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SCIENTIFIC CORRESPONDENCE

Successful plasmapheresis and immunoglobulin treatment for severe lipid storage myopathy: Doing the right thing for the wrong reason

Lipid myopathies are rare and heterogeneous multi-systemic diseases that may be hereditary or acquired and affect the skeletal muscle.[1] Four types usually demonstrate accumulation of lipids in muscle biopsy specimens that can be visualised using Oil-Red-O (ORO) stain. These comprise (i) Multiple Acyl-CoA Dehydrogenase deficiency (MADD), (ii) Primary Carnitine Deficiency (PCD), (iii) Neutral Lipid Storage Disease with Ichthyosis (NLSDI) and (iv) Neutral Lipid Storage Disease with myopathy (NLSDM).[2] MADD is an autosomal recessive disorder of fatty acid oxidation with early-onset during the neonatal period or late-onset during child- or adulthood.[3] It is caused by mutations in genes encoding electron transfer flavoprotein A, B (*ETFA*, *ETFB)* or electron transfer flavoprotein dehydrogenase (*ETFDH).*[4] Acute metabolic exacerbation may occur, leading to potentially life-threatening but treatable complications. While most early-onset forms of MADD are lethal at an early age, treatment of late-onset forms, including dietary adjustments (low-fat diet), supplementation of riboflavin and the avoidance of certain well-known trigger factors such as prolonged fasting improve symptoms and disease severity.[2] We report the case of a young female patient with *ETFDH*-associated lipid myopathy in whom we observed an unexpected good treatment response to plasmapheresis (PPh) and intravenous immunoglobulin (IVIg) infusion. The patient showed a multifaceted and atypical severe clinical course and rapid, albeit transient, recovery from respiratory failure after repeated PPh treatment. Stable improvement of her muscle strength was only observed after riboflavin and coenzyme Q10 supplementation was initiated.

After normal childhood development and adolescence, the disease manifested itself in the Turkish patient at the age of 23 years. She suffered from headache, dizziness, nausea, and vomiting. At that point, neurological examination yielded normal results, except for a mildly elevated creatine kinase (CK) activity of 237 U/L (N < 167) that was considered nonspecific. At age 27 years, she presented to the department of rheumatology due to subacute-onset of proximal tetraparesis, myalgia, and severe weight loss (of 10 kg in 3 months) as well as amenorrhea and prominent loss of hair (diffuse *Alopecia areata*). CK (1105 U/L, N < 190) and transaminases (ALT 139 U/L, N < 31; AST 221 U/L, N < 35) were markedly elevated. Differential diagnoses included atypical subacute myositis, and a muscle biopsy sample from the right *vastus lateralis* muscle was obtained 21 days after onset of symptoms, showing mild non-specific myopathic changes with increased fibre size variation and sarcoplasmic elongated vacuoles without any signs of specific inflammation (Figure 1A,D; Figure S1). The patient's condition further deteriorated continuously and she lost her ability to walk and developed urinary incontinence. Three months later, she was admitted to the neurological ward and a second muscle biopsy, this time from the *caput mediale* of the *gastrocnemius* muscle, was performed. Histological analysis showed an increased number of large lipid droplets as compared to the first biopsy (Figure 1B,E), and multiple necrotic fibres were present (Figure 1B). Conventional transmission electron microscopy (TEM) and large-scale digitisation *via* scanning transmission electron microscopy (STEM)[5] confirmed a large number and increased size of lipid droplets in the sarcoplasm as well as signs of mitochondrial damage with paracristalline inclusions (Figure 1G–I, K,L). Of note, large-scale EM showed extensive groups of numerous individual lipid droplets in some fibres (Figure 1J). On cryostat sections, these groups resembled 'empty' areas within the fibres (Figure 1B), that are probably residual spaces resulting from washed-out lipid droplets, but difficult to discern due to their similarity with ice crystal artefacts. Furthermore, the Oil-red-O stain (Figure 1E) showing many small and large fat droplets that can be more precisely visualised with the large-scale dataset. In addition, we identified varying degrees of ultrastructural mitochondrial abnormalities in all three biopsy samples such as overt ones with paracristalline inclusions but also swollen mitochondria with rarified cristae and initial stages of crystalline inclusions. Besides the fibre pathology we noticed numerous prominent endomysial capillaries, and several endothelial cells with signs of activation (Figure 1J), which might be part of a compensatory mechanism. These findings would imply an accompanying activation of the immune system as highlighted by MHC upregulation and lymphomonocytic infiltration at the peak of the disease (Figure S1). Investigation of the OXPHOS activities in muscle revealed a severe combined deficiency of all respiratory chain complexes [Complex I 1.0 U/g NCP (N: 15.8–42.8), Complex II/III 4.1 U/g NCP (N6.0–25.0), Complex IV 60.1 U/g NCP (112–351).

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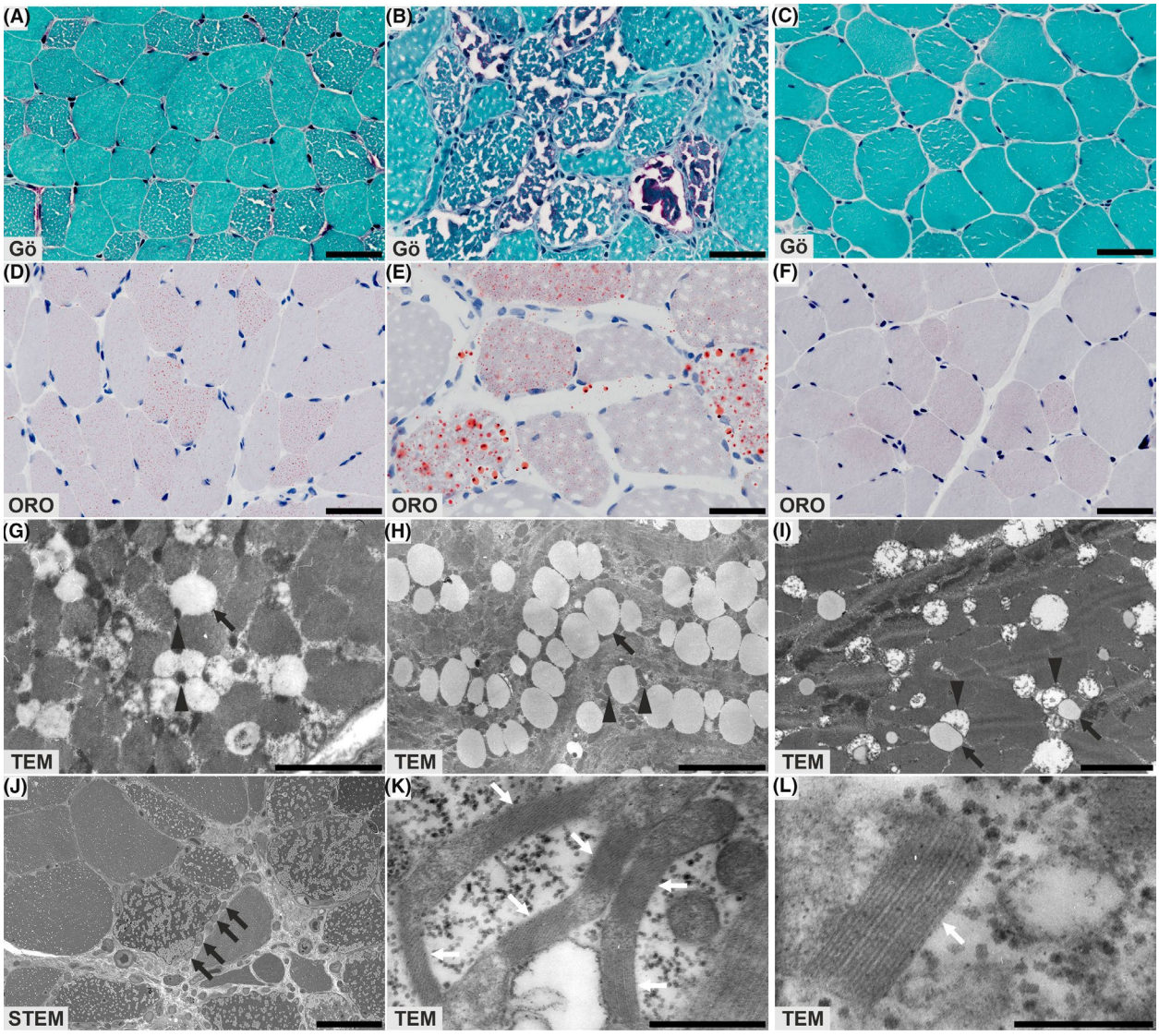

FIGURE 1 Histopathological and electron microscopic features of the three muscle biopsy specimens of our patients. (A, D, G) first, (B, E, H, J–L) second and (C, F, I) third biopsy. Myofibres with sarcoplasmic elongated vacuoles are scattered (A–C, modified Gömöri trichrome stain: Gö). Lipid droplets are prominent in the second biopsy sample while they are almost invisible in the third sample (D–F, Oil Red O stain: ORO). A considerably increased number and size of lipid droplets (arrows) are observed in the second biopsy sample as compared to the first and third one (G–I; transmission electron microscopy: TEM). Most of the lipid droplets are adjacent to mitochondria (arrowheads). (J) Note the number and distribution of lipid droplets on the micro-anatomical level that is particularly well preserved in an ultrathin section that was recorded in its entirety by large-scale digitisation *via* scanning transmission electron microscopy (STEM) as compared to cryostat sections (A–F). Some muscle fibres with numerous lipid droplets were surrounded by prominent capillaries (J; arrows) as compared to less affected fibres; several endothelial cells showed signs of activation and about 10 capillary cross sections per fibre suggesting capillary proliferation. Abnormal mitochondria containing paracristalline inclusions (K, L; TEM). The large-scale dataset for internet browser-based pan-and-zoom examination can be freely accessed via:www.nanotomy.org. Scale bars: A–F, J 50 μm, G, I 2.5 μm, H 5 μm, K 500 nm, L 250 nm

In line with these results, SDH enzyme histochemical stains showed a severely decreased activity resulting in a blue-grey staining of the myofibres (data not shown). Carnitine and acylcarnitine analysis were performed after the third muscle biopsy. The analysis revealed reduced levels of free [10.1 μmol/L, N 20.0–53.0] and total carnitine [25.4 μmol/L, N 30.0–70.9]. Mass-spectroscopy of acylcarnitines showed an increase of medium-chain C8- and C10-, and C16:2-acylcarnitines as well as mildly increased ratios between C8/C2, C8/C12, C14:1/C2 and C14:1/C16 acylcarnitines, which would be indicative of MADD. Urine organic acids analysis was done at the same, but had yielded normal results; in retrospect a not unusual finding in patients with MADD. The patient's medical history did not reveal any exposure to toxins or drugs that could lead to toxic myopathy. The clinical suspicion of atypical necrotizing myopathy with a probable autoimmune background despite the non-specific results of the first biopsy, led to initiation of treatment with high-dose

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steroids (5 × 1000 mg methylprednisolone intravenously), however, without clinical improvement. Due to further deterioration of her condition, she was subsequently treated with intravenous immunoglobulins (IVIg), which markedly improved her clinical condition, and she regained the ability to walk over a period of weeks. Six weeks later, she was readmitted to hospital due to worsening tetraparesis, bulbar syndrome and incipient hypoventilation syndrome. She subsequently required non-invasive ventilation (NIV) and became wheelchair dependent. Repeated echocardiograms did not show any signs of cardiac involvement. At that point, the diagnosis was still unclear with potential differential diagnoses of hereditary myopathy, autoimmune disease or toxic myopathy. Based on the temporary clinical improvement after IVIg treatment, a therapeutic trial of PPh was performed. After seven cycles of PPh, we saw a significant improvement of strength and walking ability as well as normalisation of CK, transaminase levels and respiratory function. Due to this favourable treatment response and under the hypothesis of an antibody-mediated disease, we initiated treatment with rituximab. However, the in-depth analysis of inflammatory features of both skeletal muscle biopsy samples did not reveal any significant sarcolemmal staining of major histocompatibility complexes I and II. We saw a few T-cells, but did not find sarcolemmal C5b-9 complement deposition or a p62-positive granular staining pattern on non-necrotic fibres (Figure S1) – findings that would argue against an immune-mediated pathogenesis.

At discharge, the patient could walk and had overall improved significantly. In the following months we continued both PPh and rituximab treatment while her condition stabilised. As the molecular diagnosis was still unknown, we performed a third muscle biopsy from the *adductor magnus* muscle. Histology showed marked improvement also on the morphological level with only mild myopathic changes and some sarcoplasmic vacuoles, but without overt lipid droplet accumulation on light microscopy (Figure 1C,F). Of note, sarcoplasmic lipid storage compatible with lipid-storage myopathy was only observed in the muscle biopsy specimen taken at the height of her symptoms (second biopsy) by Oil-Red-O staining and on ultrastructural examination. Meanwhile, genetic testing (Whole Exome Sequencing) had revealed a homozygous missense mutation in the gene encoding the Electron Transfer Flavoprotein Dehydrogenase (*ETFDH*, chr4:g.159,624,588 T>C (GRCh37) | NM_004453 c.1130 T>C | p.(L377P)] which had been reported previously as a disease-causing variant for MADD / riboflavin-responsive lipid myopathy (rs387907170). Both parents have Turkish ancestry were heterozygous for the mutation. The same mutation has previously been reported in a case series of 4 patients with MADD of Turkish/Kurdish descent.[6] Immunomodulatory treatment was stopped, and the patient was put on riboflavin and coenzyme Q_{10} supplementation. Her condition has stabilised since, with presently only mild residual tetraparesis.

We present the complicated course of a late-onset riboflavin-responsive MADD (RR-MADD) in a young woman, manifesting with acute metabolic decompensation, caused by a rare mutation in *ETFDH*. To the best of our knowledge, this is the first article reporting an impressive, albeit temporary response to immunomodulatory treatment (IVIg and PPh) in the context of RR-MADD. Our patient experienced a remarkable clinical recovery with regard to muscle strength and pulmonary function after PPh, thus setting clinicians on the wrong track by strengthening the hypothesis of an immune-mediated disease/myositis, despite the fact that muscle biopsy results had not substantiated this suspicion. A similar phenomenon had already been reported where glucocorticoid treatment had a positive short-term effect on muscular strength and CK levels in patients with *ETFDH*-associated myopathy, likewise leading to the misdiagnosis of myositis.[7,8] Patients, however, did not show a clinical significant response to glucocorticoid treatment at the long term.[4] There were no dietary changes nor other medications given at the time of PPh treatment in our patient. Morphological analysis of a muscle biopsy specimen could thus be of much help in these cases. In our case though, the first biopsy was not specific, showing only very mild lipid droplet accumulation and no significant accumulations of mitochondria on light microscopy. The second biopsy specimen was characterised by severe myofibre necrosis, albeit without the hallmarks of immune-mediated necrotising myopathy.[9] Eventually, ultrastructural studies were key in confirming the lipid myopathy and mitochondrial abnormalities. To the best of our knowledge, the latter, and the findings on large-scale digitisation of scanning transmission electron microscopy have not been described in the context of *ETFDH* associated myopathy.[5] Some authors have reported abnormal mitochondria by EM without specifying their findings [10,11], light microscopic hints for mitochondrial dysfunction such as the presence of "ragged red fibres" or of COX-negative fibres.[12,13] In contrast, light microscopic features of the disease are well established. [11,14] The, in retrospect unexpected, good treatment response to PPh, especially in the most critical phase of her disease, might be related to the removal of toxic intermediates [2,15] as previously performed through dialysis in severe cases of the disease.[12] Normalisation of insulin and metabolic changes towards an anabolic state in order to decrease plasma levels of fatty acids might be major factors to stabilise acute exacerbations in MADD and indicate that MADD is more complex than previously thought. Therefore, further research into the pathophysiology of the disease is needed, and guidelines for treatment of acute exacerbations should be refined, as this might be the manifestation of an often treatable but potentially lethal form of late-onset disease.

KEYWORDS

multiple acyl coenzyme A dehydrogenase deficiency, mitochondrial myopathy, lipid storage myopathy, electron microscopy

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CONFLICT OF INTEREST

The authors in this article have no conflict of interest to disclose. The Editors of Neuropathology and Applied Neurobiology are committed

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to peer-review integrity and upholding the highest standards of review. As such, this article was peer-reviewed by independent, anonymous expert referees and the authors (including WS) had no role in either the editorial decision or the handling of the paper.

ETHICS STATEMENT

The study was approved by the institutional ethics review board of the Charité (EA2/107/14) and was undertaken in accordance with the declaration of Helsinki.

PEER REVIEW

The peer review history for this article is available at https://publons.com/publon/10.1111/nan.12731.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy and ethical restrictions.

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