Hepatic Presentation of Late-Onset Multiple Acyl-CoA Dehydrogenase Deficiency (MADD): Case Report and Systematic Review

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Diagnosis of pediatric steatohepatitis is a challenging issue due to a vast number of established and novel causes. Here, we report a child with Multiple Acyl-CoA Dehydrogenase Deficiency (MADD) presenting with an underrated muscle weakness, exercise intolerance and an atypically severe steatotic liver involvement. A systematic literature review of liver involvement in MADD was performed as well. Our patient is a 11-year-old otherwise healthy, non-obese, male child admitted for some weakness/asthenia, vomiting and recurrent severe hypertransaminasemia (aspartate and alanine aminotransferases up to ×20 times upper limit of normal). Hepatic ultrasound showed a bright liver. MRI detected mild lipid storage of thighs muscles. A liver biopsy showed a micro-macrovacuolar steatohepatitis with minimal fibrosis. Main causes of hypertransaminasemia were ruled out. Serum aminoacids (increased proline), acylcarnitines (increased C4-C18) and a large excretion of urinary glutaric acid, ethylmalonic, butyric, isobutyric, 2-methyl-butyric and isovaleric acids suggested a diagnosis of MADD. Serum acylcarnitines and urinary organic acids fluctuated overtime paralleling serum transaminases during periods of illness/catabolic stress, confirming their recurrent nature. Genetic testing confirmed the diagnosis [homozygous c.1658A > G (p.Tyr553Cys) in exon 12 of the ETFDH gene]. Lipid-restricted diet and riboflavin treatment rapidly ameliorated symptoms, hepatic ultrasonography/enzymes, and metabolic profiles. Literature review (37 retrieved eligible studies, 283 patients) showed that liver is an extramuscular organ rarely involved in late-onset MADD (70 patients), and that amongst 45 patients who had fatty liver only nine had severe presentation.

Conclusion: MADD is a disorder with a clinically heterogeneous phenotype. Our study suggests that MADD warrants consideration in the work-up of obesity-unrelated severe steatohepatitis.

Keywords: steatohepatitis, hypertransaminasemia, fatty liver, MADD, case report

INTRODUCTION

Multiple acyl-CoA dehydrogenase deficiency (MADD, MIM #231680), also known as Glutaric aciduria type II, is a rare autosomal recessive inherited disorder of fatty acid, amino acid, and choline metabolism. It is caused by deficiency of either an electron-transfer flavoprotein (ETF, encoded by ETFA and ETFB genes) or an electron-transfer flavoprotein dehydrogenase (ETFDH, encoded by ETFDH gene). The metabolic defects result in impaired adenosine triphosphate (ATP) biosynthesis, excessive lipid accumulation in different organs and insufficient gluconeogenesis (1).

The genetic heterogeneity correlates with different clinical phenotypes that can be divided into three types: (1) neonatal onset with congenital anomalies (MADD type I): the symptoms appear during the first 24 h of life and patients usually die within the first week of life; (2) neonatal onset without anomalies (MADD type II), with symptoms arising within the first 24–48 h of life and the death often occurs within the first weeks of life; (3) mild and/or late onset (MADD type III) with course and age at presentation extremely variable; in adolescents and adults muscular or cardiac symptoms are usually first features suggestive for MADD (2). Extramuscular symptoms such as fatty liver and recurrent vomiting have been reported more rarely (3). Patients with late-onset MADD carry at least one missense variation with minor amounts of residual ETF/ETFDH activity. This seems to be sufficient to prevent embryonic development of congenital anomalies. Instead, homozygosity for null mutations is usually associated with MADD type I (1).

Diagnosis is based on both the urinary organic acids profile and the blood acylcarnitine pattern. Urine presents elevations of glutaric, lactic, ethylmalonic, adipic, suberic, sebacic, butyric, and isovaleric acids, with elevation of 2-hydroxyglutaric acid being considered pathognomonic. The plasma acylcarnitine profile demonstrates generalized elevations in most of short, medium, and long chain acylcarnitines such as C4, C5, C5-DC, C6, C8, C10, C12, C14:1, C16, and C18:1. Biochemical diagnosis of patients with type III MADD is often very challenging, because elevation of urine organic acids may be incomplete, subtle, intermittent, or elevated only during an acute metabolic crisis (4).

Here, we report a 12-year-old male child presenting some muscle weakness and exercise intolerance along with an unusual extremely severe recurrent liver involvement, which has hitherto been rarely reported in type III MADD. A systematic literature review of liver involvement in MADD has been performed as well.
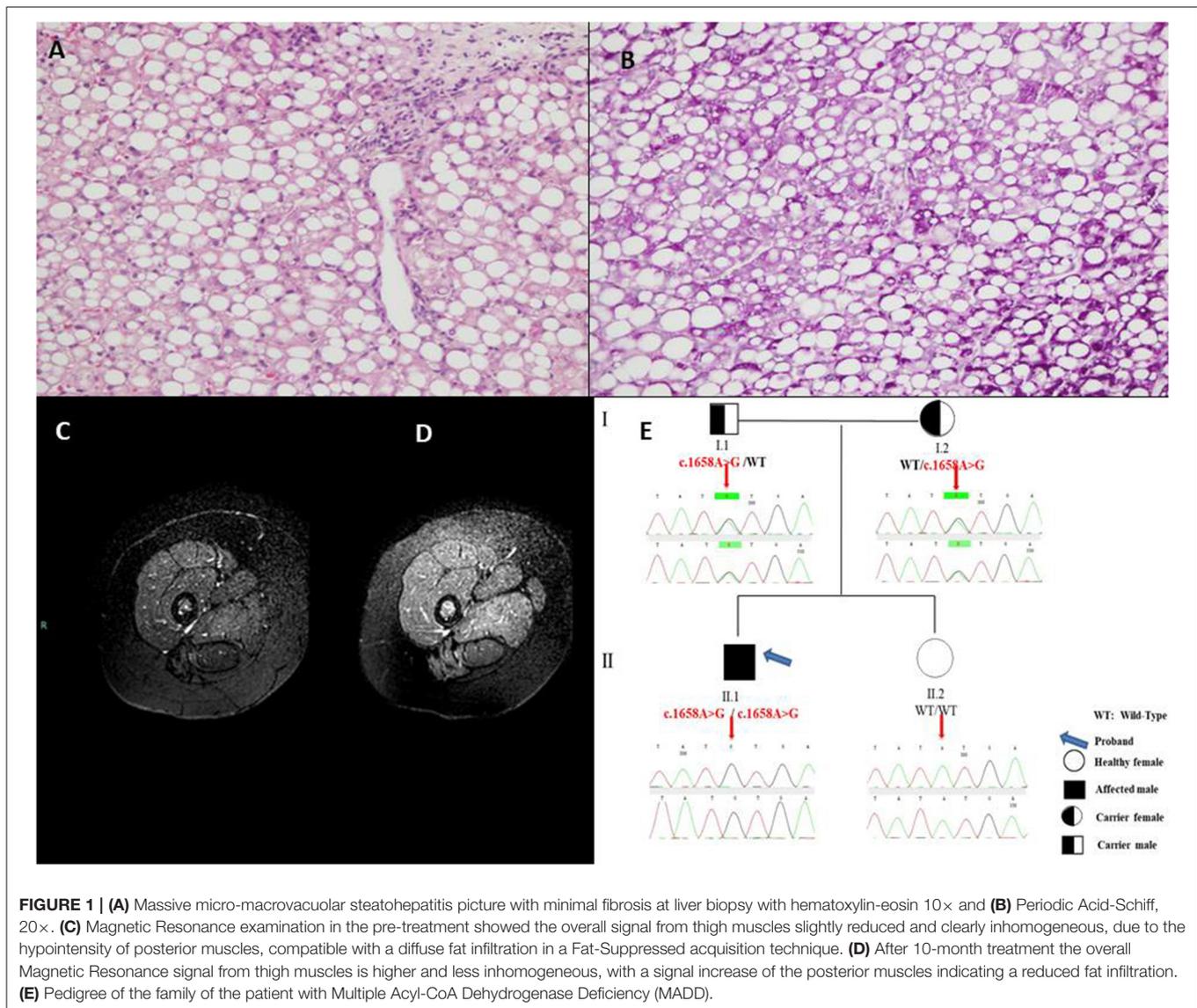
CASE REPORT

The proband was a 12-year-old otherwise healthy child admitted for some weakness/asthenia along with episodes of vomiting, and recently discovered severe hypertransaminasemia [alanine aminotransferase (ALT) and aspartate aminotransferase (AST) × 20 times upper limit of normal (uln)]. He was born at term after a normally conducted pregnancy. Previous regular checkup of laboratory tests including transaminases were normal at age 1, 7, and 8 years old. His early neurodevelopment was reported as normal with walking at 1 year of age and talking in phrases at 18 months of age.

He had a first access to a pediatric ward on July 2017 at age 11 for vomiting and asthenia. Laboratory showed hypertransaminasemia and increased serum values of creatine phosphokinase (CK) and lactic dehydrogenase (LDH) (ALT × 30 uln, AST × 20 uln, CK × 13 uln, and LDH × 6 uln), neutropenia (values at the entrance: WBC 3310/μL, N 650/μL) and positive serology of Parvovirus B19 infection [IgG 52,7 (positive if >11,5); IgM 98,5 (positive if >11,5)]. He was discharged after 10 days, following a sharp reduction of the values of serum enzymes and neutrophils normalization.

In June 2019, he was admitted to our ward for a new episode of vomiting, asthenia, and hypertransaminasemia and neutropenia. His physical examination included a weight of 37 kg (50–75th percentile), height of 146.8 cm (50–75th percentile) and body mass index of 17.2 (50th percentile). There was no facial dysmorphism or significant skin finding. Muscle mass was normal with normal strength and tone. No tremor, ataxia, or abnormal movements were present. At entry laboratory data confirmed hypertransaminasemia (ALT × 30 uln; AST × 20 uln; CK × 10 uln; LDH × 4 uln). Hepatic ultrasound showed a severe bright liver. Cardiac sonography was normal. Due to a persistent elevation of serum enzymes, a liver biopsy was performed and it showed a massive micro-macrovacuolar steatohepatitis picture with minimal fibrosis without signs of inflammation (**Figures 1A,B**).

During the hospitalization, main viral [Hepatitis A, B, and C and minor hepatotropic viruses serology (e.g., Epstein Barr Virus and Cytomegalovirus)], autoimmune [anti - nuclear, smooth muscle, liver and kidney microsomes, liver cytosol, endomysium, tissue transglutaminase antibodies], toxic and common metabolic (Wilson disease, hereditary fructose intolerance) causes of hypertransaminasemia were ruled out by appropriate tests and anamnesis. Blood glucose, gases, lactate, ammonia were repeatedly found normal. Asthenia was evaluated by 6 min walking test which resulted positive showing fatigue after only 1.49 min (with oxygen saturation 78%, heart frequency 180 beats per minute, meters traveled 160). Magnetic Resonance

FIGURE 1 | (A) Massive micro-macrovacuolar steatohepatitis picture with minimal fibrosis at liver biopsy with hematoxylin-eosin 10× and (B) Periodic Acid-Schiff, 20×. (C) Magnetic Resonance examination in the pre-treatment showed the overall signal from thigh muscles slightly reduced and clearly inhomogeneous, due to the hypointensity of posterior muscles, compatible with a diffuse fat infiltration in a Fat-Suppressed acquisition technique. (D) After 10-month treatment the overall Magnetic Resonance signal from thigh muscles is higher and less inhomogeneous, with a signal increase of the posterior muscles indicating a reduced fat infiltration. (E) Pedigree of the family of the patient with Multiple Acyl-CoA Dehydrogenase Deficiency (MADD).

Imaging [1.5 Tesla Philips MR equipment, Turbo Spin Echo Fat Suppressed T2w technique (TR 7014 ms, TE 100 ms, FA 90°), using 5 mm thick slices and 2 excitations] was indicative of lipid storage of thighs muscles (mild, **Figure 1C**), and confirmed liver steatosis (severe) as well. At hospital discharge after 11 days CK was normal (112 U/L), AST × 1.2 uln; ALT × 4 uln; LDH × 2 uln.

The determination of urinary organic acids and the search for serum amino acids and acyl carnitines were requested. Serum aminoacids showed increased proline; most of serum acylcarnites were increased (C4–C18) (**Supplementary Table 1**). Urinary mass spectrometric analysis showed a large excretion of glutaric acid, ethylmalonic, butyric, isobutyric, 2-methyl-butyric, and isovaleric acids suggesting a possible diagnosis of MADD. During follow-up, amounts of serum acylcarnitines and urinary organic acids fluctuated paralleling serum transaminases, with highest values manifest during periods of illness (e.g., flu) or catabolic stress (e.g., protracted fasting), confirming the

recurrent nature of episodes in MADD (**Supplementary Table 1** and **Figures 2A,B**).

After informed consent from the parents, a genetic test to confirm the diagnosis and to identify the carrier subjects in the family was therefore requested. Molecular analysis of genomic DNA isolated from peripheral whole blood was performed by Sanger sequencing of coding regions, intron/exon boundaries and 5′ and 3′ UTR of *ETFA, ETFB*, and *ETFDH* genes, using previously described protocol (5). Genetic results revealed the presence of the novel missense variant c.1658A > G (p.Tyr553Cys) in exon 12 of the *ETFDH* gene in the patient, at homozygous state. Both parents were heterozygous for the mutated allele. The proband's sister did not show the variant (**Figure 1E**).

Lipid restricted diet and Riboflavin treatment (100 mg thrice/day) ameliorated dramatically the asthenic symptoms. Repeat 6 min walking test showed no/minimal fatigue after 2 months (oxygen saturation 100%, heart frequency 150 beats per
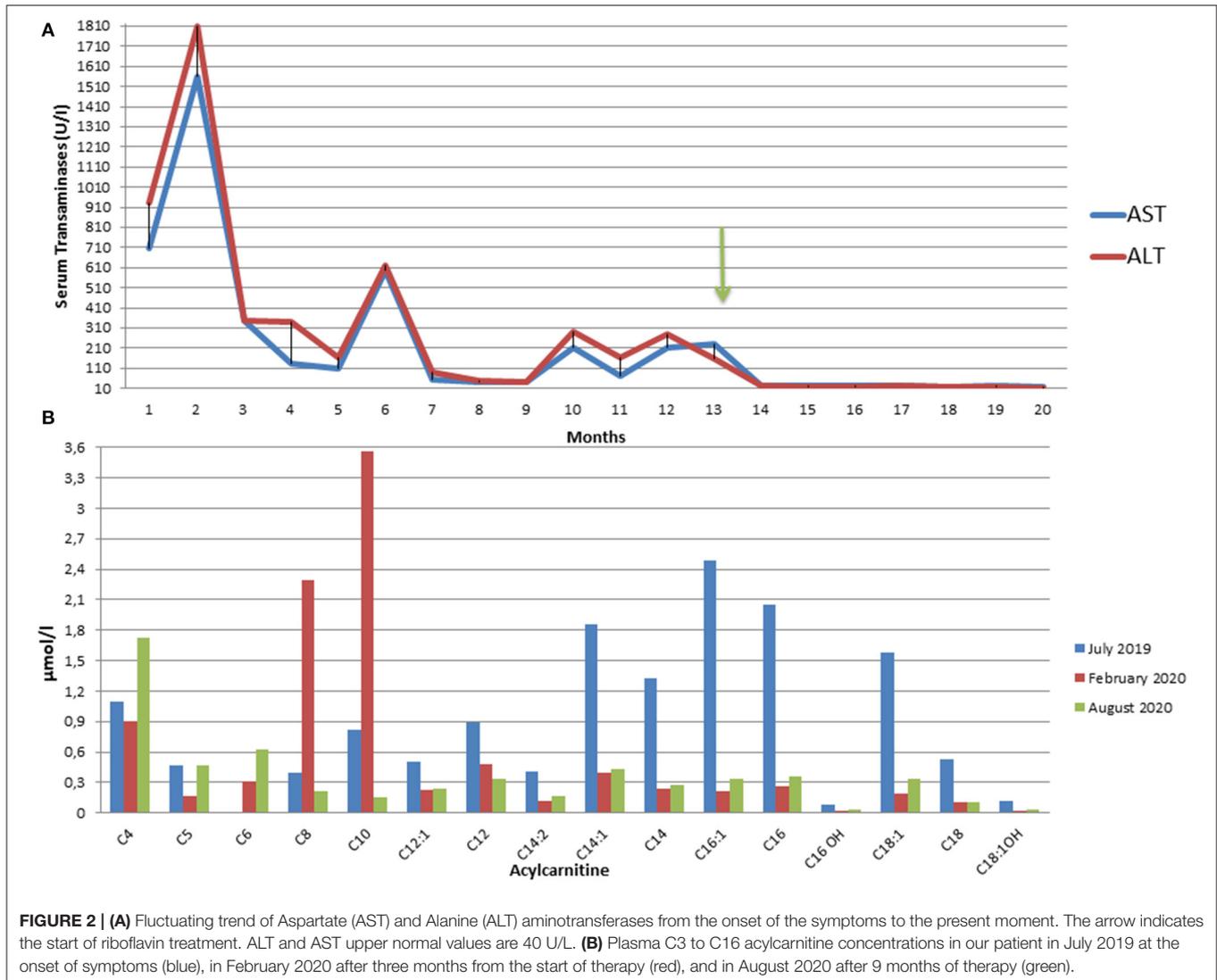
FIGURE 2 | (A) Fluctuating trend of Aspartate (AST) and Alanine (ALT) aminotransferases from the onset of the symptoms to the present moment. The arrow indicates the start of riboflavin treatment. ALT and AST upper normal values are 40 U/L. (B) Plasma C3 to C16 acylcarnitine concentrations in our patient in July 2019 at the onset of symptoms (blue), in February 2020 after three months from the start of therapy (red), and in August 2020 after 9 months of therapy (green).

minute, 560 m traveled). Liver brightness at ultrasonography, liver function tests, along with the whole hepatic, muscular and metabolic laboratory profiles improved remarkably and persistently (**Supplementary Table 1**). Control Magnetic Resonance Imaging, after 10 months of therapy, showed absent/sharp decrease of lipid storage of thighs muscles (**Figure 1D**).

LITERATURE REVIEW

We searched within the PubMed, Scopus, and Cochrane Library academic medical databases. The database search strategy was formulated around terms for MADD AND several other text words reported in **Supplementary Table 2.** Text words were chosen based on the existing literature and were obtained from related bibliographies. The earliest publication chosen date was January 2000 and the search ended in February 2021. Systematic search of literature was performed with no language restrictions.

To be eligible for inclusion, studies had to describe a case of MADD associated with liver disease.

Study details and quality characteristics were independently extracted by three of the authors for all the articles and in a stepwise approach, first by reading the title, then by reviewing the abstract, and finally by revising the full text, where appropriate (**Supplementary Figure 1**). At the end of revision, 37 studies were selected.

Out of 270 reported patients, 70 had liver involvement. Fatty liver (45 cases) and recurrent vomiting represents rare extra-muscular symptoms of late onset MADD, and it has been describsed especially in mainland Chinese patients (3). **Table 1** shows literature cases reported with fatty liver and/or liver disease as compared with our patient. The ages of the patients included ranged from six months to 68 years at diagnosis. Only 9 out of the 45 patients had severe fatty liver either at imaging or at liver histology. One patient showed early stages of liver failure, one recurrent pancreatitis, and one recurrent rhabdomyolysis and acute renal failure after the age of 46.

TABLE 1 | Summary of the systematic review of the literature with studies reporting liver involvement in patients with Multiple Acyl-CoA Dehydrogenase Deficiency (MADD).

Reference	Cases with hepatopathy /Total	Age onset Gender	ETFDH gene mutations	Main symptoms	Liver involvement	Reported Laboratory anomalies
Al-Essa et al. (6)	1/7	7 y M	n.r.	Two to three metabolic crises per year, after meals rich in fat or protein.	US enlarged liver with normal echogenic pattern.	CK and hepatic profile (serum transaminases): normal
Liang et al. (7)	1	22 y F	n.r.	Recurrent pancreatitis; exercise intolerance, generalized muscle weakness with difficult walking, swallowing, and controlling the head. Four limbs areflexia.	US fatty liver, hepatomegaly, and enlarged pancreas with calcification. Liver biopsy: marked fatty metamorphosis, cytoplasm with prominent cell borders (plantlike appearance).	↑ CK ↑ AST, ALT ↑ 2-hydroxylglutaric, pyruvic, ethylmalonic, hippuric, adipic, and suberic acids. ↑ acylcarnitines
Gempel et al. (8)	1/7	34 y F	c.1367 C > T; c.1768 A > G	Insulin-dependent diabetes mellitus diagnosed at age 14. Proximal muscle weakness, exercise intolerance, hepatopathy.	Hepatopathy	↑ CK ↑ AST, ALT ↑ acylcarnitines
Yotsumoto et al. (9)	3/11	1 y 10 m, 5, 13 y 3F	(1) 1096C > T; 1675C > T (2) c.1096C > T (3) c.524G>A; 1774T > C	(1) Vomiting, hypoglycemia, and liver dysfunction. (2) Convulsion, hypoglycemia, and liver dysfunction. (3) Vomiting, hypotonia, and liver dysfunction.	n.r	n.r.
Liang et al. (10)	1/4	10 y F	c.250G > A; c.380T > A	Mild weakness	Lipid storage disease at liver biopsy	↑ CK ↑ AST, ALT ↑ 2-hydroxylglutaric, ethylmalonic, glutaric and suberic acids. ↑ acylcarnitines
Ohkuma et al. (11)	2/4	5, 6 m 1M, 1F	(1) c.1519T > G (2) c.1208C > T	Muscle weakness, hepatomegaly	Hepatomegaly	n.r.
Wen et al. (12)	1/17	15 y M	n.r.	Muscle weakness, muscle pain	US Moderate Fatty liver	↑ CK ↑ urinary organic acids ↑ acylcarnitines
Lan et al. (13)	3/9	7, 20, 18 y 2M,1F	(1) c.250G > A (2) c.250G > A c.524G > A (+ PNPLA2 c.863C > G) (3) n.r.	(1) Encephalopathy, liver function impairment, lactic acidosis. Dysphagia. Myalgia and limb weakness. (2) Vomiting, liver function impairment. Myalgia and limb weakness. (3) Myalgia	Pt 3. Fatty liver	↑ CK ↑ acylcarnitines
Tojo et al. (14)	1	6 month M	n.r.	Severe muscle weakness (lipid storage myopathy).	Fatty liver	↑ CK ↑ urinary organic acids
Wolfe et al. (15)	1	22 y M	c.820 G > T (exon 7) c.1601 C > T (exon 12)	Severe hypoglycemia and lethargy during an acute illness at 4 months of age. At age 4 years: recurrent vomiting, mild hyperammonemia and dehydration requiring frequent hospitalizations; generalized weakness associated with acute illnesses and growth spurts. Exercise intolerance and proximal muscle weakness.	Approximately 30% of the liver specimen demonstrated mixed macromicro-steatosis (biopsy).	↑ CK ↑ urinary organic acids
Xi et al. (16)	10/35	25 ± 13.6 y 12 F/18 M	n.r.	Muscle weakness and wasting, dysphagia, palpitation, vomiting.	US Fatty liver	n.r.
Izumi et al. (17)	1	54 y M	c.1211T > C	Recurrent rhabdomyolysis and acute renal failure after the age of 46; fatigability and weakness of neck and lower limbs.	US Fatty liver	↑ CK ↑ urinary organic acids ↑ acylcarnitines

(Continued)

TABLE 1 | Continued

Reference	Cases with hepatopathy /Total	Age onset Gender	ETFDH gene mutations	Main symptoms	Liver involvement	Reported Laboratory anomalies
Lammer et al. (18)	1	41y M	n.r.	General weakness	n.r.	↑ AST, ALT
Scheicht et al. (19)	1	22 y M	n.r.	Progressive reduction of physical overall performance, muscle weakness of the extremities, diarrhea.	Early stages of liver failure	↑ AST, ALT ↑ CK
Fitzgerald et al. (20)	1	22 y F	c.1367C > T	Cyclic vomiting syndrome diagnosed at 9 years and chronic fatigue. At 22 years, cardiovascular collapse, raised anion gap metabolic acidosis and non-ketotic hypoglycaemia. Post-mortem diagnosis of MADD (lipid storage involving the skeletal muscle, heart muscle, liver and cerebellum).	US and CT fatty infiltration of the liver with ascites	n.r.
Zhu et al. (3)	4/13	26, 32, 36, 38 y 4M	(1) c.518T > G; c.1211T > C (2) c.1399G > A (3) c.715G > A; c.1810G > T (4) c.295C > T; c.821G > A	Muscle weakness, soreness, vomiting	US Severe Fatty liver	↑ CK
Xi et al. (21)	9/90	n.r.	n.r.	Muscle weakness, exercise intolerance, vomiting or diarrhea, palpitation.	US Fatty liver	n.r.
Rosenbohm et al. (22)	1	25 y F	c.1544G > T	Mild fluctuating weakness	US steatohepatitis, and hepatomegaly	↑ AST, ALT ↑ CK ↑ urinary organic acids ↑ acylcarnitines
Zhuo et al. (23)	1	9 y F	c.389A > T; c.736G > A	Muscle weakness, soreness, vomiting	Hepatomegaly	↑ AST, ALT ↑ CK ↑ urinary organic acids ↑ acylcarnitines
Ersoy et al. (24)	1	19 y F	n.r.	Progressive muscle weakness; intensive care unit with respiratory failure and acute renal failure.	Hepatomegaly	↑ AST, ALT ↑ CK ↑ acylcarnitines
Fu et al. (25)	2/3	23, 68 M	(1) c.250G > A; c.892C > T	(1) Muscle weakness; intermittent nausea and vomiting in the morning. Subacute premature fatigue, exercise intolerance, and weakness of shoulder, hip, neck, and masticatory muscles, general malaise and myalgia resembling polymyositis.	US Fatty liver	↑ AST, ALT ↑ CK ↑ urinary organic acids ↑ acylcarnitines
			(2) c.250G > A; c.449-453delTAACA	(2) Chronic or recurrent abdominal pains. Proximal limb and truncal muscle weakness, fatigue, myalgia, dysphagia, exertional and nocturnal dyspnea, and choking.		

(Continued)

TABLE 1 | Continued

Reference	Cases with hepatopathy /Total	Age onset Gender	ETFDH gene mutations	Main symptoms	Liver involvement	Reported Laboratory anomalies
Dai et al. (26)	1	13.83 y F	c.250G > A; c.353G > T	Muscle weakness	US Severe Fatty liver	↑ myocardial enzymes ↑ total cholesterol ↑ lactic acid ↑ serum free fatty acids
Yamada et al. (27)	2	58/31 y M	n.r.	Myalgia, muscle weakness	Liver dysfunction	
Behin et al. (28)	2/13	18, 22 y M/F	(1) c.1366C > T; c.1366C > T (2) c.877C > G; c.1691-3C > G	(1) "Transient hepatitis" with vomiting, abdominal pain, fatigue and myalgia during pregnancy. Exercise intolerance. (2) Delirium, vomiting, asthenia, exercise intolerance, progressive severe proximal and axial, weakness, bulbar signs.	Pt 2. Steatosis (liver biopsy)	↑ CK; ↑ C8 toC16; ↑ 2hydroxyglutaric acid, no acylglycines ↑ C4 to C18 ↑ Ethylmalonicacid
Cui et al. (29)	1	11 y M	n.r.	Muscle weakness, unconsciousness	US Fatty liver Hepatomegaly	↑ AST, ALT ↓ glucose Metabolic acidosis
Angelini et al. (30)	1/6	23 y F	c.451A > G exon 4 c.1649T > G exon 12	Progressive lower limb weakness and myalgias for 4 years; exercise intolerance, intermittent hypoglycaema.	US slight hepatomegaly	↑ CK
Missaglia et al. (2)	1	54 y F	c.1285 + 1G > A; c.560C > T	Recurrent episodes of vomiting, drowsiness, appetite loss, asthenia, and acetonemic breath; progressive arm, lower limb and neck flexors weakness.	Mild swelling of hepatocytes (liver biopsy)	↑ CK
Van der Westhuizen et al. (31)	2/3	2 y F 4 y F	(1) c.1067G > A; c.1448C > T (2) c.1067G > A; c.1448C > T	(1) Progressive muscle weakness. Hepatomegaly. Migraine. Exercise intolerance. Hypermobility (2) Progressive muscle weakness. Hepatomegaly. Migraine	Pt 2. macrovacuolar steatosis	↑ AST, ↑ ALT ↑ urinary organic acids ↑ acylcarnitines ↑ amino acids
Fan et al. (32)	2/4	30, 27 y 2M	(1) c.524G > A; c.1450T > C (2) c.1157G > A; c.1450T > C	(1) Muscle weakness (2) Non-ketotic hypoglycemia, metabolic acidosis, electrolyte disturbances, and increased muscle enzymes.	Pt 1. US fatty liver Pt 2. severe liver injury	↑ CK, ↑ AST ↑ urinary organic acids ↑ acylcarnitines
Chen et al. (33)	2 (Brothers)	19/13 y M	c.250 G > A	Exercise-induced myalgia, progressive proximal muscle weakness.	n.r.	↑ AST, ALT ↑ CK
Hu et al. (34)	1	6 m F	c.1812delG; c.579A > G	Muscle weakness. Crying faintly, vomiting and appetite loss.	TC scan fatty liver	↑ AST, ALT ↑ CK
Soldath et al. (35)	1	22 y M	c.1074G > C	Mild exercise intolerance; muscle weakness and pain (lipid storage myopathy). Crises of hypoglycemia and metabolic decompensation. Recurrent Clostridium difficile infection and septicemia.	Initially steatosis, then cirrhosis and acute liver failure.	↑ AST, ALT ↑ CK ↑↑ acyl-carnitines of all lengths
Santananukarn et al. (36)	1	40 y F	c.[250G > A]; [524G > A]	Subacute severe weakness of bulbar and limb muscles.	US Fatty liver Liver biopsy: severe steatosis	↑ AST, ALT ↑ CK

(Continued)

TABLE 1 | Continued

Reference	Cases with hepatopathy /Total	Age onset Gender	ETFDH gene mutations	Main symptoms	Liver involvement	Reported Laboratory anomalies
Pan et al. (37)	1	17 y F	c.250G > A; c.872T > G	Exercise intolerance and muscle weakness; palpitation and shortness of breath after exercise. Transient loss of consciousness. Muscle biopsy: lipid storage.	US Fatty liver	↑ AST, ALT ↑ acyl-carnitines
Xiao et al. (38, 39)	1	23 y M	c.665A > C; c.964G > T	Progressive exercise intolerance accompanied by predominantly lower proximal muscle weakness and pain.	US Fatty liver	↑ AST, ALT, ↑CK ↑ urinary organic acids ↑ acylcarnitines
Nilipour et al. (40)	2/29	3, 35 y 2F	c.1130T > C; c.92C > T	Muscle weakness	Hepatomegaly	↑CK
Lian et al. (41)	1	n.r.	c.807A > C	Recurrent abdominal pain, vomiting, and impaired consciousness.	Fatty liver	n.r.
Present case	1	11 y M	c.1658A > G	Vomiting, asthenia	US, CT scan and histologic fatty liver (severe)	↑ AST, ALT ↑ urinary organic acids ↑ acylcarnitines ↑ amino acids
TOTAL	70/283				46 fatty liver, of which 10 severe	

↑ Increased, ETFDH, Electron-transfer flavoprotein dehydrogenase; ALT, Alanine amino transferases; AST, Aspartate amino transferases; CK, Creatine kinase; m, Month; Y, Year; M, Male; F, Female; US, Ultrasound; CT, Computerized tomography; n.r., Not reported.

DISCUSSION

Differently from adult age, where the causes of hypertransaminasemia and/or fatty liver other than obesity-related liver disease are limited, the pediatric diagnostic approach may be more complex. In addition to nonalcoholic fatty liver disease (NAFLD)/metabolic dysfunction-associated fatty liver disease (MAFLD) children, in fact, may present individually rare genetic/metabolic conditions which collectively end up into a relevant and challenging ≥30% group of heterogeneous pediatric onset liver diseases (42–44). These, therefore, still require a cautious exclusion, so that pediatric fatty liver disease (PeFLD) with some subtypes has been proposed to be a better terminology to use and to be subject to revisitation upon reaching adulthood (45). In our patient urinary organic acids, serum amino acids and acyl carnitine patterns were crucial to suggest the diagnosis and pave the way to the subsequent confirmatory tests of a late onset MADD-related fatty liver disease. The latter is a condition which is more frequently characterized by prevalent metabolic and muscular signs rather than by severe fatty liver as confirmed by our systematic review of the literature (**Table 1**).

Molecular analysis of *ETFA*, *ETFB*, and *ETFDH* genes indicated that our patient was homozygous for the missense variant c.1658 A>G (p.Tyr553Cys) in exon 12 of the *ETFDH* gene. This variant is absent in the public database of mutations and polymorphisms. Interestingly, in the literature a pathogenic missense mutation (c.1657T > C) is reported in the same codon, determining a different amino acid substitution (p.Tyr553His). Including this observation in the pathogenetic evaluation, performed according to the American College of Medical Genetics and Genomics (ACMG) guidelines for variant interpretation (38), the novel variant c.1657T > C (p.Tyr553Cys) is classifiable as likely pathogenetic. Therefore, we identified a novel missense mutation in the *ETFDH* gene associated to late-onset riboflavin-responsive MADD.

The clinical phenotype of type III MADD is highly variable and ranges from acute, in some cases even fatal, metabolic crises in infancy to asymptomatic adults. This intriguing variability may probably be explained by the observation that even minute amounts of residual ETF/ETFDH activity seem to be sufficient to prevent embryonic development of congenital anomalies. Studies of an Asp128Asn mutation of the *ETFB* gene, identified in a patient with type III disease, showed that the residual activity of the enzyme could be rescued up to 59% of that of wild type activity when ETFB-(Asp128Asn)-transformed E. coli cells were grown at low temperature. This suggests that the environmental factors such as cellular temperature and stress may influence the enzymatic phenotype (46) in agreement with the case history of our patient. Although most patients become symptomatic within the first two decades, onset of symptoms ranges from the second month of life to late adulthood. Decompensations are characterized by acidosis, hypoglycemia, elevated activities of transaminases, rhabdomyolysis with raised creatine kinase activity, and, eventually, hyperammonemia. These episodes are usually triggered by catabolic states, either due to infections and febrile illnesses or to a reduced energy supply (1). In

our case, the clinical presentation was prevalently characterized by some vomiting episodes and fatigue and recurrent severe hypertransaminasemia with a histological correlate of massive micro-macrovacuolar steatohepatitis with minimal fibrosis. Lipid restricted diet and riboflavin treatment dramatically ameliorated the clinical symptoms, liver brightness and LFTs, and metabolic profiles as reported in literature for the late onset MADD, which is therefore also called *riboflavin-responsive MADD* (RR-MADD) (3, 47). Riboflavin, the precursor of the coenzyme FAD, acts as a molecular chaperone that promotes folding and steady state levels of misfolded ETF-QO proteins in early stages and stabilizes folding intermediates or membrane-inserted proteins in later stages (48).

CONCLUSIONS

RR-MADD is a treatable but rare disease and its diagnosis is difficult due to its high clinical phenotypic heterogeneity (13). Prevalent hepatic disease rather than isolated lipidic myopathy is a much less common presentation of the late-onset form. Our study is significant as it suggests that one should have consideration for this condition in the work-up of an otherwise orphan diagnosis of obesity-unrelated severe steatohepatitis with recurrent hypertransaminasemia. Correct diagnosis of MADD type III may allow timely initiation of a sometimes lifesaving riboflavin treatment and improve outcome.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin. Written informed consent was obtained from the minor(s)' legal guardian/next of kin for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

MAS and CM conceived the original idea and took the lead in writing the manuscript with input from all authors. LN, GI, and GPC followed the clinical course of the patient over time. CB made the histopathology study. ST and FDS made MRI studies. FB, CM, MR, and GF carried out biochemical and molecular studies. DM and PV gave substantial intellectual contribution. PV is the guarantor of the study. All authors provided critical feedback and helped shape the study, discussed the results, and contributed to the final manuscript.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fped.2021.672004/full#supplementary-material

Supplementary Figure 1 | Flowchart of literature search results. Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flowchart of literature search results. From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(7):e1000097.

Supplementary Table 1 | Serum acylcarnitines, aminoacids, and urinary organic acids at presentation and during the follow-up.

Supplementary Table 2 | Key words used for PUBMED, Scopus and Cochrane library search.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.