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.

To cite this version:
Caroline Michot, Laurence Hubert, Michele Brivet, Linda De Meirleir, Vassili Valayannopoulos, et al.. LPIN1 gene mutations: a major cause of severe rhabdomyolysis in early childhood.. Human Mutation, Wiley, 2010, 31 (7), <10.1002/humu.21282>. <hal-00552397>
LPIN1 gene mutations: a major cause of severe rhabdomyolysis in early childhood.

<table>
<thead>
<tr>
<th>Journal:</th>
<th>Human Mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manuscript ID:</td>
<td>humu-2010-0028.R2</td>
</tr>
<tr>
<td>Wiley - Manuscript type:</td>
<td>Mutation in Brief</td>
</tr>
<tr>
<td>Date Submitted by the Author:</td>
<td>23-Apr-2010</td>
</tr>
</tbody>
</table>
| 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, pediatic 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
**LPIN1 gene mutations: a major cause of severe rhabdomyolysis in early childhood.**

Caroline Michot¹, Laurence Hubert¹, Michèle Brivet², Linda De Meirleir³, Vassili Valayannopoulos¹, Wolfgang Müller-Felber⁴, Ramesh Venkateswaran⁵, Hélène Ogier⁶, Isabelle Desguerre¹, Cécilia Altuzarra⁷, Elizabeth Thompson⁸, Martin Smitka⁹, Angela Huebner⁹, Marie Hussen¹⁰, Rita Horvath¹¹,¹², Patrick Chinnery¹², Frederic M. Vaz¹³, Arnold Munnich¹, Orly Elpeleg¹⁴, Agnès Delahodde¹⁵, ‘Yves de Keyzer¹, Pascale de Lonlay¹⁶.

¹Paris Descartes University, INSERM U781 and Ref Center of Metabolic Diseases, Necker Hospital, Paris, France; ²Dpt Biochemistry, Kremlin-Bicêtre Hospital, Kremlin-Bicêtre, France; ³Pediatric neurology-metabolic diseases, UZ Brussel, Brussels, Belgium; ⁴Dpt Pediatrics, Ludwig-Maximilians-University, Munich, Germany; ⁵Dpt Pediatrics, Newcastle General Hospital, Newcastle upon Tyne, UK; ⁶Ref Center of Metabolic Diseases, Robert-Debré Hospital, Paris, France; ⁷Dpt Pediatrics, Besançon C.H.U., Besançon, France; ⁸SA Clinical Genetics, Women's & Children's Hospital, North Adelaide, Australia; ⁹Dpt Neuropaeiatrics, Children's Hospital, Technical University Dresden, Dresden, Germany; ¹⁰Dpt Pediatrics, Bordeaux C.H.U., Bordeaux, France; ¹¹Friedrich-Baur-Institut, Ludwig-Maximilians University, Munich, Germany; ¹²Mitochondrial Research Group, Institute for Aging and Health, Newcastle University, Newcastle upon Tyne, UK; ¹³Dpt Clinical Chemistry, Academic Medical Center, Amsterdam, the Netherlands; ¹⁴Dpt Genetics and Metabolic Diseases, Hadassah-Hebrew University Medical Center, Jerusalem, Israel; ¹⁵Paris-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 acid 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 Δpah1 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. ©2010 Wiley-Liss, Inc.

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

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

© 2010 WILEY-LISS, INC.
INTRODUCTION

Myoglobinuria is a rare condition resulting from the destruction of skeletal muscle fibers (rhabdomyolysis). Hereditary myoglobinurias have been ascribed to mitochondrial fatty acid β-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) (Peterly, 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 differentiation 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 myoglobinuria, 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 1 and 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’: GTGGTCGACTTTCACTACAGTCTCACATA 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’: AAAGGTCTGGCACATCTTGT 3’ (intron 17) ; 5’: AATCCCATTTAGCCACCGACTCAG 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 Δpah1 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 Δpah1 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.

<table>
<thead>
<tr>
<th>N°</th>
<th>Age</th>
<th>Sex</th>
<th>Ethnic origin</th>
<th>Con san guinity</th>
<th>Myolysis episodes (triggering event)</th>
<th>Nucleotide</th>
<th>Amino Acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>1*</td>
<td>5y</td>
<td>M</td>
<td>Mauritania</td>
<td>Yes</td>
<td>2 at 27 and 30m (fever)</td>
<td>c.192+2T&gt;C</td>
<td>p.Cys30LeufsX3</td>
</tr>
<tr>
<td>2*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>c.192+2T&gt;C</td>
<td>p.Cys30LeufsX3</td>
</tr>
<tr>
<td>3*</td>
<td>10y</td>
<td>F</td>
<td>France</td>
<td>No</td>
<td>3 between 18m and 10y (fever)</td>
<td>c.1441+2T&gt;C</td>
<td>p.Asn417LysfsX22</td>
</tr>
<tr>
<td>4</td>
<td>10y</td>
<td>M</td>
<td>France</td>
<td>No</td>
<td>2 at 16m and 8y (fever)</td>
<td>c.377_380dup</td>
<td>p.Met128GlnfsX45</td>
</tr>
<tr>
<td>5</td>
<td>18m</td>
<td>M</td>
<td>Belgium</td>
<td>No</td>
<td>1 at 1y</td>
<td>c.2295-866_2410-30del</td>
<td>p.Glu766_Ser838del</td>
</tr>
<tr>
<td>6</td>
<td>3y</td>
<td>F</td>
<td>France-Asia</td>
<td>No</td>
<td>2 at 2y and 2,5y (fever)</td>
<td>c.944C&gt;G</td>
<td>p.Ser315X</td>
</tr>
<tr>
<td>7</td>
<td></td>
<td>M</td>
<td>Belgium</td>
<td>No</td>
<td>1 at 2,5y (fever)</td>
<td>c.2295-866_2410-30del</td>
<td>p.Glu766_Ser838del</td>
</tr>
<tr>
<td>8</td>
<td></td>
<td>F</td>
<td>Belgium</td>
<td>No</td>
<td>1 at 4y (fasting)</td>
<td>c.2295-866_2410-30del</td>
<td>p.Glu766_Ser838del</td>
</tr>
<tr>
<td>9</td>
<td></td>
<td>M</td>
<td>Egypt</td>
<td>No</td>
<td>1 at 5m (fasting)</td>
<td>c.2513+1G&gt;A</td>
<td>p.Asp804ValfsX6</td>
</tr>
<tr>
<td>10</td>
<td></td>
<td>F</td>
<td>England</td>
<td>No</td>
<td>2 at 14m and 4y (fever)</td>
<td>c.2295-866_2410-30del</td>
<td>p.Glu766_Ser838del</td>
</tr>
<tr>
<td>11</td>
<td>6y</td>
<td>M</td>
<td>North Africa</td>
<td>Yes</td>
<td>1 at 5y (general anesthesia)</td>
<td>c.1162C&gt;T</td>
<td>p.Arg388X</td>
</tr>
<tr>
<td>12</td>
<td>8y</td>
<td>F</td>
<td>North Africa</td>
<td>Yes</td>
<td>1 at 2y (fasting)</td>
<td>c.1162C&gt;T</td>
<td>p.Arg388X</td>
</tr>
<tr>
<td>13</td>
<td>8y</td>
<td>F</td>
<td>Germany</td>
<td>No</td>
<td>10 between 2 and 8y (fever)</td>
<td>c.2295-866_2410-30del</td>
<td>p.Glu766_Ser838del</td>
</tr>
<tr>
<td>14</td>
<td>23y</td>
<td>F</td>
<td>France</td>
<td>No</td>
<td>5 between 4 and 16 y (fever, fasting with local anaesthetic)</td>
<td>c.1259delC</td>
<td>p.Pro420LeufsX3</td>
</tr>
<tr>
<td>15</td>
<td>15y</td>
<td>M</td>
<td>France</td>
<td>No</td>
<td>7 between 4 and 16 y (fever, fasting with local anaesthetic)</td>
<td>c.2513+1G&gt;A</td>
<td>p.Asp804ValfsX6</td>
</tr>
<tr>
<td>16</td>
<td>18y</td>
<td>M</td>
<td>France</td>
<td>No</td>
<td>6 between 4 and 16 y (fever, fasting with local anaesthetic)</td>
<td>c.2513+1G&gt;A</td>
<td>p.Asp804ValfsX6</td>
</tr>
<tr>
<td>17</td>
<td>4y</td>
<td>F</td>
<td>Germany</td>
<td>No</td>
<td>2 at 3y and 4 y</td>
<td>c.2253_2254del</td>
<td>p.Leu752AlafsX17</td>
</tr>
</tbody>
</table>

*: 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).
For Peer Review

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.](image)

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 Δpahl 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 Δpahl 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, Δpahl 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 Δpah1 derivatives on either glucose (YPD) or glycerol (YPG) medium at 28°C (left panels) and 36°C (right panels). Δpah1 was transformed with the human wild-type LPIN1 (LPIN1) or mutant (lipin1Δ) 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 anesthesia 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 Δpah1 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 as 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 Δpah1 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 Princes 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


<LPIN1 mutations and severe rhabdomyolysis of early childhood>