Novel α-tropomyosin gene (TPM3) in an infant with Nemaline myopathy

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Abstract

Nemaline myopathies (NEM) are heterogeneous congenital muscle disorders that cause skeletal muscle weakness and in the most severe cases, death.We describe a neonatal patient presenting with hypotonia and muscle biopsy showing nemaline myopathy. Genetic testing identified a de novo variant c.43G>C (p.Asp15His) in theTPM3 gene not previously described

Key Clinical Message:

Here we report a case of neonatal nemaline myopathy with a de novo mutation c.43G>C (p.Asp15His) in theTPM3 gene, which can be considered a pathogenic mutation given the supporting clinical scenario and histological picture.

Introduction:

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 fibre-type disproportion (CFTD)[1]. Recently, Marttila et al. summarized the findings in 35 clinically and histologically characterized families with 22 different *TPM3* variants and found that majority of the families(30/35) had missense mutations segregating in an autosomal dominant fashion 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 [5]. Generally, NEM incidence is unknown, although two studies, one in Finlandestimated the incidence to be 1 in 50,000 live births, and one in an American Ashkenazi Jewish population estimated an incidence of 1/500, suggesting a genetic founder effect [6].It accounts for approximately 20 % of cases of all congenital myopathies [6].

We report herein a case of neonatal NEM with a de novo c.43G>C (p.Asp15His) *TPM3* gene variant localized to the long arm of chromosome 1(chromosome 1q21.2) which was classified as likely pathogenic, ACMG category 2, presenting in an autosomal dominant fashion .NEM with *TPM3* gene mutation is a very rare condition with few cases reported.

Clinical report:

The patient presented at 8-month-old boy, who was the fifth pregnancy of non-consanguineous Canadian (European) parents, with an uneventful pregnancy and delivery. 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 a 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 shown signs of hypotonia since birth and at three months he was admitted to hospital 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 exam 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 showed no pathogenic mutations,andalpha-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.Histologically,the muscle demonstrated fairly dramatic fibre-size variability with both atrophic/hypotrophic ('small') and hypertrophic fibres seen (including scattered fibre-splitting). Fibrosis was increased among both the endomysium and perimysium. The Gomoritrichrome staining revealed numerous subsarcolemmal and sarcoplasmic 'red' granules and rods, consistent with nemaline rods, primarily in the small fibres but also noted in the histologically 'normal' fibres.

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

Prominent sarcoplasmic inclusions were noted among the atrophic fibres 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 fibres, but also noted among the more 'normal' appearing fibres.

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

Molecular genetics :

Clinicopathological correlation via genetic testing (Nemaline myopathy panel, Invitae) showed 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* 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 asynonymous VUS, c.17619C>T (p.Gly5873=) identified in *NEB* . The*NEB* gene is only known to be associated with autosomal recessive nemaline myopathy 2 (NEM2). The third was a missense VUS, c.43G>C (p.Asp15His) identified in *TPM3* . This variant was not present in population databases including ExAC and gnomAD. Algorithms developed to predict the effect of missense changes on protein structure and function (SIFT,

PolyPhen-2,Align-GVGD andMutationTaster) all suggest that this variant is likely to be disruptive. The *TPM3* gene is associated with autosomal dominant or recessive NEM1and congenital myopathy with fibre-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.

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 exome sequencing analysis, we revealed 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 occurred *de novo*and suggests a pathogenic mutation associated with autosomal dominant NEM1.

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 their 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 line 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 an accordance 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 fibres which were confirmed to be nemaline rods by electron microscopy [12]. Additionally, with the modified Gomori trichrome stain, Tsujihataet al [13] found numerous dark red granular deposits in many fibres. In the current case, and as judged by myosin and ATPase stains, the small fibres were of histochemical type 1 and the normal-sized fibres consisting of a mixture of type 2 (predominantly) and type 1, and the rod bodies were observed in both type 1 and scattered type2 fibres [13].

Tan and colleagues [14] 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 fibre hypotrophy, mild predominance of type 2 fibres, and nemaline bodies were only present in type 1 fibres. In contrast to the current case, that case showed no feeding problems. Kiiski et al reported that muscle biopsy showed fibre size and rod variations in a population of hypotrophic muscle fibres expressing slow myosin, often with internal nuclei, and abnormal immune labelling that revealed many hybrid fibres [11].

Family history and clinical study of the parents helped in establishing a diagnosis in the current 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 ruled out and current literature estimates the recurrence risk for *de novo* variants as 0.011% to 28.5% (PMID 30397338). Genetic counseling is both important and recommended to advice on future pregnancies as is thorough monitoring with repetitive ultrasound examinations to assess fetal parameters.

CONCLUSION:

Here we report a case of neonatal nemaline myopathy with a *de novo* mutation c.43G>C (p.Asp15His) in the*TPM3* gene, which can be considered a pathogenic mutation given the supporting clinical scenario and histological picture.

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I certify 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.

Ethical approval:

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

References:

1. Malfatti E, Schaeffer U, Chapon F, Yang Y, Eymard B, Xu R, et al. Combined cap disease and nemaline myopathy in the same patient caused by an autosomal dominant mutation in the TPM3 gene. Neuromuscular Disorders. 2013 Dec 1;23(12):992–7.
2. Marttila M, Lehtokari VL, Marston S, Nyman TA, Barnerias C, Beggs AH, et al. Mutationupdateandgenotype-phenotypecorrelationsofnoveland previously described mutations in TPM2 and TPM3 causing congenital myopathies. Hum Mutat. 2014;35(7):779-790.
3. Sztal TE, McKaige EA, Williams C, Oorschot V, Ramm G, Bryson-Richardson RJ. Testing of therapies in a novel nebulinnemaline myopathy model demonstrate a lack of efficacy. ActaNeuropathologica Communications. 2018 May 30;6(1):40.
4. Bucher HU, Boltshauser E, Briner J (1985) Neonatal nemaline myopathy presenting with multiple joint contractures. Eur J Pediatr 144:288-290

5. Vendittelli F, Manciet-Labarchède C, Gilbert-Dussardier B. Nemaline myopathy in the neonate: two case reports. Eur J Pediatr. 1996 Jun 1;155(6):502–5.
6. https://rarediseases.org/rare-diseases/nemaline-myopathy/
7. Schmalbruch H, Kamieniecka Z, ArroeM (1987) Early fatal nemalinemyopathy:case report and review. Developmentalmedecine and child neurology29:784-804
8. Cassandrini D, Trovato R, Rubegni A, Lenzi S, Fiorillo C, Baldacci J, et al. Congenital myopathies: clinical phenotypes and new diagnostic tools. Ital J Pediatr. 2017 Nov 15;43(1):101.
9. Lehtokari VL, Kiiski K, Sandaradura SA, Laporte J, Repo P, Frey JA, et al. Mutation update: The spectra of nebulin variants and associated myopathies. Hum Mutat. 2014;35(12):1418-1426
10. Yin X, Pu CQ, Wang Q, Liu JX, Mao YL. Clinical and pathological features of patients with nemaline myopathy. Molecular Medicine Reports. 2014 Jul 1;10(1):175–82.
11. Kiiski K, Lehtokari V-L, Manzur AY, Sewry C, Zaharieva I, Muntoni F, et al. A Large Deletion Affecting TPM3, Causing Severe Nemaline Myopathy. J Neuromuscul Dis. 2015 Sep 21;2(4):433–8.
12. Kiiski K, Lehtokari V-L, Manzur AY, Sewry C, Zaharieva I, Muntoni F, et al. A Large Deletion Affecting TPM3, Causing Severe Nemaline Myopathy. J Neuromuscul Dis. 2015 Sep 21;2(4):433–8.
13. Tsujihata M, Shimomura C, Yoshimura T, Sato A, Ogawa T, Tsuji Y, et al. Fatal neonatal nemaline myopathy: a case report. Journal of Neurology, Neurosurgery & Psychiatry. 1983 Sep 1;46(9):856–9.
14. Tan P, Briner J, Boltshauser E, Davis MR, Wilton SD, NorthK, et al. Homozygosityfora nonsense mutation in the alpha-tropomyosin slow geneTPM3 in a patient with severe infantile nemalinemyopathy.NeuromusculDisord. 1999;9(8):573-579.