Didem Ardicli[1*], Anna Sarkozy[1*], Irina Zaharieva[1], Charu Deshpande[2], Istvan Bodi[3], Ata Siddiqui[4], Jean Marie U-King-Im[4], Amy Selfe[5], Rahul Phadke[1], Lucy Feng[1], Heinz Jungbluth[6-8*] and Francesco Muntoni[1-9*] [*]*joint contribution*

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

[1]Dubowitz Neuromuscular Centre, UCL Great Ormond Street Institute of Child Health & MRC Centre for Neuromuscular Diseases, London, UK; [2]Department of Clinical Genetics, Guys and St. Thomas' NHS Foundation Trust, London, UK; [3]Department of Clinical Neuropathology, King's College Hospital, London, UK; [4]Department of Neuroradiology, Guys and St. Thomas' NHS Foundation Trust, London, UK; [5]Department of Neuropsychology, and [6]Department of Paediatric Neurology, Neuromuscular Service, Evelina's Children Hospital, Guy's and St. Thomas' Hospital NHS Foundation Trust, London, UK; [7]Randall Division for Cell and Molecular Biophysics, Muscle Signaling Section, King's College, London, UK; [8]Department of Basic and Clinical Neuroscience, IoPPN, King's College London, London, UK; [9]NIHR Great Ormond Street Hospital Biomedical Research Centre, Great Ormond Street Institute of Child Health, University College London, & Great Ormond Street Hospital Trust, London, UK

Corresponding Author:

Professor Francesco Muntoni

Institute of Child Health

University College London

30 Guilford Street

London WC1N 1EHE-mail: f.muntoni@ucl.ac.uk

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 progressive neurological component at least in some patients, in keeping with the mitochondrial role of the defective protein.

Key words: muscular dystrophy, ataxia, MSTO1, cerebellar atrophy; progressive cerebellar involvement

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 distinguished phenotypes.

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

Case Report

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.

On examination at age 13 years 7 months (Figure 1), weight was 43.94kg (>25th centile) and height was 145.70cm (>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 degrees 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.

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.

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 to 1614 IU/L. Cardiac assessment 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 and ophthalmology 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 (Figure 2A); when repeated at 12 years of age, there was mild progression of cerebellar atrophy and additional supratentorial sulcal prominence suggestive of volume loss (Figure 2B).

Muscle MRI obtained at 13 years of age (Figure 3, F-J) showed marked involvement of the glutei within the pelvis (F). There was diffuse involvement within the thigh (G-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 (Figure 4; A, C). Few fibres showed empty, mostly subsarcolemmal non-rimmed vacuoles without reinforcement of sarcolemmal proteins (Figure 4; B). There was an overall slow fibre predominance, and many larger fibres showed prominent central pallor with reduced mitochondrial staining (Figure 4; D). There was no (immune)histochemical evidence of mitochondrial Complex I/Complex IV deficiency. Several fetal myosin-positive fibres of all sizes and intensities were present (Figure 4; E). Overall, the picture was in keeping with a chronic, moderately severe muscular dystrophy. Initially, testing with a comprehensive immunopanel of dystrophy-associated proteins showed no convincing abnormalities. Retrospective immunolabelling performed following genetic testing showed profound reduction of MSTO1 labeling in this biopsy (Figure 4; G), as well as the biopsy of the previously reported patient with pathogenic *MSTO1* variants (Figure 4; H). Respiratory chain enzyme studies were normal.

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 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 both parents were carriers of one heterozygous *MSTO1* variant each, confirming these to be *in trans* in the patient.

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. However, our patient was the first in whom upper motor signs and substantial clinical progression were noted, suggesting a more complex progressive neurological phenotype. Pigmentary retinopathy was detected in 2 previously reported patients at age 16 and 13 years, respectively, but was not present in our case at the same age. Considering the possibility of variable onset, we cannot exclude a later occurrence of this complication in further individuals and thus recommend regular ophthalmic reviews for all *MSTO1*-RD patients. Dysmorphic features (triangular face, sunken eyes) and thick hair were reported in 3 previous cases but those were not prominent in our case. Of note, CK levels were elevated in all cases with recessive *MSTO1*-RD but levels were highly variable, ranging from between 430 up to 5420 U/l.

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. Sequential brain MRI

from the present case obtained at an interval of 7 years however indicated mild progression of cerebellar atrophy over time, corresponding to evolution of additional neurological, in particular cerebellar and upper motor neuron signs, over the same period (Figure 2).

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 (Figure 3). Also considering a degree of variability between patients, review of further cases is needed to further clarify this pattern.

Muscle biopsy findings in *MSTO1*-RD have been reported variably as being myopathic or dystrophic, suggesting a spectrum of pathological severity. We noted similarities in the pathology of the present case and the previously reported patient from our centres, both showing an overall moderately dystrophic pattern with minimal active necrosis/regeneration, striking fatty infiltration and fibrosis, and a small number of fibres featuring non-rimmed vacuoles.

MSTO1 labeling of frozen muscle sections utilizing a rabbit polyclonal MSTO1 antibody (PA5-21641, Thermofisher) with the epitope mapping to a region between amino acids 34-347 of the protein was markedly reduced in our patient (Figure 4; G) and the previously reported case (Figure 4, H), and, interestingly, in a case of MICU1-RD, compared to labeling of an unaffected control that produced a discernible mitochondrial staining pattern with subsarcolemmal accentuation (Figure 4, F). MSTO1 labeling was also retained in the two other disease controls; CHKB-RD (Figure 4; J) and TK2-RD (Figure 4; K), in both cases, the abnormal distribution of MSTO1 mirroring the mitochondrial staining. In view of these findings, MSTO1

immunoanalysis could be helpful in directing molecular testing and/or interpretation of variants within the appropriate clinical context, bearing in mind that secondary changes of MSTO1 immunolabelling may be observed 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 arborization 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 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 (Figure 3), in keeping with a diagnosis of a muscular dystrophy. Secondary mitochondrial dysfunction is not unique for *MSTO1* gene related muscular dystrophies. For example, loss-of-function variants in the *MICU1* gene have been associated with a progressive brain and muscle disorder, characterized by raised CK and dystrophic muscle pathology, with alterations in mitochondrial calcium signaling (Logan et al, 2016). 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 progression of neurological involvement, comprising progressive

cerebellar but also additional 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.

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Declaration of Conflicting Interests and consents

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Informed consent for publication and permission for use of clinical photographs and radiological images were obtained from the patient and his parents. All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with Helsinki Declaration of 1975, as revised in 2000.

References

[1] Bonnemann CG, Wang CH, Quijano-Roy S, et al. Diagnostic approach to the congenital muscular dystrophies. Neuromuscular disorders : NMD 2014;24:289-311.

[2] Iwama K, Takaori T, Fukushima A, et al. Novel recessive mutations in MSTO1 cause cerebellar atrophy with pigmentary retinopathy. Journal of human genetics 2018;63:263-270.

[3] Gal A, Balicza P, Weaver D, et al. MSTO1 is a cytoplasmic pro-mitochondrial fusion protein, whose mutation induces myopathy and ataxia in humans. EMBO molecular medicine 2017;9:967-984.

[4] Nasca A, Scotton C, Zaharieva I, et al. Recessive mutations in MSTO1 cause mitochondrial dynamics impairment, leading to myopathy and ataxia. Human mutation 2017;38:970-977.

[5] Kimura M, Okano Y. Human Misato regulates mitochondrial distribution and morphology. Experimental cell research 2007;313:1393-404.

[6] Phadke R. Myopathology of Adult and Paediatric Mitochondrial Diseases. Journal of clinical medicine 2017;6.

[7] Fukumitsu K, Hatsukano T, Yoshimura A, Heuser J, Fujishima K, Kengaku M. Mitochondrial fission protein Drp1 regulates mitochondrial transport and dendritic arborization in cerebellar Purkinje cells. Mol Cell Neurosci 2016;71:56-65

[8] Waterham HR, Koster J , van Roermund CWT, et al. A lethal defect of mitochondrial and peroxisomal fission. New Eng J Med 2007;356: 1736-1741.

[9] Vila MC, Rayavarapu S, Hogarth MW et al. Mitochondria mediate cell membrane repair and contribute to Duchenne muscular dystrophy. Cell Death Differ 2017;24:330-342.

Figures

Figure 1: Patient with *MSTO1*-related RD at 13 years of age

Figure 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.

Figure 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.

Figure 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 MTCO1 (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 previously reported patient with pathogenic MSTO1 variants (H) and a case of MICU1-myopathy. Labeling is retained and mirrors the abnormal mitochondrial distribution pattern in a case of CHKB-muscular dystrophy (J) and TK2-myopathy (K) Scale bar (A-K): 100 µm

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