High frequency of mutations in the human lipoprotein lipase gene in pregnancy-induced chylomicronemia: possible association with apolipoprotein E2 isoform

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Abstract Partial deficiency in lipolysis usually results in only mild disturbances of lipid levels. However, when this is associated with impairment of the uptake of remnant particles and increased production of triglyceride-rich lipoproteins stimulated by environmental factors such as during normal pregnancy, chylomicronemia may ensue. We have previously reported a patient who had approximately 12% of normal LPL activity and developed severe chylomicronemia during pregnancy (Ma et al. 1993. *J. Clin. Invest.* **91:** 1953–1958). Here we report four new patients with pregnancy-induced chylomicronemia. In the nonpregnant state, these patients had mild to modest elevation of triglyceride levels ranging from 80 to 623 mg/dl (0.9–7.0 mmol/l) but during the third trimester they became severely chylomicronemic with triglyceride levels ranging from 2314 to 14596 mg/dl (26 to 164 mmol/l). Three of these four patients had partial lipoprotein lipase (LPL) deficiency. The molecular characterization of the LPL gene in these three patients with partial LPL deficiency revealed four novel unpublished mutations. Patient #1 is a compound heterozygote for Leu252Arg and Ala261Thr mutations which are associated with 25% of normal LPL activity. In addition, she has an apoE3/2 genotype. Patient #2 is a heterozygote for a Asn291Ser substitution with 69% of LPL activity and also has an apoE3/2 genotype, while patient #3 is a heterozygote for a Trp382Stop mutation with 54% of normal LPL activity and has an apoE4/2 genotype. The fourth patient (#4) with pregnancy-induced chylomicronemia does not have LPL deficiency and has an apoE3/3 genotype. The previously reported patient (#5) who had 12% of normal LPL activity due to homozygosity for a Ser172Cys mutation also has an E3/3 genotype. Our data suggest that mutations in the LPL gene that cause partial LPL deficiency might be a frequent factor in the pathogenesis of pregnancy-induced chylomicronemia.—**Ma, Y., T. C. Ooi, M-S. Liu, H. Zhang, R. McPherson, A. L. Edwards, I. J. Forsythe, J. Frohlich, J. D. Brunzell, and M. R. Hayden.** High frequency of mutations in the human lipoprotein lipase gene in pregnancy-induced chylomicronemia: possible association with apolipoprotein E2 isoform. *J. Lipid Res.* 1994. **35:** 1066–1075.

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The levels of plasma triglyceride and cholesterol are controlled by many different genetic and environmental factors (1). The genes influencing plasma triglyceride levels include *a)* factors controlling the synthesis and secretion of triglyceride-rich lipoproteins such as chylomicrons and very low density lipoproteins (VLDL); *b)* enzymes involved in lipolysis such as lipoprotein lipase (LPL) and its activator apolipoprotein C-II, and hepatic lipase; and *c)* lipoprotein receptors involved in the uptake of the remnant particles such as the LDL receptor and LDL receptor-related protein (LRP).

LPL is the rate-limiting enzyme in the hydrolysis of the triglyceride core in circulating chylomicrons and very low density lipoproteins (VLDL) (2). The action of this enzyme has a major effect on the level and lipid composition of many lipoproteins including chylomicrons, VLDL, low (LDL), and high (HDL) density lipoproteins. Patients with complete LPL deficiency often have no or extremely low LPL activity and fasting plasma triglyceride levels above 1500 mg/dl (17 mmol/l). This is frequently associated with recurrent episodes of abdominal pain, pancreatitis, and eruptive xanthomas from early childhood (2). These patients have been shown to be either homozygotes for a single mutation or compound heterozygotes for two different mutations in the LPL gene.

Abbreviations: apo, apolipoprotein; VLDL, very low density lipoprotein; LPL, lipoprotein lipase; LRP, LPL receptor-related protein; LDL, low density lipoprotein; HDL, high density lipoprotein; PCR, polymerase chain reaction; BMI, body mass index; PL, pancreatic lipase.

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More than 30 mutations in the LPL gene have been reported in patients with LPL deficiency and in almost all cases these mutations were found to cause a completely catalytically defective LPL protein (3).

Partial LPL deficiency can be due to homozygosity or compound heterozygosity for LPL gene mutations associated with residual LPL activity, or heterozygosity for a mutation causing completely catalytically defective LPL. Individuals with partial LPL activity may have reduced adipose tissue and postheparin plasma LPL activity and mild hypertriglyceridemia. This mild hypertriglyceridemia may be aggravated when the partially defective lipolytic system in these patients is challenged by other environmental factors such as normal pregnancy which increases the production of triglyceride-rich lipoproteins. We have previously reported a female patient with only mild hypertriglyceridemia in the nonpregnant state who developed severe chylomicronemia and pancreatitis for the first time at 33 weeks of gestation (4). DNA analysis of her LPL gene revealed homozygosity for a Ser172Cys mutation which produced a mutant LPL protein with approximate 12% of normal LPL activity in postheparin plasma.

The effect of partial LPL deficiency can also be modulated by other genes involved in the metabolism of triglyceride-rich lipoproteins such as apolipoprotein E (apoE). ApoE is present on the surface of chylomicron remnants and serves as one of the ligands for the hepatic remnant receptor and plays a key role in the clearance of chylomicron remnants after lipolysis. One of the three apoE variants, apoE2, has been shown both in vitro and in vivo to have a much lower binding affinity to the hepatic receptor (5, 6). Population studies of apoE polymorphisms have indicated that the apoE2 allele is associated with higher plasma triglyceride levels (7) and the frequency of the apoE2 allele is significantly higher in persons with hypertriglyceridemia (8). Therefore, plasma triglyceride levels reflect the balance between production of triglyceride-rich particles, lipolysis, and uptake of remnants. Overproduction, defective lipolysis, or defective removal of remnants can cause hypertriglyceridemia. Furthermore, mild defects in one process are likely to be compounded by mild changes in another pathway.

We hypothesized that individuals with both partial deficiency of LPL and having an apoE2 allele may manifest with mild hypertriglyceridemia in the fasting state. However, in the presence of other genetic or environmental factors, such as pregnancy, which further challenge the dynamic equilibrium of triglyceride metabolism, these individuals may develop severe hypertriglyceridemia. In this report, we describe four new randomly and consecutively ascertained patients who developed severe chylomicronemia during pregnancy. Three of these four patients had partial LPL deficiency. We performed DNA analysis of the LPL gene for the three patients with partial LPL deficiency, in vitro mutagenesis, and apoE genotyping to evaluate the contributions of both LPL gene mutations and apoE2 isoform to this phenotype.

MATERIALS AND METHODS

Patient ascertainment

We have previously reported a patient who developed severe chylomicronemia and pancreatitis during pregnancy (4). Following this report, six randomly, consecutively selected women with pregnancy-induced chylomicronemia and pancreatitis were sent to us for analysis. Our selection criteria were pregnancy-induced chylomicronemia and pancreatitis. Patients with history of type I chylomicronemia or history of secondary hypertriglyceridemia were not selected for this study. Among the six patients we received, two were found to have a history of diabetes which explained their chylomicronemia during pregnancy (2). In addition, the postheparin LPL mass and activity levels in these two patients were normal which is consistent with the diagnosis of secondary hypertriglyceridemia. The other four patients had no or mild hypertriglyceridemia before pregnancy but developed severe chylomicronemia during pregnancy. None of these four patients had a history of diabetes, obesity, or heavy alcohol intake.

Patient #1 is a 37-year-old Chinese woman who presented with chylomicronemia and severe pancreatitis at 29 weeks of gestation of her first pregnancy. Patient #2 is a 37-year-old Caucasian woman of English descent who developed chylomicronemia without pancreatitis at 35 weeks of her second pregnancy. Patient #3 is a 24-year-old Caucasian woman with a history of hypertriglyceridemia which was aggravated during her second pregnancy. Patient #4 is a 32-year-old Chinese woman who developed chylomicronemia and pancreatitis at 35 weeks of her first pregnancy. The previously reported patient (listed as #5 in this study) is an East Indian woman who had no history of hyperlipidemia and developed severe chylomicronemia and pancreatitis for the first time at 33 weeks of gestation at age 30 (4).

Normal control selection

LPL mass and activity levels in the postheparin plasma were determined for 37 healthy subjects (19 women, 32 ± 9 years old, and 18 men, 28 ± 4 years old). The mean levels of LPL mass and activity from these individuals were used as normal controls in this study. These individuals had normal concentrations of plasma total, LDL- and HDL-cholesterol, triglyceride, and apoB. In addition, they met the following criteria: *1)* a history of good health, *2)* abstinence from drugs known to affect serum lipids, *3)* a stable body weight that is below 120% of ideal body weight, and *4)* absence of excessive regular consumption of alcohol.

Lipid and lipoprotein measurements

Fasting plasma triglyceride, total cholesterol, and HDL-cholesterol levels were measured as previously described (9–11).

Measurement of LPL mass and catalytic activity

Measurements of LPL mass and activity were performed in the non-pregnant state for all five women. Blood samples were collected from the patients after a 12–16 h overnight fast. An intravenous injection of heparin was given (60 U/kg body mass) and the postheparin blood samples were obtained 10 min after the injection. Plasma was separated after centrifugation at 3000 *g* for 10 min at 4°C. LPL mass in pre- and postheparin plasma samples was measured by an ELISA method (12) using a monoclonal antibody (5D2) that recognizes an epitope located at residue 400 of human LPL (13). For the LPL and hepatic lipase (HL) activity assays, the plasma samples were diluted 100-fold before assay to avoid any effect by endogenous substrate. LPL and HL lipolytic activities were measured using a radiolabeled tri-[1-^{14}C]oleate emulsion as previously described (14).

DNA analysis

Genomic DNA was isolated from white blood cells of the patients as previously described (15). Each of the 10 exons of LPL was individually amplified from 0.5–1 μg of genomic DNA from the probands using the polymerase chain reaction (PCR) as previously described (16, 17). The amplified exons were then purified and sequenced either directly or after cloning into a TA cloning vector (Invitrogen Inc.).

In vitro site-directed mutagenesis and expression in COS cells

A 1.6-kb cDNA fragment containing the entire coding sequence of the LPL gene was cloned into a dual function vector (CDM8) for both mutagenesis and expression (18). The sequences of the mutagenesis oligonucleotides were: Leu252Arg:5'-CATCGACTCTCGGTTGAATGAAGAA-3'; Leu252Pro: 5'-CATCGACTCTCCGTTGAATGAAGAA-3'; Ala261Thr:5'-AATCCAAGTAAGACCTACAGGTGCA-3'. Mutagenesis and expression in COS-1 cells were performed as previously described (19).

ApoE genotyping

ApoE genotyping was performed initially using a mismatch PCR method as previously described by Main et al. (20). All apoE genotyping was confirmed by another method using mismatch PCR followed by cleavage with *HhaI* restriction enzyme as described (21). Both methods detect mismatch at residue 112 and 158 of the apoE gene.

RESULTS

Clinical investigation

The four newly described patients with pregnancy-induced chylomicronemia had no history of diabetes, obesity, or alcohol intake. Their body mass indexes (BMI) were within the normal range of 18–23 (kg/m^2).

Patient #1 developed severe pancreatitis with plasma triglyceride level of 14411 mg/dl (162 mmol/l) and serum amylase level of 210 U/l (normal 30–100 U/l) at 29 weeks of gestation of her first pregnancy. Her blood glucose, liver, thyroid, and renal functions were found to be normal. She had no personal or family history of hyperlipidemia or pancreatitis and was not on any medication known to elevate serum triglyceride levels.

Because of the severe degree of hypertriglyceridemia, plasmapheresis was performed that together with nasogastric suction and intravenous infusion, significantly reduced the triglyceride level to 5154 mg/dl (58 mmol/l). The patient was then put on a 10% fat diet that further reduced the triglyceride levels to 1750–3500 mg/dl (20–39 mmol/l). Labor was induced at 37 weeks and a healthy female infant was delivered. Four days after delivery, the patient's plasma triglyceride levels fell sharply from 3500 mg/dl (39 mmol/l) to 87.5 mg/dl (0.98 mmol/l) on a 20% fat diet.

In the first two trimesters of her second pregnancy, plasma triglyceride levels were maintained between 230–975 mg/dl (2.6–10.9 mmol/l) with a 20% fat diet. During the third trimester, the triglyceride levels continued to increase to levels of 3675–4423 mg/dl (41.3–49.7 mmol/l) despite dietary fat restriction to 10%. Labor was induced at 37 weeks and a healthy boy was delivered.

Patient #2 developed chylomicronemia at 35 weeks of her pregnancy. The triglyceride levels were 1922–4850 mg/dl (21.6–54.5 mmol/l) and she was treated with intravenous dextrose followed by a 10% fat diet. Her condition responded well to the treatment and a baby was delivered at term. During her subsequent pregnancy, a 10% fat diet was instituted throughout the pregnancy. She was hospitalized at 39 weeks for abdominal pain with a triglyceride level of 2225 mg/dl (25 mmol/l). She was treated with a 10% fat diet and delivered a baby at term.

The mother of this patient had mild hypertriglyceridemia (196–401 mg/dl, 2.2–4.5 mmol/l) but no history of pancreatitis during pregnancy. The patient's brother with a history of smoking and obesity died of myocardial infarction suddenly at age 37. His lipid levels were not recorded.

Patient #3, a 24-year-old Caucasian, had a miscarriage at 13 weeks of gestation with no specific diagnosis. During her second pregnancy she had an episode of eruptive xanthomas at 28 weeks of gestation and severe pancreatitis with plasma triglyceride level of 2270 mg/dl (25.5 mmol/l) and serum amylase level of 566 U/l at 32 weeks of gestation. Labor was

induced at 35 weeks of gestation and a healthy male infant was delivered. Her plasma triglyceride level fell from 2270 mg/dl (25.5 mmol/l) to 801 mg/dl (9 mmol/l) 5 days after delivery. The triglyceride level was reduced to 623 mg/dl (7 mmol/l) 3 months after delivery.

This patient was adopted and was not aware of any family history of hypertriglyceridemia. The male infant had normal lipid levels.

Patient #4 was a 32-year-old Chinese woman in good health who developed chylomicronemia and pancreatitis at 35 weeks of her first pregnancy. The pregnancy ended with fetal death at 35 weeks of gestation. At 25 weeks of her second pregnancy, she again developed severe chylomicronemia associated with pancreatitis. The triglyceride level was 3471 mg/dl (39 mmol/l) while serum amylase level was normal. She was treated with a 10% fat diet which reduced the plasma triglyceride levels to 890–1780 mg/dl (10–20 mmol/l). By following the strict low-fat diet, her triglyceride levels were maintained at 1068–1780 mg/dl (12–20 mmol/l) and a baby was delivered at term. The patient had no family history of pancreatitis or lipid disorders.

Biochemical measurements

The results of fasting plasma triglyceride, total cholesterol, and HDL-cholesterol lipid levels in the non-pregnant and pregnant state for the four new patients and a previously reported patient (listed as patient #5) (ref. 4) are summarized in **Table 1.** In all five women, the third trimester of their pregnancies was associated with chylomicronemia during which the triglyceride levels were 3- to 60-fold higher than levels seen in the non-pregnant state. All had low HDL levels (< 10th percentile) in the non-pregnant state.

The postheparin plasma LPL activities were measured in the non-pregnant state and reduced LPL activity levels were found in three of the four new patients (**Table 2**). LPL mass levels were reduced to 28% of the normal for patient 1, 52% in patient 2, and 27% in patient 3. Hepatic lipase activity and apoC-II levels in all four new patients were normal. These data suggested that defects in the LPL gene were likely to be one of the underlying causes for hypertriglyceridemia in these patients.

DNA analysis

DNA sequence analysis of 10 exons of patient #1 revealed two different missense substitutions both in exon 6 of the LPL gene. One mutation is a T to G transversion at the second base of codon 252 resulting in a leucine (CTG) to arginine (CGG) substitution (**Fig. 1A**). The other mutation is a G to A transition at the first nucleotide of codon 261 causing a alanine to threonine substitution (Fig. 1B). Several independent PCR amplifications of exon 6 and subsequent DNA sequencing of both the coding and noncoding strand were performed to confirm the presence of these two mutations. Because of the possibility that these two mutations might occur on the same LPL allele, the PCR-amplified exon 6 was cloned into a TA cloning vector and eight positive clones were sequenced individually. The Leu252Arg and Ala261Thr mutations were found in separate clones, indicating that patient #1 is a true compound heterozygote rather than double heterozygote for these mutations. The DNA sequence of the nine remaining exons and the exon–intron junctions of the LPL gene were found to be normal.

DNA sequence analysis of the LPL gene of patient #2 revealed heterozygosity for an A to G transition at the second nucleotide of codon 291 in exon 6. This alteration results in an asparagine to serine substitution and this Asn291Ser mutation was also identified in another group of patients with a different form of hypertriglyceridemia (Y. Ma et al., manuscript in preparation). No other mutation was found in any other exons and exon–intron boundaries.

Patient #3 was found to be heterozygous for a G to A transition at the second nucleotide of codon 382 resulting in

TABLE 1. Lipid levels in the non-pregnant and pregnant state in patients

		Non-Pregnant			Pregnant (the third trimester)		
Patient	Age	TG	T-Chol	HDL-C	TG	T-Chol	HDL-C
	yr		*mg/dl*			*mg/dl*	
1	37	174 ± 67	132 ± 27	29 ± 4	3676–14411	308–970	12–31
2	37	201	209	37	1922–4851	832–970	n.a.
3	24	712 ± 125	320	17	2270	328	n.a.
4	32	171	169	42	3471	n.a.	n.a.
5	30	272 ± 19	174 ± 16	25 ± 3	2465–2510	343–443	19–31
Normal (n = 23)[a]	28 ± 4	71 ± 23	205 ± 23	64 ± 12	222 ± 60	251 ± 32	64 ± 9

All measurements were done on fasting samples. TG, plasma total triglycerides; T-Chol, total cholesterol; HDL-C, HDL cholesterol; n.a., data not available.

[a] Reference data from Knopp et al. (39).

TABLE 2. LPL mass and activity in the non-pregnant state, LPL gene mutation, and apoE genotype in patients

Patient	Postheparin Plasma LPL		Mutation	LPL Activity in Vitro	ApoE
	Mass	Activity			
	ng/ml	*nmol/min/ml*		*% of normal*	
1	56 (28%)[a]	43 (25%)[a]	Leu252Arg	0	E3/2
			Ala261Thr	32–36	
2	104 (52%)	118 (69%)	Asn291Ser	50	E3/2
			Normal	100	
3	53 (27%)	93 (54%)	Trp382Stop	0	E4/2
			Normal	100	
4	294 (147%)	222 (129%)	n.d.	n.d.	E3/3
5	n.a.	20.3[b] (12%)	Ser172Cys	6	E3/3
			Ser172Cys	6	
Normal (n = 37)	200 ± 91 (100%)	171 ± 66 (100%)			

n.d., DNA sequencing of the LPL gene not done because this patient did not have partial LPL deficiency; n.a., data not available.

[a] Percent of normal.

[b] Corrected value. The LPL activity level was determined in a different system using a polyclonal antibody. The LPL activity level for this patient was 33.3 nmol/min per ml, while the levels for six normal controls were 280 ± 51.7 (ref. 4).

a Trp(TGG)382→Stop(TAG) nonsense mutation in exon 8 (Fig. 1C). No other DNA alteration was found in the nine remaining exons and exon-intron boundaries of the LPL gene.

Mutation analysis in LPL gene for patient #4 was not performed because both the LPL mass and activity levels were normal (Table 2). The lack of partial LPL deficiency in this patient suggests that genetic defect(s) other than LPL are likely to be the cause for the observed pregnancy-induced chylomicronemia in the this patient. We have previously reported that patient #5 is a homozygote for a Ser172→Cys substitution in exon 5 (4).

In vitro site-directed mutagenesis and expression in COS cells

To determine whether the Leu252Arg and Ala261Thr substitutions are responsible for the reduced LPL activity observed in patient #1, we performed in vitro site-directed mutagenesis studies to reproduce each of the two mutations. Mutant specific clones were identified by oligo-hybridization and the substitutions were confirmed by DNA sequencing.

Phagemid DNA from each of the two mutants and from a wild-type (wt) LPL cDNA clone were purified and used to transfect COS-1 cells. LPL mass and activity were assayed in the transfected COS cell medium for each set of control and mutant cDNA. In the single transfection experiments using each of the two mutant LPL cDNA separately, the Leu252Arg mutant LPL was associated with reduced LPL mass (32% of the normal) and no LPL catalytic activity (0%) (**Table 3**). However, the LPL mass level for the Ala261Thr mutant was approximately 67% of the normal and the activity was approximately 32% of the normal.

To investigate further whether the positively charged arginine at residue 252 causes the loss of LPL activity in the Leu252Arg mutant, we generated another Leu252Pro mutant by in vitro mutagenesis. The LPL mass level of this mutant was reduced (71%) comparing to wtLPL but was higher than that of the Leu252Arg mutant. Nevertheless, the new mutant remained catalytically inactive (Table 3). These data suggest that Leu252 is critical in maintaining LPL activity.

LPL is active as a dimer (22–25). Thus, individuals who are compound heterozygotes for two different mutations such as patient #1 are likely to have three different populations of mutant LPL dimers (homodimers for each mutation and heterodimers). We therefore performed co-transfection experiments using equal amounts of Leu252Arg and Ala261Thr cDNA. The results are shown in **Table 4.** The LPL activity levels for individual Leu252Arg and Ala261Thr mutant LPL were 0.9% and 36% of the normal, respectively. These levels are similar to that in a separate set of experiments shown in Table 3. The co-transfections of both Leu252Arg and Ala261Thr were found to produce mutant LPL mixture with 20% of LPL activity as compared to the wild-type LPL which is similar to that seen in the patient in vivo.

In vitro site-directed mutagenesis revealed that the Asn291Ser mutation seen in patient #2 was found to be

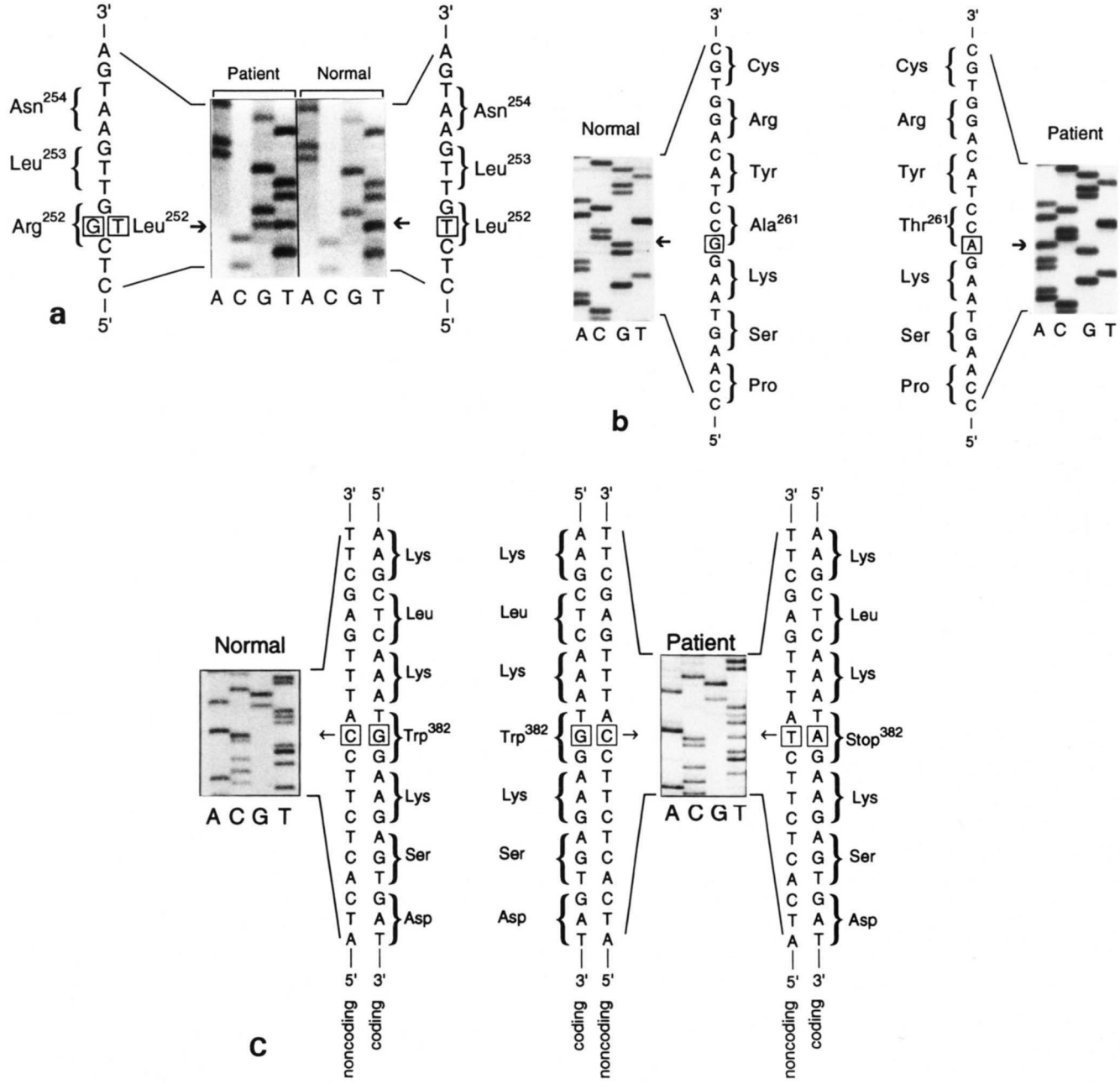

Fig. 1. DNA sequence of PCR-amplified exons 6 and 8 spanning residue Leu^{252}, Ala^{261}, and Trp^{382}. Exon 6 from patient #1 and exon 8 from patient #3 were PCR-amplified individually and sequenced either directly or after cloning into a TA vector. For patient #1, plasmid DNA was individually purified from eight positive clones for each exon and sequenced for both coding and noncoding strands. (a) Direct DNA sequencing of PCR-amplified exon 6 from patient #1 and from a control normal subject. A T→G transversion resulting in a substitution of arginine for leucine at residue 252 is indicated in the boxes. This patient is a heterozygote for this mutation. (b) DNA sequence of a exon 6 clone from patient #1. A G→A transition resulting in an Ala261→Thr substitution is indicated in boxes. DNA sequencing was performed for eight clones. These clones were found to carry either the Ala261Thr or Leu252Arg mutation, indicating that this patient is a compound heterozygote for these two mutations. (c) Direct DNA sequencing of PCR-amplified exon 8 from patient #3 and from a normal control. A G→A alteration in the coding strand which causes Trp382Stop nonsense mutation is indicated. This patient is a heterozygote for this mutation.

associated with 50% reduction of LPL mass and activity as compared with wild-type LPL (Y. Ma et al., manuscript in preparation). The Trp382Stop nonsense mutation in patient #3 is expected to produce a truncated mutant LPL protein that cannot be detected by the monoclonal antibody (5D2) in our mass assay which recognizes an epitope at residue 400 (13). However, a similar Trp382Stop mutation was found to cause complete LPL deficiency in a Japanese patient homozygous for this mutation (26).

TABLE 3. LPL mass and activity in COS medium in single transfection experiments

Mutation	LPL Mass	%	LPL Activity	%	Specific Activity	%
	ng/ml		*nmol/min/ml*			
Wild-type LPL	515 ± 35.4	100	246.7 ± 3.5	100	0.48	100
Leu252Arg	165 ± 7.1	32	0.2 ± 0.2	0	0.00	0
Leu252Pro	365 ± 49.5	71	0.4 ± 0.1	0	0.00	0
Ala261Thr	343 ± 10.6	67	79.3 ± 2.5	32	0.23	48

ApoE genotyping

Apolipoprotein E2 isoform is another factor that may influence plasma triglyceride levels. We therefore performed apoE genotyping on all five patients (Table 2). Patients 1 and 2 are E3/2 heterozygotes. Patient 3 has a E4/2 genotype. Patients 4 and 5 are E3/3 homozygotes. The frequencies of apoE2, E3, and E4 in the general population are approximately 10%, 75%, and 15%, respectively (7). The probability that three out of five women taken at random from the general population would have at least one apoE2 allele is 0.08, which may suggest that the over-representation of the apoE2 allele in these five women is not likely due to chance.

DISCUSSION

Here we describe five women (four new and one previously reported) with pregnancy-induced chylomicronemia. Four of the five women have variable degrees of partial LPL deficiency. Our results clearly show that mutations in the LPL gene were a significant factor in the pathogenesis of pregnancy-induced chylomicronemia in 4/5 of these patients. We also show, however, that partial LPL deficiency does not account for all causes of pregnancy-induced chylomicronemia because patient #4 had normal LPL mass and activity levels. Despite the fact that these patients were selected only by the criteria of pregnancy-induced chylomicronemia and did not have diabetes or a history of alcohol intake, some bias of ascertainment might still have resulted in some over-representation of partial LPL deficiency in these patients.

The four patients with partial LPL deficiency had variable levels of LPL activity, yet all presented with pregnancy-induced chylomicronemia. The patients with the high levels of LPL activity (25–69%) were deficient in apoE3 and had a E3/2 (patients 1 and 2) or E4/2 apoE genotype (patient 3). The patient who had the lowest LPL activity (12%) had a E3/3 genotype (patient #5). These data suggest that in the non-stressed state outside of pregnancy and in the absence of apoE2 isoform, individuals may tolerate as low as 15% of normal LPL activity without the development of chylomicronemia. However, the presence of a single copy of apoE2, which delays the clearance of remnant particles, might reduce the tolerance for partial LPL deficiency and unmask chylomicronemia in the presence of other environmental triggers such as pregnancy.

After lipolysis, the remnant particles are taken up by hepatic receptors, in part through the apoE ligand located on the surface of the remnants. One of these remnant receptors is the low density lipoprotein receptor-related protein (LRP) (27). ApoE2, one of the three common isoforms, has been shown to be defective in its binding to both LDL receptor (5) and LRP (27). In a recent study of dietary fat clearance in E3/2 heterozygotes, Weintraub, Eisenberg, and Breslow (28) showed that the rate of the remnant clearance in E3/2 individuals was only one-half the rate in subjects without the apoE2 allele. This clearance defect is mainly seen for apoB-48-containing particles. Numerous association studies in different populations have been performed in an attempt to determine the effect of a single apoE2 allele on plasma triglyceride levels and there have been conflicting results. Recently, a meta-

TABLE 4. LPL mass and activity in COS medium in co-transfection experiments

Mutation	LPL Mass	%	LPL Activity	%	Specific Activity	%
	ng/ml		*nmol/min/ml*			
Wild-type LPL	68.8 ± 8.5	100	50.8 ± 2.3	100	0.85	100
Leu252Arg	17.3 ± 2.8	25	0.5 ± 0.3	0.9	0.02	2
Ala261Thr	46.2 ± 5.6	67	18.5 ± 0.5	36	0.40	47
Co-transfection: Leu252Arg Ala261Thr	47.6 ± 5.6	69	10.2 ± 0.4	20	0.21	25

analysis including 27 independent studies from 17 countries concluded that triglyceride levels in E3/2 individuals are significantly higher than those in E3/3 subjects (7). Taken together, these data may suggest that the presence of a single E2 allele is likely to aggravate or precipitate hypertriglyceridemia.

The role of another apoE variant, apoE4, in triglyceride metabolism is not very clear. In vitro, apoE4 has the same normal receptor binding activity as apoE3 (5). However, an association between apoE4 and plasma triglyceride level has been reported (7). The apoE4 allele has also been suggested to be associated with severe hypertriglyceridemia by two groups of investigators when ascertained through the high triglyceride levels (29, 30). However, in 50 patients selected who had triglyceride levels over 2000 mg/dl we were unable to demonstrate such an association (J. D. Brunzell and M. R. Hayden, unpublished data). In the present study of five patients with pregnancy-induced chylomicronemia, only one patient had one apoE4 allele (1 in 10 alleles). The frequency of the E4 allele in the general population is about 15%. This suggests that the observed E4 allele in one of the five patients is likely to be a random association. In contrast, three of the five patients are heterozygotes for apoE2 which has a frequency of 10% in the general population and this suggests a possible non-random association of the E2 allele with pregnancy-induced chylomicronemia.

As the rate-limiting enzyme in the hydrolysis of both exogenous and endogenous triglyceride-rich lipoproteins, LPL plays a crucial role in maintaining the homeostasis between circulating triglyceride-rich lipoproteins. Complete LPL deficiency as seen in patients with type I hyperlipoproteinemia results in a marked elevation of triglyceride levels and very low levels of both LDL and HDL (2). However, partial LPL deficiency due to a single mutation in one LPL allele results in variable lipoprotein profiles. Many of these heterozygotes have fasting triglyceride levels within the normal range although the triglyceride levels in the carriers tend to be higher than non-carriers (12). This suggests that approximately 50% of normal LPL activity is sufficient to hydrolyze triglyceride-rich lipoproteins and maintain near normal triglyceride levels in the fasting state. However, because of their partially defective lipolytic system, the postprandial triglyceride levels in these individuals can be significantly higher than that of normal. Indeed, a recent study by Miesenbock et al. (31) demonstrated that in a 16-member kindred, 8 heterozygous carriers for a Gly188Glu mutation in the LPL gene had, on average, a 38% reduction of postheparin LPL activity as compared to non-carriers. These carriers had close to normal fasting triglyceride levels but had pronounced postprandial lipemia. In another large kindred carrying the Gly188Glu mutation in the LPL gene, Wilson et al. (32) reported that 29 heterozygous carriers were prone to developing a form of familial hypertriglyceridemia characterized by increased levels of fasting triglyceride, VLDL cholesterol, apoB, and decreased levels of LDL and HDL cholesterol. However, the expression of hypertriglyceridemia appeared to be age-modulated and significant differences in lipid levels were found only in carriers over age 40. In a separate study, Wilson et al. (33) reported that individuals with partial LPL deficiency who also had diabetes were also more likely to manifest with severe hypertriglyceridemia. These data provide further evidence that impaired lipolytic capacity due to partial LPL activity is one of the preconditions for hypertriglyceridemia and interaction between partial LPL deficiency and environmental factors such as diet, age, or acquired secondary disorders such as diabetes may interact to result in severe hypertriglyceridemia.

LPL is a member of a gene family of lipases which includes hepatic (HL) and pancreatic lipases (PL) (34, 35). The high degree of amino acid sequence homology among these lipases, especially in a region encoded by exons 4, 5, and 6 of human LPL, suggests that their three-dimensional structures are likely to be similar. Thus, the known 3D structure of PL has been previously used to examine the effects of numerous naturally occurring mutations and in vitro engineered substitution on the structure and function of LPL (36). The leucine residue at position 252 in exon 6 of human LPL is conserved both in bovine and mouse LPL and in human and rat HL. In dog, pig, and human PL, the Leu252 residue is conservatively replaced with an isoleucine residue that has a similar size and hydrophobicity. The neighboring amino acid sequence, especially residues 250 and 251, is also highly conserved and we have previously reported that substitutions in both Asp250 and Ser251 result in completely catalytically inactive LPL (37). In this study, we have shown by in vitro mutagenesis that both the Leu252Arg mutation found in patient #1 and the additional Leu252Pro substitution cause completely defective LPL. Based on the sequence homology between LPL and PL, the corresponding residue for Leu252 in PL is Ile274, which is situated in a highly conserved alpha-helix segment in the N-terminal domain containing the Asp176...His263...Ser152 catalytic triad of PL. The replacement of Ile274 either with the hydrophilic, positively charged arginine or with a proline which often generates a turn between two local secondary structures is likely to disrupt the proper folding of LPL, causing a loss of enzymatic activity.

Unlike Leu252 residue, the Ala261 nine residue downstream appears to be less conserved. Although the Ala261 is conserved in bovine and mouse LPL and in human HL, this residue is replaced with a serine residue in dog PL. In addition, many nonconservative replacements can be found in the amino acid sequence surrounding Ala261, suggesting that substitutions in this region are less likely to cause a complete loss of lipase activity. In human PL, the corresponding residue for Ala261 is Gly282, which is also located in a less conserved segment with flexible secondary

structure. Indeed, our in vitro mutagenesis showed that the Ala261Thr mutation, which is also found in patient #1, results in a mutant LPL protein with approximately 32–36% of normal LPL activity. In vivo, patient #1 with the Leu252Arg and Ala261Thr mutations has approximately 25% of normal catalytic activity.

The Trp382Stop nonsense mutation found in patient #3 is due to a G-to-A substitution at the second nucleotide of codon 382. A similar Trp382Stop mutation has been previously reported in a Japanese patient (26). However, in the Japanese patient, the DNA alteration is a G-to-A mutation affecting the third nucleotide of codon 382. Different nucleotide substitutions affecting the same residue do not seem to be rare in LPL. We have previously reported two cases in which separate mutations affecting the first and second nucleotide of codon Asp156 and codon Arg243 cause LPL deficiency (19, 38).

More than 30 different mutations causing complete inactive LPL have been identified as the cause of type I chylomicronemia (3). Because partially reduced LPL activity is likely to be sufficient to maintain near normal triglyceride levels in the fasting state, the frequency of mutations causing partial LPL activity is likely to have been underestimated. Patients with LPL gene mutations resulting in partial deficiency of LPL activity may manifest with chylomicronemia when exposed to environmental stress such as pregnancy, diabetes, or alcohol. In addition, some patients may be particularly susceptible to these environmental triggers if they are also have an apoE2 allele that affects the uptake of remnant particles.

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