

Case report

Severe congenital actin related myopathy with myofibrillar myopathy features

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Abstract

Mutations in *ACTA1* have been associated with different pathologic findings including nemaline myopathy, intranuclear rod myopathy, actin myopathy, cap myopathy, congenital fiber type disproportion, and core myopathy. Myofibrillar myopathies are morphologically distinct but genetically heterogeneous muscular dystrophies arising from mutations in Z-disk related proteins. We report a 26-month-old boy with significantly delayed motor development requiring mechanical ventilation and tube-feeding since birth. The muscle biopsy displayed typical features of myofibrillar myopathy with abnormal expression of multiple proteins. Whole exome sequencing revealed two-amino-acid duplication in *ACTA1*. In cell culture system, mutant actin was expressed at ~11% of wild-type, and mutant actin formed pleomorphic cytoplasmic aggregates whereas wild-type actin appeared in filamentous structures. We conclude that mutations in *ACTA1* can cause pathologic features consistent with myofibrillar myopathy, and mutations in *ACTA1* should be considered in patients with severe congenital hypotonia associated with muscle weakness and features of myofibrillar myopathy.

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1. Introduction

Myofibrillar myopathies (MFM) represent a group of muscular dystrophies with a similar morphologic phenotype characterized by a distinct pathologic pattern involving disintegration of the Z-disk followed by degradation of myofibrils, accumulation of myofibrillar degradation products, and ectopic expression of multiple proteins and sometimes congophilic material [1,2].

Mutations in skeletal muscle α -actin (*ACTA1*) gene have been associated with diverse congenital myopathies including nemaline myopathy [3], intranuclear rod myopathy [3], actin myopathy [3], cap myopathy [4], fiber type disproportion [5], and core myopathy [6]. In some patients multiple pathologic features can coexist. This report describes a child with severe muscle weakness with pathologic features consistent with MFM in muscle caused by a two-amino-acid duplication in *ACTA1*.

2. Patient and methods

2.1. Patient

The patient was investigated at age 26 months. The left vastus lateralis muscle was biopsied. Genomic DNA was isolated from the frozen muscle specimen. All studies were in accord with the guidelines of the Mayo Institutional Review Board.

2.2. Histochemistry and immunostains

Conventional histochemical studies were performed and congophilic deposits were visualized as described [7].

Six to 10- μ m-thick cryostat sections were treated with monoclonal antibodies against desmin (Dako, Carpinteria, CA), α B-crystallin (Stressgen, Ann Arbor, MI), myotilin (Novocastra, Bannockburn, IL), dystrophin (Novocastra), and neural cell adhesion molecule (NCAM) (Cell Sciences, Canton, MA). The immunoreactive sites were then visualized with appropriate secondary antibodies using immunoperoxidase and immunofluorescence methods, as previously described [8]. Adjacent sections in the series were stained with trichrome. Controls consisted of replacement of antibodies with nonimmune IgG of the same subclass and concentration as the primary antibody.

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2.3. Mutation analysis

Exome sequencing of genomic DNA was performed at the Mayo Clinic. The paired-end libraries were prepared following the manufacturer's protocol (Illumina and Agilent) using 1.1 µg of genomic DNA. Whole exome capture was carried out using the protocol for Agilent's SureSelect Human All Exon v.2 kit. The identified putative variants were scrutinized with Ingenuity Variant Analysis software (Qiagen, Redwood City, CA). Variants at intergenic and intronic sites, and genes not expressed in skeletal muscle and spinal cord based on the GEO database (<http://www.ncbi.nlm.nih.gov/geo/>) were excluded. The identified mutation was confirmed by Sanger sequencing. Nucleotides of *ACTA1* cDNA were numbered according to GeneBank accession number [NM_001100.3](#).

2.4. Expression studies

cDNA isolated from the patient's frozen muscle was used to amplify the entire coding region of *ACTA1* and to clone it into the pmCherry-N1 vector (Clontech, Mountain View, CA) with the mCherry-tag at the C-terminal. COS-7 and C2 cells were transfected with wild-type and mutant *ACTA1* containing plasmids using Lipofectamine LTX with Plus reagent (Thermo Fisher Scientific, Waltham, MA). COS-7 cells were harvested after 24 and 48 hours of transfection, and C2 cells were harvested after 24 hours of transfection and 2–4 days of differentiation. All experiments were done in triplicates. For the morphologic assessment, COS-7 and C2 cells were plated on 2% gelatin coated glass coverslips and transfected with mutant or wild type pmCherry-tagged *ACTA1* cDNA. The C2 cells were differentiated one day after transfection and evaluated at days 2–4 of differentiation after rinsing with PBS and fixing with 2% paraformaldehyde. The preparations were examined with a Zeiss Axiovert epifluorescence microscope using apotome optics, AxioVision 4.4 software, and a 63× objective (numerical aperture 1.4).

Extracts of transfected cells were immunoblotted with an mCherry (Clontech) antibody and with a GAPDH antibody (EMD Millipore, Billerica, MA) to control for loading. The blots were developed by the alkaline phosphatase method and quantitated using NIH Image 1.63.

3. Results

3.1. Clinical findings

A 26-month-old boy presented with hypotonia, significant delay in motor milestones, and marked muscle weakness since birth with mild improvement over time. His arms were more severely affected than the legs. He was mechanically ventilated since birth and G-tube fed. He tolerated being off the ventilator for up to 8 hours during the day. His speech was delayed but he was able to repeat words and communicate with his parents. Previous evaluations included two muscle biopsies; one was interpreted as displaying type 2 fiber atrophy and the other to show neurogenic atrophy. These specimens were not available for review. The patient had a mild generalized aminoaciduria. The brain MRI, and the serum creatine kinase level,

karyotyping, chromosomal microarray, analysis of the myotonic dystrophy and survival motor neuron genes, as well as the Prader–Willi methylation test were negative or normal. The patient was treated with pyridostigmine for a possible congenital myasthenia. On examination, he had cup-shaped low-set ears and contractures at the metacarpophalangeal and proximal interphalangeal joints involving most fingers. He had bifacial diplegia, high arched palate, but good head control, and could sit with minimal support. The tendon reflexes were hypoactive. The EMG showed prominent fibrillation potentials and rapidly recruiting small motor unit potentials with occasional complexity. The patient died at the age of 3 years and 2 months.

3.2. Histochemistry and immunostaining findings

A repeat muscle biopsy at 26 months of age showed abnormal variation in fiber size, fiber splitting, an increase in internal nuclei, vacuolar change, focal areas of myofibrillar disorganization or hyaline structures (Fig. 1A), several fibers with “punched-out” regions of oxidative enzyme activity (Fig. 1B), scattered necrotic and regenerating fibers, and marked increase of endomysial and perimysial fibrous connective tissue (Fig. 1A). Many structurally abnormal fibers displayed ectopic or abnormal expression of desmin (Fig. 1C), αB-crystallin (Fig. 1D), myotilin (Fig. 1E), dystrophin (Fig. 1F) and NCAM.

3.3. Mutation analysis

Whole exome sequencing revealed a previously published in-frame two-amino-acid insertion c.437_442dupCCTCCG predicting p.146_147dupAlaSer in *ACTA1*. The observed variant is not present in NHLBI GO Exome Variant Server (NHLBI GO Exome Sequencing Project (ESP), Seattle, WA, 10/2013; URL: <http://evs.gs.washington.edu/EVS>).

3.4. Expression studies

Although the mutant cDNA isolated from patient muscle was present in 6 out of 8 clones, the expression of the mutant-actin at the protein level was ~11% of the wild-type-actin in both COS7 and C2 cells (Fig. 2). Despite its low level of expression, the mutant actin displayed a strong fluorescent signal emanating from large pleomorphic cytoplasmic aggregates (Fig. 3B) whereas cells expressing the wild-type actin displayed mostly filamentous structures (Fig. 3A).

4. Discussion

Most MFM patients present after the fourth decade and worsen slowly, but patients carrying the Pro209Leu mutation in BAG3 [9] present in childhood, and some with desmin mutations present in the first or second decade and can die early due to cardiomyopathy [10]. More recently, recessive mutations in *CRYAB* were found to cause the fatal infantile hypertonic muscular dystrophy [11,12]. The affected infants present shortly after birth with progressive limb and axial muscle stiffness, develop severe respiratory insufficiency, and most die in the first year of life. Their muscle specimens show typical

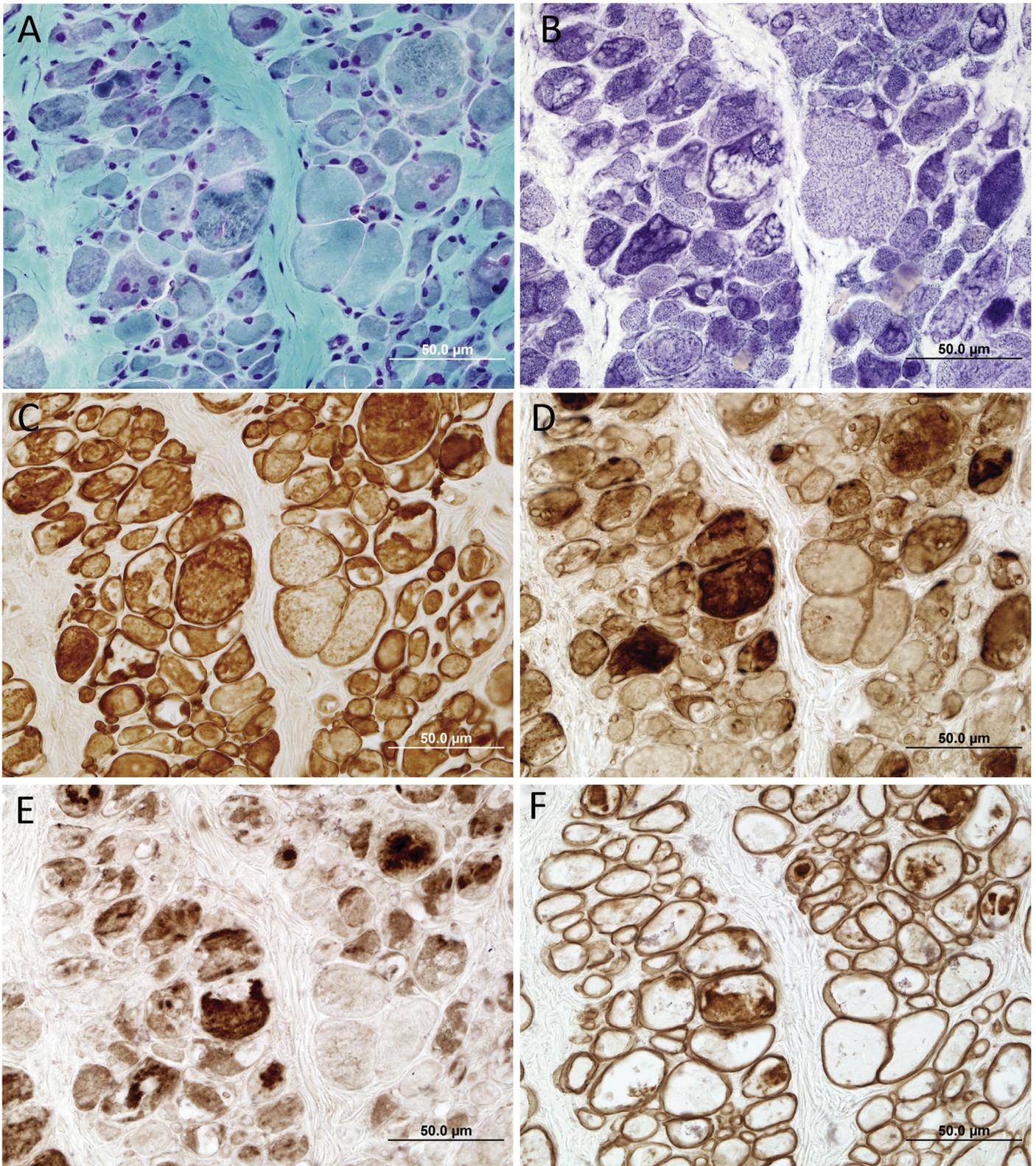


Fig. 1. Characteristic structural abnormalities in trichrome (A) and NADH dehydrogenase (B) stained sections. Nonconsecutive sections in the same series are immunoreacted for desmin (C), α B-crystallin (D), myotilin (E), and dystrophin (F). Note abnormal accumulation of each protein in the structurally abnormal fibers.

features of MFM but α B-crystallin expression is absent. Our patient presented at birth with severe muscle weakness, respiratory insufficiency and dysphagia. Because of the typical features of MFM in the muscle biopsy, we first sequenced the

previously identified MFM disease genes but found no mutation. Next, using the exome sequencing, we identified a two-amino-acid duplication mutation in *ACTA1*. This duplication was previously reported in a similarly affected

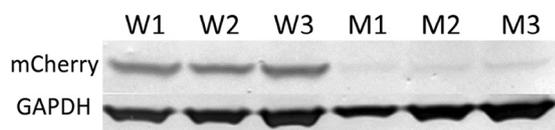


Fig. 2. Triplicate immunoblots demonstrating expression of wild-type (W) and mutant (M) ACTA1 transcripts in C2 cells. Expression levels are normalized for GAPDH. Note markedly reduced expression of the mutant-actin.

infant who died at age 5 months of respiratory failure. That patient, like our patient, had severe muscle hypotonia, bifacial weakness, and arthrogyrosis but also showed hirsutism, micrognathia, and ventricular hypertrophy. The muscle fibers had large deposits of thin filaments that did not react for desmin or vimentin. Therefore this case was later classified as actin myopathy [13,14]. Our patient displayed abnormal and ectopic expression of multiple proteins including desmin as well as necrotic and regenerating fibers and increased connective tissue consistent with the diagnosis of MFM.

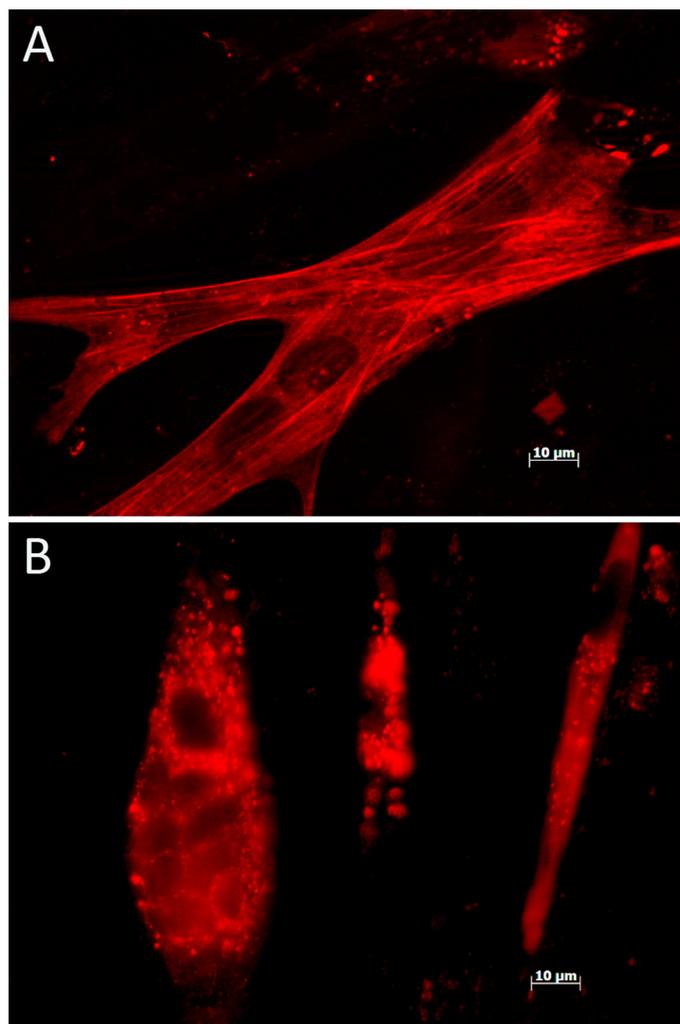


Fig. 3. C2 cells transfected with mCherry-labeled wild-type (A) and mutant ACTA1 (B) and differentiated for 3 days. Wild-type actin forms a filamentous network; mutant actin appears in pleomorphic granules. Apotome optics, 0.43 µm slice distance.

Skeletal muscle α -actin comprises 20% of the total muscle mass and consists of monomeric globular G-actin, and polymeric filamentous F-actin. The dynamic equilibrium between these two conformations plays an important role in cell motility, and location and transport of proteins within the cell. G-actin is composed of two domains and each domain consists of two subdomains. Subdomains 1 (residues 1–32, 70–144, and 338–372) and 2 (residues 33–69) constitute the small domain and subdomains 3 (145–180 and 270–337) and 4 (181–269) represent the large domain of actin. The subdomains are connected by the two hinge regions (137–150 and 333–338) that render the protein flexible [15]. The 146_147dupAlaSer mutation is located at the start of subdomain 3 and within the hinge region. The two extra amino acids in this region likely affect the flexibility of the protein and may hinder its interaction with other proteins. At the Z-disk, F-actin from two sarcomeres is cross-linked by α -actinin homodimers, positioned in an antiparallel fashion. The actin binding site for α -actinin is located in subdomain 1 [16], and the mutation in the hinge region may affect this interaction. α -Actinin is the major backbone of the Z-disk where it interacts with many proteins. As MFM is caused by mutations in Z-disk related proteins, the abnormal interaction of α -actinin with F-actin in our patient likely triggers an abnormal accumulation of multiple proteins.

In cell culture studies, the mutant 146_147dupAlaSer-pmCherry ACTA1 expressed at a significantly lower level than the wild-type protein. Also, wild-type ACTA1 formed filamentous structures whereas the mutant protein formed large aggregates, thus mimicking the structural lesions observed in the patient's muscle. Low expression in cell culture of some missense ACTA1 mutants was previously attributed to increased cell death by the mutant ACTA1 [17–19]. Actin accumulation in our patient muscle was not directly demonstrated due to lack of EM specimen and of muscle tissue for actin immunostaining.

The findings reported here highlight that mutations in *ACTA1* can cause features consistent with MFM pathology in infancy with abnormal and ectopic expression of multiple proteins. A search for mutations in *ACTA1* should be considered in patients with severe congenital hypotonia and muscle weakness and muscle biopsy features consistent with MFM.

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