LPIN1 gene mutations: a major cause of severe rhabdomyolysis in early childhood.

Caroline Michot, Laurence Hubert, Michele Brivet, Linda De Meirleir, Vassili Valayannopoulos, Wolfgang Müller-Felber, Ramesh Venkateswaran, Helene Ogier de Baulny, Isabelle Desguerre, Cécilia Altuzarra, et al.

Human Mutation

LPIN1 gene mutations: a major cause of severe rhabdomyolysis in early childhood.

Journal:	*Human Mutation*
Manuscript ID:	humu-2010-0028.R2
Wiley - Manuscript type:	Mutation in Brief
Date Submitted by the Author:	23-Apr-2010

Complete List of Authors:	Michot, Caroline; INSERM, U-781; Necker Hospital, Metabolic Diseases Hubert, Laurence; INSERM, U-781 BRIVET, michele; APHP hopital de Bicetre, biochemistry De Meirleir, Linda; UZ Brussel, Pediatric neurology- metabolic diseases Valayannopoulos, Vassili; Hospital Necker Des Enfants Malades, Department of Neuro-Metabolism Müller-Felber, Wolfgang; Ludwig-Maximilians-University, Department of Pediatrics Venkateswaran, Ramesh; Newcastle General Hospital, Pediatrics Ogier de Baulny, Helene; AP-HP hopital Robert Debre, pediatric metabolic unit Desguerre, Isabelle; Hospital Necker Des Enfants Malades, Department of Neuro-Metabolism Altuzarra, Cécilia; Besançon C.H.U., Pediatrics Thompson, Elizabeth; Women's & Children's Hospital, SA Clinical Genetics Smitka, Martin; Children's Hospital, Technical University Dresden, Neuropaediatrics Hübner, Angela; Children's Hospital, Technical University Dresden, Neuropaediatrics Husson, Marie; Bordeaux C.H.U., Department of Pediatrics Horvath, Rita; Newcastle University, Mitochondrial Research Group, Institute for Aging and Health Chinnery, Patrick; University of Newcastle upon Tyne, Mitochondrial Research Group Vaz, Frederic; Academic Medical Center, Department of Clinical Chemistry Munnich, Arnold; INSERM, U-781 Elpeleg, Orly; Hadassah Medical Center Delahodde, Agnès; Paris-Sud University, CNRS-UMR8621, Institut de Génétique et Microbiologie De Keyser, Yves; INSERM, U-781

	De Lonlay, Pascale; INSERM, U-781; AP-HP hopital Necker, pediatric metabolic unit; Hospital Necker Des Enfants Malades, Department of Neuro-Metabolism
Key Words:	rhabdomyolysis, LPIN1, founder effect, intragenic deletion

HUMAN MUTATION Mutation in Brief #____ (2010) Online

MUTATION IN BRIEF

HUMAN MUTATION

LPIN1 gene mutations: a major cause of severe rhabdomyolysis in early childhood.

Caroline Michot[1], Laurence Hubert[1], Michèle Brivet[2], Linda De Meirleir[3], Vassili Valayannopoulos[1], Wolfgang Müller-Felber[4], Ramesh Venkateswaran[5], Hélène Ogier[6], Isabelle Desguerre[1], Cécilia Altuzarra[7], Elizabeth Thompson[8], Martin Smitka[9], Angela Huebner[9], Marie Husson[10], Rita Horvath[11,12], Patrick Chinnery[12], Frederic M. Vaz[13], Arnold Munnich[1], Orly Elpeleg[14], Agnès Delahodde[15], Yves de Keyzer[1], Pascale de Lonlay[1*].

[1]Paris Descartes University, INSERM U781 and Ref Center of Metabolic Diseases, Necker Hospital, Paris, France; [2]Dpt Biochemistry, Kremlin-Bicêtre Hospital, Kremlin-Bicêtre, France; [3]Pediatric neurology-metabolic diseases, UZ Brussel, Brussels, Belgium; [4]Dpt Pediatrics, Ludwig-Maximilians-University, Munich, Germany; [5]Dpt Pediatrics, Newcastle General Hospital, Newcastle upon Tyne, UK; [6]Ref Center of Metabolic Diseases, Robert-Debré Hospital, Paris, France; [7]Dpt Pediatrics, Besançon C.H.U., Besançon, France; [8]SA Clinical Genetics, Women's & Children's Hospital, North Adelaide, Australia; [9]Dpt Neuropaediatrics, Children's Hospital, Technical University Dresden, Dresden, Germany; [10]Dpt Pediatrics, Bordeaux C.H.U., Bordeaux, France; [11]Friedrich-Baur-Institut, Ludwig-Maximilians University, Munich, Germany; [12]Mitochondrial Research Group, Institute for Aging and Health, Newcastle University, Newcastle upon Tyne, UK; [13]Dpt Clinical Chemistry, Academic Medical Center, Amsterdam, the Netherlands; [14]Dpt Genetics and Metabolic Diseases, Hadassah-Hebrew University Medical Center, Jerusalem, Israel; [15]Paris-Sud University, CNRS-UMR8621, Institut de Génétique et Microbiologie, Orsay, France.

*Correspondence to: Prof. Pascale de Lonlay, Reference Center of Metabolic Disease, Necker Hospital, 149 rue de Sèvres, 75015, Paris, France; Tel: (+33) 1 44 49 48 52; Fax: (+33) 1 44 49 48 50; Email: pascale.delonlay@nck.aphp.fr.

Communicated by <Please don't enter>

ABSTRACT: Autosomal recessive *LPIN1* mutations have been recently described as a novel cause of rhabdomyolysis in a few families. The purpose of the study was to evaluate the prevalence of *LPIN1* mutations in patients exhibiting severe episodes of rhabdomyolysis in infancy. After exclusion of primary fatty acid oxidation disorders, *LPIN1* coding sequence was determined in genomic DNA and cDNA. Among the 29 patients studied, 17 (59%) carried recessive nonsense or frameshift mutations, or a large scale intragenic deletion. In these 17 patients, episodes of rhabdomyolysis occurred at a mean age of 21 months. Secondary defect of mitochondrial fatty oxidation or respiratory chain was found in skeletal muscle of two patients. The intragenic deletion, c.2295-866_2410-30del, was identified in 8/17 patients (47%), all Caucasians, and occurred on the background of a common haplotype, suggesting a founder effect. This deleted human *LPIN1* form was unable to complement Δ*pah1* yeast for growth on glycerol, in contrast to normal *LPIN1*. Since more than 50% of our series harboured *LPIN1* mutations, *LPIN1* should be regarded as a major cause of severe myoglobinuria in early childhood. The high frequency of the intragenic *LPIN1* deletion should provide a valuable criterion for fast diagnosis, prior to muscle biopsy.

KEY WORDS: Rhabdomyolysis, *LPIN1*, intragenic deletion, founder effect.

Received <date>; accepted revised manuscript <date>.

INTRODUCTION

Myoglobinuria is a rare condition resulting from the destruction of skeletal muscle fibers (rhabdomyolysis). Hereditary myoglobinurias have been ascribed to mitochondrial fatty acid ß-oxidation defects (FAO), mitochondrial respiratory chain (RC) deficiency and inborn errors of glycogenolysis (Dubowitz and Fardeau, 1995; Tein, 1999; Tonin, et al., 1990). Metabolic investigations include plasma carnitine and acylcarnitine profiles, urinary organic acids analysis and in vitro studies of FAO in fresh lymphocytes or in cultured fibroblasts. When the latter are normal, common practice recommends performing a skeletal muscle biopsy for histological and enzymatic studies. Despite these investigations, the disease mechanism remains unknown in at least half of the patients (Ohkuma, et al., 2009).

Recently, *LPIN1* mutations (MIM *605518) have been reported as a novel cause of rhabdomyolysis (Zeharia, et al., 2008). Lipin-1 is a 890 amino acid protein predominantly expressed in muscle and adipose tissue (Donkor, et al., 2007; Reue and Brindley, 2008; Reue and Zhang, 2008), initially identified by positional cloning in the fatty liver dystrophy mouse (fld) (Peterfy, et al., 2001). It exhibits a dual role, as a phosphatidate phosphatase 1 (PAP) for triacylglycerol and phospholipid biosynthesis (Donkor, et al., 2007; Han, et al., 2006), and as a transcriptional co-activator through its association with *PPARα* and *PGC-1α* to regulate the expression of genes encoding FAO and RC enzymes (Donkor, et al., 2008; Finck, et al., 2006; Reue and Zhang, 2008; Sugden, et al., 2010). Expression of *LPIN1* is also required for adipocyte differenciation and function (Phan, et al., 2005). Lipin-1 contains two highly conserved domains, a N-LIP domain (residues 1-114) of unknown function, and a C-LIP domain (residues 673-830) which contains the canonical DXDXT motif for PAP activity and the LXXIL motif for nuclear receptor binding (Reue and Brindley, 2008; Reue and Zhang, 2008).

Studying a series of 29 cases of unexplained myoblobinuria, we found two *LPIN1* mutations in 59% of the patients. Moreover, a prevalent intragenic deletion was observed, prompting to screen for this frequent *LPIN1* deletion prior to other tests in the investigation of myoglobinuric patients.

MATERIALS AND METHODS.

Patients.

A total of 29 patients from 23 families (4 consanguineous families; 22 Eurocaucasians, 1 African, 5 Maghrebis and 1 Asian) were included in the study. Patients 1, 2 and 3 have been previously described (Zeharia, et al., 2008). Inclusion criteria were: i) episode(s) of rhabdomyolysis, ii) since early infancy (< 5 years), iii) plasma creatine phosphokinase (CK) levels above 10 000 UI/L during bouts of myoglobinuria.

Metabolic investigations.

Extensive metabolic work-up excluded primary FAO disorders, carnitine palmitoyl transferase 2 (CPT2) deficiency, glycogen storage diseases and dystrophinopathies.

In patients 1, 3 and 4, serum leptin and adiponectin levels were determined using a commercial radioimmunoassay kit (RIA, LINCO Research Inc., Saint Louis, MI, USA).

Glucose metabolism was evaluated by HbA1c level and an oral glucose tolerance test (1.75 g/kg dextrose orally administrated, dosage of blood glucose and insulin at 0, 30, 60 and 120 min) in patients 1, 3 and 4.

Body composition measurement was assessed by dual-absorptiometry (Hologic 4500W instrument) in patients 3 and 4 (Ellis, et al., 2000).

Phospholipids were assayed on muscle biopsies of patient 1 (previously described, (Zeharia, et al., 2008)) and patient 4 and on plasma samples of patients 1, 3, 4, by lipid extraction and HPLC-MS quantification, and compared to three control samples (Valianpour, et al., 2005).

RC activities were measured spectrophotometrically in skeletal muscle and cultured skin fibroblasts (Rustin, et al., 1994). CPT2 activity was studied in skeletal muscle (Isackson, et al., 2006).

Molecular analyses.

Informed consent was obtained from the patients using a form approved by the Hospital Necker ethics board committee.

Genomic DNA and total RNA were obtained from peripheral blood leukocytes and/or cultured fibroblasts using standard procedures. cDNA synthesis was performed using High capacity cDNA reverse transcription kit (Applied Biosystems).

The coding sequence of the *LPIN1* gene (GenBank NM_145693.1) was entirely determined from cDNAs or genomic DNA. Sequencing reactions were performed with the Big Dye Terminator Cycle Sequencing kit v.3.1 and analyzed on an ABI3100 sequencer (Applied Biosystems). Sequence analyses were performed using Seqscape software v2.5 (Applied Biosystems).

Parents' genotype was established to determine the allelic transmission of the *LPIN1* mutations.

Mutations numbering is based on cDNA sequence, with +1 corresponding to the A of ATG translation initiation codon (codon 1) in the reference sequence.

For the diagnosis of the intragenic deletion, a genomic DNA fragment extending from exon 17 to exon 20 was amplified by long range PCR, using specific primers (5': GCTGCTGAGTCCCAGCAGCCTCTTCTCTGC 3'; 5': GTGGTCGACTACTTCACAGAGTCTCACATA 3') with 2.5 U LA-Takara DNA polymerase (TaKaRa Bio Inc. Japan) for 30 cycles (10 sec 94°C, 30 sec 65°C and 14 min at 68°C).

The precise localization of the deletion breakpoints was established by sequencing a genomic DNA fragment amplified with the primers (5': AAAGGTCTGGCACATCTTCTGTT 3' (intron 17) ; 5': AATCCCATTTAGC CCACCGACTCAG 3' (intron 19)), with Taq DNA polymerase (Roche) for 35 cycles (30 sec 95°C, 30 sec 65°C and 2 min at 72°C).

Haplotype studies were performed in all patients carrying the genomic deletion and in their parents. We used the intragenic polymorphic markers D2S328, located in intron 16, and D2S168, D2S2377, D2S2200, W437 and D2S2199 flanking the *LPIN1* gene.

The presence of sequences known to participate in genomic rearrangements was searched for using bioinformatic tools (http://www.repeatmasker.org) as well as analysis of splicing consensus motifs and splicing enhancers/silencers (http://spliceport.cs.umd.edu/, http://genes.mit.edu/burgelab/rescue-ese/, http://rulai.cshl.edu/tools/ESE2/).

The deleterious nature of the intragenic deletion on PAP activity was examined in a yeast complementation assay, using the Δ*pah1* strain deleted for the yeast ortholog of the *LPIN1* gene (Han, et al., 2006). *LPIN1* cDNAs, normal and lacking exons 18 and 19, were obtained by RT-PCR and subcloned in the XhoI/BamHI sites of the BFG-I shuttle vector. The yeast Δ*pah1* cells were transformed with the BFG-I plasmid encoding the normal human lipin-1 or the deleted human lipin-1 (lipin-1Δ). After selection of yeast cells bearing plasmids, serial dilutions (1:10) of the cells were spotted onto YPD (complete medium, 2% glucose) and YPG (complete medium, 2% glycerol) and growth was scored after 3 days of incubation at 28 and 36°C.

RESULTS.

Molecular characterization of the patients.

More than half of our myoglobinuric patients (17/29) were homozygous or compound heterozygous for *LPIN1* mutations or intragenic deletion (Table 1). These molecular abnormalities were confirmed both on cDNA and genomic DNA and not found in 250 control chromosomes.

Table 1. Clinical and molecular characterization of patients with *LPIN1* mutations.

Patient					Clinical presentation		Mutation	
N°	Age	Sex	Ethnic origin	Consanguinity	(CK: UI/L)	Myolysis episodes (triggering event)	Nucleotide	Amino Acid
1*	5y	M	Mauritania	Yes	200 000	2 at 27 and 30m (fever)	c.192+2T>C c.192+2T>C	p.Cys30LeufsX3 p.Cys30LeufsX3
2*	died at 6y	F	France	No	100-150 000	4 between 15m and 6y (fever)	c.2401C>T c.2295-866_2410-30del	p.Arg801X p.Glu766_Ser838del
3 *	10y	F	France	No	120 000	3 between 18m and 10y (fever)	c.1441+2T>C c.2295-866_2410-30del	p.Asn417LysfsX22 p.Glu766_Ser838del
4	10y	M	France	No	120 000	2 at 16m and 8y (fever)	c.377_380dup c.2295-866_2410-30del	p.Met128GlnfsX45 p.Glu766_Ser838del
5	18m	M	Belgium	No	900 000	1 at 1y	c.2295-866_2410-30del c.2295-866_2410-30del	p.Glu766_Ser838del p.Glu766_Ser838del
6	3y	F	France-Asia	No	300-500 000	2 at 2y and 2,5y (fever)	c.944C>G c.1162C>T	p.Ser315X p.Arg388X
7	died at 2.5y	M	Belgium	No	57 000	1 at 2,5y (fever)	c.2295-866_2410-30del c.2295-866_2410-30del	p.Glu766_Ser838del p.Glu766_Ser838del
8	died at 4y	F	Belgium	No	> 20 000	1 at 4y (fasting)		
9	died <1y	M	Egypt	No	> 20 000	1 at 5m (fasting)	c.2513+1G>A c.2513+1G>A	p.Asp804ValfsX6 p.Asp804ValfsX6
10	died at 4y	F	England	No	10-450 000	2 at 14m and 4y (fever)	c.2295-866_2410-30del c.921delT	p.Glu766_Ser838del p.Gln308ArgfsX36
11	6y	M	North africa	Yes	50 000	1 at 5y (general anesthesia)	c.1162C>T / c.1162C>T	p.Arg388X p.Arg388X
12	8y	F	North africa	Yes	300 000	1 at 2y (fasting)		
13	8y	F	Germany	No	1 000 000	10 between 2 and 8y (fever)	c.2295-866_2410-30del c.946_952del	p.Glu766_Ser838del p.Asp316LeufsX26
14	23y	F	France	No	> 100 000	5 between 4 and 16 y (fever, fasting with local anaesthetic)	c.1259delC c.2513+1G>A	p.Pro420LeufsX39 p.Asp804ValfsX6
15	15y	M	France	No	> 100 000	7 between 4 and 16 y (fever, fasting with local anaesthetic)		
16	18y	M	France	No	> 100 000	6 between 4 and 16 y (fever, fasting with local anaesthetic)		
17	4y	F	Germany	No	450 000	2 at 3y and 4 y	c.2253_2254del c.57C>A	p.Leu752AlafsX17 p.Tyr19X

*: patients previously described (Zeharia, et al., 2008). CK = creatine phosphokinase.
Patients of the same family share a fused box for mutation description.
The patients 3, 5 and 9 had a sibling who died in the same condition (bout of rhabdomyolysis).

Thirteen different mutations were discovered scattered throughout the *LPIN1* coding region, including 12 nonsense mutations (either direct at residues 19, 315, 388, 801 or as a frameshift consequence) and 1 intragenic deletion (Figure 1). 9/12 nonsense mutations lead to a predicted protein totally lacking the C-LIP domain, and the 3 remaining stop mutations are located within the C-LIP domain, but do not remove the DXDXT and LXXIL motifs. The last mutation, c.2295-866_2410-30del (p.Glu766_Ser838del), is an in-frame intragenic deletion, removing a large part (residues 766-838) of the C-LIP domain, but preserves both DXDXT and LXXIL motifs.

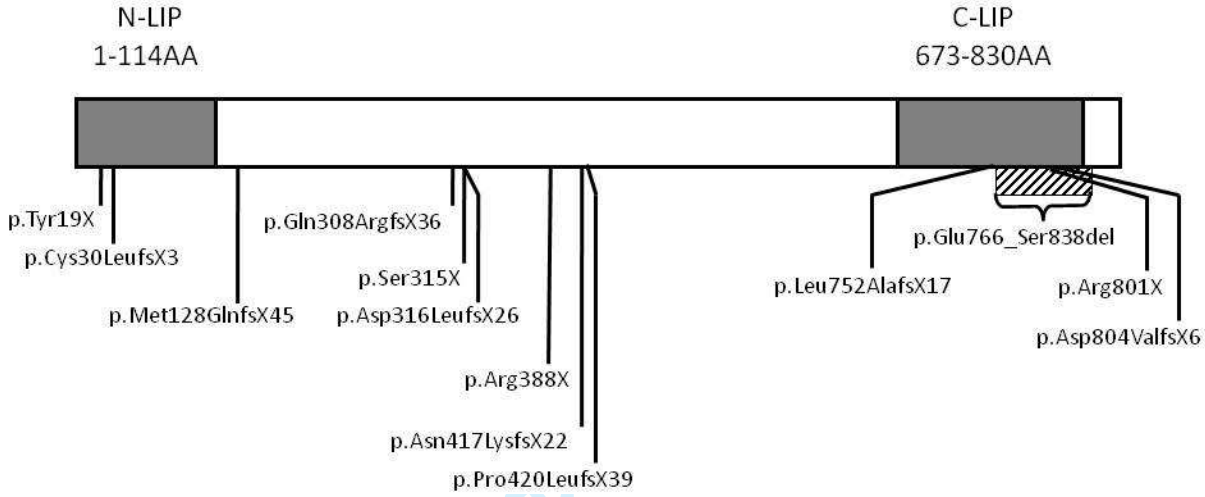

Figure 1. Schematic diagram of the mutations reported on the lipin-1 protein. The highly conserved domains are shown as shaded boxes. Mutations (as amino acid changes) are indicated with vertical lines.

Unexpectedly, this intragenic deletion was present in 8/17 patients, all Caucasians, who displayed an abnormal cDNA lacking exons 18 and 19. Long range PCR of their genomic DNA encompassing exons 17 to 20 generated an abnormal amplification product, approximately 2 kb shorter than that of controls. The breakpoints of the deletion were identical in all patients. The deletion was 1763 bp long and extended from intron 17 (nt +3377) to intron 18 (nt + 782, 30 bp upstream to exon 19), encompassing exon 18, but not exon 19.

In silico analysis of splicing enhancers and branch-point within the deleted sequence did not bring clues to understand the absence of exon 19 at the cDNA level. Similarly, no repeated elements susceptible to mediate a recurrent chromosomal rearrangement were detected by sequence analysis in the vicinity of the breakpoints. A common minimal haplotype (markers D2S328, D2S2200 and D2S2199) segregated with the intragenic deletion in the 8 children. Because all these patients were of Caucasian origin, a founder effect is highly probable.

De novo mutations were not observed in our series as all 26 parents were heterozygous for one *LPIN1* mutation, except the symptomatic father of patients 11 and 12 who was homozygous due to his parent's consanguinity. Unaffected children were homozygous or heterozygous for the wild type allele.

The functional consequences of the absence of exons 18 and 19 in the *LPIN1* mRNA were examined in a yeast complementation assay as previously reported (Zeharia, et al., 2008). The Δ*pah1* yeast strain was transformed by a plasmid encoding the human lipin-1 or its lipin-1Δ mutant. As described (Santos-Rosa, et al., 2005), the Δ*pah1* mutant strain grew more slowly than wild type cells (W303) at 28°C on glycerol and exhibited a temperature-sensitive phenotype at 36°C on glucose and glycerol (Figure 2). This phenotype was complemented by expressing the human *LPIN1* cDNA, as cells recovered a growth rate comparable to wild type cells at both 28 and 36°C. In contrast, Δ*pah1* cells expressing the human lipin-1Δ mutant (figure 2) did not grow on glycerol at 28°C and glucose at 36°C indicating that the c.2295-866_2410-30del mutation of *LPIN1* alters lipin-1 activity and is of functional significance.

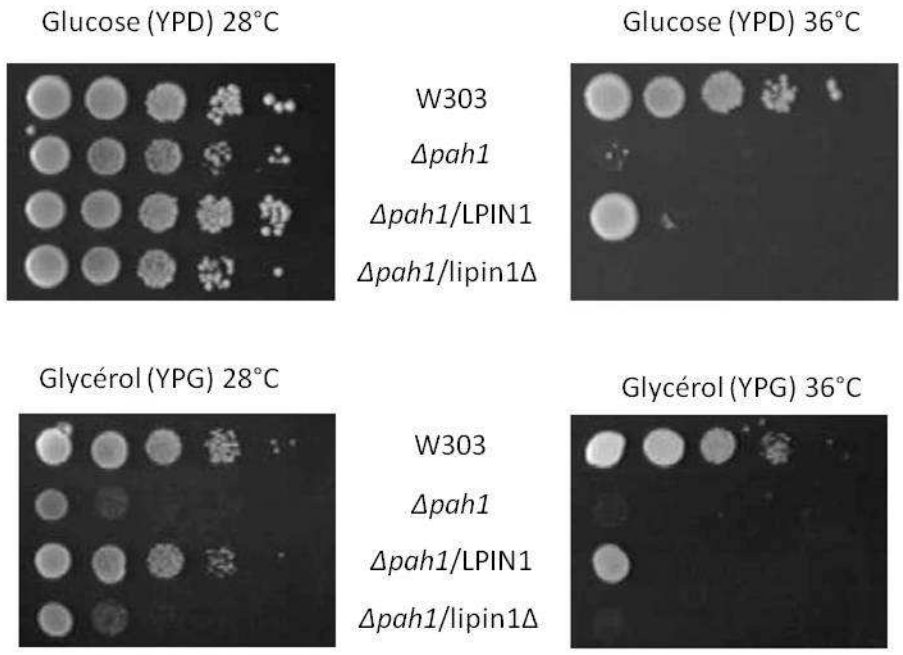

Figure 2. Functional analysis of *LPIN1* intragenic deletion by functional complementation of the yeast pah1 null mutant. Growth of the yeast wild type strain (W303) and Δ*pah1* derivatives on either glucose (YPD) or glycerol (YPG) medium at 28 (left panels) and 36°C (right panels). Δ*pah1* was transformed with the human wild-type *LPIN1* (LPIN1) or mutant (lpin1Δ) cDNAs. The five spots for each experiment correspond to decreasing dilutions of transformed yeast cells.

Clinical investigations of patients with *LPIN1* mutations.

LPIN1 mutations were detected in patients of various ethnic origins: 12/22 Caucasian, 1/1 African, 1/1 Asian and 3/5 Maghrebi patients with a sex ratio M/F=0.89.

Patients underwent their first bout of rhabdomyolysis before five years (median age = 21 months). Bouts were almost invariably precipitated by febrile illnesses and occasionally by anaesthesia or fasting (Table 1). The number of acute episodes ranged from 1 to 10 per patient. Five patients died during myoglobinuric bouts, and three siblings (not included in the study) died from similar episodes. One sibling of patient 10 died at 3 weeks of life from pneumonia. He also presented with cardiomegaly and hepatomegaly at the time of his death.

Between episodes, all patients but one (patient 4) had normal physical examination including normal muscular testing. Their basal plasma CK levels were normal or subnormal as well. The oldest patient is now 23 years old. Patient 4 exhibited writing cramps and permanent myalgia increasing with effort and requiring wheel-chair. Abnormal muscular testing was noted. Another patient (patient 1) presented with recurrent episodes of severe atopic dermatitis.

Most heterozygous parents (23/25) were asymptomatic. The two moderately symptomatic heterozygous carriers (cramps or moderate myalgias) harboured two different point mutations (mother of patient 2, c.2401C>T; and mother of patient 3, c.1441+2T>C). The healthy sister of patient 3, harbouring the *LPIN1* intragenic deletion, reported exercise-induced muscle pain. The father of patients 11 and 12, homozygous for his children's mutation, suffered of numerous bouts of myoglobinuria.

Metabolic investigations of patients with *LPIN1* mutations.

All patients including the oldest one had normal plasma levels of total, LDL and HDL cholesterol, triglycerides, and lactate. Electromyography and abdominal and heart ultrasounds were also normal; no liver steatosis was noted. Brain MRI was normal in all patients but one (patient 4) who had an arachnoid cyst.

Muscle histology was either normal or showed moderate abnormalities, including lipid inclusions, predominance of type I muscle fibers, atrophy of type II fibers and rarely subsarcolemmal aggregates of mitochondria with ragged-red fibers. Histochemical staining for cytochrome c oxidase (COX) activity and respiratory chain activities in skeletal muscle were normal in all patients but one : patient 2, whose muscle fiber staining revealed homogeneously weak COX activity (de Lonlay-Debeney, et al., 1999).

Although a complete analysis of lipid metabolism could not be performed for every patient, partial data were available for some of them. The father of patients 11 and 12 had partial CPT2 deficiency in skeletal muscle (38 mU/mg protein, controls 90 mU/mg protein), while CPT2 activity in fibroblasts and lymphocytes as well as CPT2 gene sequencing were normal (data not shown).

Analysis of phospholipids content in muscle tissues of patients 1 (Zeharia, et al., 2008) and 4 and in plasma of patients 1, 3 and 4 showed no difference in major phospholipid species compared to controls, including phosphatidic acid and lysophosphatidic acid (data not shown).

Average weight percentiles and normal fat distribution were found in all patients, confirmed by dual-absorptiometry in patients 3 and 4 (data not shown). Adiponectine and leptine plasma levels were normal in patients 1, 3 and 4, as well as oral glucose tolerance test and HbA1c level (data not shown).

DISCUSSION

Here, we report on a very high incidence of *LPIN1* mutations in a large series of young patients with severe rhabdomyolysis. Because it accounted for 59% of cases in our study and 56% of tested families, *LPIN1* mutations appear as the second cause of rhabdomyolysis of early-onset, after primary FAO defects as a whole. Moreover, since 8/17 mutated patients carried an intragenic deletion with identical breakpoints, on the background of a common haplotype, a founder effect of Caucasian origin is highly likely. The deleterious nature of this deletion, homozygous in some patients, was supported by yeast complementation assay.

The outcome of the disease was severe, as five patients died during a myoglobinuric episode, as well as three siblings. Episodes of myoglobinuria were precipitated by febrile illnesses and in a few cases by fasting. Various inflammatory inducers including lipopolysaccharides, Zymosan and proinflammatory cytokines have been recently shown to repress *LPIN1* expression, leading to decreased expression of *PPARα*, *PPARγ* and genes involved in energy metabolism (Feingold, et al., 2009; Lu, et al., 2008; Tsuchiya, et al., 2009). It is conceivable therefore that the acute-phase response induced by inflammation could cause dramatic alterations in lipid and lipoprotein metabolism (Gabay and Kushner, 1999; Kishimoto, et al., 1994) and eventually trigger episodes of myoglobinuria in lipin-1 deficient patients.

While lipodystrophy is a major feature in the natural Lpin1 mutant mouse strain fld (Peterfy, et al., 2001; Reue and Brindley, 2008; Reue, et al., 2000), neither insulin resistance, nor dyslipidemic signs were observed in our lipin-1 deficient children. The explanation of the different phenotypes presented by lipin-1 deficient mice and humans remains largely unknown, as well as the physiopathology of the muscular symptoms in man. Skeletal muscle is one of the tissues expressing the highest levels of lipin-1, which may explain why muscle is particularly affected by the *LPIN1* mutations in human. Because of the multiple roles played by lipin-1, several mechanisms leading to rhabdomyolysis, alone or combined, may be considered.

On the one hand, lipin-1 has an enzymatic PAP activity and is involved in glycerolipid biosynthesis. As it has been suggested (Zeharia, et al., 2008), lipin-1-related rhabdomyolysis could result from lyso-phosphatidate accumulation and the subsequent remodelling of membranes induced by phospholipid imbalances (Farooqui, et al., 2000), as proposed in Barth syndrome (Chicco and Sparagna, 2007; McKenzie, et al., 2006). The phospholipid content in skeletal muscle was abnormal in one previously described patient (Zeharia, et al., 2008) but not in two others, one already reported and another. Similarly, plasma phospholipid concentrations were in the normal range in three tested patients. The preliminary data available at this step do not support an important role of phospholipid imbalance alone as a trigger for massive rhabdomyolysis, also it could not be excluded. Moreover, there is growing evidence showing that triacylglycerol (TG) synthesis is coupled to fatty acid oxidation (Liu, et al., 2009). Fatty acids may have to be incorporated into TG and then turned over before they can be efficiently oxidized. Thus, low rates of TG synthesis resulting from mutations of *LPIN1* could produce a defect in fatty acid oxidation. In this view, it should be noted that the Δ*pah1* yeast complementation assay reflects PAP activity. Also bouts of rhabdomyolysis may result from the association of various functional defects linked to the PAP activity deficiency, but further studies are clearly required to determine how alterations in these pathways participate to the disease.

On the other hand, lipin-1 also plays a role of co-activator in *PPARα* and *PGC-1α*-mediated transcription, which stimulates the expression of genes related to mitochondrial energy pathways (Donkor, et al., 2008; Finck, et al., 2006; Higashida, et al., 2008). Besides, lipin-1 has been clearly demonstrated to act in exercise-induced adaptation and oxidative gene expression in muscle (Higashida, et al., 2008). Alteration of this co-activator activity could down-regulate simultaneously several metabolic pathways important for the energetic supply of muscle cells (Phan, et al., 2005). The isolated metabolic data currently available in our patients (abnormal oxidative phosphorylation activity, CPT2 activity defect) might be compatible with the hypothesis of an energy-dependent muscle disease, but need to be confirmed in more extensive studies. On this line, it should be noted that defective growth on glycerol medium of the Δ*pah1* yeast strain suggests a global impairment of aerobic/oxidative metabolism, even though it does not assess lipin-1 transcriptional activity.

Further investigations will help understand the links between deficiency of each of lipin-1 activities and the disease.

In conclusion, lipin-1 deficiency should be regarded as a major cause of severe myoglobinuria in early childhood. Since 59% of our cohort display deleterious *LPIN1* mutations, it may prompt clinicians to include the screening for *LPIN1* mutations at early stage of the metabolic work up of myoglobinurias, after exclusion of FAO defects, but certainly prior to muscle biopsy. The high frequency of the intragenic *LPIN1* deletion in the Caucasian patients provides a valuable screening test to diagnose these patients, before sequencing the full *LPIN1* sequence. Finally, we believe that metabolic investigations of severe rhabdomyolysis in young patients should include successively i) metabolic investigation of FAO, ii) research of the intragenic *LPIN1* deletion in blood samples by long range PCR, iii) full coding sequence analysis of *LPIN1* gene in blood, iv) only if all these results are normal, proceed to skeletal muscle biopsy.

ACKNOWLEDGMENTS

We thank Norma Romero, Vincent Frochot, Jean-Louis Bresson and Jean-Jacques Robert for their help in metabolic investigations, and Marc Jeanpierre for genetic analyses.

This work was supported by AFM (grants 13864 and 13988), Fondation de l'Avenir (grant 09071), Fondation pour la Recherche Médicale (fellowship CM), the Prinses Beatrix Fonds (n° WAR05-0126), the Barth syndrome foundation. Patrick Chinnery is Wellcome Trust Senior Fellow in Clinical Science. No conflict of interests has to be disclosed, nor commercial considerations.

REFERENCES

Chicco AJ, Sparagna GC. 2007. Role of cardiolipin alterations in mitochondrial dysfunction and disease. Am. J. Physiol. Cell. Physiol. 292(1):C33-44.

de Lonlay-Debeney P, Edery P, Cormier-Daire V, Parfait B, Chretien D, Rotig A, Romero N, Saudubray JM, Munnich A, Rustin P. 1999. Respiratory chain deficiency presenting as recurrent myoglobinuria in childhood. Neuropediatrics 30(1):42-4.

Donkor J, Sariahmetoglu M, Dewald J, Brindley DN, Reue K. 2007. Three mammalian lipins act as phosphatidate phosphatases with distinct tissue expression patterns. J. Biol. Chem. 282(6):3450-7.

Donkor J, Sparks LM, Xie H, Smith SR, Reue K. 2008. Adipose tissue lipin-1 expression is correlated with peroxisome proliferator-activated receptor alpha gene expression and insulin sensitivity in healthy young men. J. Clin. Endocrinol. Metab. 93(1):233-9.

Dubowitz V, Fardeau M. 1995. Proceedings of the 27th ENMC sponsored workshop on congenital muscular dystrophy. 22-24 April 1994, The Netherlands. Neuromuscul. Disord. 5(3):253-8.

Ellis KJ, Shypailo RJ, Abrams SA, Wong WW. 2000. The reference child and adolescent models of body composition. A contemporary comparison. Ann. N.Y. Acad. Sci. 904:374-82.

Farooqui AA, Horrocks LA, Farooqui T. 2000. Glycerophospholipids in brain: their metabolism, incorporation into membranes, functions, and involvement in neurological disorders. Chem. Phys. Lipids 106(1):1-29.

Feingold KR, Moser A, Patsek SM, Shigenaga JK, Grunfeld C. 2009. Infection decreases fatty acid oxidation and nuclear hormone receptors in the diaphragm. J. Lipid Res. 50:2055-2063.

Finck BN, Gropler MC, Chen Z, Leone TC, Croce MA, Harris TE, Lawrence JC, Jr., Kelly DP. 2006. Lipin 1 is an inducible amplifier of the hepatic PGC-1alpha/PPARalpha regulatory pathway. Cell Metab. 4(3):199-210.

Gabay C, Kushner I. 1999. Acute-phase proteins and other systemic responses to inflammation. N. Engl. J. Med. 340(6):448-54.

Han GS, Wu WI, Carman GM. 2006. The Saccharomyces cerevisiae Lipin homolog is a Mg2+-dependent phosphatidate phosphatase enzyme. J Biol Chem 281(14):9210-8.

Higashida K, Higuchi M, Terada S. 2008. Potential role of lipin-1 in exercise-induced mitochondrial biogenesis. Biochem. Biophys. Res. Commun. 374(3):587-91.

Isackson PJ, Bennett MJ, Vladutiu GD. 2006. Identification of 16 new disease-causing mutations in the CPT2 gene resulting in carnitine palmitoyltransferase II deficiency. Mol Genet Metab 89(4):323-31.

Kishimoto C, Kuroki Y, Hiraoka Y, Ochiai H, Kurokawa M, Sasayama S. 1994. Cytokine and murine coxsackievirus B3 myocarditis. Interleukin-2 suppressed myocarditis in the acute stage but enhanced the condition in the subsequent stage. Circulation 89(6):2836-42.

Liu L, Shi X, Choi CS, Shulman GI, Klaus K, Nair KS, Schwartz GJ, Zhang Y, Goldberg IJ, Yu YH. 2009. Paradoxical coupling of triglyceride synthesis and fatty acid oxidation in skeletal muscle overexpressing DGAT1. Diabetes 58(11):2516-24.

Lu B, Lu Y, Moser AH, Shigenaga JK, Grunfeld C, Feingold KR. 2008. LPS and proinflammatory cytokines decrease lipin-1 in mouse adipose tissue and 3T3-L1 adipocytes. Am. J. Physiol. Endocrinol. Metab. 295(6):E1502-9.

McKenzie M, Lazarou M, Thorburn DR, Ryan MT. 2006. Mitochondrial respiratory chain supercomplexes are destabilized in Barth Syndrome patients. J. Mol. Biol. 361(3):462-9.

Ohkuma A, Noguchi S, Sugie H, Malicdan MC, Fukuda T, Shimazu K, Lopez LC, Hirano M, Hayashi YK, Nonaka I and others. 2009. Clinical and genetic analysis of lipid storage myopathies. Muscle Nerve 39(3):333-342.

Peterfy M, Phan J, Xu P, Reue K. 2001. Lipodystrophy in the fld mouse results from mutation of a new gene encoding a nuclear protein, lipin. Nat Genet 27(1):121-4.

Phan J, Peterfy M, Reue K. 2005. Biphasic expression of lipin suggests dual roles in adipocyte development. Drug News Perspect 18(1):5-11.

Reue K, Brindley DN. 2008. Thematic Review Series: Glycerolipids. Multiple roles for lipins/phosphatidate phosphatase enzymes in lipid metabolism. J Lipid Res 49(12):2493-503.

Reue K, Xu P, Wang XP, Slavin BG. 2000. Adipose tissue deficiency, glucose intolerance, and increased atherosclerosis result from mutation in the mouse fatty liver dystrophy (fld) gene. J Lipid Res 41(7):1067-76.

Reue K, Zhang P. 2008. The lipin protein family: dual roles in lipid biosynthesis and gene expression. FEBS Lett 582(1):90-6.

Rustin P, Chretien D, Bourgeron T, Gerard B, Rotig A, Saudubray J, Munnich A. 1994. Biochemical and molecular investigations in respiratory chain deficiencies. Clin. Chim. Acta 228(1):35-51.

Santos-Rosa H, Leung J, Grimsey N, Peak-Chew S, Siniossoglou S. 2005. The yeast lipin Smp2 couples phospholipid biosynthesis to nuclear membrane growth. Embo J 24(11):1931-41.

Sugden MC, Caton PW, Holness MJ. 2010. PPAR control: it's SIRTainly as easy as PGC. J Endocrinol 204(2):93-104.

Tein I. 1999. Neonatal metabolic myopathies. Semin Perinatol 23(2):125-51.

Tonin P, Lewis P, Servidei S, DiMauro S. 1990. Metabolic causes of myoglobinuria. Ann Neurol 27(2):181-5.

Tsuchiya Y, Takahashi N, Yoshizaki T, Tanno S, Ohhira M, Motomura W, Tanno S, Takakusaki K, Kohgo Y, Okumura T. 2009. A Jak2 inhibitor, AG490, reverses lipin-1 suppression by TNF-alpha in 3T3-L1 adipocytes. Biochem Biophys Res Commun 382(2):348-52.

Valianpour F, Mitsakos V, Schlemmer D, Towbin JA, Taylor JM, Ekert PG, Thorburn DR, Munnich A, Wanders RJ, Barth PG and others. 2005. Monolysocardiolipins accumulate in Barth syndrome but do not lead to enhanced apoptosis. J. Lipid Res. 46(6):1182-95.

Zeharia A, Shaag A, Houtkooper RH, Hindi T, de Lonlay P, Erez G, Hubert L, Saada A, de Keyzer Y, Eshel G and others. 2008. Mutations in LPIN1 cause recurrent acute myoglobinuria in childhood. Am J Hum Genet 83(4):489-94.