


Thermo-sensitive mitochondrial trifunctional protein deficiency presenting with episodic myopathy

Marit Schwantje^{1,2} | Merel S. Ebberink² | Mirjam Doolaard² |
 Jos P. N. Ruiter² | Sabine A. Fuchs¹ | Niklas Darin³ |
 Carola Hedberg-Oldfors⁴ | Luc Régal⁵ | Laura Donker Kaat⁶ |
 Hidde H. Huidekoper⁷ | Simon Olpin⁸ | Duncan Cole^{9,10} | Stuart J. Moat^{8,9} |
 Gepke Visser^{1,2} | Sacha Ferdinandusse² 

¹Department of Metabolic Diseases, Wilhelmina Children's Hospital, University Medical Center Utrecht, Utrecht, The Netherlands

²Laboratory Genetic Metabolic Diseases, Department of Clinical Chemistry, Amsterdam UMC, University of Amsterdam, Amsterdam, The Netherlands

³Department of Pediatrics, Institute of Clinical Sciences, Sahlgrenska Academy, University of Gothenburg, Sahlgrenska University Hospital, Gothenburg, Sweden

⁴Department of Laboratory Medicine, Institute of Biomedicine, University of Gothenburg, Gothenburg, Sweden

⁵Pediatric Neurology and Metabolism Department of Pediatrics, UZ Brussel, Jette, Belgium

⁶Department of Clinical Genetics, Erasmus MC, University Medical Center Rotterdam, Rotterdam, The Netherlands

⁷Department of Pediatrics, Center for Lysosomal and Metabolic Diseases, Erasmus MC, University Medical Center Rotterdam, Rotterdam, The Netherlands

⁸Department of Clinical Chemistry, Sheffield Children's Hospital, Sheffield, UK

⁹Wales Newborn Screening Laboratory, Department of Medical Biochemistry, Immunology and Toxicology, University Hospital of Wales, Cardiff, UK

¹⁰School of Medicine, Cardiff University, Cardiff, UK

Correspondence

Sacha Ferdinandusse, Laboratory Genetic Metabolic Diseases, Amsterdam University Medical Center, Meibergdreef 9, 1105 AZ Amsterdam, The Netherlands.
 Email: s.ferdinandusse@amsterdamumc.nl

Funding information

Metakids and PNO zorg, Grant/Award Number: 2019_087

Communicating Editor: Jerry Vockley

Abstract

Mitochondrial trifunctional protein (MTP) is involved in long-chain fatty acid β -oxidation (lcFAO). Deficiency of one or more of the enzyme activities as catalyzed by MTP causes generalized MTP deficiency (MTPD), long-chain hydroxyacyl-CoA dehydrogenase deficiency (LCHADD), or long-chain ketoacyl-CoA thiolase deficiency (LCKATD). When genetic variants result in thermo-sensitive enzymes, increased body temperature (e.g. fever) can reduce enzyme activity and be a risk factor for clinical decompensation. This is the first description of five patients with a thermo-sensitive MTP deficiency. Clinical and genetic information was obtained from clinical files. Measurement of LCHAD and LCKAT activities, lcFAO-flux studies and palmitate loading tests were performed in skin fibroblasts cultured at 37°C and 40°C. In all patients (four MTPD, one LCKATD), disease manifested during childhood (manifestation age: 2–10 years) with myopathic symptoms triggered by fever or exercise.

[Click here to access the podcast for this paper.](#)

This is an open access article under the terms of the [Creative Commons Attribution-NonCommercial-NoDerivs](#) License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

© 2022 The Authors. *Journal of Inherited Metabolic Disease* published by John Wiley & Sons Ltd on behalf of SSIEM.

In four patients, signs of retinopathy or neuropathy were present. Plasma long-chain acylcarnitines were normal or slightly increased. *HADHB* variants were identified (at age: 6–18 years) by whole exome sequencing or gene panel analyses. At 37°C, LCHAD and LCKAT activities were mildly impaired and lcFAO-fluxes were normal. Remarkably, enzyme activities and lcFAO-fluxes were markedly diminished at 40°C. Preventive (dietary) measures improved symptoms for most. In conclusion, all patients with thermo-sensitive MTP deficiency had a long diagnostic trajectory and both genetic and enzymatic testing were required for diagnosis. The frequent absence of characteristic acylcarnitine abnormalities poses a risk for a diagnostic delay. Given the positive treatment effects, upfront genetic screening may be beneficial to enhance early recognition.

KEYWORDS

long-chain fatty acid oxidation disorders, long-chain ketoacyl-CoA thiolase deficiency, mitochondrial trifunctional protein complex, mitochondrial trifunctional protein deficiency, myopathy, thermo-sensitivity

Synopsis

Positive treatment effects emphasize the need for early recognition of thermo-sensitive MTP deficiency, but the frequent absence of characteristic acylcarnitine abnormalities poses a risk for a diagnostic delay and shows the importance of both genetic and enzymatic testing for a proper diagnosis.

1 | INTRODUCTION

The mitochondrial long-chain fatty acid oxidation (lcFAO) is a complex process, involving many different enzymes and carrier proteins needed for the transport of long-chain fatty acids from the cytosol into the mitochondria and the subsequent steps of lcFAO. Mitochondrial trifunctional protein (MTP) is a hetero-octameric ($\alpha\beta\beta$) multi-enzyme complex that catalyzes the last three steps of lcFAO.^{1,2} MTP harbors three different enzyme activities, namely long-chain enoyl-CoA hydratase (LCEH), long-chain hydroxyacyl-CoA dehydrogenase (LCHAD), and long-chain ketoacyl-CoA thiolase (LCKAT). LCEH and LCHAD are located on the α -subunit and are both encoded by the *HADHA* gene. LCKAT is located on the β -subunit and is encoded by the *HADHB* gene.³

MTP deficiencies are inherited disorders of lcFAO (lcFAOD), and include generalized MTP deficiency (MTPD, OMIM #609015), isolated LCHAD deficiency (LCHADD, OMIM #609016), and isolated LCKAT deficiency (LCKATD, no OMIM entry).^{4,5} In MTPD, all three MTP enzyme activities are deficient as a result of genetic variants in either *HADHA* or *HADHB*. In LCHADD or LCKATD, only one of the enzyme activities is deficient. LCHADD is caused by *HADHA* variants and LCKATD by *HADHB* variants. Although the MTP deficiencies are considered as the more severe lcFAOD, they can result in

a wide spectrum of clinical disease. Their clinical phenotypes vary from early-onset disease with cardiomyopathy, hypoketotic hypoglycemia and/or liver failure to later-onset forms with myopathy, episodes of rhabdomyolysis, and peripheral neuropathy and/or pigmentary retinopathy for MTPD and LCHADD.

Conditions leading to increased energy demand, such as exercise or febrile illness, or decreased energy supply, such as fasting or gastroenteritis, may induce a metabolic decompensation in lcFAOD-patients. In febrile illness-induced metabolic decompensation, reduced enzyme activity at higher temperatures may play an additional role. A negative effect of higher temperatures on residual enzyme activity has already been demonstrated in patients with other fatty acid oxidation disorders (FAOD), namely medium-chain acyl-CoA dehydrogenase deficiency (MCADD), very long-chain acyl-CoA dehydrogenase deficiency (VLCADD) and carnitine palmitoyl transferase 2 deficiency (CPT2D).^{6–8} This thermo-sensitivity mostly occurred in patients with relatively mild clinical phenotypes due to missense variants leading to relatively high residual enzyme activities.

For MTPD caused by mild *HADHB* variants, residual enzyme activity and MTP stability has been found to be higher at 30°C compared to 37°C.^{9,10} The lower temperature was hypothesized to stabilize the mutant subunit in protein folding and formation of the MTP-complex. To

our knowledge, effects on residual MTP enzyme activity and stability of temperatures higher than 37°C, as clinically caused by fever and physical activity, have not yet been investigated.

In this article, we describe five patients with thermo-sensitive MTP deficiency caused by *HADHB* variants. MTP enzyme activities were not or only mildly impaired at 37°C, whereas enzyme activities significantly diminished at 40°C. This temperature sensitivity was compatible with the clinical phenotype, since patients mainly developed myopathic symptoms after febrile illness or physical activity. With this case series, we aim to enhance awareness of thermo-sensitivity in inborn errors of metabolism, including MTP deficiency, and improve early recognition of these potentially severe conditions which may be alleviated by adequate preventive measures.

2 | MATERIALS AND METHODS

2.1 | Clinical outcome and consent

Clinical information was collected from the clinical files. Informed consent for publication was obtained from all patients and/or their guardians.

2.2 | Biochemical studies

Primary skin fibroblasts of the patients were cultured in Ham's F-10 medium supplemented with 10% fetal calf serum (Invitrogen), 25 mmol/L HEPES, 100 U/ml penicillin, 100 µg/ml streptomycin, and 250 µg/ml amphotericin in a humidified atmosphere of 5% CO₂. Fibroblasts were cultured at the standard temperature of 37°C. To investigate temperature sensitivity, cells were also cultured at 40°C for 2 weeks followed by enzyme or lcFAO-flux assays. For all the experiments at 40°C, two or three control cell lines were cultured and analyzed in parallel.

LCHAD and LCKAT activities were measured in fibroblast homogenates using 3-keto-palmitoyl-CoA as substrate, as described previously.¹¹ Short-chain acyl-CoA dehydrogenase (SCHAD) activity was measured essentially as described by Wanders et al.¹²

lcFAO-flux analysis was performed in fibroblasts by measuring the production of radiolabeled H₂O from [9,10-³H(N)]-oleic acid.^{13–15} The lcFAO-flux was expressed as a percentage of the mean activity simultaneously measured in three healthy control fibroblast lines cultured at the same temperature.

The palmitate loading test was performed in fibroblasts by adding 120 µmol/L [U-¹³C]palmitate and 0.4 mmol/L L-carnitine to the medium.⁷ In short, after

incubation of 96 h at respectively 37°C or 40°C, acylcarnitines in the medium were measured by tandem mass spectrometry.

2.3 | Genetic analysis

Genetic analysis was performed by either gene panel testing, whole-exome sequencing (WES), or individual gene analysis as part of the diagnostic process. Methods depended on the laboratory performing the analyses. For Patient #1, gene panel testing (for rhabdomyolysis-related disorders) was performed at Sheffield Diagnostic Genetics Service, Sheffield Children's Hospital. For Patients #2, #3a, and #4, WES was performed at BGI Tech, Shenzhen, China (Patient #2), GATC Biotech, Constance, Germany (Patient #3a), and Maastricht UMC, the Netherlands (Patient #4) and the identified variants were confirmed with Sanger sequencing. For Patient #3b, *HADHB* gene analysis was performed by Sanger sequencing at GATC Biotech, Constance, Germany. Sequence data were compared to the reference sequence NM_000183.2 (*HADHB*) for Patients #1, #2, and #4, and NM_000183.3 (*HADHB*) for Patients #3a and #3b with nucleotide numbering starting at the first adenine of the translation initiation codon ATG.

2.4 | Immunoblot analysis

Immunoblot analysis was performed with fibroblast homogenates from two controls and the patients. Fibroblasts were cultured simultaneously at 37°C and 40°C for 2 weeks. Homogenates (10 µg protein) were separated on a 10% NuPAGE Bis-Tris gel (Thermo Fisher) in a MOPS buffer and subsequently transferred onto a nitrocellulose membrane, blocked with 4% normal goat serum/PBS/0.1% Tween and probed with polyclonal antibodies raised against rat liver MTP in a 1:5000 dilution (kind gift from Prof. T. Hashimoto). As a loading control, the membranes were re-probed with a monoclonal antibody against tubulin (T6199, Sigma), used in a 1:2000 dilution. For visualization, we used the secondary antibodies IRDye 800CW goat anti-rabbit and/or IRDye 680CW donkey anti-mouse with the Odyssey Infrared Imaging System (LI-COR Biosciences).

3 | RESULTS

All five patients with thermo-sensitive MTP deficiency presented with fever- or exercise induced muscle symptoms during childhood and had long diagnostic

TABLE 1 Clinical characteristics of five patients with thermo-sensitive MTP deficiency

	Patient #1	Patient #2	Patient #3a	Patient #3b	Patient #4
Sex	Female	Male	Male	Male	Female
Current age	24y	11y	23y	16y	12y
Age at first symptoms	10y	2y	3y	3y	3y
Age at diagnosis	18y	6y	18y	9y	10y
Birth weight (in grams)	<i>Unknown</i>	3655	3210	3290	3700
Pregnancy duration (in weeks + days)	<i>Unknown</i>	A term	38 + 6	38 + 3	A term
Height (in SD compared to population)	<i>Unknown</i>	-0.67	-2.0	+0.5	+0.02
Weight (in SD compared to population)	<i>Unknown</i>	-0.67	+0.6	-0.2	+3.57
Episodic muscle symptoms	Muscle pain, rhabdomyolysis	Muscle weakness, hypotonia, abnormal gait, loss of balance, sometimes muscle pain	Muscle weakness	Muscle weakness	Muscle weakness, exercise intolerance, sometimes leg pain
Provoking factors	Prolonged exercise, alcohol, febrile illness, and giving birth	Febrile illness	Exercise, febrile illness, skipping breakfast, or high environmental temperature	Exercise, febrile illness	Exercise, febrile illness
Neuropathy/neurophysiological investigations	Normal tendon reflexes, normal NCS, EMG not performed	Absent Achilles tendon reflexes and weak patellar tendon reflexes, NCS not performed EMG: reversible proximal and bulbar muscle denervation	Absent deep tendon reflexes, normal NCS EMG: abnormalities suggestive of a proximal axonal motor neuropathy	Absent deep tendon reflexes in the lower extremities, NCS and EMG: not performed	Normal tendon reflexes, normal NCS EMG: sporadic and small motor unit potentials
Muscle biopsy	Normal histological appearance and activity of muscle mitochondrial respiratory enzymes	Isolated atrophic fibers, slightly hypertrophic fibers, glycogen depleted pre-necrotic fibers, increased intracellular lipid content in some fibers, many Type 1 and some Type 2A fibers. Normal activity of muscle mitochondrial respiratory enzymes	Normal activity of muscle mitochondrial respiratory enzymes	-	Normal histological appearance and activity of mitochondrial respiratory enzymes

TABLE 1 (Continued)

	Patient #1	Patient #2	Patient #3a	Patient #3b	Patient #4
Retinopathy/eye examinations	Mild retinopathy on slit-lamp examination, normal ERG	Not investigated	No, normal ophthalmologic investigations	No, normal ophthalmologic investigations	Not investigated

Abbreviations: EMG, electromyogram, ERG, electroretinogram; NCS, nerve conduction studies; Pt, patient; SD, standard deviation; y, years.

trajectories (range: 4–16 years), which are described below. Pregnancy and delivery were normal for all but Patient #4, for whom pregnancy was complicated with pre-eclampsia and maternal diabetes. All five patients had a normal early psychomotor development. Clinical characteristics are summarized in Table 1.

3.1 | Patient #1

Patient #1 is a now 24-year-old female. At 10 years of age, she developed exercise-induced muscle pain. Over the following years, she experienced several episodes of rhabdomyolysis requiring intensive care admission. Provoking factors included prolonged exercise, alcohol, febrile illness. Also giving birth to her first child caused rhabdomyolysis. In between episodes, creatine kinase (CK) levels were normal. Over time, she developed progressive chronic muscle pain and exercise intolerance.

Laboratory examinations showed minor elevations in long-chain acyl- and hydroxyl-acylcarnitines in plasma (C16: 0.37 $\mu\text{mol/L}$ [ref: 0–0.24], C16-OH 0.09 $\mu\text{mol/L}$ [ref: 0–0.02], C18:1-OH: 0.09 $\mu\text{mol/L}$ [ref: 0–0.01]). LcFAO-flux, CPT2 enzymology and investigations in a muscle biopsy revealed no abnormalities. At the age of 18, gene panel testing revealed compound heterozygosity for two missense variants in *HADHB*: c.397A>G (p.Thr133Ala) and c.1289 T>C (p.Phe430Ser). Enzyme activity measurements revealed a thermo-sensitive defect of LCKAT (see Section 3.7).

After confirmation of the diagnosis LCKATD, a long-chain triglycerides (LCT)-restricted diet was initiated to prevent symptoms, but was not tolerated. Although carbohydrate-rich drinks relieved muscle pain in between episodes, significant weight gain resulted in reduced use. After giving birth to her first child caused rhabdomyolysis, subsequent childbirths were successfully managed with intravenous glucose.

3.2 | Patient #2

Patient #2 is a now 11-year-old boy. From the age of 2 years, he experienced episodes of muscle weakness,

hypotonia, abnormal and deteriorating gait, loss of balance while standing and sitting, and sometimes muscle pain. Episodes were provoked by febrile illness and generally lasted several days. CK levels were always normal. At 3 years of age, severe muscle weakness caused respiratory insufficiency, requiring intensive care admission and tracheostomy. The tracheostoma could be removed at the age of 4 years. In between episodes, symptoms clearly improved, but strength and balance remained weak. Achilles tendon reflexes were absent and patellar tendon reflexes were weak. With rapid antipyretic treatment and intravenous glucose during fever, decompensations were less severe.

Laboratory examinations showed minor elevations of long-chain acylcarnitines (C14:2: 0.09 $\mu\text{mol/L}$ [ref: 0.01–0.08], C16: 2.09 $\mu\text{mol/L}$ [ref: 0.73–1.86], C18:1: 2.54 $\mu\text{mol/L}$ [ref: 0.86–2.03]) and increased CK levels (maximum: 1293 U/L [ref: <247 U/L]) during episodes of muscle pain and muscle weakness. During follow-up, plasma lactate levels were always normal. Plasma pyruvate concentration was slightly increased once. Investigations of a muscle biopsy raised suspicion of a metabolic myopathy, despite the absence of ragged red fibers, whereas neuronal atrophy could not be excluded based on the muscle biopsy (Table 1). Mitochondrial respiratory enzyme activities were normal. Initial electromyography (EMG) showed normal results, but during follow-up he developed reversible denervation of the proximal upper extremities and later also of the bulbar muscles. Magnetic resonance imaging (MRI) scan of the brain was normal.

Molecular analysis of various individual genes was performed (including *PDH-A1 α*), but no abnormalities were found. WES at the age of 6 years revealed compound heterozygosity for two variants in *HADHB*: c.209+1G>C (p.Ala37AspfsX5) and c.397A>G (p.Thr133Ala). Enzyme activity measurements showed a thermo-sensitive defect of both LCHAD and LCKAT (see Section 3.7).

After diagnosis, an LCT-restricted and carbohydrate-enriched diet during febrile illness was started. Since initiation, he has not been admitted to the hospital. Recently, he restricted LCT-intake also under healthy, non-febrile, conditions, and started supplementation with Coenzyme Q10, riboflavin, and thiamin. He now

experiences less fatigue. With an IQ of 78 (WISC-V), he recently encountered learning problems in primary school.

3.3 | Patient #3a

Patient #3a is a now 23-year-old male. From the age of 3 years onward, he experienced fever-induced episodes of muscle weakness, lasting 1–2 weeks. At 9 years of age, he was admitted to the hospital with a mild generalized muscle weakness and absent muscle tendon reflexes. Plasma CK concentrations were increased (630 U/L [ref: 0–228]), but normalized during follow-up. He had no myoglobinuria. Plasma lactate, pyruvate, liver transaminases, plasma and urinary amino acids, plasma acylcarnitines, urinary organic acids, and muscle mitochondrial respiratory enzymes were all normal. MRI scan of the brain and cerebrospinal fluid analysis showed no abnormalities.

Neurophysiological examinations were performed at the age of 9 and 14 years. Nerve conduction studies (NCS) were normal, but EMG revealed progressive abnormalities suggestive of a proximal axonal motor neuropathy in the vastus lateralis and deltoideus muscles.

At the age of 18 years, WES revealed compound heterozygosity for two missense variants in *HADHB*: c.248C>G (p.Ala83Gly) and c.694G>A (p.Ala232Thr). Enzyme activity measurements showed a thermo-sensitive defect of both LCHAD and LCKAT (see Section 3.7).

Since diagnosis, treatment consisted of an LCT-restricted, medium-chain triglycerides (MCT)-supplemented and carbohydrate-enriched diet to which he complied poorly. He still experiences muscle weakness, occurring daily to once a week. Provoking factors include exercise, febrile illness, skipping breakfast, or high environmental temperature, but muscle weakness can occur without a clear provoking factor as well.

3.4 | Patient #3b

Patient #3b, the younger brother of patient #3a, is a now 16-year-old male. Similar to his brother, he experienced febrile illness-induced muscle weakness from 3 years onward. Laboratory investigations showed normal plasma CK concentrations, liver transaminases, and plasma acylcarnitines. Following the diagnosis MTPD in his older brother, gene analysis revealed the same compound heterozygous variants in *HADHB* at the age of 9 years.

On last follow-up, he complained of exercise-induced muscle weakness lasting minutes to 24 h, depending on the intensity of exercise. Clinical examination revealed mild generalized muscle weakness (MRC +4/5) and absence of deep tendon reflexes in the lower extremities. After initiation of dietary treatment with LCT-restriction, MCT-supplementation, and carbohydrate-enrichment, he improved clinically.

3.5 | Patient #4

Patient #4 is a now 12-year-old girl, the daughter of consanguineous parents. Newborn screening was performed and showed normal OH-C16-carnitine concentrations. From the age of 3 years onward, she experienced febrile-illness-induced episodes of muscle weakness, exercise intolerance, and sometimes leg pain, without myoglobinuria. These episodes usually began when the fever dropped and generally lasted 2–4 weeks. Between episodes, she had a diminished exercise tolerance with a maximal walking tolerance of 15 min. She developed obesity from the age of 6 years onward. From the age of 9 years, muscle weakness was also induced by exercise.

Muscle weakness of the lower extremities (MRC4) was occasionally seen. Deep tendon reflexes were normal. Plasma concentrations of CK, lactate, liver transaminases, plasma and urinary amino acids, and urinary organic acids were all normal. Plasma acylcarnitines showed no abnormalities during asymptomatic periods and during an episode of exercise-induced muscle pain, but were never evaluated during febrile illness. Mitochondrial DNA analysis showed heteroplasmy of a probably pathogenic variant in the *MT-TC* gene. The mutation percentage of circa 5% was confirmed with a repeated biopsy, but deemed too low to cause clinical disease. NCS showed normal sensory nerve action potential amplitudes and nerve conduction velocities. EMG showed sporadic and small motor unit potentials (MUPs). There were no typical myopathic MUPs. MRI scan of the brain was normal.

Initial genetic tests comprised individual gene analyses and WES focusing on mitochondrial genes, and revealed no abnormalities. Because the patient's parents were consanguineous, homozygosity mapping was performed using single nucleotide polymorphism (SNP-) array, yielding *HADHA* and *HADHB* as possible candidate genes. Re-analysis of these genes in the WES data revealed homozygosity for the c.397A>G (p.Thr133Ala) variant in *HADHB* at the age of 10 years. Functional enzymatic analyses showed a thermo-sensitive defect of both LCHAD and LCKAT (see Section 3.7).

TABLE 2 Enzyme characteristics of four patients with thermo-sensitive MTP deficiency

HADHB variants	LCHAD activity		LCKAT activity		SCHAD activity		LcFAO-flux	
	nmol/(min·mg protein)	% of reference mean	nmol/(min·mg protein)	% of reference mean	nmol/(min·mg protein)	% of reference mean	nmol/(min·mg protein)	Percentage of controls
Ref	37°C 40°C	37°C 40°C	37°C 40°C	37°C 40°C	37°C 40°C	37°C 40°C	37°C 40°C	37°C 40°C
Pt #1 c.397A>G (p.Thr133Ala) c.1289 T>C (p.Phe430Ser)	46–117 50–129	74% 65%	50–99 28–141	15% 3%	68–115 68–265	60% failed	92–113% 82–134%	104% 31%
Pt #2 c.397A>G (p.Thr133Ala) c.209 + 1G>C (p.Ala37AspfsX5)	32.5 7.6	43% 9%	28.9 4.2	34% 5%	126.0 136.0	138% 98%	117% 70%	
Pt #3a c.248C>G (p.Ala83Gly) c.694G>A (p.Ala232Thr)	45.6 14.1	60% 17%	46.2 9.7	55% 12%	113.7 221.5	125% 159%	109% 12%	
Pt #4 c.397A>G (p.Thr133Ala) c.397A>G (p.Thr133Ala)	30.1 10.0	40% 12%	27.7 5.8	33% 7%	81.0 103.6	89% 74%	99% 34%	

Note: Specific activities of LCHAD, LCKAT, and SCHAD are expressed in nmol/(min·mg protein) and values are the mean of at least duplicate measurements. Percentage enzyme activity shows the residual enzyme activity as percentage of the reference mean of healthy control fibroblasts cultured at the same temperature (37°C or 40°C). LcFAO-fluxes are shown as percentage of the mean flux in three control cell lines measured in the same experiment.

Abbreviations: Pt, patient; Ref, reference range in nmol/(min·mg protein).

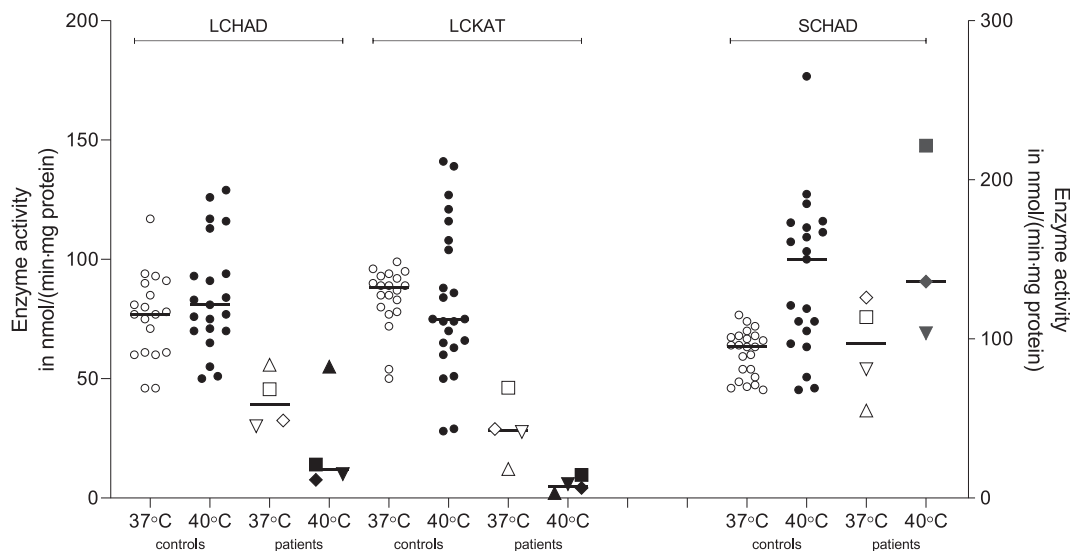


FIGURE 1 LCHAD, LCKAT, and SCHAD specific activities in patient and healthy control fibroblasts cultured at 37° and 40°C. Median (black line) and individual values (white (37°C) and black (40°C) symbols) are shown: Patient #1 = upward triangle, Patient #2 = diamond, Patient #3a = square, Patient #4 = downward triangle, healthy controls: circles. Enzyme activities are expressed as the amount of produced 3-hydroxypalmitoyl-CoA (LCHAD) or myristoyl-CoA (LCKAT) in nanomoles per minute per milligram of cellular protein (nmol/(min·mg protein)). The here displayed control values have been used for determination of the reference values at 37°C and 40°C shown in Table 2. All measurements have been performed at least in duplicate and the mean value of the measurements is shown. Except for the LCHAD activity in Patient #1, all LCHAD and LCKAT activities in patient fibroblasts were markedly reduced at 40°C compared to 37°C. The differences did not reach statistical significance

After diagnosis, preventive dietary measures during febrile illness were initiated, including an MCT-supplemented and carbohydrate-enriched diet. Clinical efficacy of these dietary measures is yet unclear, as the patient is not compliant with her diet and still gains weight.

3.6 | Cardiomyopathy and long-term complications

After confirmation of the diagnosis, additional screening for cardiomyopathy, pigmentary retinopathy, and peripheral neuropathy was performed for most of the patients, if not yet carried out during the diagnostic trajectory. None of the patients had abnormalities on echocardiography. Ophthalmological investigations were performed in Patients #1, #3a, and #3b and revealed no abnormalities in Patients #3a and #3b. Mild retinopathy was noted on slit-lamp examination in Patient #1, but electroretinograms were normal. Peripheral neuropathy in the form of absent reflexes was seen in Patients #2, #3a, and #3b. Patients #1 and #4 had normal reflexes. NCS were performed in Patients #1, #3a, and #4, and showed no abnormalities. EMG was performed in Patients #2, #3a, and #4, showing proximal abnormalities in two (Patients #2 and #3a) and reversible abnormalities in one (Patient #2) (Table 1).

3.7 | Biochemical assays

3.7.1 | LCHAD and LCKAT activities, immunoblot analysis, and lcFAO-fluxes

LCHAD and LCKAT activities were measured in four of the five patients. For Patient #3b, fibroblasts were not available. Detailed results of the biochemical analyses are summarized in Table 2 and Figure 1. LCHAD and LCKAT activities were reduced compared to the reference mean in all four patients (LCHAD: ranging from 30.1 to 55.9 nmol/(min·mg protein), median: 39.0, range ref.: 46–117, LCKAT: ranging from 12.2 to 46.2 nmol/(min·mg protein), median: 28.3, range ref.: 50–99). However, residual enzyme activities were relatively high in comparison to most other patients with MTP deficiency described in literature.^{16–18} Because temperature sensitivity is a known phenomenon in several FAOD, we studied whether culturing the fibroblasts at 37°C and 40°C would affect enzyme activities. For Patient #1, the LCKAT activity decreased at 40°C compared to 37°C, whereas the LCHAD activity remained stable. For Patients #2, #3a, and #4, both the LCHAD and LCKAT activities significantly diminished at 40°C compared to 37°C. Except for the LCHAD activity in Patient #1, all LCHAD and LCKAT activities were markedly reduced compared to the reference values at 40°C (LCHAD: 7.6–55.0 nmol/

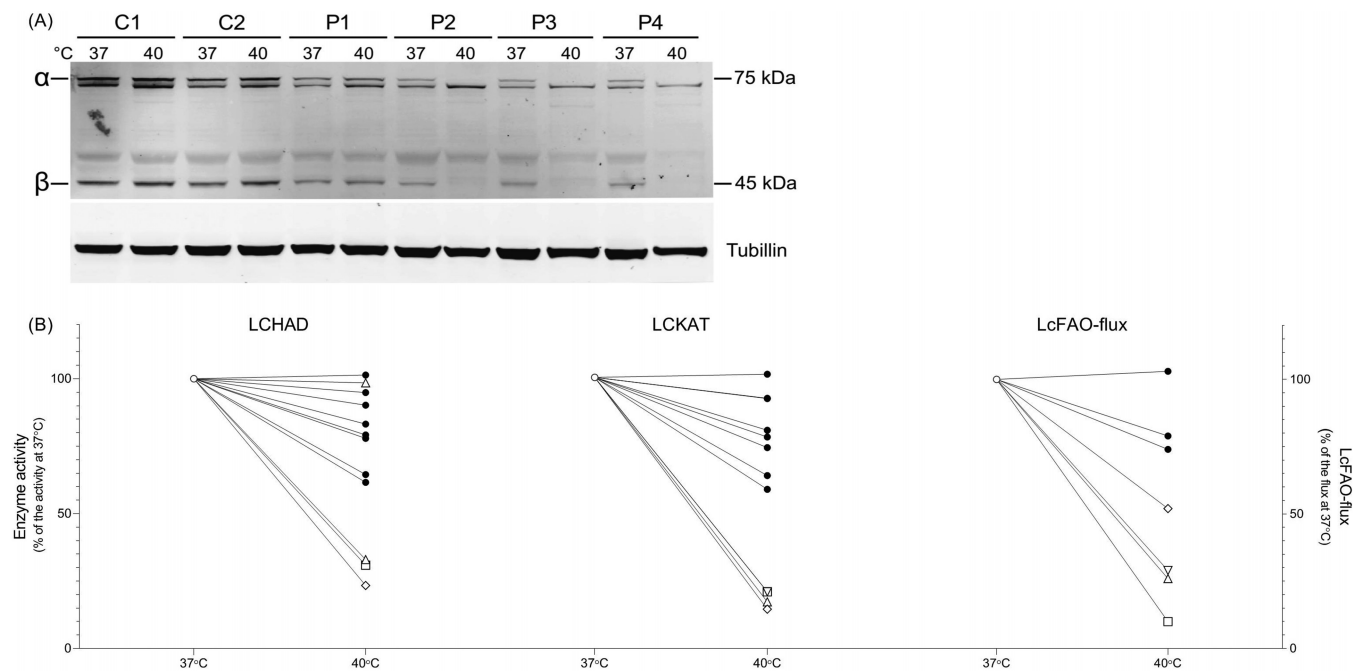


FIGURE 2 (A) Immunoblot analysis with antibodies raised against MTP of fibroblast homogenates from two controls (C1 and C2) and the patients (P1 = Patient #1, P2 = Patient #2, P3 = Patient #3a, P4 = Patient #4) cultured simultaneously at 37°C and 40°C for 2 weeks. The levels of the α - and β -subunit of MTP are reduced in all patients compared to the controls at 37°C and decreased markedly in Patients #2, #3a, and #4 at 40°C, but not in Patient #1 and the controls. (B) Temperature-dependent reduction of LCHAD and LCKAT activities, and LcFAO-fluxes in patient fibroblasts. Enzyme activities and LcFAO-fluxes at 40°C are shown as percentage of the activities in the same cell line at 37°C, which were set at 100%. Patients are shown as white symbols (Patient #1 = upward triangle, Patient #2 = diamond, Patient #3a = square, Patient #4 = downward triangle) and healthy controls as black circles. For each patient, fibroblasts were cultured at 37°C and 40°C simultaneously and subsequently enzyme activity and LcFAO-flux measurements were performed. In each experiment, two or three healthy control cell lines were cultured and measured in parallel under the same conditions

TABLE 3 Palmitate loading test in patient fibroblasts cultured at 37°C (Patients #1–4) and 40°C (Patient #1)

		Palmitate loading test ($\mu\text{mol/L}$)									
	Temp	C2	C4	C5	C6	C8	C10	C12	C14	C16	C16-OH
Ref	37°C	2.3–43.3	0–2	0–2.5	0–1.4	0.1–2.6	0.2–3.1	0–0.8	0–0.4	0–4.3	0–0.07
Pt #1	37°C	18.0	1.7	1.3	1.0	1.5	2.1	1.1 \uparrow	0.4	4.0	0.0
	40°C	50.9	3.2	0.9	2.1	1.8	2.9	1.3 \uparrow	0.7 \uparrow	5.5 \uparrow	0.2 \uparrow
Pt #2	37°C	12.7	0.8	0.9	0.4	0.5	0.6	0.3	0.1	1.1	0.0
	40°C	-	-	-	-	-	-	-	-	-	-
Pt #3a	37°C	10.0	0.7	1.0	0.6	1.3	1.6	0.3	0.2	2.0	0.0
	40°C	-	-	-	-	-	-	-	-	-	-
Pt #4	37°C	17.0	1.2	1.4	1.0	2.3	2.5	0.6	0.2	2.4	0.0
	40°C	-	-	-	-	-	-	-	-	-	-

Abbreviations: Pt, patient; Temp, temperature; Ref, reference range in $\mu\text{mol/L}$.

(min·mg protein), median: 12.0, range ref.: 50–129; LCKAT: 2.1–9.7 nmol/(min·mg protein), median: 5.0, range ref.: 28–141). In contrast to LCHAD and LCKAT

activities, SCHAD activities, measured as control, remained stable at 40°C, when compared to 37°C. Immunoblot analysis showed reduced levels of the α - and

β -subunit of MTP in fibroblast homogenates of all patients compared to controls at 37°C (Figure 2A). The levels decreased markedly in Patients #2, #3a, and #4 at 40°C, but not in Patient #1 and the controls.

The lcFAO-fluxes were normal at 37°C for all patients, but after culturing the cells at 40°C, lcFAO-fluxes were 12%–70% of the mean activity in the control cell lines measured in the same experiment (Table 2).

When comparing the LCHAD activity, LCKAT activity and lcFAO-flux at 40°C as percentage of the activity and lcFAO-flux at 37°C in the same cell line (Figure 2B), we observed a clear reduction in patient fibroblasts but not in healthy control fibroblasts, cultured and measured simultaneously. This demonstrates the significant influence of increasing the culture temperature to clinically relevant fever temperatures on MTP enzyme activities in patient fibroblasts but not in healthy control fibroblasts.

Finally, we performed the palmitate loading test at 37°C for all patients and additionally at 40°C for Patient #1. The palmitate loading test at 40°C in fibroblasts of Patient #1 showed increases of C12-, C14-, C16- and OH-C16-carnitines to 1.3 $\mu\text{mol/L}$ (ref: 0–0.8), 0.7 $\mu\text{mol/L}$ (ref: 0–0.4), 5.5 $\mu\text{mol/L}$ (ref: 0–4.3) and 0.2 $\mu\text{mol/L}$ (ref: 0–0.07), respectively (Table 3). At 37°C, no accumulation of OH-C16-carnitine or C16-carnitine was found in the medium of any of the cell lines loaded with palmitate.

4 | DISCUSSION

To the best of our knowledge, this is the first description of patients with a thermo-sensitive MTP deficiency and corresponding clinical phenotype. We report five patients including two siblings, of whom four had thermo-sensitive MTPD and one LCKATD. All presented during childhood with fever or exercise-induced episodes of muscle weakness and muscle pain. Only after a long diagnostic trajectory, genetic screening identified variants in the *HADHB* gene, leading to the diagnosis of MTP deficiency. Beneficial treatment effects emphasize the need of early recognition of thermo-sensitive MTP deficiency and thermo-sensitivity in inborn errors of metabolism in general. The possible absence of characteristic acylcarnitine abnormalities however poses a risk for a diagnostic delay, and upfront genetic screening may therefore be favorable. With this case series, we aim to enhance early recognition of thermo-sensitive diseases and expand existing knowledge of the phenotypic spectrum of MTP deficiency.

At the standard culture temperature (37°C), enzyme diagnostics in patient skin fibroblasts showed mildly decreased LCHAD and LCKAT activities, normal lcFAO-fluxes and the absence of characteristic acylcarnitine

abnormalities associated with MTP deficiency after palmitate loading. These results were not compatible with the severe symptoms patients developed after provocation. We therefore evaluated the effect of temperature on LCHAD and LCKAT activities in vitro and cultured fibroblasts at 40°C in addition to 37°C. Culturing at 40°C simulates the stress imposed by elevated temperatures. Indeed, LCHAD and/or LCKAT activities decreased significantly, whereas SCHAD activity, which is not part of the MTP-complex, remained stable. In addition, control fibroblasts showed no or only mild reduction of MTP enzyme activities and lcFAO-fluxes at 40°C. These results indicate that the thermo-sensitivity is specific for the patients' MTP-complexes, and thus a result of the *HADHB* variants.

Thermo-sensitivity of enzymes is a well-known phenomenon, reported for many genetic disorders, including other FAOD.^{6–8,19,20} Although the exact underlying mechanism leading to the thermo-sensitivity of the variant MTP-complexes have not been elucidated yet, the general effect of thermal stress on the conformational stability of proteins is the likely cause. This is supported by the marked decrease in levels of the α - and β -subunit of MTP on immunoblot at 40°C compared to 37°C in Patients #2, #3a, and #4. Correct folding of proteins is crucial for their physiological functions, and although many different factors such as temperature, pH, and ligand binding are involved, the amino-acid sequence plays a crucial role.^{21–23} Missense variants could lead to amino-acids changes with a small negative effect on protein folding and ligand binding, affecting the enzyme activities only minimally. These amino-acid changes may however affect the overall conformational stability of the protein complex. In that case, elevated temperatures may lead to relevant protein unfolding and/or aggregation, as shown for MCADD.⁸ This increases the negative effect of the amino-acid change, resulting in thermo-sensitive functional deactivation. In case of functional deactivation due to protein misfolding, unfolding, and/or aggregation at higher temperatures, pharmacological chaperones that bind to the variant MTP-complex and induce conformational stabilization could be a therapeutic option. This functional rescue combined with dietary interventions could then provide a specific treatment for patients with thermo-sensitive MTP deficiency.

The identified variants in *HADHB* included two known pathogenic variants, two previously reported as VUS and one novel variant (see Table S1 for additional information). For the c.248C>G (p.Ala83Gly) (not previously reported) and c.694G>A (p.Ala232Thr) (previously reported as VUS) variants, their pathogenicity is now confirmed by enzyme analysis. The c.397A>G (p-Thr133Ala) variant, located on the N-terminal domain of

HADHB, was previously found in three siblings (two homozygous, one heterozygous) with periodic paralyses and described in an abstract.²⁴ In these siblings, the variant's relationship to the clinical picture is questionable due to the insufficient genetic segregation combined with a phenotype atypical for MTP deficiency. Another variant in *HADHB* at the same position but resulting in a different amino acid replacement (c.397A>C (p.Thr133Pro)) has previously been found in a patient with a muscular phenotype.¹⁶ This study now enzymatically confirms that the c.397A>G (p.Thr133Ala) variant is pathogenic and, like the reported c.397A>C (p.Thr133Pro) variant, causes MTP deficiency with a mainly muscular phenotype.

The variants c.209+1G>C (p.Ala37AspfsX5) and c.1289T>C (p.Phe430Ser) were previously reported in generalized MTP deficiency and isolated LCKAT deficiency, respectively, with fatal neonatal outcome.^{17,18} However, in the patients described here, both variants were combined with the c.397A>G (p.Thr133Ala) variant, resulting in milder thermo-sensitive phenotypes. In homozygous form, this variant causes thermo-sensitive MTP deficiency, as demonstrated in Patient #4. Similarly, in Patient #2 the c.397A>G (p.Thr133Ala) variant is most likely homozygous at the mRNA (and protein) level, because the splice site variant (c.209+1G>C (p.Ala37AspfsX5)) has been shown to result in nonsense mediated mRNA decay and hence only the p.Thr133Ala allele is expressed. Also in Patient #1, the combination of this milder variant with a severe variant causes the milder thermo-sensitive phenotype.

In Patient #1, LCKAT activity diminished after culturing the fibroblasts at 40°C, while LCHAD activity remained stable and normal compared to healthy controls. This resulted in the final diagnosis of LCKATD. LCKATD has only been reported in four patients: three with cardiomyopathy and all with fatal outcome before 13 months of life.^{11,17,25} Here, we describe the first adult LCKATD patient with a relatively mild muscular phenotype, showing that LCKATD has a wider clinical spectrum than described thus far, similar to other lcFAOD.

All five patients presented during childhood with fever- or exercise-induced episodes of muscle weakness or muscle pain, in combination with signs of peripheral neuropathy in some patients. This presentation is comparable with the known neuromuscular phenotype of MTP deficiency.²⁶ Moreover, it is a recognized phenomenon in lcFAOD that (muscle) symptoms can be triggered by exercise or fever due to the increased energy demand. In the here reported patients, however, metabolic decompensations were more severe than expected based on plasma acylcarnitine concentrations and enzyme activities at 37°C. Although the patients' symptoms fit the diagnosis of MTP deficiency, the decrease of MTP enzyme

activities and as a result the decrease of the lcFAO-flux at elevated temperatures are likely to have played a major role in the severe metabolic decompensations.

The long diagnostic trajectory in all patients (range: 4–16 years) may be related to their normal or only slightly aberrant plasma acylcarnitine concentrations both at newborn screening (for Patient #4) and later in life. Hypothetically, fever or exercise may increase acylcarnitine concentrations in patients with thermo-sensitive MTP deficiency. Performing metabolic screening during symptomatic episodes may therefore increase the chance to find abnormalities and reduce the diagnostic delay. However, we found that acylcarnitine concentrations may also be normal or only minimally increased when patients have symptoms, implicating that normal metabolic screening does not rule out the presence of MTP deficiency. This increases the risk of missing the diagnosis. Therefore, genetic screening early in the diagnostic trajectory may be useful.

Preventive dietary interventions, comprising LCT-restriction, MCT-supplementation and/or carbohydrate-enrichment, were advised to all patients, either on a daily basis or only during febrile illness. Beneficial effects were reported for patients who were compliant with the diet. Additionally, glucose infusion prevented episodic symptoms during labor in Patient #1, and during febrile illness in Patient #2, when combined with antipyretic drugs. These positive treatment effects show the importance of early recognition of the disease.

The importance of early recognition of thermo-sensitive disorders not only applies to MTP deficiency, but to inborn errors of metabolism in general. Clinicians should be aware of the phenomenon of protein thermo-sensitivity, and pay attention to the onset of novel symptoms and signs in febrile patients. Early recognition is critical to allow prompt aggressive antipyretic treatment and other disease-dependent preventive measures, in order to reduce disease burden and complications.

5 | CONCLUSION

We report five patients with thermo-sensitive MTP deficiency (four MTPD, one LCKATD) who all had an episodic muscular phenotype, mainly provoked by fever or physical activity. Initial metabolic diagnostics did not lead to the proper diagnosis of MTP deficiency and only after *HADHB* variants were revealed by broad genetic screening, MTP deficiency was suspected. Extensive enzyme studies in fibroblasts at different culture temperatures showed a significant reduction of LCHAD and/or LCKAT activities at 40°C compared to 37°C, compatible

with the patients' phenotype. This case series shows that thermo-sensitive MTP deficiency is a potentially severe and disabling, but at least partly treatable condition. The possible absence of characteristic acylcarnitine abnormalities associated with MTP deficiency however poses a risk of missing patients, and therefore both genetic and enzymatic testing are required for a correct diagnosis. Moreover, the beneficial treatment effects emphasize the need of early recognition of thermo-sensitive MTP deficiencies and thermo-sensitive forms of inborn errors of metabolism in general.

ACKNOWLEDGMENTS

This study was funded by Metakids and PNO zorg. The content of the article has not been influenced by the sponsor. This work was generated within the "United for Metabolic Diseases (UMD)" and the "European Reference Network for Hereditary Metabolic Disorders (MetaBERN)." The authors are grateful for their efforts to improve care for patients with (genetic) metabolic diseases. They also thank Conny Dekker and Heleen te Brinke for their technical assistance with the immunoblot analysis.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

AUTHOR CONTRIBUTIONS

Marit Schwantje, Sacha Ferdinandusse, Sabine A. Fuchs, and Gepke Visser were involved in acquisition of data, interpretation of data and drafting of the manuscript. Sacha Ferdinandusse, Mirjam Doolaard, Merel S. Ebberink, and Jos P. N. Ruiten were involved in the biochemical analysis for all patients. Niklas Darin, Carola Hedberg-Oldfors, Luc Régal, Laura Donker Kaat, Hidde H. Huidekoper, Simon Olpin, Duncan Cole, and Stuart J. Moat were involved in diagnostics and long-term care of patients. All authors were involved in the interpretation of data and reviewing and editing the manuscript. Marit Schwantje and Sacha Ferdinandusse take responsibility for the collection of data, the interpretation and publication. All authors have given final approval of the version to be published.

ETHICS STATEMENT

Ethical approval was not required for this study, as the paper does not report on primary research but on a retrospective chart study. All data were collected as part of routine diagnostics and treatment. All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000. Informed consent for

publication was obtained from all patients and/or their guardians.

DATA AVAILABILITY STATEMENT

Additional data, not described in this article, will be shared upon reasonable request to the corresponding author (if available).

ORCID

Sacha Ferdinandusse  <https://orcid.org/0000-0002-7738-5709>

REFERENCES

- Uchida Y, Izai K, Orii T, Hashimoto T. Novel fatty acid beta-oxidation enzymes in rat liver mitochondria. II. Purification and properties of enoyl-coenzyme A (CoA) hydratase/3-hydroxyacyl-CoA dehydrogenase/3-ketoacyl-CoA thiolase trifunctional protein. *J Biol Chem.* 1992;267(2):1034-1041.
- Carpenter K, Pollitt RJ, Middleton B. Human liver long-chain 3-hydroxyacyl-coenzyme a dehydrogenase is a multifunctional membrane-bound beta-oxidation enzyme of mitochondria. *Biochem Biophys Res Commun.* 1992;183(2):443-448. doi:10.1016/0006-291x(92)90501-b
- Kamijo T, Aoyama T, Miyazaki J, Hashimoto T. Molecular cloning of the cDNAs for the subunits of rat mitochondrial fatty acid beta-oxidation multienzyme complex. Structural and functional relationships to other mitochondrial and peroxisomal beta-oxidation enzymes. *J Biol Chem.* 1993;268(35):26452-26460.
- Knottnerus SJG, Bleeker JC, Wust RCI, et al. Disorders of mitochondrial long-chain fatty acid oxidation and the carnitine shuttle. *Rev Endocr Metab Disord.* 2018;19(1):93-106. doi:10.1007/s11154-018-9448-1
- Bennett MJ, Rinaldo P, Strauss AW. Inborn errors of mitochondrial fatty acid oxidation. *Crit Rev Clin Lab Sci.* 2000;37(1):1-44. doi:10.1080/10408360091174169
- Olpin SE. Pathophysiology of fatty acid oxidation disorders and resultant phenotypic variability. *J Inher Metab Dis.* 2013;36(4):645-658. doi:10.1007/s10545-013-9611-5
- Diekman EF, Ferdinandusse S, van der Pol L, et al. Fatty acid oxidation flux predicts the clinical severity of VLCAD deficiency. *Genet Med.* 2015;17(12):989-994. doi:10.1038/gim.2015.22
- Jank JM, Maier EM, Reibeta DD, et al. The domain-specific and temperature-dependent protein misfolding phenotype of variant medium-chain acyl-CoA dehydrogenase. *PLoS One.* 2014;9(4):e93852. doi:10.1371/journal.pone.0093852
- Purevsuren J, Fukao T, Hasegawa Y, et al. Clinical and molecular aspects of Japanese patients with mitochondrial trifunctional protein deficiency. *Mol Genet Metab.* 2009;98(4):372-377. doi:10.1016/j.yimgme.2009.07.011
- Liu ZR, Dong HL, Ma Y, Wu ZY. Identification and functional characterization of mutations within HADHB associated with mitochondrial trifunctional protein deficiency. *Mitochondrion.* 2019;49:200-205. doi:10.1016/j.mito.2019.09.004
- Schwantje M, Fuchs SA, De Boer L, et al. The genetic, biochemical and clinical spectrum of patients with mitochondrial

- trifunctional protein deficiency identified after introduction of newborn screening in the Netherlands. *J Inherit Metab Dis*. 2022;45(4):804-818. doi:10.1002/jimd.12502.
12. Wanders RJ, Ruiten JP, IJlst L, Waterham HR, Houten SM. The enzymology of mitochondrial fatty acid beta-oxidation and its application to follow-up analysis of positive neonatal screening results. *J Inherit Metab Dis*. 2010;33(5):479-494. doi:10.1007/s10545-010-9104-8
 13. Manning NJ, Olpin SE, Pollitt RJ, Webley J. A comparison of [9,10-3H]palmitic and [9,10-3H]myristic acids for the detection of defects of fatty acid oxidation in intact cultured fibroblasts. *J Inherit Metab Dis*. 1990;13(1):58-68. doi:10.1007/BF01799333
 14. Olpin SE, Manning NJ, Pollitt RJ, Bonham JR, Downing M, Clark S. The use of [9,10-3H]myristate, [9,10-3H]palmitate and [9,10-3H]oleate for the detection and diagnosis of medium and long-chain fatty acid oxidation disorders in intact cultured fibroblasts. *Adv Exp Med Biol*. 1999;466:321-325. doi:10.1007/0-306-46818-2_37
 15. Olpin SE, Manning NJ, Pollitt RJ, Clarke S. Improved detection of long-chain fatty acid oxidation defects in intact cells using [9,10-3H]oleic acid. *J Inherit Metab Dis*. 1997;20(3):415-419. doi:10.1023/a:1005358802096
 16. Spiekerkoetter U, Sun B, Khuchua Z, Bennett MJ, Strauss AW. Molecular and phenotypic heterogeneity in mitochondrial trifunctional protein deficiency due to beta-subunit mutations. *Hum Mutat*. 2003;21(6):598-607. doi:10.1002/humu.10211
 17. Das AM, Illsinger S, Lucke T, et al. Isolated mitochondrial long-chain ketoacyl-CoA thiolase deficiency resulting from mutations in the HADHB gene. *Clin Chem*. 2006;52(3):530-534. doi:10.1373/clinchem.2005.062000
 18. Boutron A, Acquaviva C, Vianey-Saban C, et al. Comprehensive cDNA study and quantitative analysis of mutant HADHA and HADHB transcripts in a French cohort of 52 patients with mitochondrial trifunctional protein deficiency. *Mol Genet Metab*. 2011;103(4):341-348. doi:10.1016/j.yimgme.2011.04.006
 19. Sharma M, Benharouga M, Hu W, Lukacs GL. Conformational and temperature-sensitive stability defects of the delta F508 cystic fibrosis transmembrane conductance regulator in post-endoplasmic reticulum compartments. *J Biol Chem*. 2001;276(12):8942-8950. doi:10.1074/jbc.M009172200
 20. Imamura A, Tamura S, Shimozawa N, et al. Temperature-sensitive mutation in PEX1 moderates the phenotypes of peroxisome deficiency disorders. *Hum Mol Genet*. 1998;7(13):2089-2094. doi:10.1093/hmg/July
 21. Somero GN. Proteins and temperature. *Annu Rev Physiol*. 1995;57:43-68. doi:10.1146/annurev.ph.57.030195.000355
 22. Rehman I, Kerndt CC, Botelho S. Biochemistry, tertiary protein structure. *StatPearls*. StatPearls Publishing; 2022. <https://www.ncbi.nlm.nih.gov/books/NBK470269/>
 23. Nasser I, Mohsen AW, Jelesarov I, Vockley J, Macheroux GS. Thermal unfolding of medium-chain acyl-CoA dehydrogenase and iso(3)valeryl-CoA dehydrogenase: study of the effect of genetic defects on enzyme stability. *Biochim Biophys Acta*. 2004;1690(1):22-32. doi:10.1016/j.bbadis.2004.04.008
 24. Remiche G, Baudin P, Buon C, et al. Trifunctional protein beta subunit (HADHB) mutations associated with periodic paralysis phenotype. *Neuromusc Disorders*. 2017;27(S164):228. doi:10.1016/j.nmd.2017.06.258
 25. Sander J, Sander S, Steuerwald U, et al. Neonatal screening for defects of the mitochondrial trifunctional protein. *Mol Genet Metab*. 2005;85(2):108-114. doi:10.1016/j.yimgme.2005.02.002
 26. Dagher R, Massie R, Gentil BJ. MTP deficiency caused by HADHB mutations: pathophysiology and clinical manifestations. *Mol Genet Metab*. 2021;133(1):1-7. doi:10.1016/j.yimgme.2021.03.010

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

How to cite this article: Schwantje M, Ebberink MS, Doolaard M, et al. Thermo-sensitive mitochondrial trifunctional protein deficiency presenting with episodic myopathy. *J Inherit Metab Dis*. 2022;45(4):819-831. doi:10.1002/jimd.12503