Available online at www.sciencedirect.com

ScienceDirect

Neuromuscular Disorders 29 (2019) 108–113

www.elsevier.com/locate/nmd

Case report

Long-term follow-up and characteristic pathological findings in severe nemaline myopathy due to *LMOD3* mutations

Eva Michael[a,*], Carola Hedberg-Oldfors[b], Philip Wilmar[a], Kittichate Visuttijai[b], Anders Oldfors[b], Niklas Darin[a]

[a] *Department of Pediatrics, Sahlgrenska Academy, Institute of Clinical Sciences, Sahlgrenska University Hospital, University of Gothenburg, Gothenburg, Sweden*

[b] *Department of Pathology and Genetics, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden*

Received 8 July 2018; received in revised form 1 December 2018; accepted 17 December 2018

Abstract

We describe the long-term follow-up of a patient with severe nemaline myopathy due to a novel homozygous mutation in the Leiomodin 3 (*LMOD3)* gene and describe the histopathological characteristics of the disease. The patient presented at birth with hydrops fetalis, multiple joint contractures, severe generalized muscle weakness, no movement, and respiratory insufficiency. At eight years of age, she had bilateral ophthalmoplegia, visual impairment, multiple contractures, and scoliosis, and is dependent on a home mechanical ventilator and gastrostomy. Except for slight head nodding, she has no voluntary movements. Whole-exome sequencing revealed a homozygous one-base duplication in the *LMOD3* gene (c.882dupA, p.Asp295Argfs*2), which would result in a truncated protein. Muscle biopsy in the girl and an unrelated patient homozygous for *LMOD3* p.Glu357* showed characteristic morphology of the nemaline rods. Many rods appeared as fragments of thickened Z-discs, frequently in pairs, which were interconnected by short thin filaments. Although not specific, this may be a morphological hallmark of *LMOD3*-associated nemaline myopathy.

Keywords: Nemaline myopathy; Severe congenital form; LMOD3; Novel mutation; Morphological hallmark.

1. Introduction

Nemaline myopathy (NM) is one of the most common forms of congenital myopathy and typically presents with non-progressive, symmetrical muscle weakness and hypotonia, affecting mainly the proximal muscles. The disease manifestations range from a severe form with fetal akinesia, contractures and respiratory insufficiency at birth, to a very mild form with onset in adulthood. The diagnostic characteristic of the disease is an accumulation of nemaline bodies, which are Z-disc-derived rod-like inclusions located inside the myofibers, which can be detected by Gomori trichome staining [1,2].

Thirteen genes have been associated with nemaline myopathy to date, but in a proportion of patients the diagnosis is still without genetic verification [3–5]. The most common genes implicated in nemaline myopathy are Nebulin (*NEB)* (50%), followed by Actin 1 (*ACTA1)* (15–25%), whereas other genetic causes are much more rare [6–8]. Leiomodin-3 is an important component of the contractile apparatus, promoting actin polymerization by converting G-actin to F-actin [9,10]. Mutations in the *LMOD3* gene have recently been found to cause a severe form of nemaline myopathy that at birth, presents with severe generalized hypotonia and muscle weakness, respiratory insufficiency, contractures, and sometimes bulbar weakness [11].

* Corresponding author.
E-mail address: eva.michael@vgregion.se (E. Michael).

https://doi.org/10.1016/j.nmd.2018.12.009
0960-8966/

Table 1
Clinical and laboratory characteristics of the patients.

Characteristic	Family 1: II:3	Family 2: II:3	Family 2: II:4
Sex	Female	Male	Male
Polyhydramniosis	Present	Present	Present
Fetal movements	Diminished	Diminished	Diminished
Joint contractures at birth	Multiple	Multiple	No information
Edema	Severe	No	No information
Gestational age, weeks	37+4	37+3	36+3
Birth weight, g (SD)	2620 g (-3 SD)	Not available	2095 g (-3 SD)
Apgar score at 1, 5, 10 min	2, 4, 6	3, 7, 7	2, 5, 6
Myopathic features at birth	Myopathic face, high arched palate, open mouth, no swallowing reflex, dolicocephaly	Myopathic face, high arched palate, open mouth, no swallowing reflex	No information
Serum CK	Normal	Normal	No information
Eyes	Bilateral ptosis and complete ophthalmoplegia	Bilateral ptosis	No information
Lung function	Initially CPAP, on respirator from 2 months of age	CPAP	No information
Heart	ASD	ASD	Not investigated
CNS malformations	No	No	Not investigated
Follow-up	Alive at 8 years of age but motor function restricted to slight head nodding	No voluntary movements at birth. By 2 months, able to move his fingers and feet slightly. Died at 5 months of age due to an infection	Died 6 h from birth due to respiratory insufficiency
EMG and nerve conduction studies	MUPs with very low amplitudes and short durations	Normal amplitudes to sensory stimuli but no potentials could be registered during motor stimuli	Not performed

Abbreviations: CPAP, continuous positive airway pressure; ASD, atrial septum defect; CK, creatine kinase; EMG, electromyography; MUP, motor unit potential; CNS, central nervous system.

Our aim in this report is to describe the long-term follow-up of a patient with a novel homozygous mutation in the *LMOD3* gene, and also to describe the characteristic pathological features of muscle seen in *LMOD3*-related nemaline myopathy in two unrelated patients.

2. Case report

2.1. Patients

2.1.1. Family 1

This girl, born to healthy consanguineous Turkish parents, with two older healthy siblings, had no family history of neuromuscular disorders or other neurodevelopmental disorders (Fig. 1(B)). She was profoundly weak at birth and needed immediate respiratory assistance. Her clinical features and the clinical work-up performed are listed in Table 1.

Active treatment was pursued since the family opted for full medical treatment. The patient had suffered from severe respiratory insufficiency since birth and required ventilator support, and at two months of age she received a tracheostomy and became fully dependent on a respirator. She also has severe bulbar weakness with no suckling or swallowing, and since two months of age she has been fed using a percutaneous endoscopic gastrostomy (PEG). At 8 months of age, she was discharged from hospital.

The patient is now 8 years of age and has severe muscle weakness with essentially no motor function and no voluntary mobility, except that she can nod her head slightly to answer yes/no questions. From birth, she has no external eye movements and cannot close her eyelids. This has led to several ocular infections and corneal ulcerations, and she now has severe visual impairment. There are contractures in all her joints that have remained relatively unchanged over time, but many muscle groups in her upper and lower extremities have become stiffer. She has developed a progressive right convex scoliosis and also a thoracic and lumbar kyphosis, which are now 28 and 113°, respectively.

She needs a sitting corset to support and stabilize her torso, foot orthoses both day and night against contractures, and the use of a standing frame on a daily basis. Assistance around the clock is needed. Cardiological follow-up has been normal except for an atrial septum defect (ASD) secundum.

The patient has developed osteopenia but has had no skeletal fractures. She has daily supplementation with vitamin D. Home mechanical ventilation (HMV) is currently given with heated, humidified air with settings Ipap 12/Epap 6 cm H_2O-(inspiratory/expiratory positive airway pressure). Her current treatment also includes nebulized hypertonic saline and salbutamol, the frequent use of cough-assist, prophylactic antibiotics, and intermittent use of nebulized colistimethate every other month. A cuffed tracheostomy tube with a suction duct is used because of a continuous problem with leakage of fluid out of the tracheostomy stoma. Over the years, she has been vulnerable to lower respiratory tract infections (LRTIs) and has had frequent hospitalization for treatment with intravenous antibiotics. Consequently, she has developed a chronic atelectasis in the left lower pulmonary lobe.

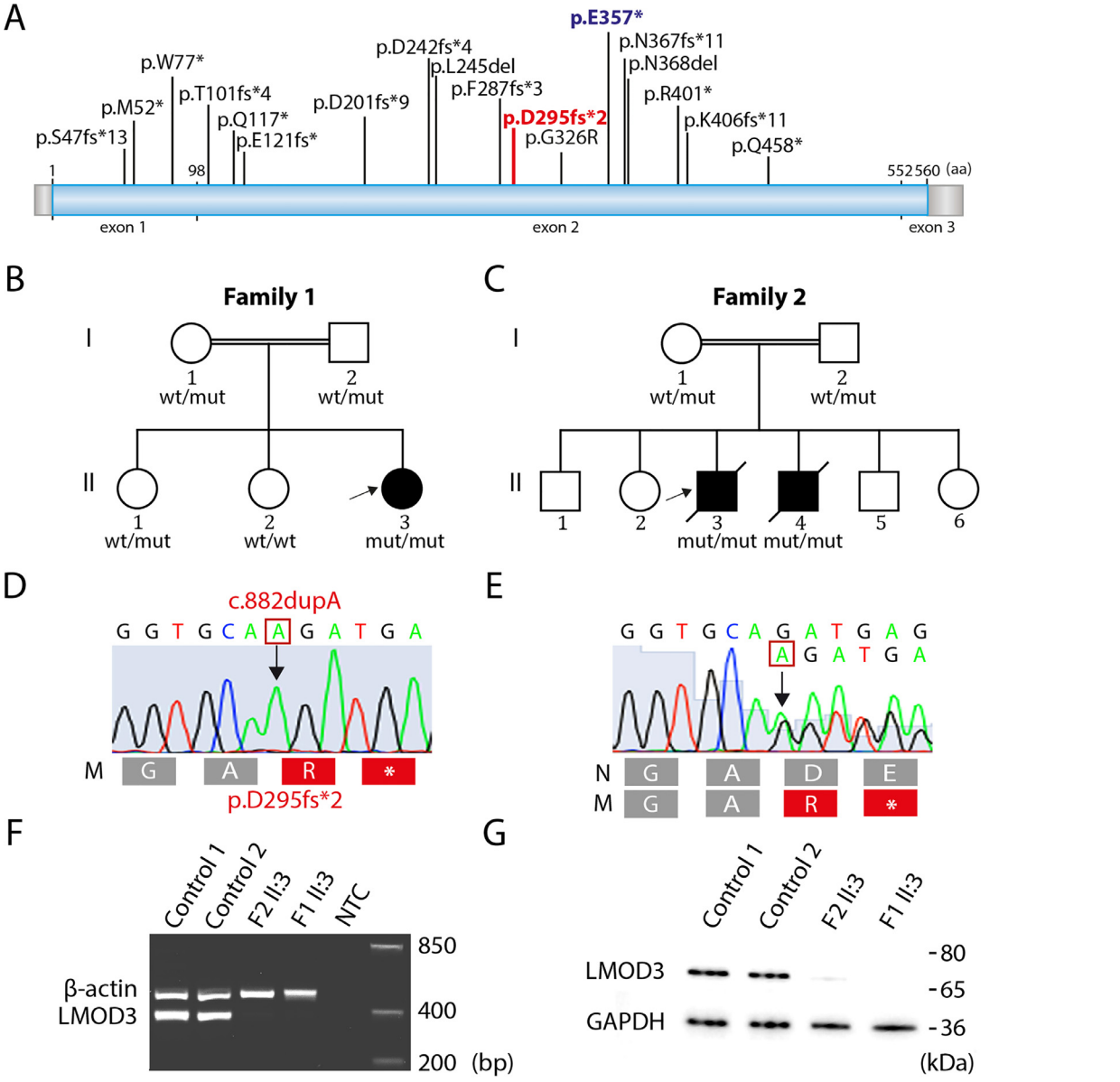

Fig. 1. Molecular genetics. (A) Illustration showing all the identified pathogenic variants in *LMOD3* (NM_198271.4). The novel variant identified in this study (p.D295fs*2) is marked in red and the previously described one is marked in blue. (B) Pedigree of family 1 with the novel c.882dupA; p.D295fs*2 variant (filled symbol indicates affected individual, mut = mutated sequence, wt = normal sequence). (C) Pedigree of family 2 with the c.1069G>T; p.E357* variant. (D) and (E) Chromatograms demonstrating the homozygous variant c.882dupA, p.D295fs*2 in the patient (D) and heterozygosity in one of the parents (E) (N = normal sequence, M = mutated sequence). (F) The semi-quantitative PCR analysis showed loss of *LMOD3* transcripts in the index patients relative to the controls. LMOD3 was co-amplified with β-actin (NM_001101.3), using cDNA as template. NTC = no-template control. (G) Western blot analysis of leiomodin-3 expression using protein extracted from skeletal muscle biopsy specimens from the index patients in both families, showing loss of leiomodin-3. GAPDH was used as loading control. Index patients are marked with arrows in panels B and C. (F1 = family 1, F2 = family 2).

2.1.2. *Family 2*

Family 2 has two affected brothers (Fig. 1(C)). Clinical and genetic data from these two brothers have already been described briefly [11]. In the present article, we have summarized the clinical findings in Table 1.

2.2. *Molecular analysis*

In patient II:3 in family 1, whole-exome sequencing (WES) was performed on DNA extracted from blood, and a homozygous one-base duplication (c.882dupA, p.Asp295Argfs*2) was identified in the leiomodin-3 gene (*LMOD3*) (NM_198271.4) (Fig. 1(A), (B) and (D)). The variant was not found in publicly available databases of human genetic variation (dbSNP version 146, www.ncbi.nlm.nih.gov/project/SNP/; ExAc, http://exac.broadinstitute.org/; NHLBI Exome Variant Server, http://evs.gs.washington.edu/EVS/; 1000 Genomes database, http://www.1000genomes.org/); the Genome Aggregation Database (gnomAD), http://gnomad.broadinstitute.org/). Most called variants

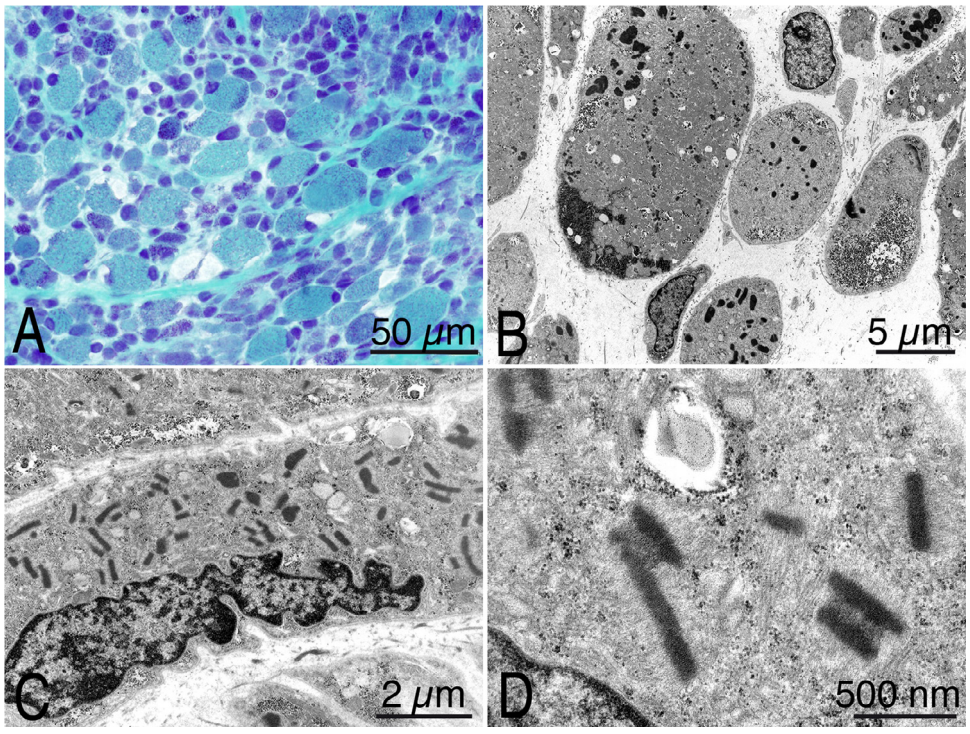

Fig. 2. Muscle biopsy from the quadriceps muscle of patient II:3 in family 1. (A) Gomori trichrome staining showing a marked variation in fiber size with a large proportion of hypoplastic fibers and numerous fibers with nemline rods, in addition to increased interstitial connective tissue (modified Gomori trichrome). (B) Electron micrograph showing hypoplastic muscle fibers with marked disorganization of the sarcomeres and nemaline rods in several fibers. (C) and (D) Electron micrographs showing a muscle fiber with multiple nemaline rods appearing as thickened Z-disc fragments, frequently in doublets. These rods were rimmed by short (50–200 nm long), thin filaments sticking out parallel to the short axis of the discs and creating a halo-like appearance or interconnecting the doublets.

identified in genes known to be associated with nemaline myopathy after filtering were predicted to probably be benign or to be of uncertain significance according to the computed ACMG Guidelines classification. No other variants that would probably affect function were detected. Genetic analysis of the parents revealed that they were both heterozygous for the variant in *LMOD3*, as was the oldest sister, whereas the other sister had normal sequences relative to the reference sequence (Fig. 1(B)).

In family 2, patients II:3 and II:4 were both identified to be homozygous for a nonsense variant in the *LMOD3* gene (c.1069G>T, p.E357*). Genetic analysis of the parents revealed that they were both heterozygous for the variant sequence in *LMOD3*.

Expression analysis of RNA and protein extracted from muscle tissue from patient II:3 in family 1 and patient II:3 in family 2 showed loss of both *LMOD3* transcripts and leomodin-3 protein in the patients relative to the controls (Fig. 1(F) and (G)).

2.3. Muscle pathology

Muscle biopsy from the vastus lateralis of the quadriceps muscle was performed in two patients (II:3 in family 1 at 3 weeks of age and II:3 in family 2 at 7 weeks of age). The specimens were snap-frozen in propane chilled with liquid nitrogen, for histochemical investigations, which were performed according to standard procedures [12]. For electron microscopy, specimens were fixed in glutaraldehyde, post-fixed in OsO_4, and embedded in resin. Ultrathin sections were stained with lead citrate and uranyl acetate.

In both individuals, we observed similar and characteristic pathological changes (Figs. 2 and 3). Many fibers appeared hypoplastic, and there was a marked increase in interstitial connective tissue (Figs. 2(A) and (B) and 3(A) and (B)). The majority of the muscle fibers showed a marked disorganization of the myofibrils, which completely lacked sarcomeric structures. Instead, randomly distributed filaments and nemaline rods were apparent in the majority of fibers. A characteristic finding in a proportion of fibers was the occurrence of many rods that appeared as fragments of thickened Z-discs with short (50–200 nm), thin filaments attached to each side (Figs. 2(C) and (D) and 3(C) and (D)). These special rods frequently appeared in pairs interconnected by thin filaments.

3. Discussion

Here we have described the long-term follow-up of an 8-year-old girl with severe nemaline myopathy associated with a novel mutation in the *LMOD3* gene. Ninety per cent of previously reported patients with *LMOD3*-associated nemaline myopathy have had a similar, severe form of the disease but have died or have been lost to follow-up before 19 months

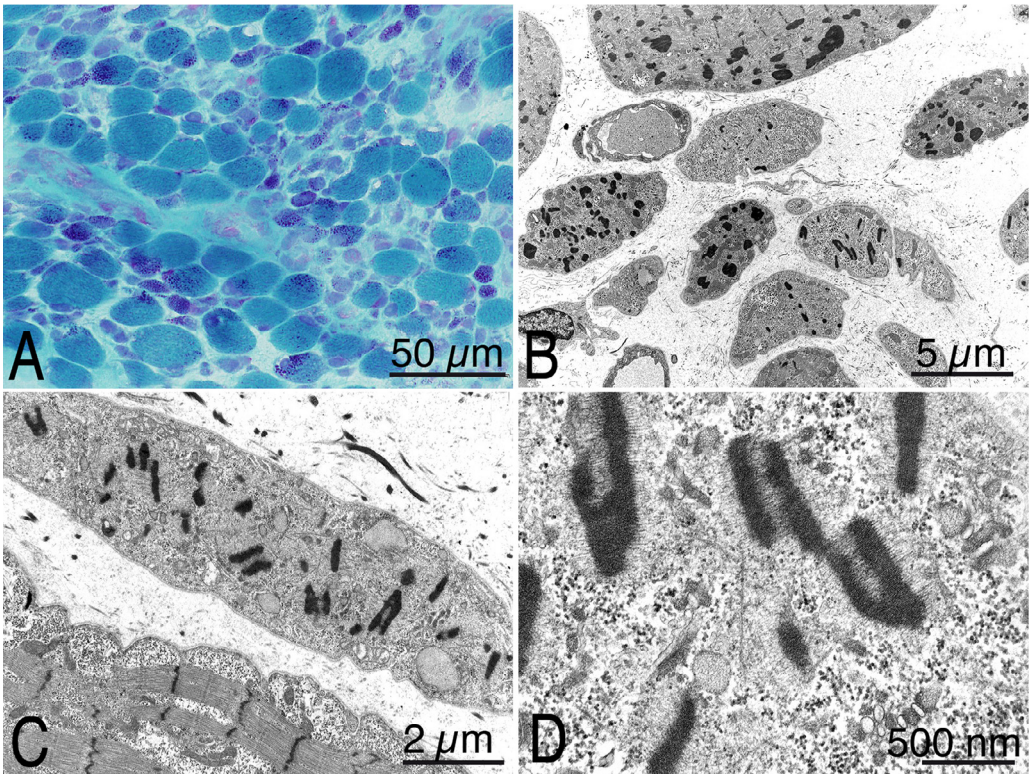

Fig. 3. Muscle biopsy from the quadriceps muscle of patient II:3 in family 2. (A) Gomori trichrome staining showing a marked variation in fiber size with numerous fibers with nemline rods. There was also an increased amount of interstitial connective tissue and frequent hypoplastic fibers (modified Gomori trichrome). (B) Electron micrograph showing hypoplastic muscle fibers with nemaline rods and loss of normal sarcomeres. (C) Electron micrograph showing a small muscle fiber with nemaline rods appearing as thickened Z-disc fragments, frequently in pairs interconnected by thin filaments. Short, thin filaments stick out parallel to the short axis of the discs, forming a rim on both sides. (D) Higher magnification of a small fiber in panel B demonstrating characteristic rods that appear as thickened Z-discs, frequently in pairs and associated with thin filaments.

of age. Our patient is severely affected with multiple contractures, progressive scoliosis, and stiffness, with the need for a respirator and gastrostomy. She has bilateral ophthalmoplegia and visual impairment, and no voluntary movements except for slight head nodding, with restriction of motor function and communication. She has an ASD which hasn't been described in patients with *LMOD3*-associated nemaline myopathy. The two patients from family 2 had a similar, severe phenotype but both succumbed, one to infection at 5 months of age and one to respiratory insufficiency only 6 h after birth. The symptoms in our patients are similar to what has been described previously [11], with prenatal onset characterized by polyhydramniosis seen in 62% of cases, reduced or absent fetal movements seen in 48%, and multiple contractures seen in 48%. External ophthalmoplegia has also been described in 29% of previous cases, but is usually absent in the other genetic forms of nemaline myopathy. Only two sibling patients are known to have survived into preschool or school age. They had a milder phenotype classified as typical childhood nemaline myopathy, which was caused by compound heterozygous mutations that resulted in some protein expression. Both were ambulatory, neither had cardiac involvement, and both needed non-invasive ventilator support at night [11].

Muscle biopsy in both our patients led to identification of a distinct ultrastructure of many of the nemaline bodies, resembling thickened Z-disc remnants, often in pairs and interconnected by very short, thin filaments. This was also described in all six patients in the original publication [11] and appears therefore to be a characteristic finding in severe *LMOD3*-associated nemaline myopathy, although they have been reported to occur also in a case of *KLHL40*-associated nemaline myopathy [8]. The cause is defective functioning of the leiomodin-3 protein, a tropomodulin-like protein that binds to both actin and tropomyosin. Leiomodin-3 promotes polymerization of actin by converting G-actin to F-actin [9,10], which has been studied in *LMOD3*-knockout mice [10] and also in a *LMOD3*-knockdown zebrafish model [11], showing that both animal models suffered from failure-to-thrive and abnormal skeletal muscle organization.

There are several lines of evidence for the previously undescribed homozygous 1-base duplication in *LMOD3*, c.882dupA, p.Asp295Argfs*2, in patient 1 causing the disease. Firstly, by introducing a premature stop codon the variant is clearly deleterious. Secondly, the variant segregates with the disease in the family. Thirdly, it has not been reported in population databases such as gnomAD, ExAc, and EVS, so it is not common in the population. Fourthly, it is associated with the characteristic morphological findings in the muscle

of our two patients with available biopsies. Fifthly, no expression of *LMOD3* mRNA or leiomodin-3 protein could be found in our patients. Lastly, the clinical phenotype is similar to previously described severe phenotypes in patients with *LMOD3* frameshift and nonsense variants [11].

In conclusion, based on long-term follow-up (eight years) of one patient with a severe form of *LMOD3*-associated nemaline myopathy, the prognosis is very poor with extreme muscle weakness and round-the-clock dependence on a respirator since the age of two years. We have also identified a novel variant of the *LMOD3* gene and, based on muscle biopsy investigations in two unrelated patients, have confirmed the characteristic morphology of many nemaline rods in *LMOD3*-associated nemaline myopathy.

Acknowledgments

We are grateful to the families who contributed to this study. We also thank Brith Leidvik for technical assistance. The study was supported by the Research Fund for Neuromuscular Disorders in West Sweden, by the Swedish Research Council (grant number 2012-02014, to AO), and by the AnnMari and Per Ahlqvist Foundation (to ND).

References

[1] Wallgren-Pettersson C, Sewry CA, Nowak KJ, Laing NG. Nemaline myopathies. Semin Pediatr Neurol 2011;18:230–8. doi:10.1016/j.spen.2011.10.004.

[2] Romero NB, Sandaradura SA, Clarke NF. Recent advances in nemaline myopathy. Curr Opin Neurol 2013;26:519–26. doi:10.1097/WCO.0b013e328364d681.

[3] Gupta VA, Ravenscroft G, Shaheen R, Todd EJ, Swanson LC, Shiina M, et al. Identification of KLHL41 mutations implicates BTB-Kelch-mediated ubiquitination as an alternate pathway to myofibrillar disruption in nemaline myopathy. Am J Hum Genet 2013;93:1108–17. doi:10.1016/j.ajhg.2013.10.020.

[4] Miyatake S, Mitsuhashi S, Hayashi YK, Purevjav E, Nishikawa A, Koshimizu E, et al. Biallelic mutations in MYPN, encoding myopalladin, are associated with childhood-onset, slowly progressive nemaline myopathy. Am J Hum Genet 2017;100:169–78. doi:10.1016/j.ajhg.2016.11.017.

[5] Sandaradura SA, Bournazos A, Mallawaarachchi A, Cummings BB, Waddell LB, Jones KJ, et al. Nemaline myopathy and distal arthrogryposis associated with an autosomal recessive TNNT3 splice variant. Hum Mutat 2018;39:383–8. doi:10.1002/humu.23385.

[6] Malfatti E, Romero NB. Nemaline myopathies: state of the art. Rev Neurol (Paris) 2016;172:614–19. doi:10.1016/j.neurol.2016.08.004.

[7] Jungbluth H, Treves S, Zorzato F, Sarkozy A, Ochala J, Sewry CA, et al. Congenital myopathies: disorders of excitation-contraction coupling and muscle contraction. Nat Rev Neurol 2018;14:151–67. doi:10.1038/nrneurol.2017.191.

[8] Sewry CA, Wallgren-Pettersson C. Myopathology in congenital myopathies. Neuropathol Appl Neurobiol 2017;43:5–23. doi:10.1111/nan.12369.

[9] Sandaradura S, North KN. LMOD3: the "missing link" in nemaline myopathy? Oncotarget 2015;6:26548–9. doi:10.18632/oncotarget.5267.

[10] Cenik BK, Garg A, McAnally JR, Shelton JM, Richardson JA, Bassel-Duby R, et al. Severe myopathy in mice lacking the MEF2/SRF-dependent gene leiomodin-3. J Clin Invest 2015;125:1569–78. doi:10.1172/jci80115.

[11] Yuen M, Sandaradura SA, Dowling JJ, Kostyukova AS, Moroz N, Quinlan KG, et al. Leiomodin-3 dysfunction results in thin filament disorganization and nemaline myopathy. J Clin Invest 2014;124:4693–708. doi:10.1172/jci75199.

[12] Dubowitz V, Sewry CA, Oldfors A. Muscle biopsy: a practical approach. 4th ed; 2013. 2013, Philadelphia: Elsevier.