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CASE REPORT

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α-tropomyosin gene (TPM3) mutation in an infant with nemaline myopathy

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Abstract

We report a case of neonatal nemaline myopathy with a de novo *TPM3* mutation, which has been classified as a likely pathogenic mutation. With the expanding use of genetic testing in congenital myopathies, genotype-phenotype descriptions of novel variants are important to inform clinical care, diagnosis, genetic counseling, and management of disease.

KEYWORDS

congenital myopathy, nemaline myopathy, neuromuscular disease, TPM3, tropomyosin

1 | INTRODUCTION

Nemaline myopathies (NEM) are heterogeneous congenital muscle disorders that cause skeletal muscle weakness and, in the most severe cases, death in the neonatal period. NEM due to a α-tropomyosin gene (*TPM3*) mutations are very rare conditions with few cases reported. Here, we describe a neonatal patient presenting with early onset of hypotonia and developmental delay with muscle biopsy showing nemaline myopathy. Genetic testing identified a likely de novo variant c.43G > C (p.Asp15His) in the *TPM3* gene, which is clinically classified as a likely pathogenic mutation.

Pathogenic mutations in the slow α-tropomyosin gene (*TPM3*) have been associated with three distinct histological entities: nemaline myopathy (NM, NEM1), cap disease (CD), and congenital fiber-type disproportion (CFTD).[1] Recently, Marttila et al summarized the findings in 35 clinically and histologically characterized families with 22 different *TPM3*

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variants and found that majority of the families(30/35) had missense variants segregating in an autosomal dominant inheritance manner or arising de novo.[2] Nemaline myopathies are heterogeneous congenital muscle disorders that cause skeletal muscle weakness and, in some cases, death immediately after birth.[3] Mild progressive proximal muscular weakness is the most common manifestation of nemaline myopathy (NEM).[4] In neonates, NEM is rarely reported in the literature, its diagnosis is difficult to establish and as a result, performing a muscle biopsy is instrumental for the correct diagnosis to be established.[5] Generally, NEM incidence is unknown, although two studies, one in Finland, estimated the incidence to be 1 in 50 000 live births[6], and another study in an American Ashkenazi Jewish population, estimated an incidence of 1 per 500, suggesting a genetic founder effect.[15] Collectively, NEM account for approximately 20% of all congenital myopathy cases.

We report herein a case of neonatal NEM with a heterozygous de novo variant, c.43G > C (p.Asp15His) in the *TPM3* gene within the long arm of chromosome 1 (chromosome 1q21.2), which was recently classified as likely pathogenic, ACMG category 2, and was also previously observed by three independent clinical genomics laboratory per ClinVar. Overall, NEM due to a*TPM3* gene mutation are a very rare condition with few cases reported.

2 | CLINICAL REPORT

The patient presented as an 8-month-old boy, who was the fifth pregnancy of non-consanguineous Canadian (European descent) parents. The pregnancy was uneventful with normal fetal movements and amniotic fluid volume; following an unremarkable delivery, there was no concern of respiratory distress or swallowing difficulties. He presented with a history of hypotonia, developmental delay, and failure to thrive. He was severely delayed in motor milestones but appropriate in social and language development. There was a significant family history of a paternal first cousin with an undifferentiated muscular dystrophy, the details or diagnosis of which could not be confirmed. There were no other familial neuromuscular, genetic, or congenital diseases.

The patient had displayed signs of hypotonia since birth, and at 3 months, he was admitted for failure to thrive. Upon presentation to the neuromuscular clinic, he continued to demonstrate difficulty with head movement, including holding up his head. He was, however, able to spontaneously move all extremities against gravity.

His general examination was normal. On neurological examination, he was hypotonic with evidence of poor muscle bulk to his extremities, truncal hypotonia, and head lag. There were no obvious dysmorphic features or evidence of tongue fasciculations. Sensation appeared intact. He was a reflexic with downgoing toes. His mobility was limited to side-to-side rolling.

The patient underwent multiple admissions for respiratory difficulties and additional investigations. Given the significant hypoventilation and inability to increase respiratory effort, the decision was made for the patient to initiate nighttime BiPAP. At age 9 months, he had a tracheostomy placed and has been stable on intermittent BiPap via tracheostomy.

The creatine kinase (CK) level was normal. Echocardiography was normal. Sequencing of the *SMN1* gene did not yield any pathogenic mutations, and alpha-Glucosidase DBS(dried blood spot) enzyme test was normal. EMG and nerve conduction studies demonstrated spontaneous activity in proximal muscles, consistent with possible myopathy. An MRI of the thigh and pelvic girdle muscles showed no obvious abnormalities.

The patient underwent a quadriceps skeletal muscle biopsy shown in Figure 1. Histologically, the muscle demonstrated fairly dramatic fiber-size variability with both atrophic/hypotrophic ("small") and hypertrophic fibers seen (including scattered fiber-splitting; range 3-40 μm). Fibrosis was increased among both the endomysium and perimysium. The Gomori trichrome staining revealed numerous subsarcolemmal and sarcoplasmic "red" granules and rods, consistent with nemaline rods, primarily in the small fibers but also noted in the histologically "normal" fibers. Focally, at least 50% of the small fibers contain nemaline rods.

Enzyme histochemistry demonstrated that the small fibers were type 1 (ATPase 4.2 and MHC-s immunohistochemistry) and that the type 2 fibers (and scattered type 1 fibers) were of relatively normal size. Overall, there was type 2 fiber predominance with many fibers co-expressing both slow (MHC-s) and fast (MHC-f) myosin heavy chains.

Prominent sarcoplasmic inclusions were noted among the atrophic fibers on semi-thin Toluidine blue-stained sections. Ultrastructural analysis demonstrated abundant and robust Z-line-like electron densities with lattice morphology. These were seen in highest density among the small fibers, but also noted among the more "normal" appearing fibers.

Generally, it is thought that in alpha-tropomyosin NEM cases, the nemaline inclusions are largely restricted to type 1 fibers and that the majority of these fibers are atrophic/hypotrophic (small).

3 | MOLECULAR GENETICS

Clinicopathological correlation via genetic testing (Nemaline myopathy panel, Invitae) initially revealed three variants of uncertain significance (VUS) identified in *MEGF10*, *NEB,* and *TPM3*, and all of them were heterozygous. The first was a missense VUS, c.2974G > A (p.Glu992Lys) identified in *MEGF10*. The *MEGF10*

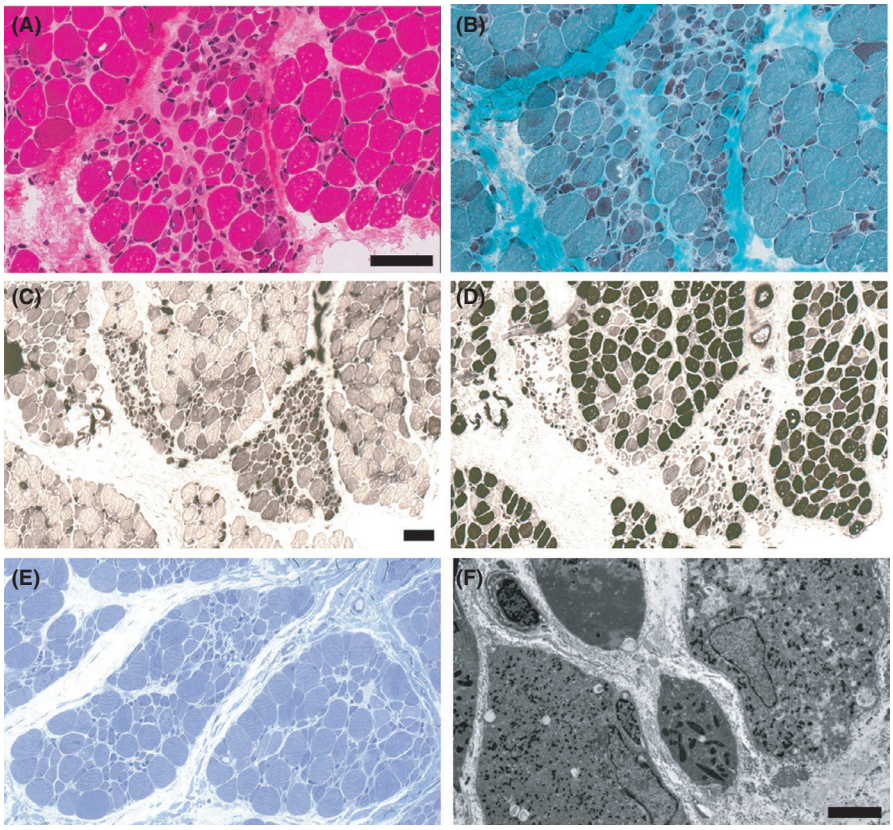

FIGURE 1 Skeletal muscle biopsy. A, The well-populated fascicles demonstrated fibre-size variability with both atrophic and hypertrophic fibres (HPS stain). There were several groups of polygonal atrophic fibres. There was increased perimysial and endomysial fibrosis. B, Gomori Trichrome demonstrated numerous sarcoplasmic "red' granules or rods uniformly distributed in the atrophic fibres and patchy in non-atrophic fibres. C, ATPase 4.2 demonstrated type 1 atrophy/hypotrophy (grouped or single) with the type 2 fibres (ATPase 9.4; D, comprising the majority of normal diameter fibres. E, Toluidine blue sections revealed prominent dark staining 'inclusions' noted most prominently within the atrophic fibres but also pale subsarcolemmal and sarcoplasmic "core-like" staining seen in non-atrophic fibres. F, Ultrastructural examination demonstrated abundant and robust Z-line-like electron densities with a 'lattice' appearance interpreted as nemaline rods.These were seen in highest densities among the atrophic fibres but also noted among the more 'normal' appearing fibres. Scale bar A-E is 50 μm; F is 4 μm

gene is known to be associated with autosomal recessive early-onset myopathy, areflexia, respiratory distress, and dysphagia (EMARDD), but not nemaline rod histology on muscle biopsy. The second was a synonymous VUS, c.17619C > T (p.Gly5873=) identified in *NEB*. The *NEB* gene is known to be primarily associated with autosomal recessive nemaline myopathy 2 (NEM2). The third was a missense VUS, c.43G > C (p.Asp15His) identified in *TPM3*. Importantly, this variant classification has recently been updated to be likely pathogenic per ClinVar. This variant was not present in population databases such as gnomAD. Algorithms developed to predict the effect of missense changes on protein structure and function (SIFT, PolyPhen-2,Align-GVGDandMutationTaster) all suggest that this variant is likely to be disruptive. The *TPM3* gene is associated with autosomal dominant or recessive NEM1 and congenital myopathy with fiber-type disproportion (CFTD). Absence of a second causative variant in the autosomal recessive *MEGF10* and *NEB* genes suggested it is unlikely that these are causative variants. In contrast, genetic analysis confirmed that neither of the parents were carriers for the pathogenic *TPM3* mutation in peripheral blood, suggesting a de novo mutation in the affected child in a gene with known dominant inheritance.

4 | DISCUSSION

In this case report, we describe an infant with an early-onset NEM and a morphological phenotype characterized by the presence of nemaline rods, which was confirmed ultrastructurally. Using next generation sequencing analysis, we observed a heterozygous missense mutation, c.43G > C (p.Asp15His), in the *TMP3* gene. This mutation was not present in both parents indicating that the mutation has likely occurred de novo and has been classified as a likely pathogenic mutation associated with autosomal dominant NEM1. Functional modeling of the mutation would be needed to definitely understand the pathophysiologic implications of this mutation on TPM3.

Childhood NEM is associated with a wide range of phenotypes from more benign congenital conditions, which present early and either progresses slowly or not at all[7,8] to severe weakness and debilitating functional impairment. The *TPM3* gene is one of ten genes (*TPM3, NEB, ACTA1, TNNT1, TPM2, CFL2, KBTBD13, KLHL40, KLHL41*, and *LMOD3*) currently associated with NEM.[9] In contrast to our case which showed a normal pregnancy history with normal fetal movements, it has been reported that hydramnion and decreased fetal movements are the most frequent symptoms of NEM during pregnancy.[5] At birth, the clinical findings of NEM are inconsistent and nonspecific; however, severe hypotonia, especially involving the proximal limb muscles and those of the face, neck, and trunk, may be noted.[5] Respiratory difficulties can be a prominent and worrisome finding at birth due to diaphragmatic muscle weakness.[4,5]In a paper that studied the clinical and pathological features of 28 Chinese patients with NEM, Yin and colleagues demonstrated that hypotonia was observed in most patients.[10] This is in keeping with our case, which showed signs of hypotonia since birth, but with the ability to spontaneously move all limbs in response to external stimuli. Additionally, respiratory problems appear to be a consistent finding at birth due to diaphragmatic muscle weakness.[4,5] In parallel with these 28 cases of NEM,[10] the current case showed normal creatine kinase level. Side-to-side rolling was the most complex volitional motor function achieved in our case and in a similar case reported by Kiiskiet al.[11]

In the case reported by Kiiski et al, the Gomori trichrome stain identified red-staining inclusions in several fibers which were confirmed to be nemaline rods by electron microscopy.[11] Additionally, with the modified Gomori trichrome stain, Tsujihataet al[12] found numerous dark red granular deposits in many fibers. In the current case, and as judged by myosin and ATPase stains, the small fibers were of histochemical type 1 and the normal-sized fibers consisting of a mixture of type 2 (predominantly) and type 1, and the rod bodies were observed in both type 1 and scattered type 2 fibers.[12]

Tan P et al[13] described a NEM case with a homozygous nonsense mutation in *TPM3* with severe NEM1 phenotype, showing extremely delayed and impaired motor development, except for rolling over. This patient showed type 1 fiber hypotrophy, mild predominance of type 2 fibers, and nemaline bodies were only present in type 1 fibers. In contrast to the current case, that case showed no feeding problems. Kiiski et al reported that muscle biopsy showed fiber size and rod variations in a population of hypotrophic muscle fibers expressing slow myosin, often with internal nuclei, and abnormal immune labeling that revealed many hybrid fibers.[11]

Family history and clinical evaluation of the parents helped in establishing a diagnosis in this case. There were no known cases of muscle disease in the immediate family, and the parents' clinical investigations and genetic testing were normal. The possibility of germline mosaicism in either parent has not been excluded and current literature estimates the recurrence risk for de novo variants as 0.011%-28.5%.[14] Genetic counseling is both important and recommended to advice on future pregnancies as is thorough monitoring with repetitive ultrasound examinations to assess fetal parameters.

5 | CONCLUSION

Here, we report a case of neonatal nemaline myopathy with a heterozygous de novo mutation c.43G > C (p.Asp15His) in the *TPM3* gene, which has been classified as likely pathogenic given the supporting clinical scenario and histological picture Figure 1.

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CONFLICT OF INTEREST

Icertify that they have NO affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge, or beliefs) in the subject matter or materials discussed in this manuscript.

AUTHOR CONTRIBUTIONS

SA: served as a primary author and performed the medical writing. JH: provided information and language editing. KL: served as a pathologist and provided the details of muscle biopsy and images. L-CA: served as a pathologist and provided the details of muscle biopsy and images. CC: served as a corresponding author and performed the final review, editing, and follow-up of all steps in preparing the manuscript.

ETHICAL APPROVAL

This paper is a case report and no need to have ethic approval in our institute, and we have written consent from parents to report the case.

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