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Case report

Variants in tropomyosins *TPM2* and *TPM3* causing muscle hypertonia

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ABSTRACT

Patients with myopathies caused by pathogenic variants in tropomyosin genes *TPM2* and *TPM3* usually have muscle hypotonia and weakness, their muscle biopsies often showing fibre size disproportion and nemaline bodies. Here, we describe a series of patients with hypercontractile molecular phenotypes, high muscle tone, and mostly non-specific myopathic biopsy findings without nemaline bodies. Three of the patients had trismus, whilst in one patient, the distal joints of her fingers flexed on extension of the wrists. In one biopsy from a patient with a rare *TPM3* pathogenic variant, cores and minicores were observed, an unusual finding in *TPM3*-caused myopathy. The variants alter conserved contact sites between tropomyosin and actin.

1. Introduction

Muscle hypotonia and weakness are common characteristics of nemaline myopathy (NM) and other myopathies caused by pathogenic variants in one of the tropomyosin genes *TPM2* and *TPM3*. Muscle biopsy findings often include small type 1 fibres and larger type 2 fibres, as well as the presence of nemaline bodies [1].

Here, we describe seven patients in three families who presented with high, not low muscle tone, in some of them causing contractures and trismus, and in most patients accompanied by significant fatigability and/or stiffness hampering their daily lives. Histological features were often unspecific, most of the patients had no nemaline bodies observed on biopsy, and one patient with a pathogenic *TPM3* variant showed a few cores and minicores on biopsy.

2. Case reports

Family 1: Patients 1 and 2

Patient 1 was a 51-year-old female. She was born at term, receiving 8 Apgar points and weighing 3260 g. Since birth, moderately increased muscle tone has been evident in the proximal upper and lower limb muscles. She was followed up at the pediatric neurology unit because of her high muscle tone, early muscle hypertrophy and limitation of joint movements, including the shoulders, hips, wrists and ankles, i.e. a mild arthrogryposis. An electromyography (EMG) showed motor units of short duration in the upper limbs and some fibrillation in the palmaris longus muscle, whilst nerve conduction velocities were normal. A muscle biopsy taken at the age of 3 years from the rectus femoris muscle showed predominance of type 1 fibres.

Her motor milestones were normal, however she maintained toe walking, necessitating Achilles tendon surgery twice. Also, in her twenties, she underwent bilateral foot surgery several times because of her club feet.

The patient was diagnosed with breast cancer at the age of 38 and was found to a carry a variant in one of the breast cancer genes, *BRCA2*.

Neuromuscular examinations performed at age 21 included a second muscle biopsy, showing normal findings. At her first muscle magnetic resonance imaging (MRI) examination, no abnormalities were found in the lower limb muscles. Creatine kinase (CK)

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concentrations were normal, as they had been in childhood, or mildly elevated. Several EMG analyses also gave normal results, but at the age of 35, EMG showed mild myopathic alterations in distal lower limb muscles.

Muscle MRI at age 45 showed disease progression and bilateral fatty infiltration in the soleus muscles, but no confluent degeneration areas. Muscle bulk was small both in the distal and the proximal musculature, compared with the thighs and axial muscles. Mild fatty infiltration was noted in the gastrocnemius, extensor digitorum longus and peroneus muscles.

At neurological examination, the patient was found to have muscle stiffness in the axial muscles and around the mandibular joints, resulting in limitation of mouth opening, trismus. The palate was normally arched. Stiffness and restricted mobility were observed in every joint; there were bilateral contractures of the elbows and in the spine, pelvic region, and both Achilles tendons, especially the left. Active shoulder mobility was 130–140°. The distal joints of her fingers flexed on extension of the wrists. Muscle strength was weaker than normal, especially in the extensors of the upper limbs, apparently partly due to restricted mobility.

Patient 2, the 76-year-old mother of Patient 1, was born with a unilateral club foot and treated with an orthopedic cast. At age 16, she underwent foot surgery. In childhood, she often had jaw lock and pain in the mandibular joint, and at age 20, she underwent bilateral condylotomy. She had felt a slight stiffness of her body all her life and regularly experienced tightness of the hamstring muscles, e.g. on climbing stairs and walking uphill. She had always engaged actively in sports, and felt she required frequent stretching and massage to keep her muscles agile.

At the age of 32, she underwent physical examination because of the unspecified muscle disorder in her daughter. Her spine was noted to be unusually straight, i.e. to lack the normal curvature of lordoses and kyphoses, but had a slight scoliosis in the lumbar region, also seen at X-ray examination. At age 54, lumbar decompressive surgery at level L4–5 was performed.

Neurological examinations were done at age 63, an EMG showing a mild neurogenic pattern in the in the right lower limb, consistent with L5/S1 compression, and no myopathic signs.

Since age 71, Patient 2 noted tremor. Early Parkinson's disease was diagnosed and medication initiated, with good response. A beta-CIT single photon emission computed tomography (SPECT) examination, however, gave normal results. Neurological examination showed the post-operative state of the unilateral club foot, with atrophic changes in the left foot, and limited ankle mobility. Joint stiffness and mild contractures were observed in the elbows. Muscle MRI showed fatty infiltration in the left semimembranosus and biceps femoris muscles, with bilateral hamstring tendon avulsion and oedema. Atrophic changes were observed bilaterally in the hamstring, left gastrocnemius and soleus muscles. CK concentrations were mildly elevated, 399 IU/l

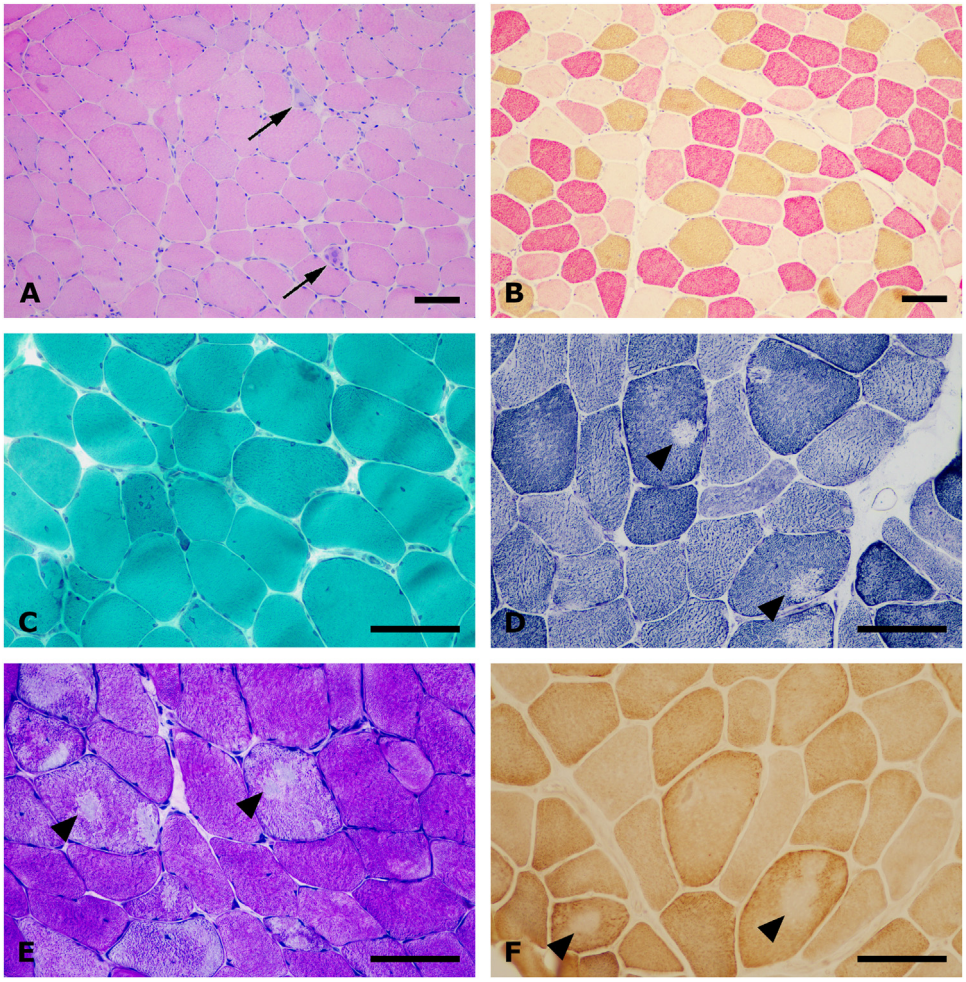

Fig. 1. (a) A muscle biopsy of Patient 3 shows fiber size variation and a few regenerative fibres (arrows)(H&E). (b) Normal fibre typing pattern on immunohistochemical myosin heavy chain double staining (slow fibres DAB, fast fibres red). (c) Gomori trichrome. (d-f) Some fibres showed core-like areas (arrowheads) devoid of enzyme activity and glycogen (d NADH-TR, e PAS, f COX). Scale bar 100 μm.

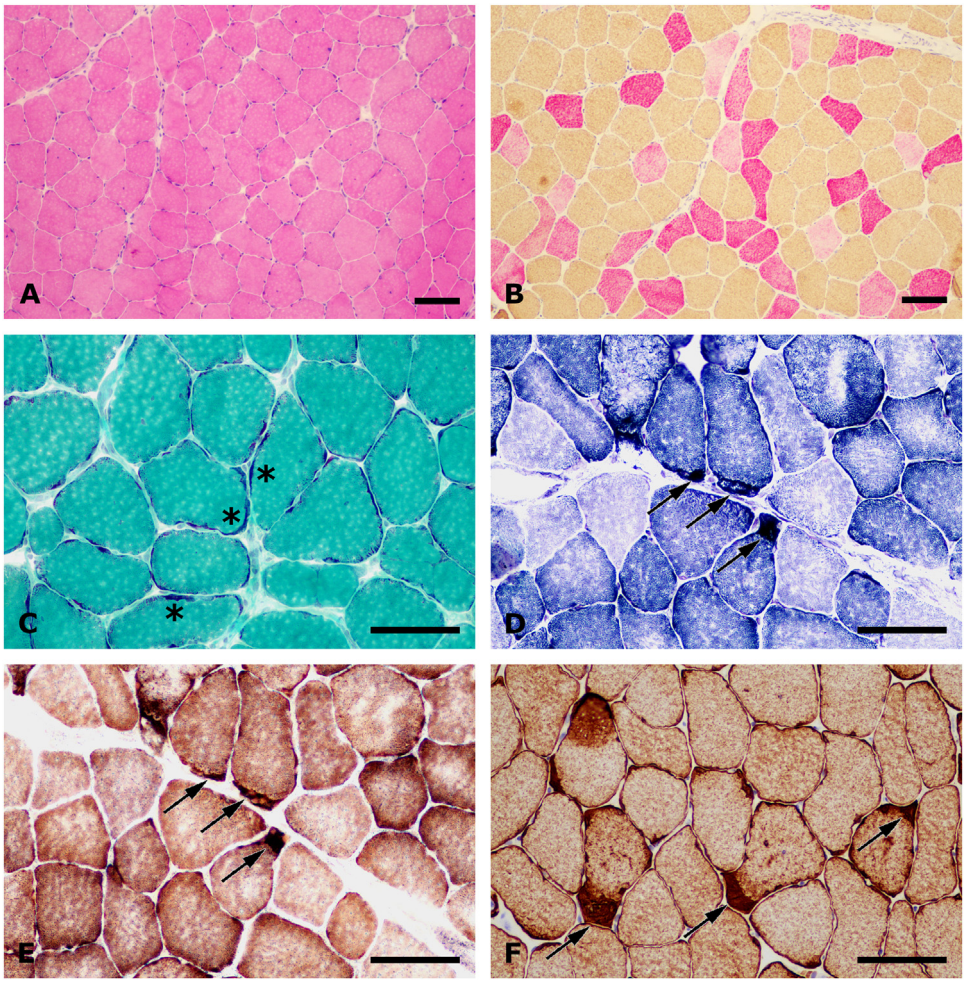

Fig. 2. (a) A muscle biopsy of Patient 6 shows mild fibre size variation (H&E). (b) Immunohistochemical myosin heavy chain double staining shows a normal fibre typing pattern (slow fibres DAB, fast fibres red). (c) Prominent subsarcolemmal mitochondria (asterisks) are seen in some fibres (Gomori trichrome). (d-f). Subsarcolemmal cap structures (arrows) in histochemical stains (d NADH-TR, e COX-SDH, f desmin). Scale bar 100 μm.

(reference values 35–210 IU/l). The patient was still an active golf-player, doing regular exercises.

Family 2: Patients 3–6

Patient 3 was born with bilateral hip luxation. Since childhood, she was followed up because of delayed motor milestones, and tightness of both the hip flexors and Achilles tendons. At the age of 2 years, her walking was described as stiff and clumsy. At the age of 3, her CK was 1710 IU/l, and in later samples between 372 and 683. She also had attention deficit disorder, for which she used regular methylphenidate medication.

At neurological examination at the age of 24, her major symptoms were stiffness, fatigue and pain on exertion. Walking was more difficult in cold weather. She was able to walk some 30 m before having to stop because of muscle stiffness, and after a substantial meal, her chewing muscles were sore. The patient was able to ride a bicycle the 1 km distance to work.

Stooping forward was only possible by flexing the hips with her back straight. Facial expressions were stiff and lumbar mobility was restricted by muscle hypertonia, noted in the thigh and hamstring muscles also. She had trismus. The palate was normally arched. There was hypermobility of the finger joints, while mobility was normal in the other joints. She had mild weakness of the abdominal muscles and mild distal weakness. The grip force in the right hand was 33 kg and on the left 30 kg, i.e. mildly weak. She also had distal muscle weakness in the lower limbs, walking with her legs rotated inwards.

A muscle biopsy was taken at the age of 7 years, and another at the age of 23 years. The latter, from the gastrocnemius muscle, showed chronic myopathic findings, and, on oxidative staining, a few cores and minicores, mainly in type 1 fibres. There were no nemaline bodies (Fig. 1). EMG examination gave normal results.

Patients 4–6

These were the patient's mother, maternal grandmother and sister, presenting with similar features. The mother's biopsy showed ring fibres and nemaline bodies, while the sister's biopsy showed mild myopathic features including variability in fibre size and subsarcolemmal cap structures (Fig. 2). Electron microscopy showed splitting of myofibrils, Z-band streaming and some ring fibres. The grandmother was not available for examination.

Family 3

Patient 7 was a 53-year-old, ambulant female with a congenital myopathy manifesting as mild proximal weakness and fatigability. She had congenital hip dislocations, initially treated conservatively with casting. Gross motor milestones were mildly delayed and she started walking after the age of two years. She was always less athletic than her peers and had trouble swimming and doing her hair because of upper limb muscle fatigability.

In adulthood, total hip replacements were performed because of pain and stiffness due to hip arthritis. Currently, she is unable to get up from a squat position without using her hands, requiring a handrail when climbing stairs. On manual muscle testing she had weakness of hip abduction and neck flexion (Medical Research

Council Scale (MRC) grade 3/5), while shoulder abduction and forearm extension were MRC 4/5. She had trismus and limited flexion of the lower back, but no other contractures. Muscle tone was slightly higher than normal (MDS-UPDRS grade 2), symmetrically in the wrists, ankles, elbows and knees.

A biopsy from the soleus muscle showed variability in fibre size, numerous internal nuclei, some cytoplasmic bodies and no nemaline bodies.

Molecular genetic findings

Family 1: The genetic analysis of Patient 1 revealed an NM_213,674.1:c.541_542inv, NP_001288156.1:p.(Glu181Arg) in the *TPM2* gene, which had been previously published for the same patient [2]. Here, we found the same variant in Patient 2. The variant is not present in The Genome Aggregation Database (gnomAD) and is predicted to be pathogenic. Combined Annotation Dependent Depletion (CADD 1.6) score for c.541G > *A* is 28.4, and the affected amino acid is highly conserved.

Family 2: Using the HUS Diagnostic Center gene panel for muscular dystrophies and myopathies, a missense variant, NM_152,263.4:c.301G > *A*, NP_689,476.2:(p.Asp101Asn), was identified in the *TPM3* gene in Patient 3. It is predicted to be pathogenic (CADD 1.6: 25.3, Rare Exome Variant Ensemble Learner REVEL: 0.69). The variant was analyzed and subsequently identified in the samples of all the affected family members.

Family 3: A neuromuscular gene panel, Myocap, was performed in Patient 7 and a different nucleotide change, altering the same amino acid as that in Family 2, was identified in the *TPM3* gene (CADD 1.6: 27.6, REVEL 0.963), leading to a different amino acid change (c.301G > *T*, p.Asp101Tyear). Segregation analysis using samples from the healthy parents confirmed that the variant had arisen *de novo*.

Neither of the *TPM3* variants were found in gnomAD.

3. Discussion

We describe a small series of patients with tropomyosin variants in whom muscle tone was high, not low, as is usually the case in patients with pathogenic variants in these genes for sarcomeric proteins.

In a paper compiling the clinical details of all patients then known with pathogenic variants in *TPM2* or *TPM3*, trismus and stiffness were very rare features of *TPM2*-mutated patients, and not seen with *TPM3* variants [3]. A further clinical feature, unusual in tropomyosin disease, was the bilateral congenital hip luxation in two patients.

The *TPM2* variant p.(Glu181Arg) identified in Family 1 has not been noted in NM previously, but another variant affecting the same codon (p.Glu181Lys) has been seen in a patient with NM, who also had a hypercontractile molecular phenotype [4,5]. Glu181 in tropomyosin is known to interact with Arg147, Lys326 and Lys328 in actin [6]. Further *TPM* variants causing hypercontractility have been reported, including single glutamic acid deletions in *TPM3* and the recurrent variant K7del in *TPM2* [7,8]. Interestingly, a p.Lys328Asn variant in the skeletal muscle actin gene, *ACTA1,* has been reported to cause NM with muscle hypertonia [9].

In *TPM3*, the p.(Asp101Asn) variant identified in Family 2 changes the residue Asp101, also known to interact with Lys328 in actin [6]. Another variant in *TPM3*, p.(Arg168His), described by Munot et al., caused stiffness of the spine, but the stiffness was not worsened by cold [10] as in Patient 3 of Family 2. Arg168 in tropomyosin is known to interact with Asp25 in actin [6].

Patient 3 had a small number of cores in her biopsy, however not warranting the classification of core-rod myopathy. To our knowledge, no other patients with pathogenic *TPM3* variants have been observed to have cores and minicores on biopsy, while core-rod myopathy has been previously described in one patient with *TPM2*-caused myopathy [11]. Commonly, patients with pathogenic tropomyosin variants have had nemaline bodies and fibre type disproportion, whereas in this series, these features were only present in one of the patients. Ring fibres, rare in NM, were present in two of the patients. A common denominator for the myopathy patients with high muscle tone described herein is that their tropomyosin variants reside in contact sites between actin and tropomyosin, causing a hypercontractile molecular phenotype.

Declaration of competing interest

The authors declare no competing interests.

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