A novel amino acid substitution (His183→Gln) in exon 5 of the lipoprotein lipase gene results in loss of catalytic activity: phenotypic expression of the mutant gene in a heterozygous state

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Abstract We have identified a hitherto unrecognized mutation of the lipoprotein lipase gene (LPL) in a Finnish family with Russian and Swiss ancestors. A single base pair substitution of a guanine for cytosine in codon 183 of exon 5 of the LPL gene results in a change of histidine to glutamine in the mature enzyme protein. Expression of a mutant cDNA construct in COS cells resulted in secretion of inactive LPL enzyme protein confirming the functional significance of the mutation. The proband, a 50-year-old female and her two daughters were all heterozygous for the His183→Gln mutation. Clinically, the proband was characterized by variable and occasionally severe hypertriglyceridemia, obesity, hypertension, coronary heart disease and non-insulin-dependent diabetes mellitus. The daughters, aged 24 and 19 years, were also obese but had milder hypertriglyceridemia. In conclusion, we have identified a novel LPL mutation that results in the synthesis of an inactive enzyme protein. Although the assessment of a causative link between the mutation and hyperlipidemia awaits further studies, our data suggest that heterozygosity for a functional defect of LPL should be considered in patients presenting with the metabolic dyslipidemic syndrome, "syndrome-X."—**Tenkanen, H., M-R. Taskinen, M. Antikainen, I. Ulmanen, K. Kontula, and C. Ehnholm.** A novel amino acid substitution (His183→Gln) in exon 5 of the lipoprotein lipase gene results in loss of catalytic activity: phenotypic expression of the mutant gene in a heterozygous state. *J. Lipid Res.* 1994. **35:** 220–228.

Supplementary key words lipoprotein lipase • point mutation • dyslipidemia • non-insulin-dependent diabetes mellitus • metabolic syndrome

Lipoprotein lipase (LPL: EC 3.1.1.34) plays a central role in triglyceride metabolism regulating the catabolism of triglyceride-rich lipoprotein particles (1–3). Hypertriglyceridemias constitute a heterogenous group of lipid disorders that can be due to primary metabolic abnormalities or may manifest secondarily due to a variety of other diseases. The best documented example of primary hypertriglyceridemia is Type I hyperlipoproteinemia, which occurs as a result of functional LPL deficiency. Familial LPL deficiency is inherited as an autosomal recessive trait with an estimated frequency of less than one per million (4). The plasma lipoprotein pattern is characterized by fasting chylomicronemia, normal or marginally increased VLDL levels, and decreased low density (LDL) and high density (HDL) lipoprotein concentrations. First-degree relatives of probands with Type I hyperlipoproteinemia, obligate carriers of a mutated LPL gene, have been reported to have variable lipoprotein patterns and variable levels of measurable LPL activity in serum (5, 6).

The human LPL gene has been assigned to chromosome 8p22 (7). It extends over 30 kb, and consists of 10 exons that code for a 475-amino acid protein including a 27-amino acid signal peptide (8–10). Exon 10 specifies the 3' noncoding region of the LPL mRNA. A variety of mutations of the LPL gene have been reported in patients with primary LPL deficiency (reviewed in ref. 11). These include missense and nonsense single nucleotide alterations, insertions, deletions as well as splice site mutations.

In this paper we report a previously unknown missense mutation of the LPL gene in a patient with transient severe hypertriglyceridemia, and demonstrate by in vitro expression of the mutant cDNA that this nucleotide change results in a nonfunctional LPL enzyme protein.

Abbreviations: PH-LPL, postheparin lipoprotein lipase; PH-HL, postheparin hepatic lipase; PCR, polymerase chain reaction; SSCP, single-strand conformational polymorphism; NIDDM, non-insulin-dependent diabetes mellitus; LDL, low density lipoprotein; HDL, high density lipoprotein.

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MATERIALS AND METHODS

Subjects

The proband, a female text operator, was born 1939 in Kangasniemi, Finland. Her mother was of Russian descent, and died at the age of 68 yr in an acute heart attack. The proband's father had Swiss ancestry and died at the age of 69 yr, probably of an ischemic cerebrovascular event. The husband of the proband is healthy and non-obese. The couple had two daughters, born in 1965 and 1970.

In March 1977 the patient was referred to the Helsinki University Central Hospital because of eruptive skin xanthomatosis. Her weight was 111 kg (relative body mass index, BMI, 38.4 kg/m²) and blood pressure 160/100 mmHg. Serum cholesterol was 19.6 mmol/l, triglycerides 72.5 mmol/l, and fasting blood glucose 14.0 mmol/l. Plasma postheparin lipoprotein lipase activity was 10.70 μmol FFA · $ml^{-1} \cdot h^{-1}$ and postheparin hepatic lipase activity was 27.2 μmol FFA · $ml^{-1} \cdot h^{-1}$. Analysis of a biopsy sample revealed a low lipoprotein lipase activity in skeletal muscle (0.268 μmol FFA · $g^{-1} \cdot h^{-1}$, normal 0.62 ± 0.06 μmol FFA · $g^{-1} \cdot h^{-1}$ (3)). A low caloric diet was instituted, which resulted in a reduction of serum triglyceride, cholesterol, and glucose concentrations and disappearance of skin eruptions within a few months. From 1977 to 1979 the patient weighed about 84–89 kg, serum cholesterol was 5–6 mmol/l, triglycerides 0.8–1.6 mmol/l, and fasting glucose 5.1–5.8 mmol/l.

From 1980 to 1984 her weight gradually increased from 89 to 101 kg. Serum triglyceride level rose up to 8 mmol/l and the glycemic control deteriorated. In March 1987 the patient suffered an acute anteroseptal myocardial infarction. Coronary angiography revealed narrowings of 90–99% in left descending and right coronary arteries, respectively. A three-vessel bypass-operation was performed in August 1988. The patient was put on a low-calorie, low-fat diet and gemfibrozil was instituted. Data of laboratory examinations in 1989 are summarized in **Table 1.**

Even though she maintained her body weight around 84 kg, the metabolic parameters gradually deteriorated in 1990: serum triglycerides ranged from 19 to 38 mmol/l and cholesterol from 10 to 15.5 mmol/l. The fasting serum C-peptide concentration was 0.34 nmol/l in July 1991 (reference values for healthy controls, 0.33–0.67 nmol/l). Insulin treatment was initiated in July 1991. The patient developed claudication, and angiographic examination disclosed widespread atherosclerotic changes in abdominal aorta as well as in iliac and femoral arteries down to the popliteal level. A reconstructive operation was carried out in October 1991, with a placement of an Y-type vascular prosthesis. In 1992, the patient was symptomless and her body weight was 88 kg. She received insulin, clofibrate, guar, metoprolol, digoxin, furosemide, and aspirin. Serum cholesterol was 4.5 mmol/l, HDL-cholesterol 0.76 mmol/l, triglycerides 3.1 mmol/l, and fasting glucose levels ranged from 10.7 to 13.4 mmol/l.

Daughter 1 of the proband was born in 1965. In 1977, serum cholesterol was 6.8 mmol/l and triglycerides 1.70 mmol/l. Re-examinations were conducted in 1989 (Table 1). She had no hypertension. Due to obesity and hyperlipidemia, a program of dietary advice and weight reduction was instituted. She lost 11 kg of weight but her serum triglyceride concentration remained elevated. Clofibrate was started in September 1991, with a significant response: in March 1992 the concentrations of serum triglycerides, total cholesterol, and HDL-cholesterol were 2.2 mmol/l, 6.0 mmol/l, and 1.16 mmol/l, respectively.

Daughter 2 of the proband was born in 1970. Data from

TABLE 1. Clinical and laboratory data for the family members (recorded in 1989)

Variable	Mother	Daughter 1	Daughter 2
Age	50	24	19
BMI (kg/m²)	32.5	29.7	30.8
Cholesterol (mmol/l)	5.7	6.1	5.0
Triglycerides (mmol/l)	6.90	5.28	1.68
HDL-cholesterol (mmol/l)	0.65	0.72	0.99
Fasting glucose (mmol/l)	10.0	5.8	4.3[a]
PH-LPL[b] (μmol FFA · $ml^{-1} \cdot h^{-1}$)	10.63	22.81	23.58
LPL mass[c] (ng/ml)	414	342	—[d]
PH-HL[e] (μmol FFA · $ml^{-1} \cdot h^{-1}$)	39.04	32.49	29.09
ApoE phenotype	3/3	3/3	3/3

Abbreviations: apoE, apolipoprotein E; PH-LPL, postheparin lipoprotein lipase; PH-HL, postheparin hepatic lipase; FFA, free fatty acids.

[a] Measured during pregnancy in 1992.

[b] Normal, 22.8 ± 1.1 μmol FFA · $ml^{-1} \cdot h^{-1}$ (3).

[c] Measured 1992, normal, 269 ± 48 ng/ml (25).

[d] Not measured due to pregnancy.

[e] Normal, 19.5 ± 1.0 μmol FFA · $ml^{-1} \cdot h^{-1}$ (3).

clinical and laboratory examinations in 1989 are presented in Table 1. The daughter was pregnant at the time of this study. Serum lipids were measured on the 36 week of gestation and were as follows: cholesterol 5.9 mmol/l, HDL-cholesterol 1.02 mmol/l, and triglycerides 4.2 mmol/l. The fasting blood glucose level was normal and she had no hypertension.

Amplification of the exons of the lipoprotein lipase gene by the polymerase chain reaction

The first 9 exons of the LPL gene were amplified by the polymerase chain reaction technique (PCR) (12). PCR primers, homologous to intron sequences flanking the exons of the LPL gene and containing 22–27 nucleotides, were designed based on published LPL gene structure (13) (exon 5, I: 5'-CCT GCT TTT TTC CCT TTT AAG GCC-3', II: 5'-AAT TCG CTT CTA AAT AAT ATT TAC CTC-3'). The primers were synthesized on an Applied Biosystems Model 381A DNA synthesizer (14). The PCR was carried out as described (15) in a total volume of 50 μl, except that 1 μCi of [α-^{32}P]dCTP (Amersham) was included in each reaction. The cycles of denaturation (1 min at 95°C), annealing (1 min at 57°C), and elongation (2.5 min at 72°C) were repeated 30 times.

Analysis of single-strand conformation polymorphism (SSCP)

The PCR products were diluted 1:5 in 0.1% SDS, 10 mM EDTA, and mixed with an equal volume of 95% formamide, 20 mM EDTA, containing 0.05% bromophenol blue and 0.05% xylene cyanol. The samples were denatured at 90°C for 2 min and cooled on ice. Aliquots of 2–4 μl were analyzed for SSCPs (16) on nondenaturing 5% polyacrylamide gel containing 10% glycerol at 360 V for 18 h at room temperature. After electrophoresis, the gel was transferred to Whatman 3 MM paper and dried in a vacuum slab dryer. Autoradiographs were developed for 1–3 days at −70°C using Kodak XAR film (Rochester, NY).

DNA sequencing

The exons of the LPL gene were amplified without labeled dCTP for sequencing. The PCR products were sequenced by the dideoxy chain termination method (17) with modifications (18).

Solid-phase minisequencing

The principle of the method has been described by Syvänen et al. (19). The upstream PCR primer was the same as that used for the amplification of exon 5 of the LPL gene (primer I). The downstream primer (5'-TCA ACA TGC CCA ACT GGT TTCT-3') was biotinylated as described (20). The use of these primers results in the amplification of 172 bp DNA fragment of the LPL gene. Five ng of DNA was amplified under conditions given for the amplification of exons of the LPL gene except that the biotinylated primer was used at 0.2 μM and the annealing temperature was lowered to 55°C.

For each minisequencing reaction a 10-μl aliquot of the PCR mixture and 40 μl of 20 mM sodium phosphate buffer, pH 7.5, containing 0.1% Tween 20 were added to microtitration wells (Maxisorb Nunc) that had been coated with streptavidin. The samples were incubated with gentle shaking at 37°C for 1.5 h and washed three times with 200 μl of 40 mM Tris-HCl, pH 8.8, 1 mM EDTA, 50 mM NaCl, 0.1% Tween 20, at room temperature. The wells were treated twice with 100 μl of 50 mM NaOH for 5 min at room temperature and washed as above. A reaction mixture consisting of 0.2 μM detection primer (5'-GCA GAT TTT GTA GAC GTC TTA CA-3') and 0.4 μM [^{3}H]dCTP (TRK 625, 67 Ci/mmol, Amersham) to detect C at the third position of codon 183 or 0.4 μM [^{3}H]dGTP (TRK 627, 37 Ci/mmol) to detect G at this position was added to each well. The samples were incubated at 50°C for 10 min and washed as above. The microtitration plates were treated with 60 μl of 50 mM NaOH for 5 min at room temperature and the eluted radioactivity was measured in a liquid scintillation counter.

Site-directed mutagenesis of lipoprotein lipase cDNA

Human LPL cDNA (LPL35) was kindly provided by Dr. Michael C. Schotz (8). The LPL cDNA (1-1653) was amplified by the PCR (12). Sac I and BamHI restriction sites (underlined below) were added to the primers (Primer I: 5'-ACG TGA GCT CCC CCT CTT CCT CCT CCT CAA G-3'; Primer II: 5'-ACG TGG ATC CCA TTC TTC ACA GAA TTC ACA TGC C-3'). The PCR product was subcloned to the Sac I–BamH I site of the replicative form of M13mp18. Oligonucleotide directed site-specific mutagenesis of LPL-cDNA in M13mp18 single-stranded DNA was carried out by the phosphorothioate DNA selection method (21) using a commercial kit (Amersham, RPN 1523). The oligonucleotide used for mutagenesis was 5'-GGT GAA TGT C*TG TAA GAC GTC TAC -3'. Mutants were verified by dideoxynucleotide sequencing (17).

Expression of the wild-type and mutant LPL cDNAs in COS-7 cells

Wild-type and mutant LPL cDNAs were inserted into the Sac I and BamH I sites of expression vector pSVL (Pharmacia). COS-7 cells (ATCC, CRL 1651), maintained in Dulbecco's modified Eagle's medium supplemented with antibiotics and 10% fetal calf serum, were seeded 1 day prior to transfection at a confluence of 10^6 cells/10 cm petri dish. The cells were transfected with 5 μg of the pSVL plasmid DNA constructs by the liposome transfection method (22) using lipofectin (Lipofectin reagent, BRL) as described (23). For LPL activity and

mass determinations, culture medium containing heparin (20 U/ml) was collected at 48 and 72 h after transfection. Cells were collected, washed in PBS, solubilized in 1 ml of 50 mM NH_3/NH_4Cl (pH 8.1) containing heparin, and sonicated. Media and cell extracts were stored at −70°C until assayed for LPL (24, 25). The transfection efficiency of the constructs was confirmed by co-transfections of the LPL constructs with 5 μg of pGL-control plasmid containing the luciferase gene (Promega). The luciferase activity in transfected cells was determined using the Luciferase Assay System (Promega). Two separate expression procedures were carried out.

Lipoprotein lipase activity and mass determinations

Postheparin plasma LPL and HL activities were determined with an immunochemical method (24). The mass of LPL protein was determined by sandwich enzyme-linked immunosorbent assay (25). A polyclonal affinity purified chicken anti-bovine LPL antibody (kindly donated by G. Bengtsson-Olivecrona and T. Olivecrona) was absorbed onto microtiter plates. As second antibody a monoclonal anti-LPL antibody (Monoclonal Antibody LPL(Ab-1), Cat N:o LP01, Lot N:o 25910201, Oncogene Science, New York) was used.

Lipid and lipoprotein analyses

Serum lipid and lipoprotein analyses were carried out on blood samples obtained after 12 h of fasting. Lipoprotein fractions were isolated by sequential ultracentrifugation (26). Cholesterol concentrations were determined by an enzymatic method (27) using a commercial kit (No. 187313, Boehringer Mannheim, Germany). Triacylglycerol concentrations were determined enzymatically (28) with a commercial kit (No. 297771, Boehringer Mannheim, Germany). ApoE phenotyping was accomplished by isoelectric focusing and immunoblotting (29).

RESULTS

Clinical characteristics of the proband

The proband had significantly reduced postheparin lipoprotein lipase activity while the LPL mass was not affected (Table 1). The skeletal muscle lipase activity was clearly diminished (see Materials and Methods). In addition to dyslipidemia she had overweight, non-insulin-dependent diabetes mellitus (NIDDM), and mild hypertension. She displayed several episodes of multiple exacerbations of severe hypertriglyceridemia connected with weight gain and deterioration of glycemic control between the years 1977 and 1990. When the glycemic control was good and she had lost weight, her lipoprotein profile returned to normal. The lipoprotein profile is consistent with a pattern commonly observed in cases heterozygous for LPL deficiency (5, 30, 31). The two daughters of the proband were also obese and showed mild elevations of serum triglycerides and/or lowering of serum HDL (Table 1).

Single-strand conformation polymorphism (SSCP) analysis and sequencing of the exons of the LPL gene

All the nine coding exons of the LPL gene were amplified and analyzed for SSCPs. Amplification of exon 5 of the mother and her two daughters generated PCR products with aberrant migration on electrophoresis (**Fig. 1**). Sequencing of the PCR products derived from exon 5 of the mother (**Fig. 2**) and her two daughters revealed a C to G change at the third base of codon 183. This change causes a substitution of glutamine for histidine at this position (Fig. 2). The proband and her two daughters were heterozygous for this mutation. In addition, daughter 2 had aberrantly migrating DNA fragments derived from amplification of exon 9. Sequencing of exon 9 revealed that these were due to a C to G change at the second base of codon for Ser447 (TCA) leading to a premature stop codon (TGA) (data not shown). The father and the younger daughter (daughter 2) were both heterozygous for this mutation which has been characterized previously (32, 33). Sequencing of the nine coding exons of the LPL gene of the proband and her daughters revealed no further nucleotide changes.

Solid phase minisequencing

The solid phase minisequencing method has been described by Syvänen et al. (19) and validated in the diagnosis of several inherited diseases (34, 35). In the minisequencing reaction, a detection step primer, annealing immediately adjacent to the site of mutation, is elongated by a DNA polymerase with a single dNTP corresponding to the nucleotide at the site of the mutation. To identify the normal nucleotide (C) and the mutant nucleotide (G) at the third position of codon 183, [^{3}H]dCTP is included in one minisequencing reaction and [^{3}H]dGTP in another reaction. A positive signal is obtained from both reactions in samples of heterozygous subjects. The ratio between

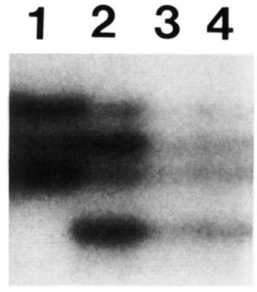

Fig. 1. SSCP analysis of exon 5 of the LPL gene. Lane 1, DNA from a healthy control; lane 2, DNA from the proband; lane 3, DNA from the daughter 1, lane 4, DNA from the daughter 2.

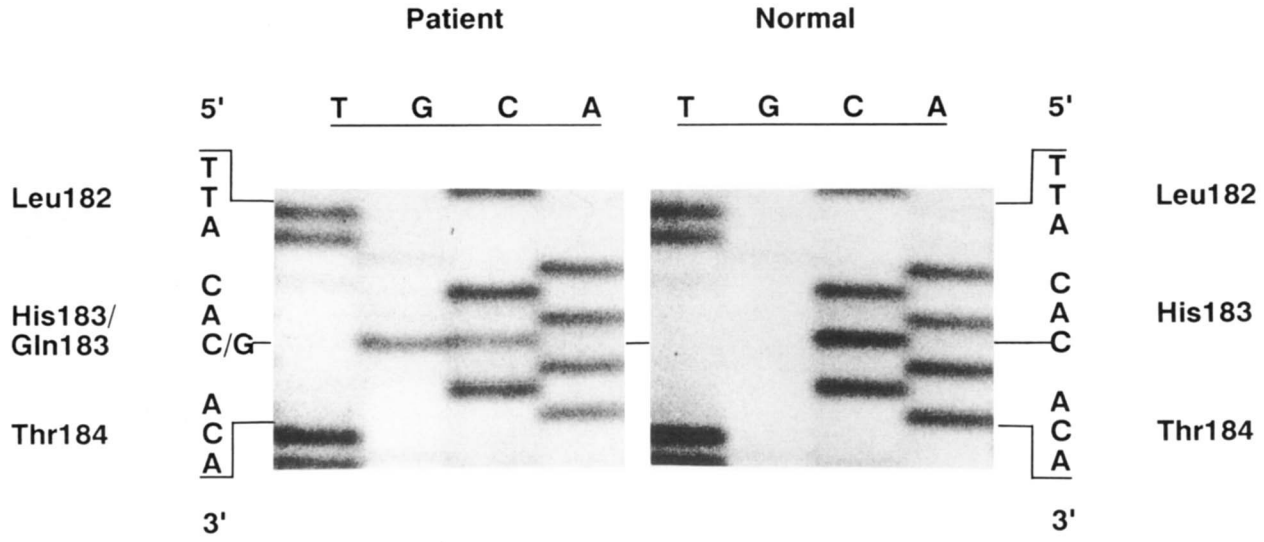

Fig. 2. Sequencing gel of a portion of exon 5 of the LPL gene from the proband (on the left) and a healthy control (on the right) demonstrating the location of the G for C substitution causing a His to Gln change at the amino acid 183. Numbering of the amino acid positions is according to Wion et al. (8).

the incorporated nucleotides directly reflects the ratio between the two sequences present in the PCR product.

DNA samples from the mother, her two daughters, her husband, and three control subjects were analyzed by minisequencing technique with respect to His183→Gln change (**Table 2**). The mother and her two daughters were found to be heterozygous for the C to G transition at the third base of codon 183. The husband and the three control subjects appeared to be homozygous for the C at this position, which was clearly established by the ratio of the incorporated nucleotides (Table 2).

Functional significance of the His183→Gln substitution

Mutant cDNA construct containing the nucleotide change corresponding to Gln183 was generated by in vitro mutagenesis from wild type LPL cDNA. These constructs were transiently expressed in COS cells, and the cells and culture media were analyzed for LPL enzyme activity and immunoreactive protein mass (**Fig. 3**). LPL activity was readily detectable in COS cells transfected with the plasmid construct containing the wild type LPL cDNA (Fig. 3). Compared with this activity, LPL activity was only one-tenth in the cells transfected with the His183→Gln construct, whether measured in the media or intracellularly (Fig. 3). To ensure that the reduced enzyme activity was not caused by the failure of production of the mutant LPLs, the amount of immunoreactive LPL produced by COS cells was measured by an ELISA. COS cells transfected with the mutant construct produced about equal amounts of protein in comparison to the cells transfected with the wild type LPL cDNA (Fig. 3). However, in the media of these cells the mutant lipase mass detected was only about 25% as compared to the wild type lipase mass (Fig. 3). This suggests decreased secretion of the mutant protein in addition to the low hydrolytic activity. The specific activity of the wild type LPL was 0.30 nmol/min per ng in the medium and 0.19 nmol/min per ng in the cell homogenate. The specific activity of the mutant LPL was 0.06 nmol/min per ng in the medium and 0.02 nmol/min per ng in the cells.

TABLE 2. Detection of His183→Gln change by the minisequencing technique

	Radioactivity Incorporated (cpm)		Ratio (R)
Subject	G	C	G/C
Mother	1718	4276	0.4018
Daughter 1	4033	8742	0.4613
Daughter 2	2326	5867	0.3964
Husband	37	14384	0.0026
Control 1	30	16453	0.0018
Control 2	39	20604	0.0019
Control 3	30	18391	0.0016

DISCUSSION

This study reports the characteristics of a novel LPL mutation that is due to a single nucleotide alteration in exon 5 of the LPL gene. This mutation results in a substitution of glutamine 183 for histidine in the mature enzyme protein. Expression of the mutant cDNA in COS

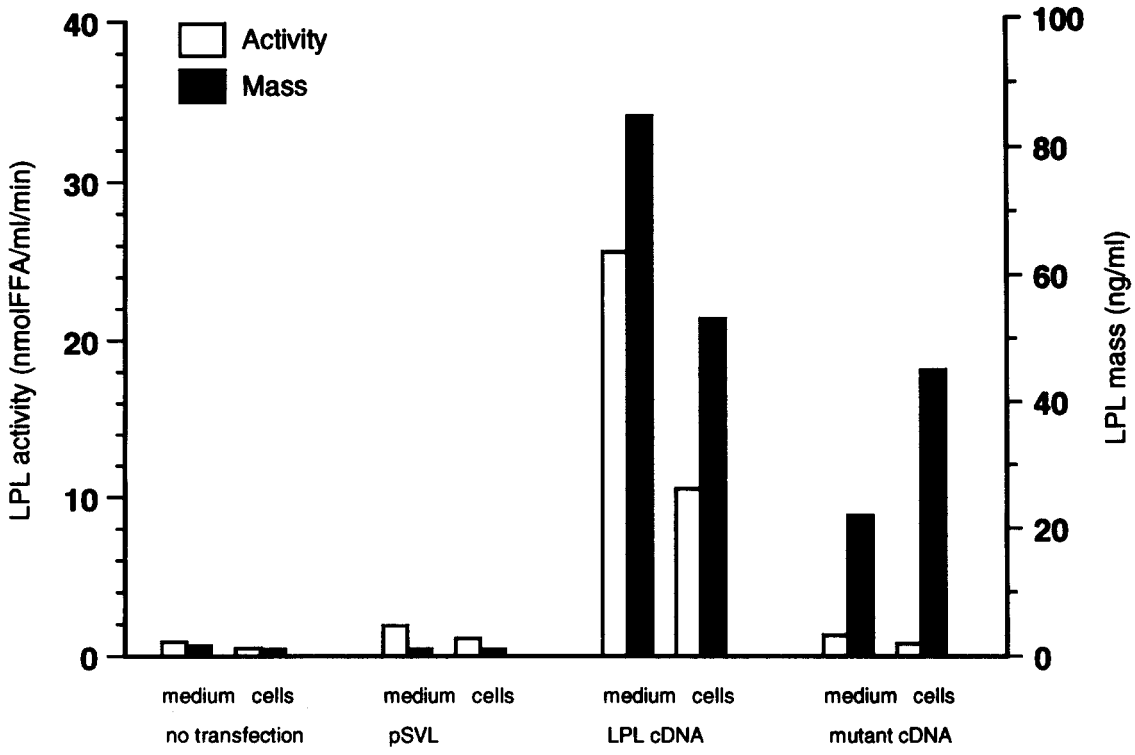

Fig. 3. Expression of His183→Gln substitution in the COS-7 cells. LPL activity (light columns) and immunoreactive mass (dark columns) was measured in the culture medium and in cell homogenates. The data represent means of two separate experiments.

cells established the production of an enzymatically inactive protein that was not efficiently released to the culture medium. These results suggest that the His183→Gln change effect on the tertiary structure of the LPL molecule leads to a functionally deficient protein. The proband and her two daughters were all heterozygous for this mutation. The racial origin of this mutation remains to be clarified. It is noteworthy that the mother of the proband had clinical features similar to those of the proband and died of myocardial infarction, pointing to the possibility that the mutated gene originates from Russia. As clinical data available from the father is scanty, we cannot exclude the possibility that the mutation is of Swiss origin.

Exon 5 codes for the amino acids 154 to 231 of the mature LPL enzyme protein (10, 13). This domain of the molecule is highly conserved among different lipolytic enzymes within and between species (36). In fact, there is complete structural homology extending from amino acid 178 to 210 between the human (8), bovine (37), mouse (9), and guinea pig (38) lipoprotein lipases. Furthermore, LPL of these species share complete homology with human and rat hepatic lipases between residues 182–187 (36). This high degree of conservation strongly suggests that this domain is important for the catalytic activity of these enzymes. In line with this idea are previous studies that have shown that naturally occurring missense mutations in this portion of the LPL molecule, i.e., Asp156→Asn (39), Asp156→Gly (39, 40), Pro157→Arg (41), Ala176→Thr (42), Gly188→Glu (43-45), Ile194→Thr (46, 47), and Pro207→Leu (31), all lead to loss of LPL activity. The His183→Gln mutation characterized in the present study provides further evidence for the functional significance of this region of the molecule. This mutation of His183, an amino acid fully conserved among all mammalian hepatic and lipoprotein lipases characterized so far (36, 48), leads to an alteration in the charge of the LPL molecule. This charge alteration may affect the conformation of this critical area of the LPL molecule and lead to synthesis of an inactive enzyme protein.

Another LPL mutation could also be demonstrated in the family. The husband of the proband and daughter 2 were both found to be heterozygous for a relatively common mutation in exon 9, a C–G transversion, which creates a premature termination codon (Ser447→Ter) and thereby eliminates the C-terminal dipeptide Ser-Gly. In all mammalian LPL sequences reported, the carboxy-terminal end of the LPL molecule is well conserved, implying a functional significance for this region. However, in vitro expression of cDNA carrying this mutation has revealed that the truncated form of LPL has normal lipolytic activity (40). The frequency of the Ser447→Ter mutation has been reported to be lower in subjects with primary hypertriglyceridemia compared to normolipidemic healthy subjects (32, 33) suggesting that this mutation

may have a protective effect against development of hypertriglyceridemia.

Familial hyperchylomicronemia, Type I hyperlipoproteinemia, is a very rare inherited disorder and has therefore limited clinical significance. The determination of the structure of the human LPL gene (10, 13) and the fast progress in DNA technology has made it possible to unravel the mechanisms underlying clinically more common forms of dyslipidemia and hypertriglyceridemia. A recent report (5) clearly demonstrated that heterozygosity for a mutation in the LPL gene is an important precondition for hypertriglyceridemia. Other factors such as age, obesity, hyperinsulinemia, and insulin resistance may also be important modulators of the phenotypic expression of the mutant gene.

Our three patients were all heterozygous for the His183→Gln mutation which results in the production of functionally defective LPL. The LPL activity measurable in postheparin plasma was decreased in the propositus while the two daughters had values that fell within the normal range. This is in line with previous studies (5, 30) that have shown normal or slightly decreased lipolytic activity in presumed heterozygotes.

The constellation of the metabolic disorders, i.e., insulin resistance, glucose intolerance, hyperinsulinemia, increased level of VLDL triglyceride, decreased levels of HDL cholesterol, and hypertension, has been defined "syndrome X" (49). The proband in our study fulfilled the criteria for "syndrome X," which is associated with a high risk for CHD (50). In addition to dyslipidemia and obesity the proband had NIDDM and hypertension. Her two daughters were also obese and showed mild elevations of triglycerides and/or lowering of HDL. Whether relative LPL deficiency due to defective LPL genes is one of the reasons underlying "syndrome-X" remains to be established.

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