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Case report

Severe nemaline myopathy associated with consecutive mutations E74D and H75Y on a single *ACTA1* allele

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ABSTRACT

Nemaline myopathy is among the most common congenital myopathies. We describe for the first time a novel double *de novo* mutation in two adjacent codons resulting in two amino acid changes E74D and H75Y in the *ACTA1* gene. The hypotonic male infant was the first son of healthy unrelated parents with no family history of neuromuscular disorders. Pregnancy was complicated: decreased fetal movements were noted on the 25th week of gestation, premature labour pains were present from the 29th week onwards and because of breech presentation a Caesarian section was carried out in the 39th week. The patient presented with multiple congenital fractures and joint contractures. He was dependent on ventilatory support until his death at 2 months.

Muscle biopsy revealed severely atrophic and rounded muscle fibers with considerable variation in diameter and pronounced disorganization of the myofibers. Electron microscopy indicated a distinct disturbance of the myofibrillar architecture and nemaline rods. In view of previously described cases carrying different single missense mutations of the amino acid residues E74 or H75, we suggest that the particular genotype E74D/H75Y is compatible with the severity of the patient's phenotype. The possibility of germ cell mosaicism should be taken into account in genetic counseling.

1. Introduction

Nemaline myopathy (NM) is a congenital muscle disease characterized by signs of muscle weakness accentuated in the proximal muscles, as well as facial, bulbar and respiratory impairment. Morphological hallmarks of this myopathy include variations in size and architecture of the muscle fiber types, threadlike cytoplasmic and intranuclear rods and actin aggregates in the muscle biopsy [1]. Based on the severity and onset of disease symptoms, NM is clinically classified into six types [2].

Several genes may cause NM when mutated, including the slow α-tropomyosin gene (*TPM3*), the Nebuline gene (*NEB*), the slow troponin T gene (*TNNT1*), the β-tropomyosin gene (*TPM2*) and the alpha-actin gene (*ACTA1*) [3] but these genetic defects show overlapping phenotypes and clinical features.

The *ACTA1* gene is mutated in 20–25% of patients presenting with NM [2]. Patients carrying a mutation in this gene tend to present a more severe clinical phenotype than patients who carry a mutation in the nebulin gene, even though the latter is responsible for the majority of NM cases [4]. Here we report a double *de novo* mutation in adjacent amino acids (Glu74Asp and His75Tyr) of the *ACTA1* gene that causes a severe clinical phenotype in the patient.

2. Case report

A hypotonic male infant was born to a primigravida mother. A younger sister is healthy who was born nearly two years later. The patient was the first son of healthy unrelated parents with no family history of neuromuscular disorders. Pregnancy was complicated: decreased fetal movements were noted on the 25th week of gestation, premature contractions were present from the 29th week onwards and because of breech presentation a Caesarian section was carried out in the 39th week.

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Weight and head size were normal (3300 g and 36 cm, respectively) but height was below normal range (47 cm, 1 cm below third percentile). Of note were the lack of facial expression and the absence of spontaneous movements (Fig. 1A). Congenital fractures of both femurs (Fig. 1B) and the right arm, bilateral chylothorax, contractures of hands and shoulder joints were noted. A cerebral haemorrhage and a subdural haematoma in the left hemisphere were observed on cerebral MRI (data not shown).

Creatine kinase was normal and EMG analysis showed no spontaneous activity. Following surgical treatment of the fractures and draining of the chylothorax, intubation and artificial respiration were needed due to respiratory failure. The patient died at 2 months.

In order to establish a diagnosis, a muscle biopsy from the right quadriceps muscle was performed (Fig. 2). Routine histology and histochemistry analyses (NADH, ATPase, SDH, COX, MADA, SP, AP, Oil red O, PAS) showed severely atrophic and rounded muscle fibers with considerable variation in diameter (3–15 μm). There was no inflammatory infiltrate but marked increase of endomysial connective tissue (Fig. 2A). Pronounced disorganization of the myofibrils was noted, which tended to aggregate. Ragged red fibers were absent. Electron microscopy revealed a distinct disturbance of the myofibrillar architecture and nemaline rods (Fig. 2B). Intranuclear rods were not detected.

The tentative diagnosis of a severe nemaline myopathy was made on account of the biopsy and the symptom complex. *ACTA1* gene analysis was performed. Genomic DNA was isolated from whole blood of the patient and his parents. Exons 1–7 of the *ACTA1* gene (GenBank Accession No. AF182035) and flanking intronic sequences were amplified by polymerase chain reaction (PCR). Primer sequences of exon 2 F (forward), 3 F and 4 F as well as exon 1 R (reverse), 4 R, 6 R were used as described [5] except for the prolongation of exon 5 R with an adenine at the 5′ end of the sequence. The remaining primers were designed as follows: exon 1 F, 5′-GGCTCTAGTGCCCAACACCCAA-3′; exon 2 R, 5′-ACGTAGGAATCTTTCTGACCC-3′; exon 3 R 5′-GCCCTCATAAATGGCACGTTGTGGG-3′; exon 5 F, 5′-TCACTGAGCGTGGCTACTCC-3′; exon 6 F 5′-TCCTCCCTGGAAAAGAGCTACGAGCTGCCA-3′; exon 7 F 5′-AGCACCATGAAGATCAAGG-3′, exon 7 R 5′-CTGTGTCAGTTTACGATGGCAGC-3′.

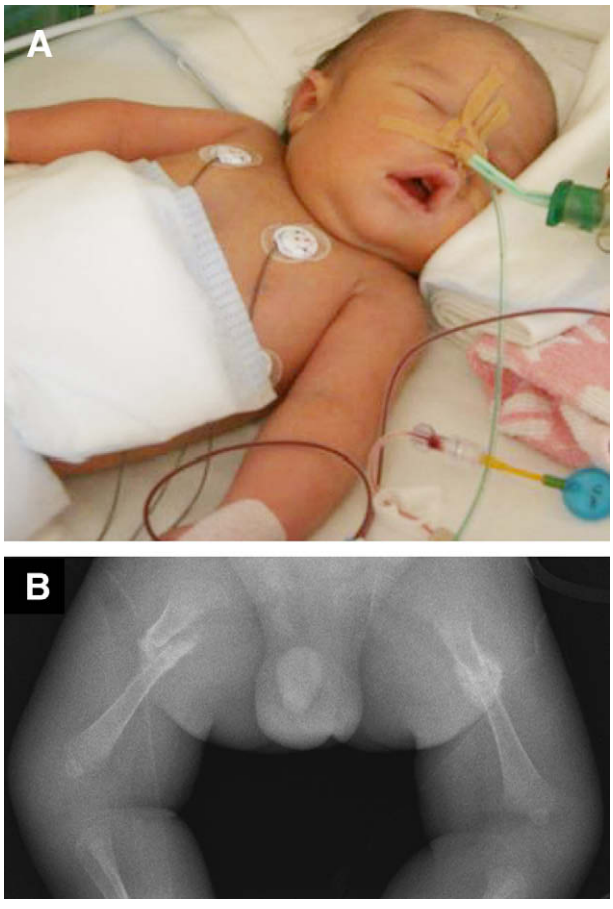

Fig. 1. (A) Patient at 3 weeks of age. Of note were the lack of facial expression and the absence of spontaneous movements. (B) X-ray examination revealed congenital fractures of both femurs.

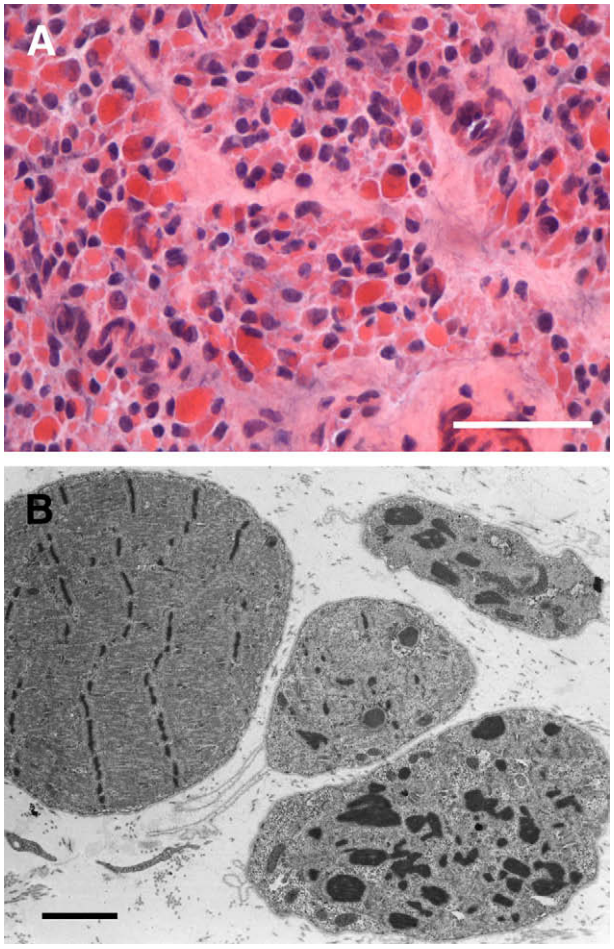

Fig. 2. Muscle histopathology: (A) H&E staining showed severely atrophic and rounded muscle fibers with considerable variation in diameter (3–15 μm). An inflammatory infiltrate was absent but there was marked increase of endomysial connective tissue. Pronounced disorganization of the myofibrils was detected, which tended to aggregate. Size bar 50 μm. (B) Electron microscopy revealed a pronounced disturbance of the myofibrillar architecture and nemaline rods. Size bar 2 μm.

PCR cycling conditions are available from the authors on request. PCR products were analyzed by direct sequencing.

We identified two heterozygous missense mutations in adjacent nucleotides in exon 3 g.2007G > T (c.222G > T) and g.2008C > T (c.223C > T) (Fig. 3), affecting adjacent codons and resulting in two amino acid changes: glutamic acid to aspartic acid (E74D) [6] and histidine to tyrosine (H75Y) [3]. Either mutation has been described previously. However, to our knowledge this is the first time that they are reported in the same patient.

To verify whether the mutations were on the same allele or on different alleles, we cloned the mutated exon. The patient's exon 3 of the *ACTA1* gene was amplified by PCR and ligated into the plasmid vector pCR®2.1 using the TA Cloning® Kit (Invitrogen,