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Neuromuscular Disorders 29 (2019) 448–455

www.elsevier.com/locate/nmd

A novel case of *MSTO1* gene related congenital muscular dystrophy with progressive neurological involvement

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Received 21 December 2018; received in revised form 11 March 2019; accepted 21 March 2019

Abstract

Recessive mutations in the *MSTO1* gene, encoding for a mitochondrial distribution and morphology regulator, have been recently described in a very limited number of patients with multisystem involvement, mostly characterized by myopathy or dystrophy, cerebellar ataxia, pigmentary retinopathy and raised creatine kinase levels. Here we report an additional patient with recessive *MSTO1*-related muscular dystrophy (*MSTO1*-RD), and clinical and radiological evidence of progressive cerebellar involvement. Whole-exome sequencing identified two novel *MSTO1* missense variants, c.766C > T (p. (Arg256Trp) and c.1435C > T (p. (Pro479Ser), predicted as damaging by *in silico* tools. We also report a distinct pattern of selective involvement on muscle MRI in *MSTO1*-RD. This case confirms a consistent *MSTO1*-related neuromuscular phenotype and in addition suggests a progressive neurological component at least in some patients, in keeping with the mitochondrial role of the defective protein.

Keywords: Muscular dystrophy; Ataxia; *MSTO1*; Cerebellar atrophy; Progressive cerebellar involvement.

1. Introduction

Congenital muscular dystrophies (CMDs) and congenital myopathies (CMs) are individually rare and highly heterogeneous conditions, characterized by congenital/early onset muscle weakness and characteristic muscle biopsy findings compatible with a dystrophic or myopathic process, respectively. The clinical complexity of CMDs and CMs is mirrored by their wide genetic heterogeneity. With advances in novel diagnostic genetic technologies such as the introduction of next generation sequencing (NGS), the number of genes and disease-causing variants associated with CMDs and CMs has rapidly increased in recent years [1]. NGS and in particular whole exome (WES) or whole genome sequencing (WGS) are also facilitating diagnosis in patients with less distinct phenotypes.

Recessive mutations in the *MSTO1* gene, located at 1q22 and encoding for an evolutionarily conserved, ubiquitously expressed cytoplasmic protein with a key role in modulating

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Joint contribution.

https://doi.org/10.1016/j.nmd.2019.03.011
0960-8966/

mitochondrial dynamics, have been recently reported in 5 patients from four unrelated families [2,4]. The phenotype in these individuals was characterized by growth and motor delay, muscle weakness with raised creatine kinase (CK) levels, cerebellar hypoplasia and early-onset ataxia, and a pigmentary retinopathy. Muscle histopathology showed dystrophic features in 2 out of 3 patients, and milder myopathic changes in the remaining case [4].

Here, we report an additional patient with early-onset *MSTO1*-related muscular dystrophy, presenting with global developmental delay, proximal weakness, scoliosis, clinical and radiological evidence of cerebellar involvement and mild intellectual disability, further expanding the clinico-pathological spectrum of this recently described neuromuscular disorder.

2. Case report

2.1. Clinical history

The patient, a 13-year-old boy of non-consanguineous Caucasian parents, was born at 42^{+2} weeks gestation by normal vaginal delivery following an uneventful pregnancy. He was floppy at birth and required facial oxygen due to the umbilical cord being tightly wrapped around his neck. During the early postnatal period, he showed no feeding and breathing problems, had normal antigravity movements and no gross hypotonia. He sat at 11 months of age and started walking at 2 years of age. Scoliosis was first noted at around 2 years of age. Speech development was slower compared to his peers, and he had persistent articulation problems for which he received speech language therapy. Family history was negative for neurological or neuromuscular disease.

The patient was first seen in a specialized neuromuscular clinic at age 5 years. He was ambulant, but unable to run or climb up stairs. He showed a mild thoracolumbar scoliosis convex to the left. He had no symptoms or signs of bulbar or respiratory involvement. From the age of 8 years he started to show slow deterioration due to a combination of progressive muscle weakness and cerebellar involvement, becoming increasingly reliant on a wheelchair for longer distances. Additional neurological signs including dysarthria and intention tremor became obvious from the age of 10 years, and he subsequently also developed progressive upper motor neuron signs including brisk reflexes, sustained clonus and upgoing plantars. At the age of 12 years and 7 months, he was able to walk short distances indoors only, had frequent falls and difficulties getting up from the floor independently. He showed predominant proximal hip girdle weakness (hip flexors, abductors and adductors MRC 3; hip extension MRC 3-) with relatively better-preserved strength in the shoulder girdle (shoulder abductors MRC4, shoulder flexor and extensors MRC3). His functional difficulties were disproportionate to the degree of muscle weakness on formal MRC grading. He showed progressive tendon Achilles (TA) tightness for which ankle splints were provided. There were no other contractures. The scoliosis remained stable under conservative management with a spinal brace and regular orthopedic surgical follow-up from 8 years of age. He frequently complained of muscle pain, but had no episodes of overt rhabdomyolysis. Speech became increasingly dysarthric over the years. Over time, mild to moderate behavioral and learning difficulties became more obvious, but there was no clear evidence of neuropsychological regression. He also had recurrent problems with bowel and bladder incontinence for which no underlying cause could be identified.

Fig. 1. Patient with *MSTO1*-related muscular dystrophy at 13 years of age. Note the lack of gross facial weakness, ptosis or dysmorphisms.

On examination at age 13 years 7 months (Fig. 1), weight was 43.94 kg (>25th centile) and height was 145.70 cm (>2nd centile), respectively. BMI was 20.7 kg. The patient showed no gross facial weakness and no ptosis. Range of extraocular movements was normal. He was able to stand on his toes, heels and on one leg but with difficulties only. He was unable to jump or hop. He got up from floor with a positive Gowers' sign. He showed axial and proximal weakness, with mild asymmetry and more prominent in the lower limbs, with hip extensors on the left being the weakest muscles at MRC 2. Other MRC grades were as follows: neck and trunk flexors 3 and 3-, neck and trunk extensors 5 and 3+, shoulder flexors

and extensors 3 and 3-, shoulder abductors and adductors 3+ and 3, elbow flexors 4 and extensors 3 on the right and 3+ on the left, hip flexors 3 and extensors 3- on the right and 2 on the left, hip abductors and adductors 3-on the right and 3 on the left, knee flexors 3+ on the right and MRC4 on the left, knee extensors 5, ankle plantar flexors 4 on the right and 5 on the left, ankle dorsiflexors 5 on the right and 4 on the left. He had mild TA contractures of approximately 10–15° bilaterally, with good range of movements in other joints, and some mild laxity in hands and fingers. He showed a mild thoracic curve convex to the left, stable from previous assessment, also in keeping with a stable Cobb angle of around 44° on spinal X-rays.

He had marked dysarthria and a slight intention tremor, but no past pointing was noted. Deep tendon reflexes were brisk in both upper and lower limbs with extensor plantar responses and clonus bilaterally.

2.2. Neuropsychology assessment

Formal neuropsychology assessment at the age of 13 years and 8 months included an assessment of intellectual functioning (WISC-V, Wechsler Intelligence Scales for Children, Fifth UK Edition), and measures of language, verbal episodic memory, attention, visuomotor skills and review of reported social communication difficulties. His overall intellectual ability was low, within the bottom 10% of the normal population, but just above the level of an intellectual disability (FSIQ < 70). His clearest deficits were in the language domain, with his verbal intellectual functioning score, performance on an expressive language task and an auditory verbal working memory test all in the impaired range. Sustained attention by contrast was good, consistent with behavioural observations. Although verbal learning was hampered by poor working memory, he did not show amnesic difficulties. His fine motor abilities and shape copying were poor but there were no visuospatial matching problems. Parental and school reports (indicating problems with social communication, behavior and making and maintaining friendships) were consistent with him having difficulties on the Autistic Spectrum and he scored above cut-off on the Social Communication Questionnaire. He attended a special educational support unit within a mainstream secondary school.

2.3. Investigations

Baseline blood investigations including full blood count, liver function tests, bone and renal profile, were normal. Lactate levels were 0.7 (normal range 0.7–2.1 mmol/L). Plasma CK levels ranged between 909 and 1614 IU/L. Cardiac assessments including cardiac ultrasound were normal at 6 and 13 years. Respiratory assessments including forced vital capacity and sleep studies have been normal. He had normal hearing assessments, and his most recent ophthalmological examination at age 13 years showed no abnormalities, in particular no evidence of retinitis pigmentosa.

EMG and nerve conductions studies at 6 years of age were normal.

Brain MRI at age 5 years showed cerebellar atrophy (Fig. 2A); when repeated at 12 years of age, there was mild progression of cerebellar atrophy and additional supratentorial sulcal prominence suggestive of volume loss (Fig. 2B).

Muscle MRI obtained at 13 years of age (Fig. 3, F–J) showed marked involvement of the glutei within the pelvis (F). There was diffuse involvement within the thigh (G and H) with relative sparing of the gracilis compared to the sartorius, and the adductor longus compared to the adductor magnus. In the lower leg, the peroneal group and the gastrocnemii were the most severely affected muscle group, with variable involvement of other anterior compartment muscles, and relative sparing of the soleus.

We compared these findings to recently obtained muscle MRI findings in another previously published *MSTO1*-mutated patient (patient N.3, Table 1) [4]. The muscle MRI in this additional patient revealed a recognizable pattern of selectivity, characterized by consistent involvement of sartorius, adductor magnus and the peroneal group, with relative sparing of gracilis, adductor longus and soleus, and variable involvement of other muscle groups.

A muscle biopsy from the vastus lateralis performed at 6 years of age showed marked variability in fibre size across fascicles, with a dual fibre population of rounded and polygonal larger fibres surrounded by small fibres. There was striking fatty infiltration in between and focally within fascicles, with patchy fibrosis, and rare necrotic/regenerating fibres (Fig. 4A and C). Few fibres showed empty, mostly subsarcolemmal non-rimmed vacuoles without reinforcement of sarcolemmal proteins (Fig. 4B). There was overall slow fibre predominance, and many larger fibres showed prominent central pallor with reduced mitochondrial staining (Fig. 4D). There was no histochemical/immunohistochemical evidence of mitochondrial Complex I/Complex IV deficiency. Several fetal myosin-positive fibres of all sizes and intensities were present (Fig. 4E). Overall, the picture was in keeping with a chronic, moderately severe muscular dystrophy. Ultrastructural examination showed mild non-specific mitochondrial alterations. Initially, testing with a comprehensive immunopanel of dystrophy-associated proteins showed no convincing abnormalities. Retrospective immunolabelling was performed following genetic testing utilizing a rabbit polyclonal *MSTO1* antibody (PA5-21641, Thermofisher) with the epitope mapping between amino acids 34–347 on this patient and patient B from Nasca et al. [4]. The analysis showed profound reduction of *MSTO1* labeling in biopsies of both patients with *MSTO1* variants (Fig. 4G and H). Respiratory chain enzyme studies were normal.

2.4. Genetic analysis

Pathogenic variants in the *DYSF*, *LMNA* *FKRP* and *ANO5* genes were excluded by Sanger sequencing. Following appropriate parental consent, DNA of the patient was included

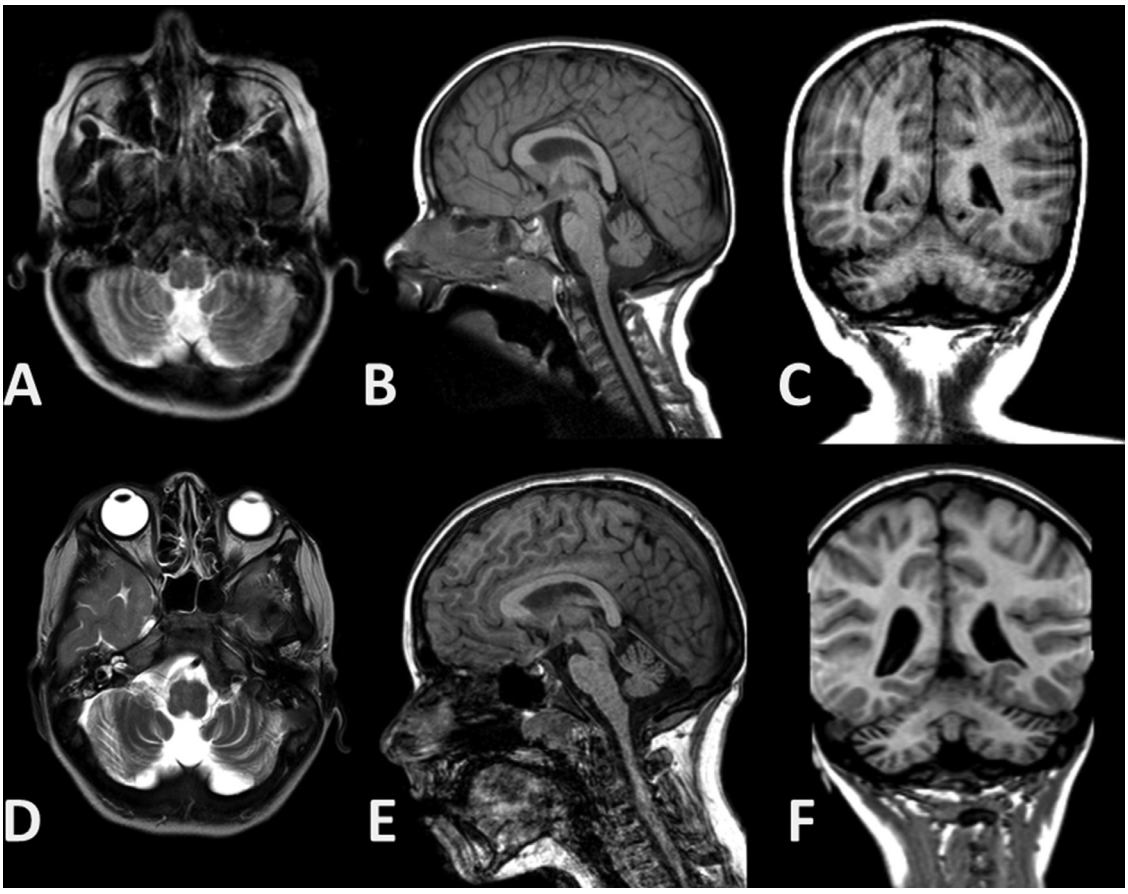

Fig. 2. Brain MRI findings in *MSTO1*-RD. Brain MRI images obtained at 5 (A–C) and 12 (D–F) years of age, axial (A,D), sagittal (B,E) and coronal (C,F) sections. There is cerebellar atrophy with evidence of mild progression between the two scans.

into the BBMRI-LPC project. WES analysis was carried out in the National Center for Genomic Analysis (CNAG), Barcelona, Spain. Data analysis was performed using the RD-Connect Genome–Phenome Analysis Platform. To identify disease-causing variants we applied several filters such as variants' frequency in Exome aggregation consortium (ExAC) and Genome aggregation database (http://gnomad.broadinstitute.org), and assessed the pathogenicity of variants identified by *in silico* prediction programs (Polyphen2 (http://genetics.bwh.harvard.edu/pph2/), SIFT (http://sift.jcvi.org) and Mutation Taster (http://www.mutationtaster.org). Variants predicted to be pathogenic by at least two *in silico* tools were selected.

The analysis revealed two missense variants in the *MSTO1* gene. The first variant c.766C>T, p. (Arg256Trp), affecting a conserved residue in the tubulin domain of the protein, is reported in the gnomAD dataset with an allelic frequency of 0.00003, while the second, c.1435C>T p. (Pro479Ser), is novel. Both variants were predicted to be damaging by all *in silico* tools (SIFT, Polyphen2 and Mutation taster). Sanger sequencing validation confirmed the presence of both variants in the patient and showed that mother was heterozygous for one *MSTO1* variant, suggesting that the variants could be *in trans* in the patient. DNA of the father was not available for analysis.

3. Discussion

In this study, we report the sixth patient affected by a neuromuscular condition caused by recessive variants in the *MSTO1* gene. We identified 2 novel *MSTO1* variants and have added to the phenotypical spectrum of this recently described clinical entity by providing details of the associated neurological and neuroradiological phenotype. In view of its considerable clinical heterogeneity, we suggest referring to this condition as *MSTO1*-related muscular dystrophy (or, for brevity, *MSTO1*-RD).

Including the current patient, most recessive *MSTO1*-RD patients show onset during the first year of life with developmental delay; one patient presented in the neonatal period with congenital arthrogryposis. Growth impairment, axial and proximal weakness, scoliosis, gait disturbances, dysmetria and tremor were the most common features (Table 1). Speech articulation problems suggestive of cerebellar dysarthria and mild cognitive impairment as seen in our patient have been previously reported only in a few cases. Pigmentary retinopathy was detected in 2 previous patients at age 16 and 13 years, respectively, but was not observed in our case at the same age. However, we cannot exclude a later occurrence of this complication and thus recommend regular ophthalmic reviews for all *MSTO1*-RD patients. Dysmorphic

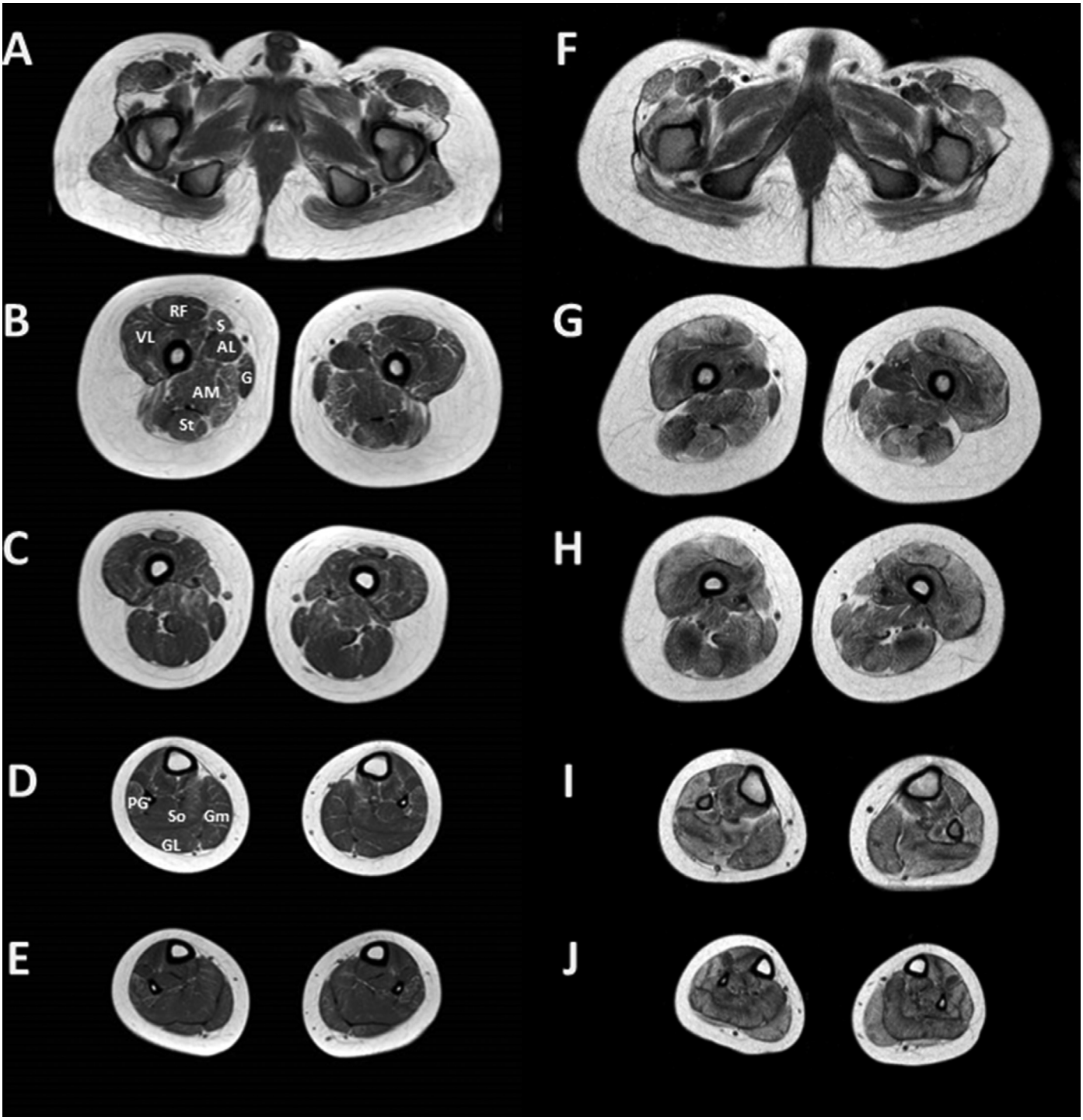

Fig. 3. Muscle MRI findings in *MSTO1*-RD. Muscle MRI images of the lower limb, T1-weighted images, transverse sections, from the patient reported in this paper obtained at 13 years of age (A–E), and from a previously reported male patient obtained at 7 years of age. Both patients show marked involvement of the glutei within the pelvis (A,F), with an overall more proximal pattern of involvement in the more mildly affected patient. Within the thigh (B-C;G-H), pattern of involvement was diffuse, with relative sparing of gracilis (G) compared to sartorius (S), and adductor longus (AL) compared to adductor magnus (AM) in both patients. Quadriceps (in particular rectus femoris, RF) and hamstring muscles were more variably affected. Within the lower leg (D-E;I-J), the peroneal group (PG) was the most severely affected muscle group in both patients. In the more severely affected patient (on the right), there was additional involvement of other anterior compartment components and the gastrocnemii, with relative sparing of the soleus. VL = vastus lateralis; So = soleus; Gm = gastrocnemius medialis, Gl = gastrocnemius lateralis.

Table 1
Phenotypic and genetic characteristics of current and reported patients with recessive *MSTO1*-RD.

Reference	Nasca et al., 2017	Iwama et al., 2018			Current patient
Pt N.	1, 2	3	4	5	6
Age (year)/ sex	17/F 13/F	7/M	13/F	3/F	15/M
Variants	c.1033 C>T, p.(Arg345Cys) c.1128 C>A, p.(Phe376Lys)	c.971 C>T, p.(Thr324Ile) c.966 +1 G>A	c.836 G>A, p.(Arg279His) c.1099–1 G>A, p.(Val367Trpfs*2)	c.836 G>A, p.(Arg279His) c.1099–1 G>A, p.(Val367Trpfs*2)	c.766C > T, p. (Arg256Trp) c.1435C > T p. (Pro479Ser)
Onset	8 months	1 years	5 months	birth	2 years
Presenting symtoms	growth and motor delay	motor and speech delay	motor delay	hypotonia, arthrogryposis	motor delay
Growth impairment	+	NA	+	+	+
Muscle weakness	NA	P>D, L>U	–	–	A, P>D, L>U
Cerebellar findings	ataxia, fine tremor, dysmetry, dysdiadochokinesia	ataxia, fine tremor, dysmetry, dysdiadochokinesia, dysarthria	dysmetry, tremor, adiadochokinesia, walking disturbance	dysmetry, tremor, adiadochokinesia, walking disturbance	ataxia, fine tremor, dysarthria
Cognitive status	N	N	delayed	delayed	delayed
Thorax/spine	PE, severe scoliosis	N	scoliosis	N	scoliosis
Ophthalmological findings	RP	N	RP, esotropia, hypermetropia	RP?	N
CK (U/L)	1200	5420	430	916	1614
EMG	myopathic	–	–	–	N
Muscle biopsy	myopathy- dystrophic	dystrophic	–	–	dystrophic
Brain MRI	Global cerebellar hypotrophy, enlarged cisterna magna	Hypoplasia cerebellar vermis and hemispheres	Atrophy of cerebellar vermis and hemispheres	Atrophy of cerebellar vermis and hemispheres, pons and tegmental area	Cerebellar atrophy, mild under-opercularisation of the left Sylvian fissure
Additional features	triangular face, sunken eyes, thick hair	TA tightness	microcephaly, triangular face, sunken eyes, thick hair	microcephaly, triangular face, sunken eyes	upper motor neuron signs

F: female, M: male, SD: standard deviation, A: axial, P: proximal, D: distal, L: lower limbs, UL: upper limbs, EMG: electromyography, MRI: magnetic resonance imaging, - not performed, PE: pectus excavatum, RP: retinitis pigmentosa, NA: not available + present, N: normal.

features (triangular face, sunken eyes) and thick hair were reported in 3 previous cases but those were not prominent in our patient.

Cerebellar hypotrophy was detected in all patients with recessive *MSTO1*-RD, with onset in early childhood [2,4]; in one previous case, short-term longitudinal neuroradiological features did not identify any progression. However, the mildly progressive cerebellar atrophy, the upper motor neuron signs and the substantial clinical progression over time, suggest a more complex and progressive neurological phenotype in our case (Fig. 2). CK levels were elevated but highly variable in all recessive *MSTO1*-RD (from 430 up to 5420 U/l).

Muscle MRI features of *MSTO1*-RD have not been reported to date. Here we report a pattern of consistent selectivity also in comparison with recently obtained muscle MRI findings from a previously published patient (Figs. 3 and 4). Also considering a degree of variability between patients, review of further cases is needed to further clarify this pattern.

Muscle biopsy findings in this and previous *MSTO1*-RD patients have been reported as myopathic or dystrophic, suggesting a spectrum of pathological severity. Interestingly, we observed marked reduction of *MSTO1* labeling in biopsies of both *MSTO1*-RD patients (Fig. 4G and H). However we also observed a similar reduction in a patient with *MICU1* gene related myopathy (Fig. 4I), compared to unaffected control (Fig. 4F) and two disease controls (Fig. 4J and K). This suggests that while MSTO1 immunoanalysis could help direct molecular testing and/or interpretation of variants within the appropriate clinical context, it is important to bear in mind possible secondary changes in non-*MSTO1*-RD. Further testing of this antibody in a variety of muscular dystrophies will be necessary to ascertain the prevalence of secondary abnormalities.

MSTO1 (or Misato 1) is a soluble protein, predominantly localized in the cytoplasm. The function of Misato1 is not yet fully understood. Misato1 appears to interact with the outer mitochondrial membrane during fusion [3,4] and to be required for mitochondrial fusion and mitochondrial network formation [5,6]. Recent data show a critical role of Misato1 in modulating mitochondrial dynamics by regulating mitochondrial morphology and distribution [4]. Of note, pathogenic variants in genes involved in mitochondrial fusion and fission have been associated with diverse genetic disorders with predominant neurological phenotypes suggesting that mitochondrial fission proteins are essential for cerebellar development [7]. For example, the mitochondrial fission protein Drp1 regulates mitochondrial transport and dendritic arborisation in cerebellar Purkinje cells, and is required for cerebellar development [7]. Pathogenic variants in the *DNM1L* gene, encoding for DrP1, cause a lethal form of encephalopathy with defective mitochondrial and peroxisomal fission, characterized by features in part overlapping what observed in *MSTO1*-RD, such as in particular the predominant

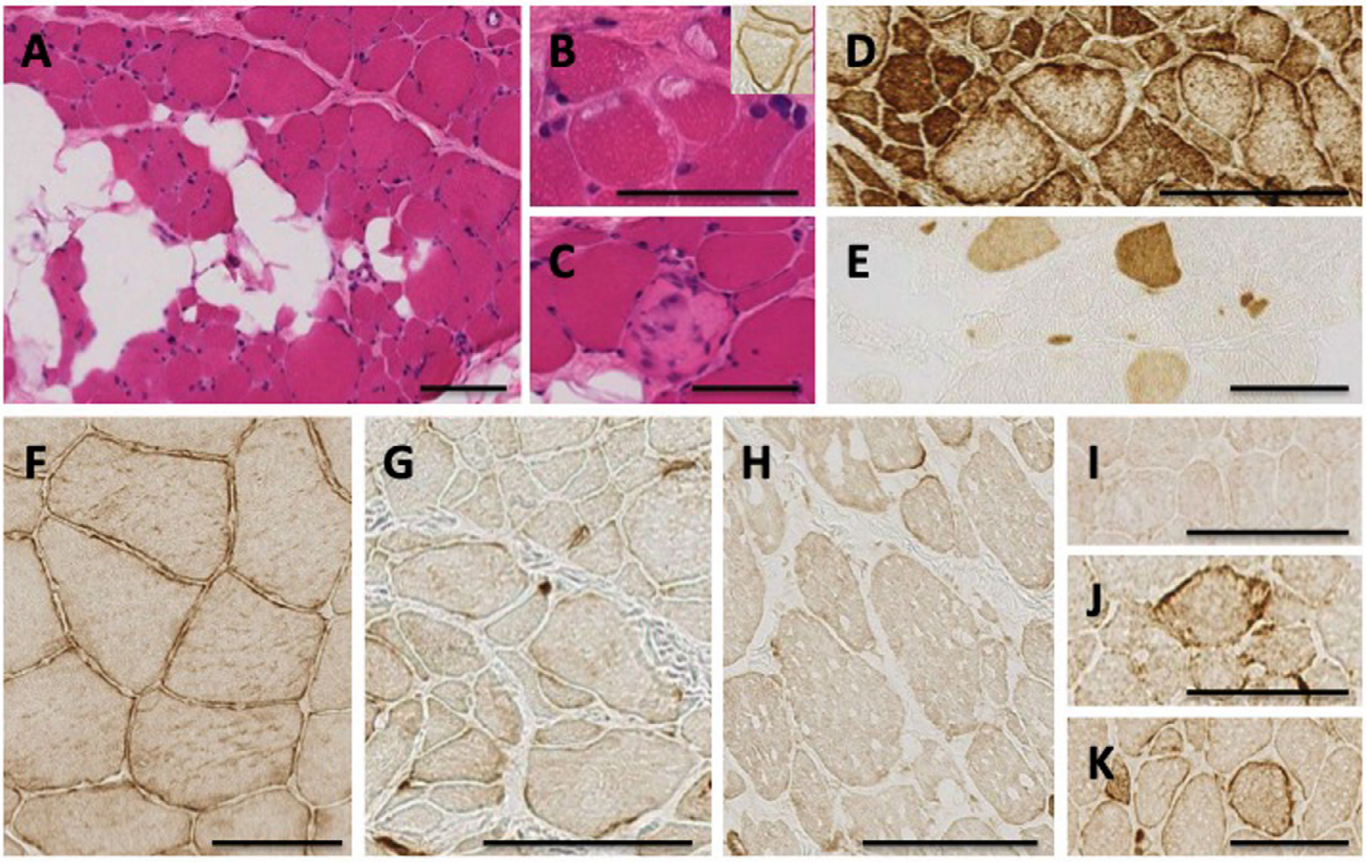

Fig. 4. Histopathological findings. Vastus lateralis biopsy performed at 6 years of age. Haematoxylin and eosin stained sections show marked variability in fibre size across fascicles, with a dual fibre population of rounded and polygonal larger fibres surrounded by small fibres. There is striking fatty infiltration in between and focally within fascicles, with patchy fibrosis, and rare necrotic/regenerating fibres (A, C). Few fibres show empty, mostly subsarcolemmal non-rimmed vacuoles (B) without reinforcement of sarcolemmal proteins (B, inset). Many larger fibres show prominent central pallor with reduced mitochondrial staining in a section immunolabeled with an antibody to mitochondrial complex IV sub-unit MTSO1 (D). Several fetal myosin-positive fibres of all sizes and intensities are present (Figure Z; E). *MSTO1* immunohistochemistry shows a discernible mitochondrial staining pattern in an unaffected control (F), with profound reduction of labeling in this patient (G), as well as the biopsy of the patient B reported in Nasca et al. [4] (H) and a case of *MICU1* gene related myopathy (I). Labeling is retained and mirrors the abnormal mitochondrial distribution pattern in a case of *CHKB* gene related muscular dystrophy (J) and *TK2*-myopathy (K). Scale bar (A–K): 100 μm.

neurological and ophthalmic involvement [8]. Analysis of *DNM1L* mutated patient fibroblasts showed elongated, tangled mitochondria, with tubular structures in particular around the nuclei [8]. While the multisystem features of *MSTO1*-RD with muscular, cerebellar, ophthalmic and skeletal involvement is similar to what seen in other mitochondrial cytopathies, the normal lactate levels and respiratory chain enzyme values are not typical of primary mitochondrial OXPHOS diseases. In contrast to primary mitochondrial cytopathies, *MSTO1*-RD patients also show raised CK values, often markedly elevated in the region of 10–20x the normal values, and muscle imaging findings (Fig. 3), in keeping with a diagnosis of a muscular dystrophy. Secondary mitochondrial dysfunction is not unique to *MSTO1* gene related muscular dystrophies. For example, loss-of-function mutations in *MICU1* cause a brain and muscle disorder linked to primary alterations in mitochondrial calcium signaling [10]. Interestingly, a recent paper showed that the mitochondrial dysfunction in the mdx mouse model of DMD compromises the repair of injured myofibers, suggesting further role of mitochondria in membrane resealing and thus in muscle pathology in dystrophinopathies [9]. Further studies on *MSTO1*-RD will be necessary to fully clarify if altered mitochondrial dynamics contribute to this specific pathological process.

Notably, Gal et al., [3] reported on a heterozygous variant in the *MSTO1* gene segregating in affected members of a dominant family, presenting with an adult-onset myopathy, distal involvement, hypoacusis, endocrine dysfunctions, psychiatric symptoms and normal CK. This phenotype appears different from what observed in recessive *MSTO1*-RD, suggesting that, if confirmed to be pathogenic, dominant *MSTO1* gene variants could associate with a different clinical presentation.

In conclusion, our report further expands the phenotypic and genetic knowledge concerning *MSTO1*-RD, in particular by reporting 2 novel pathogenic *MSTO1* variants and by

describing progressive cerebellar and upper motor neuron signs. In addition, for the first time we report muscle MRI features of this rare condition in 2 patients. Investigation of further patients will be important not only to further inform phenotype-genotype correlations, but also to provide a better understanding of the function of the protein and underlying disease mechanisms. In view of our findings, we strongly recommend investigating *MSTO1* gene variants in patients presenting with early onset myopathies/muscular dystrophies with raised CK, in particular in cases with additional cerebellar involvement.

Acknowledgements

Our patient and the parents are gratefully acknowledged. We would like to acknowledge Dr Lucy Feng and Darren Chambers for their technical assistance.

The research leading to these results has received funding (held by FM) from: the European Commission Seventh Framework Programme (FP7) grants Biobanking and Biomolecular Research Infrastructure - Large Prospective Cohorts (BBMRI-LPC) (GA no. 313010), "Integrated European –omics research project for diagnosis and therapy in rare neuromuscular and neurodegenerative diseases (NEUROMICS)" (agreement no 2012-305121), the European Union's Horizon 2020 research and innovation programme under grant agreement no 779257 "Solving the unsolved Rare Diseases (Solve-RD)", the Muscular Dystrophy Association under grant agreement MDA577346 "Novel CMD and CMY genes: Discovery and functional analysis." DM was supported by "The Science and Technical Research Council of Turkey, Scientific Human Resources Development" Grant (TUBITAK-BIDEB). This research was also supported by the NIHR Biomedical Research Centre at GOSH. The views expressed in this article do not necessarily represent the views of NIHR or NHSE, but of the authors.

Supplementary material

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.nmd.2019.03.011.

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