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Severe nemaline myopathy caused by mutations of the stop codon of the skeletal muscle alpha actin gene (*ACTA1*)

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Abstract

Most nemaline myopathy patients have mutations in the nebulin (*NEB*) or skeletal muscle α-actin (*ACTA1*) genes. Here we report for the first time three patients with severe nemaline myopathy and mutations of the *ACTA1* stop codon: TAG > TAT (tyrosine), TAG > CAG (glutamine) and TAG > TGG (tryptophan). All three mutations will cause inclusion of an additional 47 amino acids, translated from the 3′ UTR of the gene, into the mature actin protein. Western blotting of one patient's muscle demonstrated the presence of the larger protein, while expression of one of the other mutant proteins fused to EGFP in C2C12 cells demonstrated the formation of rod bodies.

Keywords: Nemaline myopathy; Skeletal muscle alpha actin (*ACTA1*); Stop codon mutations

1. Introduction

Nemaline myopathy, characterised by the presence of rod-like nemaline bodies in skeletal muscle tissue, varies from severe fatal to mild adult phenotypes [1]. Mutations in five skeletal muscle thin filament protein genes are associated with the disease: nebulin (*NEB*), α-tropomyosin (*TPM3*), β-tropomyosin (*TPM2*), troponin T (*TNNT1*) and skeletal muscle α-actin (*ACTA1*) [2]. *ACTA1* mutations are responsible for about 20% of nemaline myopathy cases, but a preponderance of severe cases [1,2], and also cause other disease pathologies [2,3]. Expression of mutant actin fused to enhanced green

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fluorescent protein (EGFP) in C2C12 muscle cells results in tissue culture models mimicking the histopathology in patients [4]. For example, mutations causing intranuclear rods in patients produce intranuclear bodies in transfected cells [4].

Until now, despite over 120 different mutations being detected in *ACTA1* ([5] and unpublished observations), no mutations have been identified affecting the *ACTA1* stop codon. Here we report three patients, one Hungarian, one Japanese and one Spanish with *ACTA1* stop codon mutations and severe nemaline myopathy. Western blotting of one patient's muscle shows the presence of a larger protein, while transfection into C2C12 muscle cells of another of the mutant proteins fused to EGFP resulted in the formation of rod bodies *in vitro*.

2. Patients and methods

2.1. Patient clinical descriptions

2.1.1. Patient A – Hungarian

During pregnancy, polyhydramnios and reduced foetal movements were noted. The male baby was born at 38 weeks of gestation by Caesarean section. Birth weight was 2490 g. Apgar scores were 0 and 1. The neonate was floppy; he was intubated and mechanically ventilated (Fig. 1). Severe hypotonia, muscular atrophy, arthrogryposis, hypoplastic thorax, high palate, open mouth and a dislocated fracture of the right humerus were noted. Only extraocular muscles appeared to be spared. A muscle biopsy was taken at 1 month of age. There was no clinical improvement within the first months of life, with the patient requiring mechanical ventilation and tube feeding. Muscular atrophy and arthrogryposis progressed. There was no family history suggestive of neuromuscular disorders. Genetic testing for spinal muscular atrophy was negative.

The patient is currently alive at 1 year of age, though he requires permanent mechanical ventilation. Enteral feeding is maintained via a PEG tube. He weighs approximately 6 kg.

2.1.2. Patient B – Japanese

This male baby was born at 33 weeks of gestation by Caesarean section. Apgar scores were 1 and 6. He was floppy and needed intubation and ventilatory support from birth as his respiration was very weak. When biopsied at eight months, he had marked muscle weakness and hypotonia, including respiratory muscle insufficiency and could only slightly move his fingers, hands, legs and feet. Tendon reflexes were diminished. Facial muscles were involved and a high-arched palate was seen. His face looked apathetic. He also had low-set ears and micrognathia. There was no family history suggestive of neuromuscular disorders. His karyotype was normal. Genetic testing for myotonic dystrophy and spinal muscular atrophy were negative. Nerve conduction velocity in the median nerve was 46.0 m/s.

2.1.3. Patient C – Spanish

This female patient was born at 33 weeks of gestation in a family with no history of neuromuscular disorders. During pregnancy polyhydramnios and an almost absence of fetal movements were noted. Birth weight was 1320 g. The neonate was floppy and needed ventilatory support. The patient had severe hypotonia and there were only minimal, distal spontaneous movements in the limbs. Facial muscles were also involved and enteral feeding was needed. Tendon reflexes were absent.

Her karyotype was normal. Genetic testing for spinal muscular atrophy and myotonic dystrophy were negative.

The patient is currently alive at 5 years of age, though she requires permanent mechanical ventilation and enteral feeding but shows a slight improvement in distal movements.

2.2. Methods

2.2.1. Pathology

The pathology laboratory associated with each patient clinical group performed their standard procedures on the muscle biopsies taken.

2.2.2. Molecular analysis

The skeletal muscle α-actin gene *ACTA1* was amplified from patient genomic DNA and sequenced as previously described [3,6,7].

2.2.3. Western blotting

Total muscle protein was extracted using SDS lysis buffer (1% SDS, 10 mM Tris pH 7.4). Lysates equivalent to 20 μg total protein were fractionated by SDS–Tricine–PAGE [8], and transferred to nitrocellulose. The membrane was probed with a mouse monoclonal antibody to sarcomeric actin (5C5, Abcam, Cambridge, UK, 1:500) followed by rabbit anti-mouse immunoglobulins coupled to horseradish peroxidase (P0260, DAKO, Denmark, 1:10,000). Specific protein bands were visualized using ECL (Amersham, Buckinghamshire, UK).

2.2.4. Tissue culture transfection studies

The construct "*ACTA1* + 47/EGFP" containing the mutation in patient B was made as described [4]. The wild-type *ACTA1* cDNA sequence was amplified from normal human muscle cDNA using a reverse primer situated over the position where the activated 3′ UTR in-frame stop codon would be (5′-GAATTCGTAA ACACTGTGTCAGTTTACG). The normal *ACTA1*