

Evidence of mild founder *LMOD3* mutations causing nemaline myopathy 10 in Germany and Austria

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Abstract

Objective

To expand the clinical and genetic spectrum of nemaline myopathy 10 by a series of Austrian and German patients with a milder disease course and missense mutations in *LMOD3*.

Methods

We characterized the clinical features and the genetic status of 4 unrelated adolescent or adult patients with nemaline myopathy.

Results

The 4 patients showed a relatively mild disease course. They all have survived into adulthood, 3 of 4 have remained ambulatory, and all showed marked facial weakness. Muscle biopsy specimens gave evidence of nemaline bodies. All patients were unrelated but originated from Austria (Tyrol and Upper Austria) and Southern Germany (Bavaria). All patients carried the missense variant c.1648C>T, p.(Leu550Phe) in the *LMOD3* gene, either on both alleles or *in trans* with another missense variant (c.1004A>G, p.Gln335Arg). Both variants were not reported previously.

Conclusions

In 2014, a severe form of congenital nemaline myopathy caused by disrupting mutations in *LMOD3* was identified and denoted as NEM10. Unlike the previously reported patients, who had a severe clinical picture with a substantial risk of early death, our patients showed a relatively mild disease course. As the missense variant c.1648C>T is located further downstream compared to all previously published *LMOD3* mutations, it might be associated with higher protein expression compared to the reported loss-of-function mutations. The apparent clusters of 2 mild mutations in Germany and Austria in 4 unrelated families may be explained by a founder effect.

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Nemaline myopathy (NEM) is a genetically heterogeneous congenital myopathy with the characteristic finding of electron-dense “nemaline bodies” in myofibers. In 2014 twenty-one patients from 14 families with a severe form of congenital NEM and disrupting mutations in *LMOD3* (MIM *616112) were reported.¹ This form is denoted as NEM10 (MIM #616165). Following the first patients series, 2 further families with fatal NEM10 were published.^{2,3}

Here we describe 4 unrelated patients with clinically, ultra-structurally, and genetically confirmed NEM10 based on 2 missense mutations within *LMOD3* presenting with a hitherto undescribed milder phenotype.

Methods

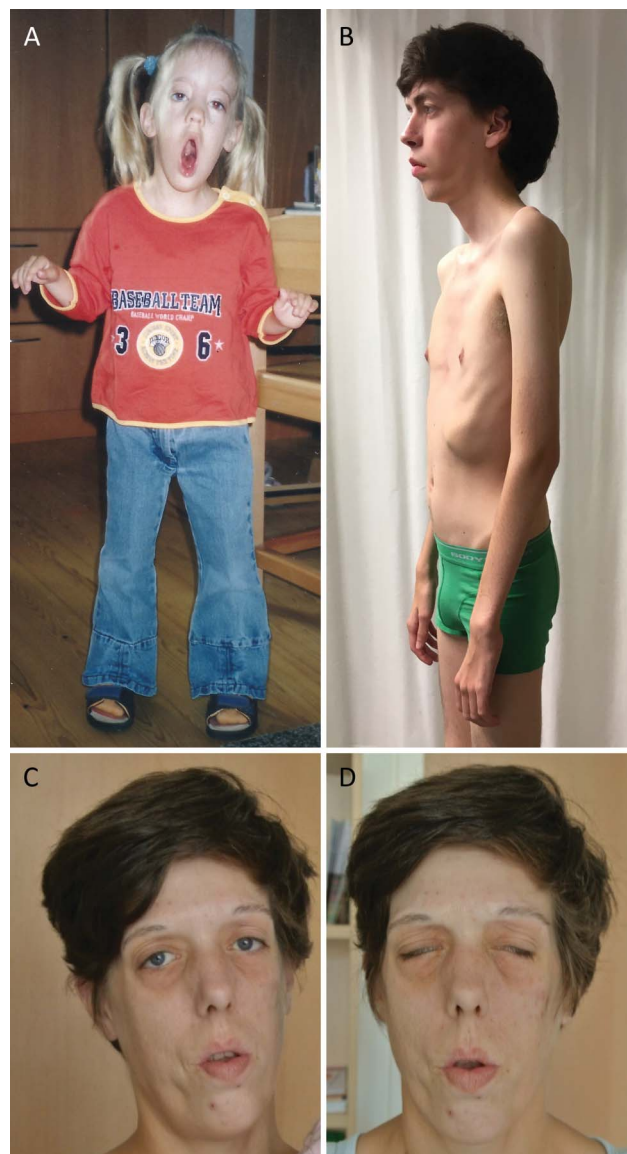
We reviewed the clinical features of 4 unrelated adolescent or adult patients with nemaline myopathy. The *LMOD3* mutations were detected by whole exome sequencing in patients 1 and 2, and by next generation sequencing-based panel analysis in patients 3 and 4. Histologic stains and transmission electron microscopy were performed according to standard protocols.

Results

In contrast to previous reports, our patients showed a mild disease course with pronounced facial weakness from birth but mostly stable muscle function otherwise (figure 1). In patient 1 reduced fetal movements were reported. Patient 2 had a history of intrauterine growth restriction and polyhydramnios resulting in Cesarean delivery. Both patients showed an impaired neonatal respiratory adaptation with resuscitation needed in patient 2. Pregnancy and delivery were normal in the remaining 2 patients. Severe weakness of facial, oral, and pharyngeal muscles was present in all patients. All had swallowing difficulties after birth making transient nasogastric tube feeding necessary in 3 of them (table). All patients had dysarthric speech. When last examined, no patient had swallowing difficulties, but chewing was still not possible in patient 2.

Despite impaired postnatal adaptation and the presence of muscular hypotonia from birth, our patients developed remarkably well and stabilized during the first year of life. Motor development was delayed in 2 patients: independent walking was achieved at age 2 years in patient 1 and at the age of nearly 6 years in patient 2. Patients 3 and 4 walked independently from the age of 16 and 14 months, respectively. Limb function was affected in all patients with lower limb weakness and a milder distally pronounced upper limb weakness. Patient 2 was non-ambulatory from age 12 due to truncal weakness and progressive thoracolumbar scoliosis. Patients 1, 3, and 4 were ambulatory at ages 18, 20, and 30 years, although patient 1 was not able to run and had difficulties in climbing stairs. Mild scoliosis was present in patient 3. No patient had a clinically relevant respiratory dysfunction; only patient 2 had a reduced forced vital capacity (45%) in lung function tests at the age of 18 years.

Figure 1 Clinical phenotype of patients with NEM10 and *LMOD3* mutations



(A) Patient 2 at the age of nearly 6 years presenting with an elongated face, and severe facial and jaw weakness. (B) Patient 3 at age 20 years with a symmetrical deep funnel chest with Harrison's groove. (C and D) Patient 4 at age 30 years showing pronounced facial weakness with incomplete eye closure.

Muscle biopsy specimens showed multiple nemaline bodies in Gomori trichrome stain. Transmission electron microscopy demonstrated Z-line streaming and multiple nemaline rods (figure 2).

The patients were offspring of 4 non-consanguineous couples with Austrian (Tyrol and Upper Austria) or German (Bavaria) provenance within a maximum geographical distance of ca. 300 km. Our patients shared 2 *LMOD3* missense mutations (NM_198271.4: c.1004A>G and c.1648C>T), either in homozygous or in compound heterozygous state (table). In all families, segregation studies were performed confirming

Table Clinical features and genotype of the 4 patients with NEM10

	Patient 1	Patient 2	Patient 3	Patient 4
Age at last presentation, y	18	18	20	30
Sex	Female	Female	Male	Female
Age at onset	Prenatal	Prenatal	Birth	Birth
Apgar-score	4/5/7	3/3/5	7/8/9	8/9/9
Postnatal feeding problems	+	+ (tube feeding until age 2 years)	+ (tube feeding until age 3 months)	+ (tube feeding until age 3 months)
Age at first walking	2 y	6 y	16 mo	14 mo
Ambulatory	+	– (From age 12 y)	+	+
Limb weakness (LL > UL)	+	+	+	+
Scoliosis	–	+ (progressive)	+ (mild)	–
Dysarthria and pronounced facial weakness	+	+	+	+
Respiratory function	Normal (age 15 years)	Reduced (FVC 45%; age 18 y)	Normal (age 19 y)	Normal (age 29 y)
<i>LMOD3</i> genotype	c.[1648C>T];[1648C>T]	c.[1004A>G];[1648C>T]	c.[1004A>G];[1648C>T]	c.[1648C>T];[1648C>T]
Provenance	Austria (Tyrol)	Austria (Upper Austria)	Germany (Bavaria)	Austria (Tyrol)

Abbreviations: FVC = forced vital capacity; LL = lower limbs; UL = upper limbs.

autosomal recessive inheritance. Both *LMOD3* variants (c.1004A>G, p.Gln335Arg and c.1648C>T, p.Leu550Phe) affect amino acid residues that are highly conserved up to *Xenopus* and zebrafish and are not listed in the in-house database (Munich Exome Server)⁴ nor in large reference datasets, such as the ExAC or the gnomAD browsers. In silico prediction is pathogenic according to MutationTaster, CADD, DANN, and fathmm. Both mutations are listed in ClinVar with an unknown clinical significance after detection in patient 3.

Discussion

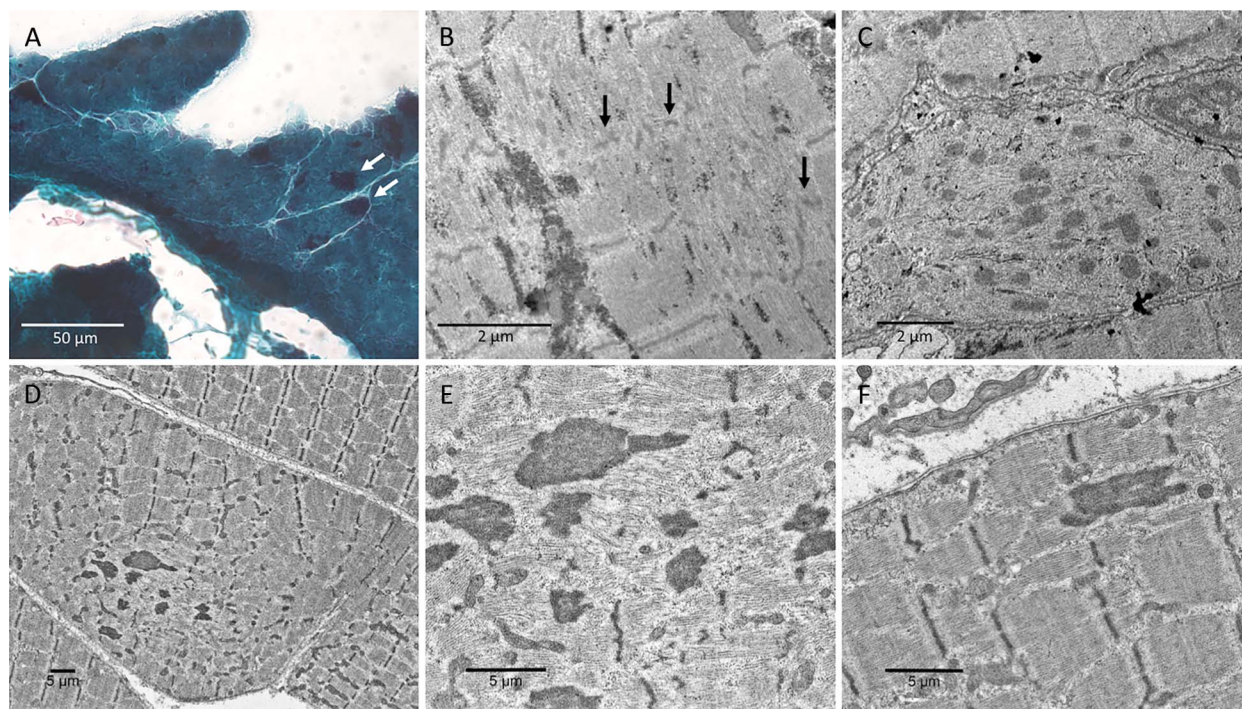
In contrast to the previously reported patients, who had a severe clinical picture with a substantial risk of early death, our patients showed a stable and much milder disease course. When NEM10 was genetically defined, the patients initially reported presented with intrauterine manifestations: polyhydramnios (62%), decreased or absent fetal movements (48%), arthrogryposis or joint contractures (48%), or fetal edema.¹ All patients had severe muscular weakness and hypotonia with a fatal outcome in most patients: 7 (33%) died from respiratory failure in the neonatal period, 6 (29%) patients died during the first year of life, and one pregnancy was terminated. Only 2 sisters were reported to be alive at the time of publication (age 10 and 4 years), after prenatal onset and a severe infantile disease course requiring gastrostomy and nocturnal noninvasive ventilation.

In 2017, 3 siblings with NEM10, born to consanguineous parents, due to the previously described frame-shift

mutation c.138dupC, p.Ser47Glnfs*13 were reported.² They showed fetal hypokinesia, had congenital fractures of long bones, and died of respiratory failure during early infancy. Recently, 2 fetuses from one family have been reported with a severe prenatal disease course especially encompassing absent fetal movements and abnormal posturing.³ Genetic diagnosis of NEM10 was made after pregnancy termination with both fetuses being compound heterozygous for the previously unknown frame-shift mutation p.Glu121Argfs*5 and the deletion p.Leu245del in *LMOD3*, respectively (no transcript information has been provided for these mutations).

LMOD3 localizes mainly to the A band of the sarcomere⁵ and is a key regulator of thin filament length in skeletal muscle.¹ It contains a tropomodulin-binding helix and 3 actin-binding domains: actin-binding helix, leucine-rich repeat domain (LRR), and Wiskott-Aldrich-syndrome protein homology 2 domain (WH2).¹ Most *LMOD3* mutations known so far are loss of function mutations. Protein expression analysis was performed for ten of the 15 mutations reported: protein expression was only present in 2 milder affected siblings who were compound heterozygous for 2 nonsense mutations in the C-terminal region of the LRR domain.¹ There was no protein expression for 6 other nonsense mutations; inconclusive results were reported for 2 mutations.¹ Only one missense mutation (c.976G>C, p.Gly326Arg) has been described so far. It was found in combination with 2 different nonsense mutations, causing a severe phenotype. The c.976G>C mutation lies within the LRR domain, as does the mutation c.1004A>G described here.

Figure 2 Muscle biopsies from 3 patients with NEM10 and *LMOD3* mutations



(A) Gomori trichrome stain (patient 3 at the age of 14 years) shows marked nemaline bodies (arrows) in highly atrophic myofibers. (B and C) Transmission electron microscopy images of patient 2 at the age of 3 weeks. (B) Large area of disorganized myofilaments, compatible with a core-like structure, containing dispersed Z-band material (arrows). (C) Multiple rod-bodies in a very small muscle fiber. (D-F) Transmission electron microscopy images of patient 1 at the age of 5 years. (D) Fiber displaying disturbed intrafusal filament texture and multiple electron dense nemaline rod structures. (E) Detail of rod textures out of (D). (F) Single rod-body spanning a sarcomere.

All 4 patients described in this study are homozygous or compound heterozygous for the variant c.1648C>T. This variant is located further downstream (within the WH2 domain) compared to all previously published *LMOD3* mutations. The physicochemical difference of the affected amino acid is predicted to be small in this mutation (p.Leu550Phe) with a Grantham distance of 22, possibly leading to a more tolerable molecular change in *LMOD3*. The functional role for the WH2 domain in *LMOD3* is not known to date. In *LMOD1* and *LMOD2* the WH2 domain acts as an actin binding domain, involved in actin nucleation, an early step in actin polymerization.⁶ In these 2 other *LMOD* proteins the equivalent to the LRR domain is the main actin binding site. The WH2 domain itself is responsible for a 2 to 3-fold increase in actin nucleation activity, and mutations within the WH2 domain are predicted to have only a mild effect on this process. This is also supported by the observation that other domains in the C-terminal region of *LMOD2* might have a stronger functional implication than the WH2 domain does. Hence, the mutation c.1648C>T might be responsible for the milder disease phenotype in the patients described here.

The observation of recurrent rare *LMOD3* variants in patients with a clinical and morphological phenotype matching NEM supports the predictably pathogenic effect of the detected mutations. The mutational spectrum in *LMOD3* remains to

be further defined, but the apparent clusters of 2 mild mutations in Germany and Austria in 4 unrelated families may be explained by a founder effect.

Author contributions

U.A. Schatz: study concept and design, acquisition of data, analysis and interpretation of data, study supervision, writing and final approval of the manuscript. S. Weiss: acquisition of data, analysis and interpretation of data, critical revision of manuscript for intellectual content. S. Wenninger: acquisition of data, analysis and interpretation of data, critical revision of manuscript for intellectual content. B. Schoser: analysis and interpretation of data, critical revision of manuscript for intellectual content. W.H. Muss: acquisition of data, analysis and interpretation of data, critical revision of manuscript for intellectual content. R.E. Bittner: acquisition of data, analysis and interpretation of data, critical revision of manuscript for intellectual content. W.M. Schmidt: acquisition of data, analysis and interpretation of data, critical revision of manuscript for intellectual content. A.S. Schossig: acquisition of data, critical revision of manuscript for intellectual content. S. Rudnik-Schöneborn: acquisition of data, analysis and interpretation of data, critical revision of manuscript for intellectual content. M. Baumann: study concept and design, acquisition of data, analysis and interpretation of data, study supervision, writing and final approval of the manuscript.

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Disclosure

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