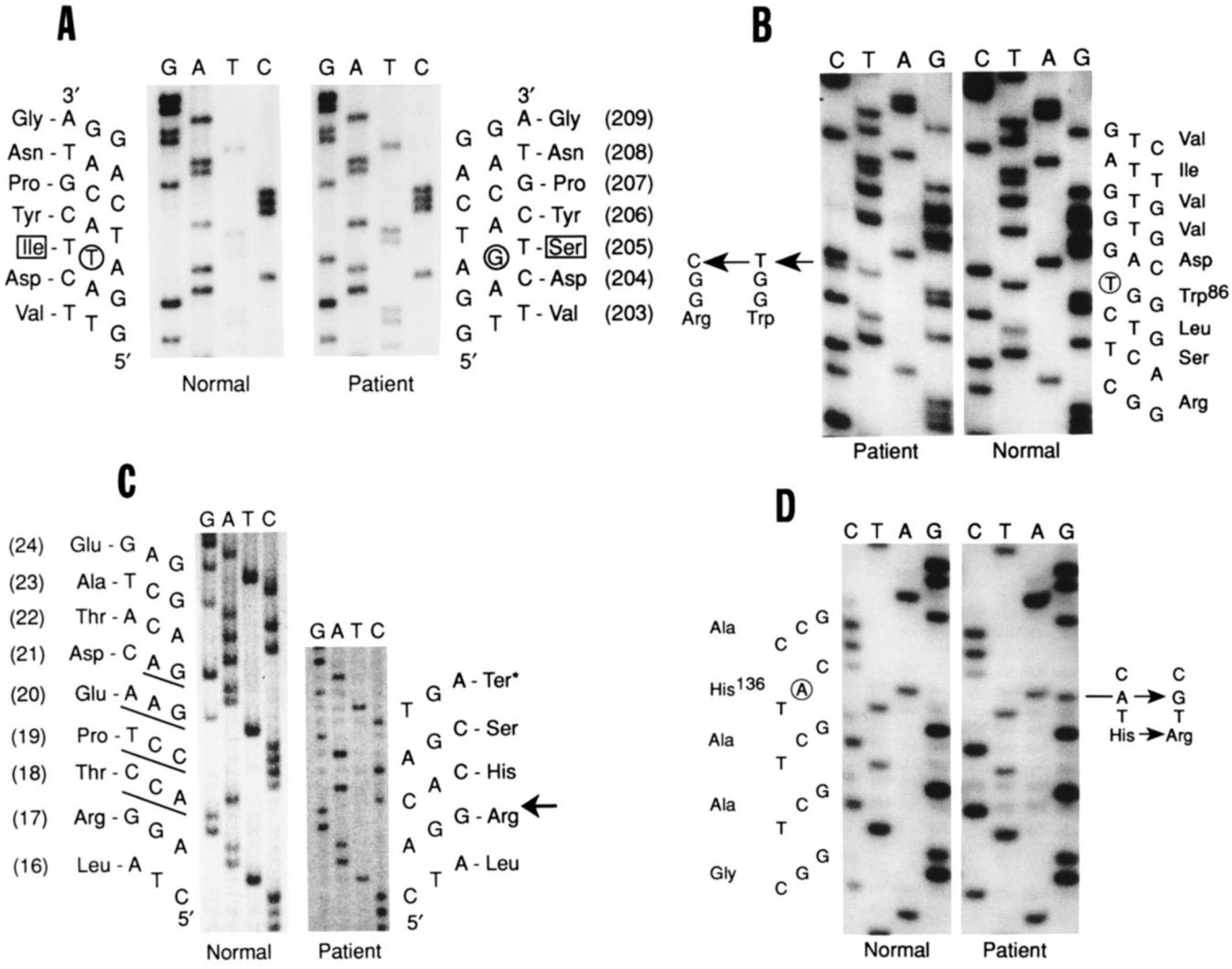

Fig. 2. Differences in sequence between normal and mutant LPL alleles. Autoradiographs of sequencing gels of PCR-amplified DNA fragments from patient and normal controls in the region of the mutations. The nucleotide and amino acid substitutions are encircled and boxed respectively. A) The residue 205 mutation. B) The residue 86 mutation. C) The 11 bp deletion in exon 2. The underlined nucleotides are deleted in the patient at the site indicated with an arrow. The termination codon is indicated with an asterisk. D) the residue 136 mutation.

(Fig. 1A). These two mutations were not detected by SSCP analysis of exons 3 and 4 in 67 control subjects.

The sequence variants outlined above were the only changes observed in the coding and exon/intron junction sequences of the LPL gene of deficient subjects. Thus, results of SSCP analysis were in complete agreement with sequence data.

Analysis of the apoC-II gene

The sequence of exons 2–4 and intron/exon junctions of the apoC-II gene of patient J. C., who was found to be apoC-II-deficient, was determined by PCR amplification and direct sequencing of both strands of DNA as described in Materials and Methods. This patient was found to be homozygous for a C→T transition that transforms codon-19 from CGA (ARG) to a chain termination codon (**Fig. 3**). This base change results in the creation of a recognition site for the restriction endonuclease Nla III. The parents of the proband were each shown to be heterozygotes for this mutation.

DISCUSSION

In this paper, we describe a rapid and reliable strategy for the identification of DNA sequence variants of the LPL gene. It consists of amplification by PCR followed by SSCP analysis and direct sequencing of the abnormal

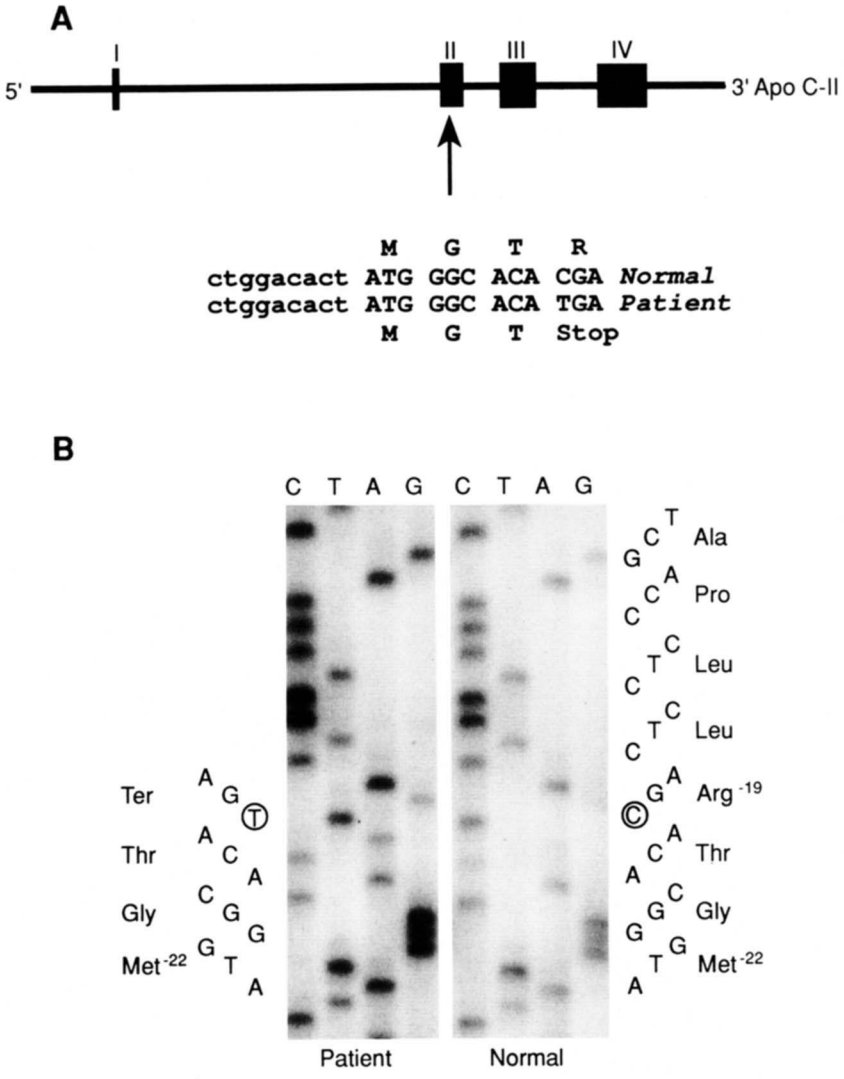

Fig. 3. Sequence of the apoC-II$_{Barcelona}$ mutant allele. Panel A: structure of the apoC-II gene showing sequence and location of the nonsense mutation. Panel B: autoradiograph showing the sequence (sense strand) of the wild type and mutant alleles.

fragments. Five missense and one deletion mutant alleles were identified in the human LPL gene in five unrelated patients with lipoprotein lipase deficiency. Subject E. V. was homozygous for a new mutation: a T→G transversion in exon 5 that led to the substitution of Ser for Ile at position 205 of the mature protein. Surprisingly, this patient had no detectable LPL immunoreactive mass in either pre- or postheparin plasma. The one previously described patient with no postheparin immunoreactive mass was a compound heterozygote for a deletion and a tandem duplication (14, 17). As the LPL mass assay used can detect only homodimer complexes of LPL, it is possible that the Ile→Ser mutation results in impairment of formation of such complexes. Alternatively, the mutant protein could have been rapidly degraded as a result of having an abnormal conformation, and thus have been undetectable by the ELISA.

Subject J. D. was homozygous for a T→C transition in exon 5 leading to a substitution of Thr for Ile at position 194. This alteration abolished catalytic activity without diminishing affinity of LPL to glycosaminoglycans on the surface of endothelial cells (Class II defect). This mutation was recently found in three unrelated patients with LPL deficiency from South Africa with French and German and Dutch ancestries (24) and in a Caucasian from the United States (25).

Two of the four LPL-deficient patients carried the Glu for Gly substitution at residue 188. This particular mutation has been previously found in 29 alleles of 17 unrelated lipoprotein lipase-deficient individuals of British,

French, Dutch, German, and East Indian decent (16–18). Most patients with the residue 188 mutation have the appearance of inactive LPL protein in plasma after intravenous heparin (16, 17) implying normal endothelial cell binding of LPL. However, one patient homozygous for this mutation had no incremental increase in protein after intravenous heparin (17), and another had a mildly decreased affinity for heparin-Sepharose in vitro (16) as did the patient with the residue 176 mutation (23). The putative heparin-binding domain (residues 290–300), which is rich in basic residues, is distant from this region (34). The above residues in exon 5 may not be at all involved in heparin binding per se, but may lead to conformational changes in the structure of the protein that affect the heparin binding site.

Subject E. G. S. was shown to be a compound heterozygote for the Gly→Glu mutation at residue 188 and a previously undescribed 11 bp deletion in exon 2. This deletion introduces an in-frame termination codon at residue 23, and predicted to result in the synthesis of a truncated mature protein expected to be 19 residues in length.

Subject G. P., who is a compound heterozygote for the previously undescribed mutations at positions 86 and 136, has detectable levels of LPL immunoreactive mass in both pre- and postheparin plasma with higher levels in postheparin plasma, indicating that at least one of the alleles encodes an inactive protein that is released into plasma with heparin.

Many of the missense mutants that result in severely diminished LPL activity have so far been found to lie within the so called central homology region (residues 120 to 216) of the protein (34) encoded by exons 4, 5, and 6 (**Fig. 4**). This region is highly conserved in four members of the lipase gene family: LPL, hepatic lipase, pancreatic lipase, and the *Drosophila* yolk protein 1 (6). Furthermore, this domain includes the proposed interfacial "recognition site" and the Ser^{132}, Asp^{156} of the catalytic triad (35).

The three-dimensional X-ray crystallographic structure of human pancreatic lipase was recently determined at 2.3 A resolution (35). Sequence homologies with respect to secondary structure motifs among human pancreatic triglyceride lipase, LPL, and hepatic lipase (36, 37) indicate that the proposed three-dimensional model of pancreatic lipase is quite likely to be similar to those of LPL and hepatic lipase, particularly at the postulated N-terminal lipolytic domain. Maximum homology was found in the strands that make up the central hydrophobic beta-sheet that forms the core pocket which contains the catalytic triad, those in helices 3, 4, and 7 which pack against the central beta-sheet, and in four loops located around the active center (37).

The positions on human pancreatic lipase that correspond to the currently known missense mutations in LPL are shown in **Fig. 5.** The majority lie either in the strands of the beta-pleated sheet or the loops neighboring the catalytic site. These mutations may therefore either directly affect residues of the catalytic triad or play an important role in polypeptide folding to establish the proper conformation of the hydrophobic core around the active site. For example, both of the Gly188Glu and Ile194Thr mutations are located in the loop that links strands 9 to 10. Because this loop lies in the neighborhood of the catalytic site, it has been proposed that these two mutations directly influence catalysis (37). On the other hand, the mutations at positions 86, 136, and 205 most likely interfere with folding of conserved domains to form the central beta-sheet. The Trp86Arg mutation lies at the C-terminal end of beta-sheet 6 and is in the vicinity of helix 3. Helix 3 packs against helix 4 and both pack against the central beta-sheet (35, 37). The introduction of a positive charge

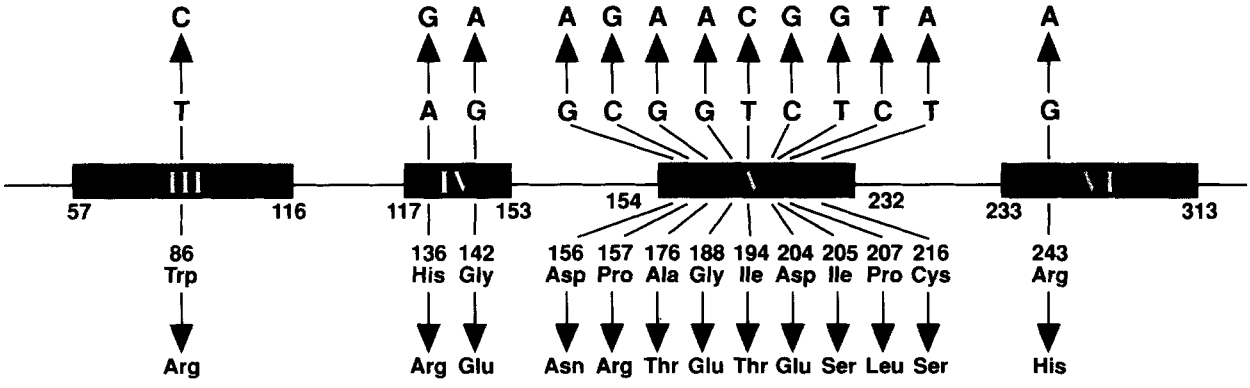

Fig. 4. The location of known missense mutations in the human LPL gene. Only three of the 10 exons are shown. Diagram is not to scale. Arrows indicate changes from wild type to mutant sequences. No missense mutations associated with lipoprotein lipase deficiency have been reported in other exons.

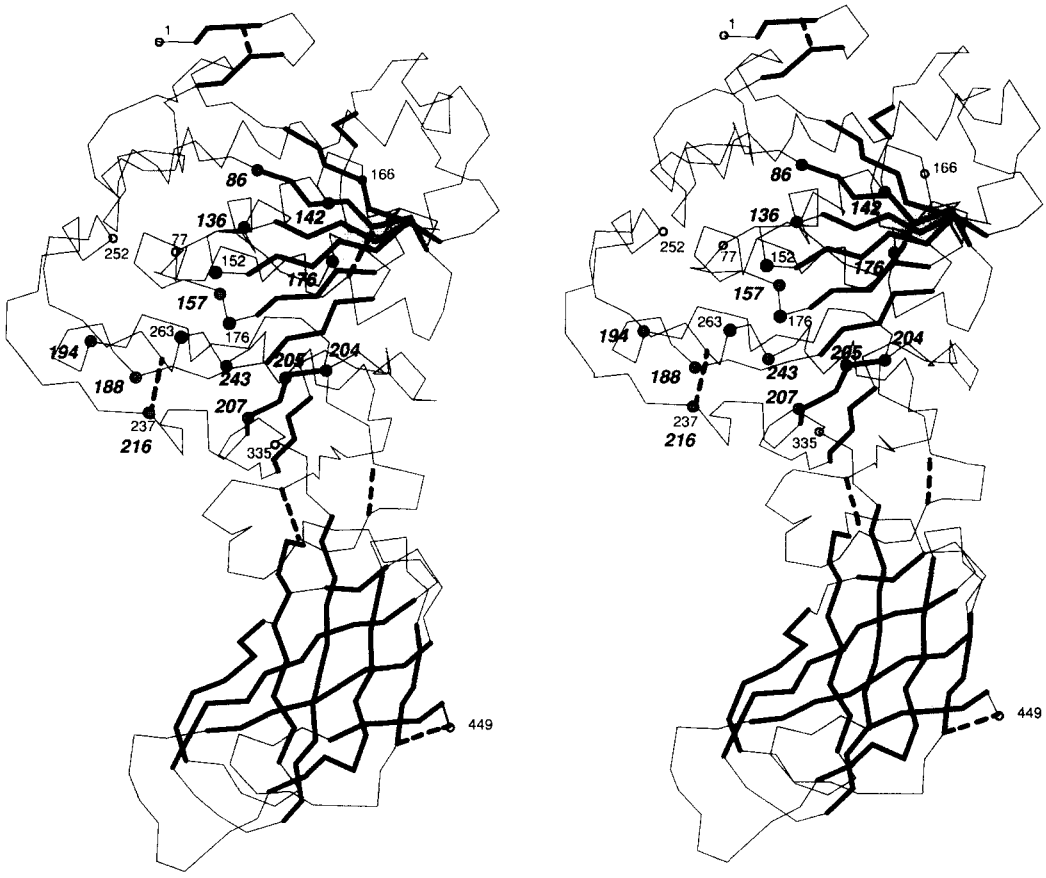

Fig. 5. Location of residues of human pancreatic lipase that correspond to LPL missense mutations. Shown is a stereo diagram of human pancreatic lipase adapted from (35) in which are indicated the positions of the Ser^{152}, Asp^{176}, and His^{263} of the catalytic triad (closed circles) as well as few other selected residues (open circles). The positions of residues that correspond to LPL mutations are indicated by hatched circles, next to which are LPL residue numbers (bold). Dashed lines represent disulfide linkages.

at position 86 could result in unfolding of the protein.

The His136Arg mutation lies in helix 4 that constitutes part of the highly conserved beta-epsilon serine-alpha folding motif of the family of serine lipases and esterases (37). Another mutation was reported in helix 4 which replaces a highly conserved Gly at position 142 with the polar and bulky Glu. These mutations are indicative of the importance of proper folding of this domain in the function of LPL.

Finally, the substitution of Ser for the highly conserved Ile at position 205 that lies in strand 10 could disrupt hydrophobic interaction of this strand with strand 11. Two additional mutations have been described in this strand, namely Pro207Leu (20) and Asp204Glu (38), pointing to the important contribution of this strand to conformation of the central catalytic domain.

Proband J. C., the apoC-II patient who had normal LPL and massive chylomicronemia, was shown to be homozygous for a previously undescribed missense mutation Arg^{3}→Term. Eight other mutations in the apoC-II gene have previously been shown to be associated with familial apoC-II deficiency, marked chylomicronemia, and chronic pancreatitis (8, 9). It is interesting to note that only one of the known mutations in apoC-II is a missense (initiation codon Met→Val). All the rest result in the synthesis of a truncated protein or no protein at all. This suggests that most missense mutations in apoC-II do not result in severe perturbation of function and therefore do not cause chylomicronemia.

This work was supported by U.S. Public Health Service Grant HL-30086 (S. D.) and DK 02456 (J. B.). M. Reina is a postdoctoral fellow of the Ministerio de Educacion y Ciencia of Spain, and is supported by an FISS grant 89/0391. We thank Reiling Peng, Steve Hashimoto, and Martha Kimura for excellent technical assistance.

Manuscript received 27 March 1992 and in revised form 26 June 1992.

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