Original Article

Lipoprotein Lipase Deficiency: Clinical, Biochemical and Molecular Characteristics in Three Patients with Novel Mutations in the *LPL* Gene

(lipoprotein lipase deficiency / hypertriglyceridaemia / hypercholesterolaemia / hepatosplenomegaly / acute pancreatitis / lipaemia retinalis)

H. KOLÁŘOVÁ[1], M. TESAŘOVÁ[1], Š. ŠVECOVÁ[1], V. STRÁNECKÝ[2], A. PŘISTOUPILOVÁ[2], T. ZIMA[3], J. UHROVÁ[3], S. Y. VOLGINA[4], J. ZEMAN[1], T. HONZÍK[1]

[1]Department of Paediatrics and Adolescent Medicine, [2]Institute of Inherited Metabolic Disorders, [3]Institute of Medical Biochemistry and Laboratory Diagnostics, First Faculty of Medicine, Charles University in Prague and General University Hospital in Prague, Prague, Czech Republic
[4]Kazan State Medical University, Kazan, Russia

Abstract. Lipoprotein lipase (LPL) deficiency, caused by mutations in the *LPL* gene, is a rare autosomal recessive disorder manifesting in early childhood with recurrent abdominal pain, hepatosplenomegaly, acute pancreatitis, lipaemia retinalis and eruptive xanthomas. Typical laboratory findings are lactescent serum, extreme hypertriglyceridaemia and hypercholesterolaemia. The diagnostics is based on post-heparin serum LPL assay and DNA analyses of the *LPL* gene. We report clinical, biochemical and molecular data of three children with LPL deficiency. One child manifested since the first week of life with recurrent abdominal pain (Patient 1), the second with abdominal distension and hepatosplenomegaly since the second month of life (Patient 3) and patient 2, asymptomatic younger brother of patient 1, was diagnosed in the first week of life. Lipaemia retinalis and splenomegaly were present in two symptomatic children, hepatomegaly in patient 3 and acute pancreatitis in patient 1. All children had lactescent serum, profound hypertriglyceridaemia (124 ± 25 mmol/l; controls < 2.2), hypercholesterolaemia (22.8 ± 7.3 mmol/l, controls < 4.2) and their LPL immunoreactive mass in serum did not increase after heparin injection. Molecular analyses revealed that both siblings are homozygous for novel mutation c.476C > G in the *LPL* gene changing the conserved amino acid of the catalytic centre. The third patient is a compound heterozygote for mutations c.604G>A and c.698A>G in the *LPL* gene, both affecting highly conserved amino acids. We conclude that LPL deficiency must be considered in neonates and young infants with abdominal pain and hypertriglyceridaemia because early treatment might prevent development of life-threatening acute pancreatitis.

Introduction

Familial lipoprotein lipase (LPL) deficiency is a rare autosomal recessive disorder caused by loss-of-function mutations in the *LPL* gene (Evans and Kastelein, 2003). The general prevalence of this disease is one in a million (Rahalkar et al., 2009) in contrast to the French-Canadian population of Québec with the carrier frequency 1 : 40 attributed to the founder effect (Gagne et al., 1989).

LPL is a key enzyme in hydrolysation of serum triglycerides (TG) in chylomicrons (CM) and very low-density lipoproteins (VLDL). The enzyme is present in capillaries of adipose tissue, skeletal muscle, adrenal glands, kidney, intestine and neonatal liver (Siafakas et al., 1999). LPL deficiency usually manifests in childhood as abdominal pain, failure to thrive, hepatosplenomegaly, lipaemia retinalis or eruptive xanthomas. The most se-

Received February 27, 2014. Accepted March 17, 2014.

Institutional support was provided by PRVOUK P49/LF1/3, PRVOUK P24/LF1/3 from Charles University in Prague, by Research Project RVO-VFN64165/2012 and UNCE 204011.

Corresponding author: Tomáš Honzík, Department of Paediatrics and Adolescent Medicine, First Faculty of Medicine, Charles University in Prague and General University Hospital in Prague, Ke Karlovu 2, 128 08 Prague 2, Czech Republic. Phone: (+420) 224 967 792; fax: (+420) 224 967 713; e-mail: tomas.honzik@vfn.cz

Abbreviations: CM – chylomicrons, GPIHBP1 – glycosylphosphatidylinositol anchored-HDL-binding protein 1, HC – hypercholesterolaemia, HDL – high-density lipoprotein, HTG – hypertriglyceridaemia, LPL – lipoprotein lipase, MCT – medium-chain triglyceride, SNP – single-nucleotide polymorphism, TG – triglycerides, VLDL – very low-density lipoproteins.

vere and life-threatening complication is acute pancreatitis (Brunzell et al., 2001). Typical laboratory characteristics are lactescent plasma/serum with milky-cream appearance, hypertriglyceridaemia (HTG) and hypercholesterolaemia (HC) with very low level of high-density lipoprotein (HDL) cholesterol < 0.5 mmol/l (Santamarina-Fojo, 1998; Rahalkar et al., 2009).

We present clinical, biochemical and molecular data of three children from two unrelated families with LPL deficiency. Three novel mutations in the *LPL* gene and our experience with the therapeutic strategy are described.

Material and Methods

Patients

Patient 1

The girl was born at term with a birth weight of 2700 g (12th percentile) and length of 48 cm (17th percentile). Her mother, mother's sister and father and maternal grandmother are clinically healthy but all have HTG (2.43, 4.85; 11; 4.4 mmol/l, respectively; reference range < 1.7). Her father is healthy, his parents have cholesterol 5.34 and 5.42 mmol/l (reference range < 5.2).

Since birth, the girl manifested an abdominal distention, tenderness and irritability assessed as infantile colics. At the age of 2.5 months, she was admitted to hospital because of loss of appetite, constant crying and abdominal distention with ascites and splenomegaly. Eruptive xanthomas were not present. Her serum had a milky-cream appearance (Fig. 1) with HC 33.1 mmol/l (reference range < 4.2) and HTG 12.3 mmol/l (reference range < 2.2). The HDL cholesterol concentration was decreased to 0.81 mmol/l (reference range 1–1.8). Aminotransferases were elevated (ALT 6.6 μkat/l, reference range < 0.85; AST 4.3 μkat/l, reference range < 0.97). She was anaemic with a need of a blood transfusion (haematocrit 0.18, erythrocytes 2.1×10^{12}/l, MCV 85.2 fl, haemoglobin concentration could not be obtained due to lactescent serum). Abdominal ultrasound revealed splenomegaly (78 mm, controls 3.46 ± 0.52 mm), enlarged kidneys (60 mm, controls 52.8 ± 0.66 mm) and ascites. Necrotic debris with exudation caused by endured pancreatitis and lipoid necrosis of the omentum were described during an exploring laparotomy. Liver biopsy revealed steatosis with mild fibrosis. Ophthalmologic examination revealed whitish veins compatible with lipaemia retinalis. ECG and echocardiography examinations were normal.

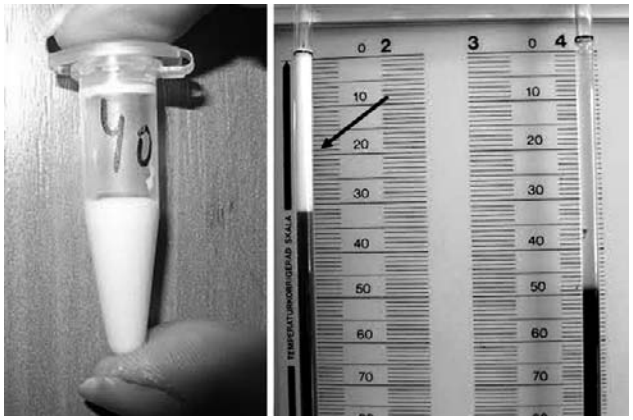

Fig. 1. Milky-cream, lipaemic appearance of the serum in P1 with LPL deficiency and hypertriglyceridaemia of 93 mmol/l.

After breastfeeding was reintroduced, a rapid increase of TG to 93 mmol/l and cholesterol to 13.4 mmol/l was documented. Spleen enlargement had diminished within days and profound HTG and HC decreased rapidly when a milk formula (Basic F® – MILUPA AG, Friedrichsdorf, Germany) with very low fat content (0.1 g of fat/100 ml) was introduced. Later on, the girl has thrived well on the low fat diet (0.6 g of fat/kg/day) supplemented with MCT oil (1 ml/kg/day), polyunsaturated fatty acids (PUFAs), vitamins A, E, D and calcium.

The girl is now 6.5-year-old, her weight is 22 kg (35th percentile) and height 122.5 cm (35th percentile). During the follow up, her TG levels are 6.4 ± 3.2 mmol/l, cholesterol 4.05 ± 0.57 mmol/l) and HDL-cholesterol 0.46 ± 0.06 mmol/l.

Patient 2

The younger brother of patient 1 was born at term with birth weight 3390 g (30th percentile) and length 51 cm (55th percentile). Due to a positive family history, laboratory analyses performed on the fifth day of life revealed a profound HTG (126 mmol/l (reference range < 2.2) and HC 17.6 mmol/l (reference range < 4.2). Low-fat diet with a smaller amount of breast milk combined with Basic F® formula was started, the daily intake of fat was approx. 0.7 g/kg and day. The diet was supplemented with fat-soluble vitamins. Cholesterol levels had normalized on the diet, but mild HTG persisted (TG 5.3 mmol/l). Aminotransferases and pancreatic amylase were both in the reference range. The cardiovascular and ophthalmologic examinations showed no pathology. The ultrasound scan revealed neither liver nor spleen enlargement, but the echogenity of both tissues was increased. Interestingly, mild enlargement of both kidneys was found, similarly to his sister (47 mm, controls 44.8 ± 0.31).

The boy is on a similar diet as his sister (0.6 g of fat/kg/day). Now, at the age of 3.5 years, his weight is 15 kg (16th percentile) and height 102 cm (24th percentile). The level of TG is 6.9 ± 3.1 mmol/l, total cholesterol 4.18 ± 0.7 mmol/l and HDL-cholesterol 0.47 ± 0.06 mmol/l.

Patient 3

The boy was born at term as a hypertrophic child with birth weight 4370 g (> 99.9th percentile) and length 58 cm (> 99.9th percentile). His mother has a normal lipid profile, the father has HC of 7.05 mmol/l (reference range < 5.2), the level of triglycerides was borderline (1.7 mmol/l; reference range < 1.7). The boy was fully breastfed for the first six months, then porridge, fruits,

vegetable puree and small portions of lean meat were added to breastfeeding. His anthropometric parameters had normalized during the first year of life; at the age of 10 months he reached 10.5 kg with a length of 73 cm.

Since the age of 2 months, the boy suffered from recurrent ear and skin infections, perianal mycosis and abdominal distension. Abdominal ultrasound revealed hepatomegaly (78 mm, controls 55.4 ± 1.06 mm) and splenomegaly (65 mm, controls 34.6 ± 0.52 mm), the size of pancreas and kidneys was normal. Ophthalmologic examination showed grey optic discs compatible with lipaemia retinalis. Echocardiography showed no pathology of the heart and coronary vessels. The laboratory analyses revealed a lactescent serum, but data of lipids were not released from the laboratory because of the effect of HTG on the results. At the age of 4 months, when the serum was 10-times diluted, the total cholesterol was 17.7 mmol/l (reference range < 4.2), TG 154 mmol/l (reference range < 2.2) and HDL-cholesterol 0.45 mmol/l (reference range 1-1.8). LDL-cholesterol and liver function tests were within the reference range.

Since the age of one year, the boy is on a low-fat diet (cereals, fruits, vegetables and lean meat) with 0.5 g of fat/kg/day. At the age of 1.5 year, his weight is 13.2 kg (79th percentile) and height 83 cm (45th percentile). The ultrasound scan showed normal size of liver and spleen; the levels of lipids have normalized (TG 2.19 mmol/l, total cholesterol 3.05 mmol/l, LDL-cholesterol 1.95 mmol/l), only HDL-cholesterol is low (0.45 mmol/l).

Ethics

Informed consent was obtained from the parents.

Methods

LPL concentration

The immunoreactive LPL concentration (mass) was measured in the serum before and 15 minutes after intravenous injection of heparin (30 IU/kg of body weight, Heparin, Léčiva™, Zentiva, Prague, Czech Republic). The samples were frozen at -70 °C and kept until analysis. LPL was analysed by a sandwich ELISA kit according to the operating instructions (Lipoprotein Lipase Elisa, ALPCO Diagnostics, Salem, NH) using anti-bovine milk lipoprotein lipase mouse monoclonal antibody coated in microtitre wells. LPL in the serum was trapped on the plate by the antigen-antibody reaction with LPL in serum samples, then reacted with antibodies. Thereafter, anti-LPL serum consisting of anti-bovine milk lipoprotein lipase chicken serum and enzyme horseradish peroxidase-labelled anti-chicken immunoglobulin G goat serum (enzyme-linked antibody) were added into the wells and incubated to initiate antigen-antibody reactions. The plates were rinsed and the peroxidase activity was measured by absorbance at 492 nm using o-phenylenediamine dihydrochloride for colour development. The reference interval of LPL mass concentration is 45–63 ng/ml in pre-heparin and 164–284 ng/ml in the post-heparin plasma. The correlation formula between plasma and serum concentration is, in compliance with the manual, N = 54, r = 0.992, y = 0.97x-1.3, showing very good concordance.

Mutation analyses

Exome sequencing was performed as described previously (Ehling et al., 2013) using 3 µg of DNA from Patients 1 and 2 and their parents. For DNA enrichment, SureSelect All Exome Kit (Agilent, Santa Clara, CA) was used according to the manufacturer's protocol. DNA sequencing was performed with the captured barcoded DNA library using SOLiD™ 4 System (Applied Biosystems, Carlsbad, CA) at the Institute for Inherited Metabolic Disorders (Prague, Czech Republic). Prioritized variants were confirmed by Sanger sequencing.

Sequencing of individual genes: all coding exons and adjacent intronic regions of the *LPL* gene (ENSG00000175445, NC_000008.10), *GPIHBP1* gene (ENSG00000182851), *APOA5* gene (ENSG00000110243), *APOC2* gene (ENSG00000234906) were amplified by PCR and analysed by direct sequencing in genetic analyser ABI 3500xL (Applied Biosystems). PCR primers are available upon request.

Results

The clinical and laboratory data of our children with LPL deficiency in comparison to 45 patients from the literature are summarized in Table 1 (Feoli-Fonseca et al., 1998; Pouwels et al., 2008). Our patients had a positive family history for dyslipidaemia. One child manifested since the first week of life with recurrent abdominal pain and one child with abdominal distension and hepatosplenomegaly since the second month of life. The third child (Patient 2), younger brother of Patient 1, was asymptomatic when diagnosed in the first week of life. Lipaemia retinalis and splenomegaly were present in two children, hepatomegaly in one and acute pancreatitis in one. All children had lactescent serum (Fig.1), profound HTG (124 ± 25 mmol/l; reference range < 2.2), HC (22.8 ± 7.3 mmol/l, reference range < 4.2) and low levels of HDL-cholesterol (Table 1). Two patients had normocytic anaemia; one of them required blood transfusion. Neither eruptive xanthomas nor intestinal bleeding were observed. The LPL mass concentration in pre-heparin serum samples was low only in one patient, but in all of them no significant increase of LPL mass was found after heparin injection (6.3, 5.6 and 4.6 ng/l respectively), corresponding to LPL deficiency. Inadequate increase of LPL mass after heparin injection was also found in the heterozygote mother of Patients 1 and 2; the other heterozygous parents had borderline increase of LPL mass after heparin injection (Fig. 2).

Mutation analysis

After direct sequencing of *APOC2*, *APOA5*, and *GPIHBP1* genes was negative, exome sequencing was performed in Patients 1 and 2 and their parents. Both siblings are homozygous for two variants: c.476C > G resulting in p.Ser159Thr amino acid substitution

Table 1. Clinical and laboratory data in three children with LPL deficiency in comparison to the patients' series from the literature (Feoli-Fonseca et al., 1998; Pouwels et al., 2008)

	Current report			Feoli-Fonseca et al., 1998	Pouwels et al., 2008
	Patient 1	**Patient 2**	**Patient 3**	**N = 16**	**N = 29**
Affected siblings	+	+	-	1/16	8/29
Gender (F/M)	F	M	M	F10/16	n.a.
Age of manifestation	1 week	*	2 months	< 1 y	2-43 y
Current age	6.5 years	3.5 years	15 months	n.a	n.a
Clinical data					
Irritability	+	-	+	7/16	n.a
Abdominal tenderness	+	-	+	2/16	n.a
Vomiting	-	-	+	3/16	n.a
Diarrhoea	-	-	+	1/16	n.a
Pancreatitis	+	-	-	7/16	16/29
Hepatomegaly	-	-	+	7/16	n.a
Splenomegaly	+	-	+	7/16	n.a
Eruptive xanthomas	-	-	-	3/16	2/29
Lipaemia retinalis	+	-	+	5/16	n.a
Lower intestinal bleeding	-	-	-	2/16	n.a
Laboratory data					
Triglycerides (mmol/l)	93	126	155[&]	↑ in16/16	↑ in 29/29
Total cholesterol (mmol/l)	33.1	17.6	17.7[&]	4.26 – 32.17	n.a
HDL-cholesterol (mmol/l)	0.8	0.47[Y]	0.45[&]	n.a	n.a
Hepatopathy	+	-	-	n.a	n.a
Haemoglobin < 95 g/l	+	-	+	10/16	n.a
Activity of LPL (ng/ml)[£]	4.6	5.6	6.4	n.a	n.a

n.a. – not available , * – younger brother of Patient 1 was asymptomatic when diagnosed at the age of 5 days, Y – on the diet, & – at the age of 4 months, £ – activity of lipoprotein lipase (LPL) expressed as the difference between pre- and post-heparin LPL mass (controls 119–220).

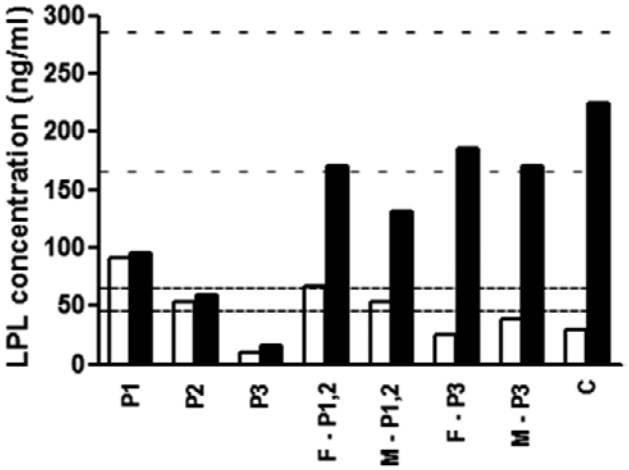

Fig. 2. LPL immunoreactive mass in pre-heparin □ and post-heparin ■ serum in three children with LPL deficiency (P1, P2, P3) compared to their parents.
F - P1,2: father of Patients 1 and 2; **M - P1,2**: mother of Patients 1 and 2; **F - P3**: father of Patient 3; **M - P3**: mother of Patient 3. **C**: healthy control. The reference range interval for pre-heparin plasma LPL mass -------- is 45-63 ng/ml, for the post-heparin plasma - - - - 164-284 ng/ml.

and c.1421C > G ending up in an early stop codon p.Ser474Term. The first mutation has not yet been described, the second variant c.1421C > G resulting in two amino acid shorter LPL protein occurs in 12 % of European alleles. Although both of the parents are heterozygous carriers for both of the mutations *in cis*, consanguinity was not confirmed. We performed genetic counselling in the family and offered prenatal diagnosis.

The *LPL* gene was sequenced in Patient 3. Two heterozygous mutations were found in exon 6: a heterozygous mutation c.604G > A resulting in amino acid substitution p.Asp202Asn, and c.698A > G resulting in amino acid substitution p.Tyr233Cys. None of these mutations has been described previously according to HGMD® Professional 2013.4 Database. Both mutations have been evaluated as pathogenic by *in silico* analysis (MutationTaster, PolyPhen2, Sift). Each of the parents is a carrier of one of these mutations.

Discussion

Three children with LPL deficiency due to three novel mutations in the *LPL* gene and early clinical manifestation during the first weeks of life are described. LPL

deficiency causes hyperchylomicronaemia (not routinely measured in our institution) and HTG (typically > 11.3 mmol/l) that leads to milky-cream appearance of the plasma (Rahalkar et al., 2009). All our patients had profound HTG (124.4 ± 25.2 mmol/l) at the time of diagnosis. As LPL is also involved in other lipoprotein metabolism, the decreased HDL-cholesterol level < 0.5 mmol/l may contribute to the unfavourable lipid profile in patients with LPL deficiency (Brunzell and Bierman, 1982; Santamarina-Fojo, 1998). All our patients had low HDL-cholesterol (0.42 ± 0.02 mmol/l). It may be of importance that the low HDL-cholesterol in our patients persisted even after long-standing dietary interventions with good compliance.

The severity of clinical symptoms seems to be proportional to the level of TG; however, asymptomatic cases with massive HTG up to 327 mmol/l were described (Brunzell et al., 1995). Therefore, LPL deficiency can be sometimes recognized only accidentally by the milky-cream appearance of the plasma or serum.

The onset of clinical symptoms in patients with LPL deficiency may vary substantially since neonatal period to adulthood (Pouwels et al., 2008; Pugni et al., 2014). The first symptoms are usually not specific (Brunzell et al., 1995; Önal et al., 2007). In childhood, recurrent attacks of abdominal pain, failure to thrive, nausea, anorexia, fever, diarrhoea and vomiting are most common (Levy and Rifkind, 1973). Neither failure to thrive nor diarrhoea was present in our patients. LPL deficiency, especially in small infants, may mimic the episodes of infantile colic or a simple abdominal tenderness, which may or may not be caused by ongoing pancreatitis. The risk for pancreatitis becomes especially apparent when the concentration of TG exceeds 10 mmol/l (Yuan et al., 2007). In our patients, recurrent abdominal pain was present in two cases and acute pancreatitis in one case. In 45 patients described by Feoli-Fonseca et al. (1998) and Pouwels et al. (2008), pancreatitis was recognized in 41 % of cases. Eruptive xanthomas, the typical yellowish skin lesions arising from foam cell deposition in patients with HTG > 22.5 mmol/l (Rahalkar et al., 2009), were not observed in our patients, although their TG levels were even higher. The 11% incidence of eruptive xanthomas in patients described by Feoli-Fonseca et al. (1998) and Pouwels et al. (2008) may indicate that long-term HTG is probably necessary for their development.

Fundoscopic examination in two of our patients revealed whitish retinal veins compatible with lipaemia retinalis. This symptom may easily be overlooked, because it is initially visible only at the retinal periphery (Zahavi et al., 2013). With increasing level of TG (> 45 mmol/l) the whole retina becomes affected and gets a salmon colour (Brunzell et al., 2001). Both liver and spleen are the target organs of lipid storage resulting in hepatosplenomegaly. Splenomegaly was present in two and hepatomegaly in one of our patients. It is worth mentioning that in our patient siblings, the enlargement of kidneys was also found, which to our best knowledge has not been described so far.

A common symptom in LPL deficiency is normocytic anaemia resulting from occult intestinal blood losses (Feoli-Fonseca et al., 1998). It has been suggested that haemolysis due to the changes in lipid composition of the erythrocyte membrane may also play an important role (Frohlich and Godin, 1986; Cantin et al., 1995). In agreement with published data, anaemia was present in two of our patients, and blood transfusion was necessary in one of them. Hyperchylomicronaemia may increase blood viscosity. Consequently, a decreased blood supply can cause a local ischaemia. Encephalopathy with convulsions or lower intestinal bleeding with bloody diarrhoea by disrupting blood circulation was described as rare symptoms in LPL-deficient patients (Simons et al., 1980; Ameis et al., 1991; Black and Sprecher, 1993; Önal et al., 2007). Intestinal bleeding did not develop in our patients and it was not common in the group of patients described by Feoli-Fonseca et al. (1998).

Mutations analyses in the LPL gene

The *LPL* gene comprises 10 exons and encodes a 448 amino acid long protein (Rahalkar et al., 2009). So far, more than 200 mutations were reported in patients with LPL deficiency. The majority of them are missense mutations in exons 5 and 6 encoding amino acids in the catalytically active site, TG-binding site and structurally relevant disulphide bridges (van Tilbeurgh et al., 1994, HGMD® Professional 2013.4 Database). Exome sequencing of our patient siblings revealed the presence of two homozygous mutations in the *LPL* gene. Mutation c.476C > G leads to substitution of serine to threonine in position 159. Ser^{159} is part of the 'catalytic triad' (three amino acids forming the catalytic centre of the LPL enzyme) and is highly conserved among species. Although serine and threonine are both polar amino acids, threonine is slightly larger and may affect conformation of the enzyme active site (Wang et al., 2013).

The second mutation/polymorphism (12% frequency in European alleles) is quite interesting, probably underlying the different biochemical phenotypes in our families. Mutation c.1421C > G causes an early stop codon on p.Ser474 (S447X) compared to the regular termination codon at position 476. At the mRNA level, the nucleotide substitution results in decreased susceptibility to translational inhibition (Ranganathan et al., 2012) and increased amount of LPL protein. In otherwise healthy carriers, the *LPL* S447X gene variant has been associated with a favourable lipid profile. The studies of gene therapy with the S447X variant in rodent models were promising in its therapeutic and protective value (Ross et al., 2004, 2005). However, this issue still remains a matter of debate as in compound heterozygotes for the S447X variant and loss-of-function mutation in the *LPL* gene, the S447X variant did not protect against HTG (Hegele et al., 2006). Although its presence leads to higher amounts of LPL in pre-heparin plasma, this released pool has altered activity and hence is not protective (Wang et al., 2007; Surendran et al., 2012).

Both of the parents are carriers of both these mutations *in cis* (c.[476C>G, 1421C>G];[=]), but they differ in laboratory results. Only part of heterozygotes with one defective allele of the *LPL* gene develop HTG. It is expected that half-normal LPL activity suffices for proper maintaining of a normal TG fasting concentration and additional genetic or environmental factors result in elevation of TG plasma levels (Hölzl et al., 2000). However, lipid metabolism is very complex and these additional factors may influence the levels of LPL itself. This may explain why the mother presented with post-heparin serum LPL masses that did not reach the upper reference interval (Fig. 2, M - P1,2). Since all the family of the mother's side have HTG, we searched for these possible supplementary causes of dyslipidaemia. It has been shown that the cumulative influence of single-nucleotide polymorphisms (SNPs) in important genes including *LPL*, *APOC2*, *APOA5*, *GPIHBP1*, *APOE2* and *APOB* may participate in HTG (Johansen and Hegele, 2011). Besides causing mutations in our patients mentioned above, there were no SNPs in the *LPL* gene. In addition, direct sequencing did not prove any polymorphism in the *APOC2*, *APOE*, *AOPA5* or *GPIHBP1* genes. In the *APOA5* gene, which encodes lipoprotein Apo A-V correlating with plasma values, we found a combination of polymorphisms that forms the common haplotype ApoA5*1. Nevertheless, this haplotype is not associated with HTG (Moreno-Luna et al., 2007). Our results did not allow us to explain the differences in lipid values of the parents; other mechanisms must play a role.

Patient 3 is a compound heterozygote for two novel missense mutations c.604G > A (p.Asp202Asn) and c.698A > G (p.Tyr233Cys). These mutations change amino acids Asp^{202} and Tyr^{233} localized in the highly conserved "central homology region" in close proximity to the catalytic site of LPL (Reina et al., 1992). Exchange of Tyr^{233} into Asp has been described in a patient with HTG (Evans et al., 2011).

Differential diagnostics

In children, fasting chylomicronaemia with extreme HTG may be caused by several factors. Most commonly, HTG is a complex result of both accumulated genetic variants and environmental factors including imbalance between caloric intake and expenditure, alcohol consumption, various diseases (obesity, renal failure, hypothyroidism, type 2 diabetes) or medications with corticosteroids, oestrogens, retinoids or β-blockers (Ballantyne et al., 2000; Yuan et al., 2007). We have already mentioned the role of SNPs involved in the lipid metabolism. Exceptionally, severe HTG is caused by monogenic disorders resulting from loss-of-function mutations in *LPL*, *APOC2*, *APOA5*, *LMF1* or *GPIHBP1* genes (Hegele, 2009; Johansen et al. 2011). Unlikely to combined HTG, these monogenic HTG usually develop already in childhood.

Although Apo C-II deficiency may also be considered as a functional deficiency of LPL taking into account the fact that biochemical and clinical phenotypes are similar (Rahalkar et al., 2009), this disorder can be recognized by addition of artificial Apo C-II to the post-heparin plasma samples. Approximately 10 mutations in the *APOC2* gene have been found in patients with familial chylomicronaemia (Connelly et al., 1987). HTG may be present in patients homozygous for mutations in *APOA5*. Apo A-V plays a focal role in hydrolysis of TG-rich lipoproteins by enhancing LPL activity (Charlton-Menys and Durrington, 2005). An extremely rare primary cause of chylomicronaemia is a mutation of the lipase maturation factor 1 (*LMF1*) gene. This gene encodes a transmembrane protein with an evolutionarily conserved domain localized in the endoplasmic reticulum and it has been suggested that it is responsible for the post-translational maturation of nascent lipase polypeptides to fully functional forms (Briquet-Laugier et al., 1999). Patients homozygous for the loss-of-function mutation in the *LMF1* gene have low expression of both LPL and hepatic lipase resulting in combined lipase deficiency (Paterniti et al., 1983; Reue and Doolittle, 1996; Peterfy et al., 2006). This disorder is responsible for extreme HTG, which may cause high blood viscosity and ischaemia (Peterfy et al., 2007). A few probands with chylomicronaemia are characterized by the presence of an endogenous circulating LPL inhibitor. These patients have extremely high LPL levels (Davignon and Dufour, 2007) and the protein function (activity) is normal. Last but not least, the discovery of glycosylphosphatidylinositol anchored-HDL-binding protein 1 (GPIHBP1) needed for integration of LPL to the endothelium surface showed that these capillary endothelial cells are not simply the passive hosts of lipolysis, but rather active participants in the lipolytic process (Beigneux et al., 2009).

Genetic variability is due to the diversity of the LPL complex in the endothelium, which includes numberless important components. In the present report we tried to summarize the most important genes and their roles in TG metabolism. This genetic basis is giving us a new perspective for distinguishing HTG phenotypes rather than a strictly biochemical approach. A variable combination of genetic mutations mentioned above is therefore responsible for hyperlipoproteinaemias classified in the Fredericson scheme (Fredericson, 1971).

Methodological notes

Reliable diagnosis of LPL deficiency requires either a post-heparin serum/plasma LPL assay or DNA analysis of the *LPL* gene (Rahalkar et al., 2009). LPL is an enzyme that binds to the endothelial surface by heparan sulphate, which is the reason why intravenous heparin elutes LPL from the endothelium to the plasma where it can be assayed (Brunzell et al., 1995). Only the mass attached to the endothelium is catalytically active (Auwerx, 1989; Brunzell et al., 1995; Hayden and Henderson, 1999). Therefore, a difference between pre- and post-heparin LPL mass reflects the enzyme activity.

A small amount of LPL is constantly present in the plasma and it has been shown that its majority consists

of inactive monomeric LPL acting as a ligand for liver receptors and cell surfaces (Williams et al., 1992). Although this pre-heparin, catalytically non-active LPL varies during the day due to food consumption, it can also be influenced by lipid-lowering agents (Totsuka et al., 2000). Lipoprotein lipase ELISA test only detects the non-denatured dimeric form of the enzyme, and therefore no alteration of inactive LPL can disturb the measurement of active LPL, which is minimally present in the plasma of normal patients as well.

Measuring the mass of LPL is a standard method for diagnosing LPL deficiency. Although LPL activity is also measured, particularly in experimental settings, quantitative determination of LPL mass is a diagnostic approach in the majority of cases. However, in some very rare cases, LPL mass could be normal regardless of its low activity resulting in false negative results of the test. Especially mutations in the *APOC2* gene encoding the important cofactor of LPL may cause this discrepancy. The addition of artificial APOC2 to the test would be necessary for the correct diagnosis (Rahalkar et al., 2009).

In our study, the LPL immunoreactive mass in the pre-heparin serum was low only in one patient, but as already mentioned, the pre-heparin LPL mass might be influenced by additional environmental or genetic factors, and hence is not convenient for the diagnostics of LPL deficiency. On the contrary, in agreement with the diagnosis, the post-heparin release of the LPL immunoreactive mass was very low in all patients. Interestingly, in their parents, all molecularly confirmed heterozygotes for LPL deficiency, the post-heparin release of the LPL immunoreactive mass was low only in one of four, suggesting that biochemical analyses in the affected families are not feasible for identification of heterozygotes.

Dietary interventions and treatment

Low-fat diet (0.5–0.7 g/kg of body weight) recommended in patients with LPL deficiency is usually sufficient for the proper growth in childhood. Oils containing medium-chain fatty acids can be used as a source of fat as they do not rely on CM formation (Rouis et al., 1997). In addition, supplementation with essential free fatty acids and lipid-soluble vitamins is advisable (Press et al., 1974). In small children, frequent feeding is necessary to avoid lipolysis.

Pharmacotherapy plays a less important role, because the lipid-lowering drugs usually up-regulate LPL activity, and therefore their TG-lowering effect is low (Rahalkar et al., 2009). Although LPL deficiency as a monogenic disorder seems to be a perfect candidate for gene therapy, its high price makes it hardly accessible. Alipogene tiparvovec (Glybera®), using a functional copy of LPL, has been recently approved by the European Commission (Haddley, 2013, http://www.uniqure.com), but its cost would make it probably the most expensive drug on the market (http://www.bionews.org).

Conclusion

Although very rare, the LPL deficiency must be considered in neonates and young infants with abdominal pain and HTG because its early treatment might prevent development of life-threatening acute pancreatitis.

References

Ameis, D., Kobayashi. J., Davis R. C., Ben-Zeev, O., Malloy, M. J., Kane, J. P. (1991) Familial chylomicronemia (Type I hyperlipoproteinemia) due to a single missense mutation in the lipoprotein lipase gene. *J. Clin. Invest.* **87**, 1165-70.

Auwerx, J. H., Babirak, S. P., Fujimoto, W. Y., Iverius, P.-H., Brunzell, J. D. (1989) Defective enzyme protein in lipoprotein lipase deficiency. *Eur. J. Clin. Invest.* **19**, 433-437.

Ballantyne, C. M., Grundy, S. M., Oberman, A. (2000) Hyperlipidemia: diagnostic and therapeutic perspectives. *J. Clin. Endocrinol. Metab.* **85**, 2089-2112.

Beigneux, A. P., Weinstein, M. M., Davies, B. S. J., Gin, P., Bensadoun, A., Fong, L. G., Young, S. G. (2009) GPIHBP1 and lipolysis: an update. *Curr. Opin. Lipidol.* **20**, 211-216.

Black, D. M., Sprecher, D. L. (1993) Dietary treatment and growth of hyperchylomicronemic children severely restricted in dietary fat. *Am. J. Dis. Child.* **147**, 60-62.

Briquet-Laugier, V., Ben-Zeev, O., White, A., Doolittle, M. H. (1999) *cld* and *lec23* are disparate mutations that affect maturation of lipoprotein lipase in the endoplasmic reticulum. *J. Lipid. Res.* **40** 2044-2058.

Brunzell, J. D., Bierman, E. L. (1982) Chylomicronemia syndrome. Interaction of genetic and acquired hypertriglyceridemia. *Med. Clin. North Am.* **66**, 455-468.

Brunzell, J. (1995) Familial lipoprotein deficiency and other causes of the chylomicronemia syndrome. In: *The Metabolic and Molecular Bases of Inherited Disease,* 7th ed., eds. Brunzell J., Scriver, C., Baudet, A., Sly, W., Valle, D., pp. 1913-1932, McGraw-Hill, New York.

Brunzell, J. D., Deeb, S. S. (2001) Familial lipoprotein lipase deficiency, Apo C-II deficiency, and hepatic lipase deficiency. In: *The Metabolic and Molecular Bases of Inherited Disease,* 8th ed., eds. Scriver, C. R., Beadet, A. L., Sly, W. S., Valle, D., Childs, B., Kinzler, K. W., Vogelstein, B., pp. 2789-2816, McGraw-Hill, New York.

Cantin, B., Boudriau, S., Bertrand, M., Brun, L. D., Gagné, C., Rogers, P. A. (1995) Hemolysis in primary lipoprotein lipase deficiency. *Metabolism* **44**, 652-658.

Charlton-Menys, V., Durrington, P. N. (2005) Apolipoprotein A5 and hypertriglyceridemia. *Clin. Chem.* **51**, 295-297.

Connelly, P. W., Maguire G. F., Little, J. A. (1987) Apolipoprotein CII St. Michael. Familial apolipoprotein CII deficiency associated with premature vascular disease. *J. Clin. Invest.* **80**, 1597-1606.

Davignon, J., Dufour, R. (2007) *Primary Hyperlipidemias: an Atlas of Investigation and Diagnosis.* Clinical Publishing, Oxford, UK.

Ehling, R., Nosková, L., Stránecký, V., Hartmannová, H., Přistoupilová, A., Hodaňová, K., Benke, T., Kovacs, G. G., Ströbel, T., Niedermüller, U., Wagner, M., Nachbauer, W., Janecke, A., Budka, H., Boesch, S., Kmoch, S. (2013) Cerebellar dysfunction in a family harboring the PSEN1

mutation co-segregating with a cathepsin D variant p.A58V. *J. Neurol. Sci.* **326**, 75-82.

Evans, D., Arzer, J., Aberle, J., Beil, F. U. (2011) Rare variants in the lipoprotein lipase (LPL) gene are common in the hypertriglyceridemia but rare in Type III hyperlipidemia. *Atherosclerosis* **214**, 386-390.

Evans, V., Kastelein, J. J. P. (2003) Mini-symposium: Lipid and protein disorders: Biochemistry and molecular genetics. *Cardiovasc. Drugs Ther.* **16**, 283-287.

Frederickson, D. S. (1971) An international classification of hyperlipidemias and hyperlipoproteinemias. *Ann. Intern. Med.* **75**, 471-472.

Frohlich, J., Godin, D. V. (1986) Erythrocyte membrane alterations and plasma lipids in patients with chylomicronemia and in Tangier disease. *Clin. Biochem.* **19**, 229-34.

Gagne, C., Brun, L. D., Julien, P., Moorjani, S., Lupien, P. J. (1989) Primary lipoprotein-lipase-activity deficiency: clinical investigation of French Canadian population. *CMAJ*, **140**, 405-411.

Haddley, K. (2013) Alipogene tiparvovec for the treatment of lipoprotein lipase deficiency. *Drugs Today (Barc.)* **49**, 161-170.

Hayden, M., Henderson, H. E. (1999) The molecular biology and genetics of human lipoprotein lipase. In: *Lipoproteins in Health and Disease.* eds. Betterige, D. J., Illingworth, D. R., Shepherd, J., pp. 247-260, Oxford University Press, London.

Hegele R. A. (2006) Gene therapy with lipoprotein lipase variant S447X. *Arterioscler. Thromb. Vasc. Biol.* **26**, 25-28.

Hegele R. A. (2009) Plasma lipoproteins: genetic influences and clinical implications. *Nat. Rev. Genet* **10**, 109-121.

Hölzl, B., Kraft, H. G., Wiebusch, H., Sandhofer, A., Patsch, J., Sandhofer, F., Paulweber, B. (2000) Two novel mutations in the lipoprotein lipase gene in a family with marked hypertriglyceridemia in heterozygous carriers. Potential interaction with the polymorphic marker D1S104 on chromosome 1q21-q23. *J. Lipid Res.* **41**, 734-741

Johansen, C. T., Hegele, R. A. (2011) Genetic bases of hypertriglyceridemic phenotypes. *Curr. Opin. Lipidol.* **22**, 247-253.

Johansen, C.T., Kathiresan, S., Hegele, R. A. (2011) Genetic determinants of plasma triglycerides. *J. Lipid. Res.* **52**, 189-206.

Levy, R. I., Rifkind, B. M. (1973). Diagnosis and management of hyperlipoproteinemia in infants and children. *Am. J. Cardiol.* **31**, 547-556.

Moreno-Luna, R., Perez-Jimenez, F., Marin, C., Perez-Martinez, P., Gomez, P., Jimenez-Gomez, Y., Delgado-Lista, J., Moreno, J. A., Tanaka, T., Ordovas, J. M., Lopez-Miranda, J. (2007) Two independent apolipoprotein A5 haplotypes modulate postprandial lipoprotein metabolism in a healthy Caucasian population. *J. Clin. Endocrinol. Metab.* **92**, 2280-2285.

Önal, H., Aktuglu-Zeybek, C., Alhaj, S., Altun, G. (2007). Encephalopathy in Type I hyperlipidemia. *Indian Pediatr.* **44**, 306-308.

Paterniti, J. R. Jr, Brown, W. V., Ginsberg, H. N., Artzt, K. (1983) Combined lipase deficiency (cld): a lethal mutation on chromosome 17 of the mouse. *Science* **221**, 167-169.

Peterfy, M., Mao, H. Z., Doolittle, M. H. (2006) The *cld* mutation: narrowing the critical chromosomal region and selecting candidate genes. *Mamm. Genome* **17**, 1013-1024.

Peterfy, M., Ben-Zeev, O., Mao, H. M., Wissglas-Volkov, D., Aouizerat, B., E., Pullinger, C., R., Frost, P. H., Kane, J. P., Malloy, M. J., Reue, K., Pajukanta, P., Doolittle, M. H. (2007) Mutations in *LMF1* cause combined lipase deficiency and severe hypertriglyceridemia. *Nat. Genet.* **39**, 1483-1487.

Pouwels, E. D., Blom, D. J., Firth, J. C., Henderson, H. E., Marais, A. D. (2008) Severe hypertriglyceridemia as a result of familial chylomicronaemia: the Cape Town experience. *SAMJ* **2**, 105-108.

Press, M., Hartop, P. J., Prottey, C. (1974) Correction of essential fatty-acid deficiency in man by the cutaneous application of sunflower-seed oil. *Lancet* **1**, 597-598.

Pugni, L., Riva, E., Pietrasanta, C., Rabacchi, C., Bertolini, S., Pederiva, C., Mosca, F., Calandra, S. (2014) Severe hypertriglyceridemia in a newborn with monogenic lipoprotein lipase deficiency: an unconventional therapeutic approach with exchange transfusion. *JIMD Rep.* **13**, 59-64.

Rahalkar, A. R., Giffen, F., Har, B., Ho, J., Morrison, K. M., Hill, J., Wang, J., Hegele, R. A., Joy, T. (2009) Novel *LPL* mutations associated with lipoprotein lipase deficiency: two case reports and a literature review. *Can. J. Physiol. Pharmacol.* **87** 151-160.

Ranganathan, G., Unal, R., Pokrovskaya, I. D., Tripathi, P., Rotter, J. I., Goodarzi, M. O., Kern, P.A. (2012) The lipoprotein lipase (LPL) S447X gain of function variant involves increased mRNA translation. *Atherosclerosis* **221**, 143-147.

Reina, M., Brunzell, J. D., Deeb, S. S. (1992) Molecular basis of familial chylomicronemia: mutations in the lipoprotein lipase and apolipoprotein C-II genes. *J. Lipid. Res.* **33**, 1823-1832.

Reue, K., Doolittle, M. H. (1996) Naturally occurring mutations in mice affecting lipid transport and metabolism. *J. Lipid. Res.* **37**, 1387-1405.

Rouis, M., Dugi, K. A., Previato, L., Patterson, A. P., Brunzell, J. D., Brewer, H. B., Santamarina-Fojo, S. (1997) Therapeutic response to medium-chain triglycerides and ω-3 fatty acids in a patient with the familial chylomicronemia syndrome. *Arterioscler. Thromb. Vasc. Biol.* **17**, 1400-1406.

Ross, C. J., Twisk, J., Meulenberg, J. M., Liu, G., van den Oever, K., Moraal, E. (2004) Long-term correction of murine lipoprotein lipase deficiency with AAV1-mediated gene transfer of the naturally occurring LPL(S447X) beneficial mutation. *Hum. Gene Ther.* **15**, 906-919.

Ross, C. J., Liu, G., Kuivenhoven, J. A., Twisk, J., Rip, J., van Dop, W. (2005) Complete rescue of lipoprotein lipase-deficient mice by somatic gene transfer of the naturally occurring LPLS447X beneficial mutation. *Arterioscler. Thromb. Vasc. Biol.* **25**, 2143-2150.

Santamarina-Fojo, S. (1998). The familial chylomicronemia syndrome. *Endocrinol. Metab. Clin. North Am.* **27**, 551-567.

Siafakas, C. G., Brown, M. R., Miller, T. L. (1999) Neonatal pancreatitis associated with familial lipoprotein lipase deficiency. *J. Pediatr. Gastroenterol. Nutr.* **29**, 95-98.

Simons, L. A., Grigor, W., Martin, H. C. O., Gibson, J. C., Gunn, A. P. (1980) Type I hyperlipoproteinaemia and recurrent scrotal pain. *Aust. NZ J. Med.* **10**, 336-339.

Surendran, R. P., Visser, M. E., Heemelaar, S., Wang, J., Peter, J., Defesche, J. C., Kuivenhoven, J. A., Hosseini, M., Peterfy, M., Kastelein, J. J., Johansen, C. T., Hegele, R. A., Stroes, E. S., Dallinga-Thie, G. M. (2012) Mutations in LPL, APOC2, APOA5, GPIHBP1 and LMF1 in patients with severe hypertriglyceridaemia. *J. Intern. Med.* **272**, 185-196.

Totsuka, M., Miyashita, Y., Ito, Y., Watanabe, H., Murano, T., Shirai, K. (2000) Enhancement of preheparin serum lipoprotein lipase mass by bezafibrate administration. *Atherosclerosis* **153**, 175-179.

van Tilbeurgh, H., Roussel, A., Lalouel, J. M., Cambillau (1994) Lipoprotein lipase. Molecular model based on the pancreatic lipase x-ray structure: consequences for heparin binding and catalysis. *J. Biol. Chem.* **269**, 4626-4633.

Wang, J., Cao, H., Ban, M. R., Kennedy, B. A., Zhu, S., Anand, S., Yusuf, S., Pollex, R. L., Hegele, R. A. (2007) Resequencing genomic DNA of patients with severe hypertriglyceridemia. *Arterioscler. Thromb. Vasc. Biol.* **27**, 2450-2455.

Wang, Z., Li, S., Sun., L., Fan, J., Liu, Z. (2013) Comparative analyses of lipoprotein lipase, hepatic lipase, and endothelial lipase, and their binding properties with known inhibitors. *PLoS One* **8**, 1-15.

Williams, K. J., Fless, G. M., Petrie, K. A., Snyder, M. L., Brocia, R. W., Swenson, T. L. (1992) Mechanism by which lipoprotein lipase alters cellular metabolism of lipoprotein (a), low density lipoprotein, and nascent lipoproteins: roles for low density lipoproteins receptors and heparan sulphate proteoglycans. *J. Biol. Chem.* **267**, 13284-13292.

Yuan, G., Al-Shali, K. Z., Hegele, R. A. (2007). Hypertriglyceridemia: its etiology, effects and treatment. *CMAJ* **176**, 1113-1120.

Zahavi, A., Snir, M., Kella, Y. R. (2013) Lipemia retinalis: case reports and review of the literature. *J. AAPOS* **17**, 110-111.