First presentation of LPIN1 acute rhabdomyolysis in adolescence and adulthood

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Abstract: LPIN1 mutations are a known common cause of autosomal recessive, recurrent and life-threatening acute rhabdomyolysis of childhood-onset. The first episode of rhabdomyolysis usually happens in nearly all cases before the age of 5 and death is observed in 1/3 of patients. Here we present two cases of acute rhabdomyolysis with a milder phenotype caused by LPIN1 mutation presenting in adolescence (11 years old) and adulthood (40 years old) after Parvovirus infection and metabolic stress, respectively. In our opinion, the mutation types, epigenetic factors, the environment exposition to triggers or the existence of proteins with a similar structure of LPIN1, may have a role in modulating the onset of rhabdomyolysis. LPIN1 should be included on a panel of genes analysed in the investigation of adult individuals with rhabdomyolysis. Metabolic and viral stressors should be included in the list of possible rhabdomyolysis precipitant.

Keywords: LPIN1, rhabdomyolysis, adult, next generation sequencing.

Introduction:

Lipin-1 (LPIN1) is an 890 aminoacid intracellular protein involved in different pathways of fatty acid metabolism (1). It belongs to the family of phosphatidate phosphatase (PAP) enzymes and its

main role is to catalyse the conversion of phosphatidate to diacylglycerol, the penultimate step of triglyceride synthesis which constitute the major energy storage in our body (2).

In humans, LPIN1 is mainly expressed in skeletal muscles and adipose tissue but lower levels have been found in the gastrointestinal tract as well (3).

LPIN1 mutations are a known cause of autosomal recessive, recurrent and life-threatening acute rhabdomyolysis of childhood-onset. The first description dates back to 2008 (4). Since then, other cases have been described in the literature (5,6) and LPIN1 mutations are now considered the second most common cause of early-onset acute rhabdomyolysis, after primary fatty acid oxidation defects (5). The episodes are usually triggered by fever, fasting or general anaesthesia (7) and the outcome is severe with death in 1/3 of patients (5). Clinically patients present with episodes characterised by myalgia and myoglobinuria; creatine kinase (CK) increases over 100,000 UI/L but can reach $1x10^6$ UI/L. Between episodes, clinical examination, CK and acyl-carnitine profile are usually normal (7).

To our knowledge, there has only been two reports of two individuals presenting with adult-onset rhabdomyolysis (6,8). We describe two cases of LPIN1 mutation with a milder phenotype presenting in adolescence and adulthood respectively. Our aim is to highlight the importance of looking for LPIN1 mutations in adults presenting with acute rhabdomyolysis.

Case report:

Case 1: An 11-year-old Caucasian girl of British origin presented to A&E with diffuse muscle pain, swelling and brown coloured urine. Her CK was 560,000 IU/L. At first, an inflammatory cause was suspected as there were no apparent precipitating factors such as exercise, infection, fasting or general anaesthesia. The autoimmune screening was negative but she was later found to have positive serology indicating Parvovirus infection despite a lack of fever. Six months later she

presented with the second episode of acute rhabdomyolysis; CK was 250,000 IU/L, once more, there was no clear precipitant. Both episodes were treated with intravenous fluids and she did not require dialysis. Her CK went back to normal after a couple of weeks on both occasions. Neurological examination was normal between the episodes.

The past medical history was unremarkable apart from a six-year history of painful calves: she described suffering from calf cramps 30 minutes after physical education at school. Motor development was normal (she walked before 1 year of age) and learning difficulties were not reported. There was no history of hypoglycaemia in infancy or worsening of symptoms with fasting. She was able to run. Family history was negative for rhabdomyolysis and muscle cramps. Parents were unrelated. Other investigations included carnitine and acylcarnitines which were normal. The electrodiagnostic study was not done. Following the first episode, a muscle biopsy was performed (Figure 1). It showed very mild myopathic changes with one macrophage cluster and one basophilic regenerating fibre per haematoxylin and eosin (H&E) level and increased lipid droplets. Electron microscopy revealed 30nm viral particles in the pinocytotic vesicles of intramuscular capillaries but not muscle fibres. Parvovirus was detected by real-time PCR of the frozen muscle homogenate. After the second episode, next-generation sequencing (NGS) panel of 30 genes commonly associated with acute rhabdomyolysis was performed which identified homozygous in-frame deletion of exon 18 (c.2295-865_2410-30del) in LPIN1. See Table 1 for clinical details and for the list of genes included in the NGS panel.

Case 2: A 40-year-old Caucasian female of Italian origin presented to the A&E department complaining of muscle pain and diffuse muscle weakness. Neurological examination was unremarkable. She was oliguric and passed low volumes of rust-coloured urine. Four days before, she had returned from a holiday in South Africa where she was Kayaking in the hot weather. The precipitant may have been stress-related since she reported a highly stressful driving lesson 12 hours before the onset of symptoms. At presentation, her CK was 102,185 IU/L with transaminitis

(ALT 1407 IU/L [NR 0-50 IU/L]) and a serum creatinine (sCr) of 158 umol/l [NR: 49-90 umol/l] reflecting impaired renal function. Over the next 48 hours, sCr peaked to 448 umol/l with a decline in urine output requiring admission in the Intensive Treatment Unit (ITU) for haemofiltration. She was transferred to the tertiary care renal unit. Her sCr peaked to 697 umol/L and required five sessions of haemodiafiltration, following which she started to recover with increasing urine output. She was discharged on day 16 at which point her sCr was 488 umol/L and CK was 160 IU/L.

One year later, she had a second less severe episode. Once again, she was very stressed after driving on a motorway; a few hours later, she developed marked shoulder and proximal upper limb pain and swelling. The CK level was 45,987 IU/L. Renal function was normal and she was treated with intravenous fluids.

A few months later, she possibly had another milder episode associated with infection when she developed a paronychia on her right index finger and noticed myalgia in her right arm for a few days, although she had no myoglobinuria.

She had no past medical history and was not on any regular medication. Her parents are of Sicilian origin and are first cousins. She was conceived naturally and born after an uneventful pregnancy. She did not have any health problems or muscle pains during childhood. She described herself as not being a particularly athletic child, but was able to participate in sport at the same level as her peers without difficulty. Her exercise levels increased as an adult, and she regularly swims and rides a mountain bike, including long distances, but had not had any abnormal muscle pains prior to the presenting episode. She has a brother who has not had any similar episodes. She has a young daughter, who is well.

Investigations included infection and autoimmune screening, which were negative. Plasma acylcarnitine was within normal limits (27 umol/l, normal values 15-53). A NGS panel of 30 genes associated with acute rhabdomyolysis showed the same mutation detected in Case1: LPIN1 homozygous deletion of exon 18 (c.2295-865_2410-30del). The muscle biopsy and the electrodiagnostic study were not performed. See Table 1 for details.

Discussion:

LPIN1 is a known cause of rhabdomyolysis in early childhood. According to literature, the first episode of rhabdomyolysis usually happens at a mean age of 21 months and in nearly all cases before the age of 5 (5). Here we present two cases, the first one with onset in adolescence (11 years old) and the second one in adulthood (40 years old).

LPIN1 protein is encoded by the LPIN1 gene located in chromosome 2p25.1 and contains 20 exons (9). Over 25 mutations in LPIN1 gene has been described so far (10) but a genotype/phenotype correlation has not been described yet. Most of them are nonsense or deletion mutations, resulting in a loss of function of the protein (10). Both our patients present with the same in-frame deletion of exon 18, which is the most common in the Caucasian population and is found in approximately 68% of patients (6). Deletion of exon 18 is not usually associated with a later onset of disease, although the presence of an in-frame deletion instead of a loss of function mutation could be the biggest contributor to the milder phenotype observed in our patients. In our opinion, other factors may have a role in modulating the onset of rhabdomyolysis, such as epigenetic factors or the environment exposition to triggers. Moreover, in mammalian, the LPIN family includes other two proteins named LPIN2 and LPIN3 (3) encoded by different genes. LPIN2 and LPIN3 share with LPIN1 a similar structure and the role as a PAP enzyme (11). For these reasons, they could help compensate for the lack of LPIN1 if this protein is mutated or non-functional. However, few data on the localisation of LPIN protein family in skeletal muscle tissue sections are available (3,11) and further studies are needed to clarify the expression patterns and any compensatory role of the other LPIN family members, including LPIN2 and LPIN3, in skeletal muscle with LPIN1 deficiency.

In heterozygous carriers, LPIN1 mutation can cause cramps, myalgia and can trigger statin-induced myotoxicity (12). However, in our cases parents of both patients were asymptomatic.

154 The pathophysiology of LPIN1-induced rhabdomyolysis still needs to be clarified. In Figure 2,
155 possible mechanisms are illustrated. According to a recent article by Vissing et al., LPIN1
156 deficiency affects lipolysis and subsequently limits the fatty acid oxidation during exercise (13).
157 Moreover, sarcoplasmic reticulum (SR) is the site of phospholipid production and SR stress has
158 been recently hypothesized to have a primary role in causing LPIN1 myopathy (14) in mouse
159 skeletal muscles. In fact, SR stress leads to the activation of lipogenesis and indirectly damage
160 mitochondrial function, an important energy pathway inside cells (15, 16). An indirect sign of
161 lipogenesis activation is the frequent finding of lipid droplets accumulation in muscle biopsies of
162 patients with LPIN1 mutations (5). However, lipid droplets should not be considered a specific
163 pathological finding in muscles of LPIN1 mutations as they can be present in a variety of other lipid
164 myopathies (17). Other possible findings are signs of mitochondrial dysfunction such as ragged red
165 fibres or cytochrome oxidase negative fibres (18). Lipid droplets have been found in the muscle
166 biopsy of our first patient. In the second patient, a muscle biopsy was not performed due to the
167 availability of an extensive and less invasive genetic panel that led to the final diagnosis.
168
169 Several triggers of rhabdomyolysis in LPIN1 have been described, including fever, exercise,
170 fasting, infections and general anaesthesia (19). All those situations require a high energy demand, a
171 need that cannot be satisfied in LPIN1 disorder due to reduced fatty acid oxidation (13).
172 Our second patient developed acute rhabdomyolysis following stressful events. During stress, there
173 is an increased sympathetic tone with catecholamine release (20). Catecholamine role includes
174 stimulation of lipolysis and release of fatty acids (21), processes that are impaired with LPIN1
175 mutation (13). Stress should also be included in the list of possible precipitant of myoglobinuria in
176 these patients. Infection-related myoglobinuria was observed in both patients, as already described
177 (5, 10).
178 Parvovirus infection may have triggered rhabdomyolysis in our first patient. Parvovirus B19 is a
179 rare but recognised cause of rhabdomyolysis in patients with underlying muscle diseases (22) and

including patients with no other medical history (23). Parvovirus B19 belongs to the family of Picornaviridae, which has an affinity for endothelial cells.

LPIN1 mutations cause severe episodes of rhabdomyolysis that can lead to death. In a case series, death was observed in 1/3 of young patients (5). In our second case, the first episode of rhabdomyolysis was severe resulting in ITU admission and dialysis while all the other episodes were less severe, requiring intravenous fluids for both patients.

Diagnosis of LPIN1 related rhabdomyolysis is important for management. According to Pichler et al. (24), management should include hyperhydration with glucose infusion to establish anabolism during rhabdomyolysis. Moreover, between episodes, it would be useful to avoid fasting and increase the caloric intake with carbohydrate during a situation that could lead to catabolism. Our patients are under follow up in a specialised clinic for rhabdomyolysis and related disorders and since receiving instructions and advice on how to avoid rhabdomyolysis, no further episodes have been reported.

In conclusion, we would like to highlight the use of next generation sequencing panels in the investigation of rhabdomyolysis. LPIN1 mutations should not be considered a cause of rhabdomyolysis in young children alone. Metabolic and viral stressors should be included in the list of possible rhabdomyolysis precipitants and episodes managed appropriately.

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Figure1

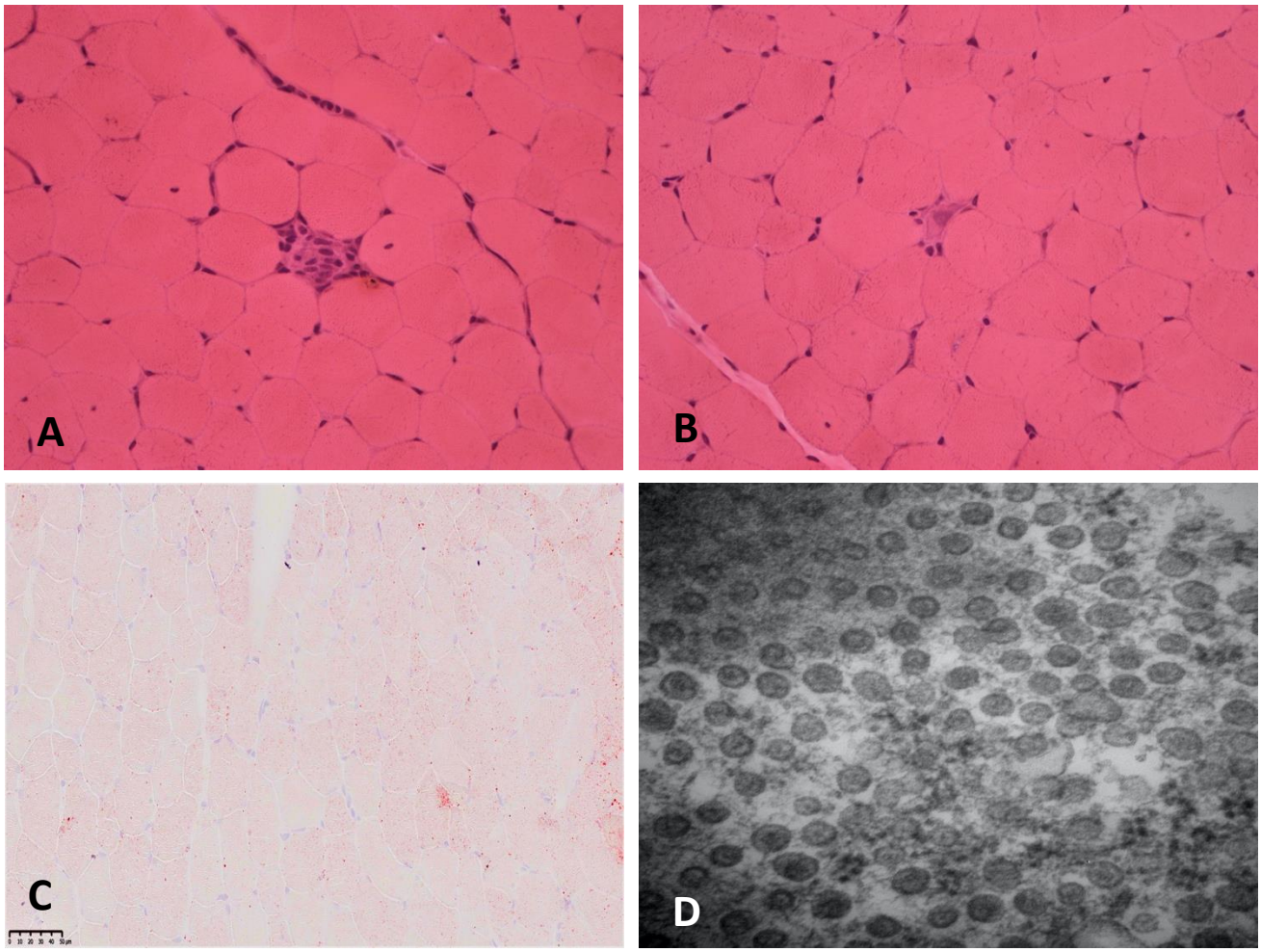

Figure 1 (intended for colour reproduction): muscle biopsy of Case 1. **A**: one macrophage cluster due to fibre necrosis, H&E stained section, magnification 40x; **B**: one basophilic regenerating fibre, H&E stained section, magnification 40x; **C**: mild excess neutral lipid, Oil Red O, magnification 40x; **D**: electron micrograph of intramuscular capillary endothelium showing ~30nm viral particles within Pinocytotic Vesicles, magnification 100,000x.

Figure2

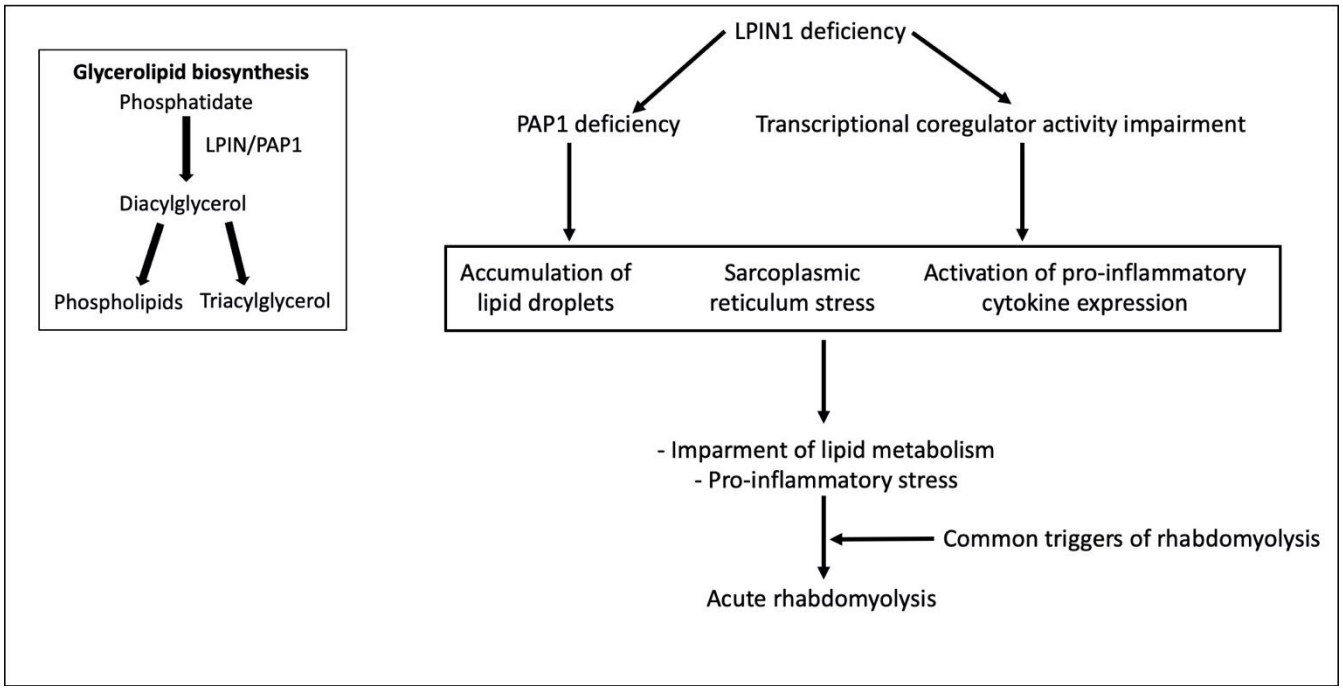

Figure 2: possible mechanisms of LPIN1-induced rhabdomyolysis. Acute rhabdomyolysis in LPIN1 deficiency couples genetic and environmental components. LPIN1 has a role in the glycerolipid biosynthesis (it catalyzes the dephosphorylation of phosphatidic acid to diacylglycerol) and in the regulation of gene expression. In the nucleus, LPIN1 interacts with transcriptional factors, such as PPARα, PGC-1α, SREBP1 or NFATc4, through which lipid metabolism, sarcoplasmic reticulum and pro-inflammatory cytokines production are regulated. The impairment of lipid metabolism together with the proinflammatory stress may lead to rhabdomyolysis in presence of common triggers. PAP1: phosphatidic acid phosphatase 1.

	Case 1	Case 2
Sex	F	F
Ethnic origin	England	Italy
Parents Consanguinity	No	Yes (first cousins)
Age at onset (years)	11	40
Number of episodes	2	3
Precipitants	1st: Parvovirus infection 2nd: not identified	1st and 2nd: stress 3rd: infection
Peak CK (normal range 25 – 200 IU/L)	560,000	102,185
CK between episodes	Normal	Normal
Fixed muscle weakness	No	No
Renal replacement therapy	No	Yes
LPIN1 mutation (nucleotide)	c.2295-865_2410-30del c.2295-865_2410-30del	c.2295-865_2410-30del c.2295-865_2410-30del
Genes tested	ACADVL, AGL, ALDOA, CAV3, CPT1B, CPT2, ENO3, ETFA, ETFB, ETFDH, FPB2, GAA, GBE1, GYG1, GYS1, HDAHA, HDAHB, ICSU, LDHA, LPIN1, PFKM, PGAM2, PGK1, PGM1, PHKA1, PHKG1, PYGM, RBCK1, SLC22A5, RYR1	

Table 1: Summary of clinical features and rhabdomyolysis description of the two patients. A list of the 30 genes tested in the NGS panel for acute rhabdomyolysis is also present.