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Child Neurology: Recurrent rhabdomyolysis due to a fatty acid oxidation disorder

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Rhabdomyolysis may result from various factors, namely trauma, exercise, medications, infections, endocrine disorders, congenital myopathies, and metabolic diseases.[1] Among the latter, mitochondrial fatty acid β-oxidation (FAO) defects frequently cause recurrent rhabdomyolysis. FAO disorders are recessively inherited and have a combined incidence of 1:9,300, estimated after implementation of newborn screening programs by tandem mass spectrometry (MS/MS).[2] Clinical manifestations of these disorders range from sudden infant death to Reye-like syndrome, nonketotic hypoglycemia, skeletal myopathy, peripheral neuropathy, and progressive cardiomyopathy. Here, we describe an 18-month-old child presenting with episodes of recurrent rhabdomyolysis related to mitochondrial trifunctional protein deficiency (MTPD), without additional manifestations of FAO defects. We discuss the diagnosis of MTPD and review the prognosis and treatments.

CASE REPORT An 18-month-old boy was referred to our pediatric department for evaluation of acute onset of hypotonia, lethargy, and poor feeding. He was the first son of healthy, nonconsanguineous, Caucasian parents; he was born at 39 weeks' gestation after an uncomplicated pregnancy. Delivery and perinatal events were unremarkable. Expanded newborn screening was not performed. At evaluation, physical growth and developmental milestones were normal. On admission, the patient had fever and herpetic gingivostomatitis, which, together with malaise, caused poor feeding. On neurologic examination, he manifested mild appendicular and axial hypotonia, as well as upper limb weakness. Muscular bulk, deep tendon reflexes, and sensation were normal. Cranial nerve examination was normal and the gag reflex was intact bilaterally. The history was negative for trauma and drug ingestion. Laboratory investigations showed elevated serum creatine phosphokinase (CPK) 40,160 U/L (reference values [RV] < 227 U/L), alanine aminotransferase 473 U/L (RV < 35 U/L), aspartate aminotransferase 1,375 U/L (RV < 45 U/L), myoglobin 10,988 U/L (RV < 70 U/L), and troponin I 0.105 ng/mL (RV 0–0.03 ng/mL). C-reactive protein and erythrocyte sedimentation rate were elevated. Blood gases, glucose, lactate, and ammonium were normal. Thyroid hormones were normal. Testing for infectious agents, including TORCH infections and H1N1 virus on nasal culture and pharyngeal swab, was negative. ECG revealed sinus tachycardia. Chest x-ray, echocardiogram, and abdominal ultrasonography were unremarkable. Blood levels of several hydroxylated and nonhydroxylated very-long-chain acylcarnitines (C14:1, C16:1, C16, C16-OH, C18:1, C18:1-OH) were elevated (table). The urine organic acid profile revealed increased excretion of several dicarboxylic and hydroxydicarboxylic acids (table). During the hospitalization, the patient received IV hydration, glucose infusion, and furosemide, as well as IV sodium bicarbonate to prevent myoglobin-induced tubular toxicity and acyclovir for herpetic gingivostomatitis. Blood CPK normalized within 15 days and the patient's hypotonia and muscle weakness resolved within 1 week.

The association of rhabdomyolysis and elevated levels of hydroxylated and nonhydroxylated very-long-chain acylcarnitines suggested a mitochondrial FAO disorder. However, a modest increase in hydroxylated and nonhydroxylated very-long-chain acylcarnitines may occur in at least 5 FAO deficiencies[3]: 1) very-long-chain acyl-CoA dehydrogenase (VLCAD) deficiency; 2) carnitine palmitoyltransferase II (CPTII) deficiency; 3) long-chain L-3-hydroxyacyl-CoA dehydrogenase deficiency (LCHADD); 4) MTPD; and 5) carnitine-acylcarnitine translocase deficiency. To investigate the etiology of our patient's symptoms, we sequenced the *ACADVL* and *CPT2* genes to assess for VLCAD and CPTII deficiencies, respectively, and both tests were negative. We next sequenced the *HADHA* and *HADHB* genes, which are associated with LCHADD and MTPD, respectively, and found that the patient was a compound heterozygote for 2 novel mutations in *HADHB* (MTP β subunit), namely c.184A>G

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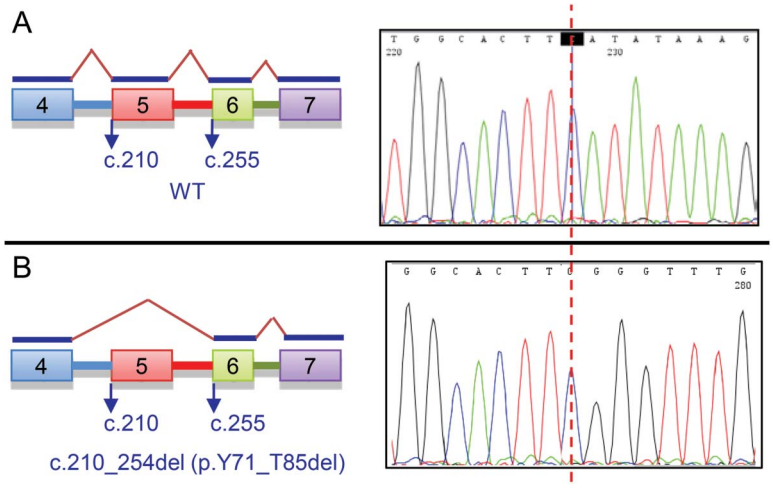
Table Plasma acylcarnitine and urine organic acid values in our patient

	Our patient	Reference values
Plasma acylcarnitine profile, μmol/L		
Tetradecenoyl carnitine (C14:1)	0.51	0.02–0.2
Palmitoyl carnitine (C16)	0.59	0.01–0.23
Hexadecenoyl carnitine (C16:1)	0.25	0.01–0.07
3-OH-hexadecanoyl carnitine (C16-OH)	0.09	0.01–0.05
Oleyl carnitine (C18:1)	0.40	0.02–0.34
3-OH-oleyl carnitine (C18:1-OH)	0.08	0.01–0.07
Urine organic acid profile, mmol/mol creatinine		
Adipic acid	658	ND–34.3
Sebacic acid	40	ND–1.4
Decanedioic acid	123	ND–3.1
3-Hydroxybutyric acid	87	ND–11.1
3-Hydroxyadipic acid	34	ND–15
3-Hydroxysebacic acid	105	ND–9
3-Hydroxydodecanedioic acid	67	ND–10

Abbreviation: ND = not detectable.

and c.254+1G>A inherited from the mother and father, respectively. c.184A>G causes replacement of threonine 62 with an alanine (T62A). This variation is absent from 600 control chromosomes and is not reported in polymorphism databases, including the 1000 Genomes project database (http://www.1000genomes.org/). T62 is highly conserved, and a deleterious effect is predicted for T62A (SIFT [http://sift.jcvi.org/] and PolyPhen-2 [http://genetics.bwh.harvard.edu/pph2] algorithms). Because the c.254+1G>A mutation is a canonic donor splicing site, we looked for an abnormal transcript in the patient's mRNA. Peripheral blood mRNA amplification revealed the normal-size wild-type band and a shorter band corresponding to a transcript lacking exon 5 that causes the p.Y71_T85 in-frame deletion (figure). Given the clinical and laboratory findings, in concert with the genetic testing, we diagnosed the patient with MTPD.

Figure Exon 5 skipping in the patient's mRNA



Exon-intron architecture, mRNA maturation, and sequence electropherogram of the wild-type (WT) *HADHB* transcript (A) and of the mutated transcript resulting from the novel deletion (B). Dashed red line indicates the sequence junctions at the 3′ end of exon 4: in (A) the 5′-end of exon 5, in (B) the 5′-end of exon 6.

After the acute episode resolved, the patient was discharged with a low-fat, high-carbohydrate diet, with limited long-chain fatty acid intake and medium-chain triglyceride supplementation. Avoidance of extended fasting and prolonged exercise was recommended. In follow-up, the patient developed 2 further episodes of acute rhabdomyolysis during infective illnesses. Similar to the first episode, he had neither hypoglycemia nor acidosis during relapses. At age 3 years, neurologic examination (including muscle bulk, tone, strength, and sensation) and developmental status were normal for age, CPK levels were within normal limits, and acylcarnitines C16-OH and C18:1-OH were slightly increased (0.07 μmol/L [RV 0.01–0.05] and 0.10 μmol/L [RV 0.01–0.07], respectively).

DISCUSSION Infection and inborn errors of metabolism are among the most common causes of rhabdomyolysis in pediatric patients.[1] Glycogen storage disorders, phosphatidic acid phosphatase deficiency, muscular dystrophies, congenital myopathies, and mitochondrial FAO defects must be considered in the differential diagnosis of patients presenting with rhabdomyolysis. Mitochondrial β-oxidation of fatty acids results in the oxidation of long-chain fatty acids and provides substrate to organs with high metabolic demands, such as heart, skeletal muscles, and liver. MTP is a heteroctamer formed by 4 α and 4 β subunits and resides in the mitochondrial inner membrane.[4] These subunits are encoded by the *HADHA* and *HADHB* genes, respectively.

MTPD impairs β-oxidation of long-chain fatty acids and affects energy supply. Patients might be asymptomatic when residual enzyme activity enables a balance between energy supply and demand. However, symptoms manifest under catabolic states such as infection, exercise, and prolonged fasting. MTPD can present with a wide array of clinical manifestations, including nonketotic hypoglycemia, cardiomyopathy, myopathy, neuropathy, retinopathy, and liver disease. During acute metabolic crises, cardiac arrhythmias increase the risk of sudden death. Up to 80% of patients with MTPD develop peripheral neuropathy and 5%–13% develop retinopathy.[5] Rhabdomyolysis or myoglobinuria are frequent complications of MTPD and are presumably caused by the toxic effect of the elevated acylcarnitines and hydroxyacyl fatty acid

metabolites on skeletal muscle. It is conceivable that our patient manifested only with bouts of rhabdomyolysis and without nonketotic hypoglycemia because the Y71_T85 in-frame deletion, localized in a region not previously described as a prone-mutation spot, likely retains residual enzymatic activity, thereby attenuating the effect of the severe p.T62A mutation.[4] MTPD is routinely diagnosed from the blood acylcarnitine profile generated by MS/MS, and plasma levels of hydroxylated long-chain acylcarnitines (C16:1-OH, C18:1-OH, C18-OH) are typically increased. A urine organic acids profile demonstrating 3-OH-dicarboxylic aciduria is also suggestive of this disorder. Plasma acylcarnitines must be tested during the acute episode because their levels can be normal during asymptomatic periods.[3] The acylcarnitine pattern is not specific for MTPD, and other FAO disorders may have an overlapping pattern of alterations. Genetic analysis to detect pathogenic mutations in *HADHA* and *HADHB* is required for a correct diagnosis, which is crucial given the implications for patients, their families, and counseling.

The combined worldwide incidence of MTPD and LCHADD by standard newborn screening is 1:110,000.[6] In Italy it is <1:100,000 and <1:75,000, respectively, for MTPD and LCHADD. Caution should be exerted in defining the prognosis of MTPD. Patients are at risk of developing fatal cardiac arrhythmias, sudden death, vision loss, and peripheral neuropathy. The overall mortality of children with MTPD is approximately 75%, with 50% of patients dying in the neonatal period and the remainder often dying before reaching 2 years.[7] Lower rates of mortality have been reported among children detected during newborn screening.[2] Cardiac involvement remains the leading cause of death. In fact, MTPD can present in the neonatal period with severe cardiac arrhythmias, especially ventricular tachycardia, supraventricular tachycardia, left bundle branch block, or sinus node dysfunction. To our knowledge, there are no reports of implantation of a cardiac device in patients with MTPD and life-threatening arrhythmias. However, this treatment might be suggested in case of failure of drug therapy. In our patient, we recommended close cardiologic follow-up (ECG and echocardiography) for early detection of arrhythmias and left ventricular hypertrophy.

An ocular examination including ophthalmoscopy, fundus photography, and electroretinography should be suggested within the first month of diagnosis and annually thereafter to detect chorioretinopathy, visual impairment, or progressive myopia.[8] Nerve conduction studies should also be included in the follow-up of patients with clinical signs or symptoms of peripheral neuropathy.

The first-line treatment for MTPD is avoidance of fasting and frequent meals, especially during catabolic states (e.g., infection, prolonged exercise, and surgical interventions). Moreover, a diet with 70% of calories from carbohydrates, 15% from protein, and 15%–20% from fat is recommended. Long-chain fatty acids should be avoided and the diet should include supplementation with medium-chain fatty acids, which are preferentially oxidized by skeletal muscle, provide essential energy, and inhibit accumulation of cytotoxic long-chain fatty acid metabolites.[9] However, to date there is no evidence that these therapeutic interventions affect the high mortality rate related to cardiac arrest in pediatric patients with MTPD.[7] L-Carnitine supplementation for treatment of FAO disorders is controversial. In patients with low blood levels of total acylcarnitines, oral supplementation with L-carnitine may correct the defect in hepatic ketogenesis and improve cardiac function and muscle strength.[10] However, L-carnitine could be detrimental during metabolic crises because it may increase the concentration of arrhythmogenic long-chain fatty acylcarnitines.

AUTHOR CONTRIBUTIONS

G. Terrone: drafting the manuscript, including medical writing for content; study design. M. Ruoppolo: interpretation of metabolic data. N. Brunetti-Pierri: drafting/revising the manuscript, including medical writing for content. C. Cozzolino: analysis and interpretation of genetic data. E. Scolamiero: analysis and interpretation of metabolic data. G. Parenti, A. Romano, G. Andria: revising the manuscript, including medical writing for content. F. Salvatore, G. Frisso: drafting/revising the manuscript, including genetic writing for content, study concept; study supervision and coordination.

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DISCLOSURE

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