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Myopathy in a woman and her daughter associated with a novel splice site *MTM1* mutation

Carola Hedberg [a,*], Christopher Lindberg [b], Gyöngyvér Máthé [a], Ali-Reza Moslemi [a], Anders Oldfors [a]

[a] *Department of Pathology, Institute of Biomedicine, Sahlgrenska Academy at University of Gothenburg, Gothenburg, Sweden*
[b] *Department of Neurology, Institute of Neuroscience and Physiology, Sahlgrenska Academy at University of Gothenburg, Gothenburg, Sweden*

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Abstract

We have investigated a woman and her daughter with an early onset, slowly progressive myopathy. Muscle biopsy showed in both cases severe atrophy with marked fatty replacement. Frequent fibers with internalized nuclei were present but no typical features of centronuclear myopathy. There were also many fibers with deep invaginations of the plasma membrane. The presence of necklace fibers provided clue to correct genetic diagnosis. Both patients had a novel heterozygous splice site mutation in the myotubularin gene, *MTM1* (c.867+1G>T). Analysis of *MTM1* cDNA revealed that the mutation resulted in aberrant splicing with variable exon skipping. The expression of normal transcripts was markedly reduced and there was reduced expression of myotubularin protein. Although the expression of the allele without the mutation was reduced we did not obtain evidence of skewed X-chromosome inactivation. Other factors than skewed X-inactivation may cause allele inactivation and manifestation of severe myopathy in heterozygous carriers of pathogenic *MTM1* mutations.

Keywords: Centronuclear myopathy; Myotubularin; *MTM1*; X-linked recessive myotubular myopathy; Manifesting carriers; Necklace fibers

1. Introduction

Centronuclear myopathy (CNM) is a group of congenital myopathies, characterized by the presence of central nuclei in the muscle fibers. Inheritance may be X-linked recessive, autosomal dominant or autosomal recessive depending on the causative gene [1]. So far identified genes associated with CNM include *MTM1* [2], *DNM2* [3] and *BIN1* [4]. X-linked recessive myotubular myopathy (XLMTM; OMIM ID #310400) is due to mutation in *MTM1*, which encodes myotubularin, a protein that belongs to a family of putative tyrosine phosphatases. Myotubularin is required for muscle cell differentiation and is also a potent phosphatidylinositol 3-phosphate (PI3P) phosphatase [2,5,6]. Myotubularin has also been reported to interact with desmin to regulate mitochondrial dynamics and morphology [7]. XLMTM usually affects boys and in most cases presents with severe neonatal onset of hypotonia and muscle weakness. Female carriers are usually asymptomatic but may show muscle weakness possibly due to skewed X-inactivation [1].

In a recent study it was demonstrated that a characteristic morphological alteration described as necklace fibers could be used as a histological marker to identify manifesting carriers of *MTM1* mutations [8].

In this study we describe a novel mutation in the *MTM1* gene in a familial myopathy with severely affected women

* Corresponding author. Address: Department of Pathology, Institute of Biomedicine, Sahlgrenska Academy at University of Gothenburg, 413 45 Gothenburg, Sweden.
E-mail address: carola.hedberg@gu.se (C. Hedberg).

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in two generations but no evidence of skewed X-inactivation. The clue to the diagnosis was the presence of necklace fibers in muscle biopsy.

2. Materials and methods

2.1. Patients

Two patients were examined clinically, a woman (Patient II:2) and here daughter (Patient III:7) (Fig. 1). The proband (Patient II:2) had mild leg weakness since childhood manifesting as frequent stumbles and falls, and onset of upper extremity weakness at age 20. She has slowly lost muscle strength in her lower extremities and had to use a roller since age 45 and has been confined to a wheelchair since age 66. Her muscle weakness was in part asymmetric, as she was significantly weaker in several of the muscles groups in her left arm and left knee extensors. Her vital capacity has been reduced and at age 69 it was below 1.0 L (35% of predicted) and she had slightly elevated nocturnal pCO2 levels. Mild dysarthria has been observed. At age 71 she was found to have atrial fibrillation. Her serum CK level was slightly elevated to 4.5 μkat/L (normal < 3.6). Genetic analysis for myotonic dystrophy type 1 and 2 were negative. Her daughter (Patient III:7) was first seen at age 43. She was then found to have mild to moderate facial, neck flexor and handgrip weakness. Her elbow flexors and extensors and the wrist extensors were weaker in her left side, but she was strong in the lower extremities. She had a mild symmetric weakness in her hands. Her vital capacity was 2.8 L (80% of predicted). Her serum CK was normal (1.3 μkat/L, normal < 3.6). Neither of the patients had ptosis, ophtalmoplegia or calf hypertrophy. They both had spontaneous muscle fibre activity (fibrillations and positive sharp waves) in combination with signs of myopathy (short motor unit action potentials) on EMG. Echocardiography has been performed in both patients with normal findings. Patient II:2 has given birth to five children and two of them died within the first hours. A girl (III:1) born at home was preterm at 34 weeks of gestation. She had severe seizures and died in the ambulance on the way to the local hospital. Three years later a boy (III:6) was born, also at home, at term, but after a very short labour. He was severely weak, and he also died in the ambulance on the way to the local hospital. No post mortem examinations were performed. Several members of the family have had cataract surgery (II:2, III:7, III:4, I:1 and two other family members not included in the pedigree). A brother of patient III:7 (individual III:3) had cataract surgery at early age but he has no muscle weakness indicating that the muscle disease is not associated with cataract in the family.

2.2. Morphological analysis

Open muscle biopsy of the deltoid muscle was performed in both patients. Specimens were either frozen in propane chilled by liquid nitrogen for histochemical analysis or fixed in glutaraldehyde for electron microscopy. Standard techniques were applied for histochemical staining of cryostat sections and for electron microscopy.

2.2.1. MTM1 gene analysis

Genomic DNA was isolated from either the muscle biopsy sample or blood sample by standard methods. The entire coding sequence and intron–exon boundaries of *MTM1* (NM_000252.2) were analyzed in patients using

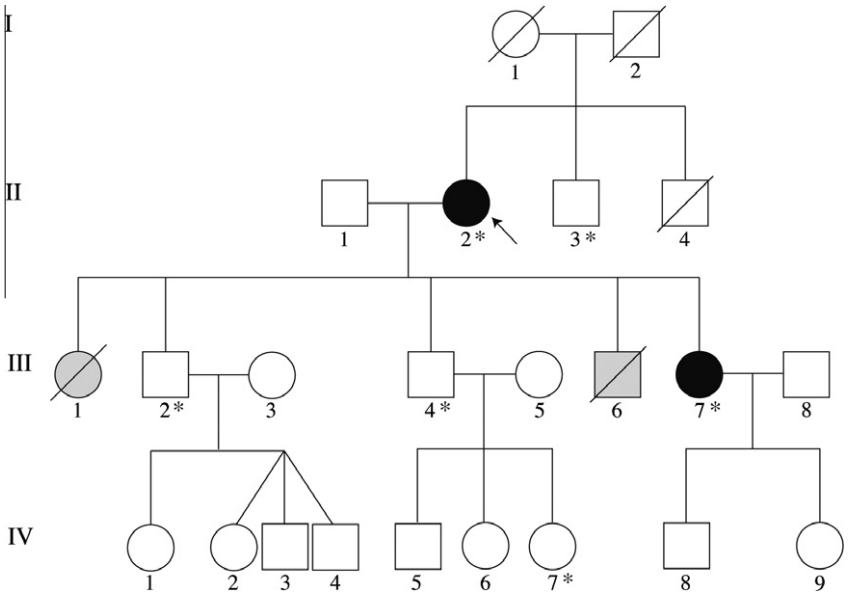

Fig. 1. Pedigree of family. Black solid symbols = affected. Proband II:2 is indicated by arrow. Grey solid symbols = individuals III:1 and III:6 how died at early infancy. *Individuals clinically tested for muscle weakness.