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Cytoplasmic body pathology in severe *ACTA1*-related myopathy in the absence of typical nemaline rods

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Abstract

Mutations in *ACTA1* cause a group of myopathies with expanding clinical and histopathological heterogeneity. We describe three patients with severe *ACTA1*-related myopathy who have muscle fiber cytoplasmic bodies but no classic nemaline rods. Patient 1 is a five-year-old boy who presented at birth with severe weakness and respiratory failure, requiring mechanical ventilation. Whole exome sequencing identified a heterozygous c.282C>A (p.Asn94Lys) *ACTA1* mutation. Patients 2 and 3 were twin boys with hypotonia, severe weakness, and respiratory insufficiency at birth requiring mechanical ventilation. Both died at 6 months of age. The same heterozygous c.282C>A (p.Asn94Lys) *ACTA1* mutation was identified by whole exome sequencing. We conclude that clinically severe *ACTA1*-related myopathy can present with muscle morphological findings suggestive of cytoplasmic body myopathy in the absence of definite nemaline rods. The Asn94Lys mutation in skeletal muscle sarcomeric α -actin may be linked to this histological appearance. These novel *ACTA1* cases also illustrate the successful application of whole exome sequencing in directly arriving at a candidate genetic diagnosis in patients with unexpected phenotypic and histologic features for a known neuromuscular gene.

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Keywords

Skeletal muscle α -actin; *ACTA1*; Congenital myopathies; Cytoplasmic bodies

1. Introduction

Skeletal muscle α -actin, encoded by *ACTA1*, is the predominant actin isoform in adult skeletal muscle, forming the core structure of the sarcomeric thin filament. It mediates binding with thick filament myosin for force generation of muscle contraction [1]. Mutations in *ACTA1* cause a group of myopathies of ever increasing clinical and histopathological heterogeneity with variable degrees of muscle weakness and a spectrum of muscle pathology including nemaline rods, cores, cap-like structures, fiber-type disproportion, type I fiber predominance, and zebra-bodies [2].

Cytoplasmic bodies are a characteristic but non-specific histopathological finding observed in muscle fibers in different disorders including inflammatory myopathies, myotonic dystrophy, periodic paralysis, myofibrillar myopathies, and neurogenic diseases such as spinal muscular atrophy (SMA) [3–5]. Their ultrastructural features suggest that cytoplasmic bodies arise from Z-disk material but are distinct from nemaline rods, spheroid bodies, and filamentous bodies. Perhaps not surprisingly, several hereditary myopathies that affect myofilament organization such as desmin-related myofibrillar myopathy, reducing body myopathy and congenital myopathies have been associated with the morphological finding of cytoplasmic bodies on muscle biopsies as well. The designation cytoplasmic body myopathy has been used when these inclusions are the defining histological feature in the biopsy [6–8]. Here we present three patients from two unrelated families with genetically confirmed, severe *ACTA1*-related myopathy, characterized by the muscle histological finding of predominant cytoplasmic bodies in the absence of definite nemaline rods by either light microscopy of Gömöri trichrome stained cryosections or electron microscopy (EM), justifying their clinico-histopathologic designation as cytoplasmic body myopathy.

2. Case report

2.1. Clinical findings

This study was approved by the Institutional Review Board of the National Institute of Neurological Disorders and Stroke (NINDS), National Institutes of Health (NIH). Patient 1 (P1) of family 1 is a five year-old boy born to a G1P1 mother at 36 weeks gestational age due to premature rupture of membranes. Pregnancy was uncomplicated, but fetal movements were reported as being reduced. At delivery, he was cyanotic with Apgar scores of 3, 4 and 4 at 1, 5 and 10 minutes, respectively, and required intubation. He was profoundly hypotonic with evidence of severe generalized weakness, rendering him with no perceivable movements other than the movements of his eyes. He also had respiratory failure and feeding difficulties. A tracheostomy was performed and a gastrostomy tube was placed on day 35 of life. He remained hospitalized until 4 months of age when he was discharged on mechanical ventilation.

P1 never acquired head control, rolling, or any other motor milestone. Extraocular movements remained intact. Over time, he developed some movement in his fingers, toes, and elbows, but all movements were subgravity. Starting at age 4 years he was able to move his head slightly. On examination at age 5 years, he had a high-arched palate, maintenance of the mouth in an open position with an inability to close (Fig. 1A). He had generalized hypotonia with significantly decreased muscle bulk throughout the upper and lower extremities. He showed some flicker-type movements of the fingers and toe flexion against gravity. He had evidence of minimal left-sided subgravity elbow extension. He was areflexic throughout the upper and lower extremities. He was not able to communicate verbally beyond “yes” and “no,” which were uttered with poor articulation. Otherwise, he communicated via eye movements. Cognition appeared intact.

Serum CK level ranged from 69 to 393 U/L. EMG was myopathic, electroencephalography (EEG) was normal, and MRI of the brain, performed on day 3 of life, was normal except for a small left-sided subdural fluid collection reported as similar to cerebral spinal fluid intensity. Magnetic resonance spectroscopy (MRS) was normal. He was initially diagnosed with phosphofructokinase (PFK) deficiency based on increased glycogen deposition in the muscle biopsy performed at age 3 weeks as well as low, but not deficient, PFK activity levels. *PFKM* sequencing was normal. Repeat biopsy was performed at age 3.5 years (see below).

Patients 2 (P2) and 3 (P3) of family 2 were fraternal twin boys born to healthy parents. Family history is shown in Fig. 2A. Pregnancy was uncomplicated, and the twins were born full term; however, fetal movements were reported as being reduced. Marked hypotonia, severe generalized muscle weakness, and respiratory insufficiency were noted soon after birth. Both twins initially required noninvasive ventilation and subsequently required a tracheostomy with mechanical ventilation. They had feeding and swallowing difficulties, initially requiring nasogastric (NG) tube feeding, and a gastrostomy tube was placed in both at age 2 months. Due to impaired oral motor function with excessive drooling, both required frequent suctioning.

Both twins had evidence of facial weakness, generalized hypotonia with marked head lag, and severe weakness with only minimal movements of the fingers and toes (Fig. 1B and C). Extraocular movements were full. There were contractures of the elbows and fingers. Spine was flexible. Electromyography (EMG) findings suggested myopathic changes. Serum creatine kinase (CK) levels were normal. Echocardiogram and magnetic resonance imaging (MRI) of the brain and spine were normal. Metabolic studies were negative. Methylation studies for Prader–Willi syndrome were normal. Genetic testing for SMA and myotonic dystrophy type I were negative. Both patients passed away at age 6 months from respiratory insufficiency.

2.2. Mutation analysis

WES on DNA from whole blood samples obtained from Patient 1 (P1) was performed at the Broad Institute of the Massachusetts Institute of Technology (MIT) and Harvard University using an Illumina rapid capture exome enrichment kit and Illumina HiSeq 2000 sequencing

instruments. Variants were analyzed using the Broad *seqr* platform (<https://seqr.broadinstitute.org>).

Whole exome sequencing (WES) on gDNA from whole blood samples obtained from P2, his fraternal twin brother (P3), and unaffected parents was performed at the NIH Intramural Sequencing Center (NISC) using a Nimblegen SeqCap EZ Exome +UTR Library version 3.0 and Illumina HiSeq 2500 sequencing instruments. Exome data were analyzed using Varsifter [9].

The *ACTA1* c.282C>A (p.Asn94Lys) missense mutation was identified in P1, P2, and P3. This mutation was previously identified as a cause of severe nemaline myopathy [1]. Exon 3 of *ACTA1* in P1, his mother, P2, P3 and their parents was then amplified by PCR and Sanger sequenced in forward and reverse direction. The mutation was confirmed in all three patients, but absent in P1's mother and the twins' parents (Fig. 2B). A paternal sample of P1 was not available for segregation testing. Exome sequencing confirmed that the twins were non-identical.

2.3. Histological findings

Muscle biopsy of the right quadriceps of P1 at age 3.5 years revealed nearly end-stage myopathic features. Wide variation in fiber size due to the presence of atrophic and hypertrophic fibers was observed in cryosections (Fig. 3A). Atrophic fibers were a mixture of type I and type II fibers; however, most type II fibers were small and most hypertrophic fibers were type I. There were scattered necrotic and regenerating myofibers but no lymphocytic inflammation. A significant increase in endomysial fibrous tissue was noted. Internally placed nuclei were present but were not numerous. No inclusion bodies, vacuoles, or ragged-red fibers were identified. On H&E staining, many fibers had increased cytoplasmic basophilic granular material in a whorled pattern (Fig. 3A). Lipid was increased in oil red O (ORO) stained sections and confirmed ultrastructurally. Oxidative stains were suggestive of disrupted sarcomeric organization, but all fibers stained positive for COX and SDH.

EM performed on the muscle biopsy of P1 (Fig. 3B and C) showed no evidence of mitochondrial inclusions or structurally abnormal cristae. Sarcomeric filaments were disrupted in many fibers; however, no cores, nemaline rods, or Z-band streaming was appreciated. Several inclusion bodies were noted, most of which had a starburst-like appearance with densely compact filaments centrally and a halo of radiating filamentous material at the periphery, consistent with cytoplasmic bodies.

Muscle biopsy of the quadriceps in P2 and P3 at age 2 months revealed marked variation in fiber size, polygonal and rounded myofiber atrophy and occasional internally located nuclei. Multiple fibers had eosinophilic deposits evident on hematoxylin and eosin (H&E) stain (Fig. 3D and G). Modified Gömöri trichome (GT) stain showed scattered fibers with multiple dark staining deposits located centrally or peripherally in the fibers (Fig. 3E and H). These deposits were only rarely found in aggregate form. Nicotinamide adenine dinucleotide (NADH) stain and succinic dehydrogenase (SDH) stain did not show definite core structures. Electron microscopy (EM) showed focally distorted myofibrillary architecture

with Z-line streaming. Central densities surrounded by less dense filaments, suggestive of cytoplasmic bodies, were observed, although they are more obvious in Fig. 3F than in Fig. 3I.

3. Discussion

Cytoplasmic bodies are a relatively rare finding in muscle biopsies that were first identified in patients with desmin-related myopathy [3,6,8]. Based on EM analysis, cytoplasmic bodies are thought to derive from Z-disk material. This muscle morphological finding is felt to be suggestive of rapid turnover or disruption of myofibrillar material [10]. Although cytoplasmic bodies are not pathognomonic for any specific neuromuscular condition, their presence is distinct and may help to guide the diagnostic workup and facilitate targeted genetic testing in certain clinical scenarios. Cytoplasmic bodies are a rare histologic finding in *ACTA1*-myopathy. Here we present three patients with severe congenital myopathy manifesting with respiratory failure, hypotonia, and profound weakness who share the same heterozygous *ACTA1* mutation c.282C>A (p. Asn94Lys). Each patient's muscle biopsy contained cytoplasmic bodies without evidence of definite nemaline rods. The morphological finding of cytoplasmic bodies in the absence of clear nemaline pathology in *ACTA1* patient muscle biopsies expands the spectrum of histologic findings in *ACTA1*-related myopathy and adds to the genetic heterogeneity associated with cytoplasmic body pathology.

ACTA1 muscle pathology is a spectrum of one, or more, overlapping findings that may include intranuclear rods, cytoplasmic nemaline rods, actin thin filament accumulations, congenital fiber type disproportion (CFTD), type I fiber predominance, core-like structures, caps, dystrophic features and zebra bodies [1]. Although not exclusive to *ACTA1*-related myopathy, and not present in all *ACTA1* biopsies, nemaline rods are the most common characteristic muscle morphological feature associated with *ACTA1* mutations [2,11,12]. Notably, in the three *ACTA1* patients reported here, we did not identify clear nemaline rods either histologically (on GT staining) or ultrastructurally (on EM). The absence of nemaline rods in our patients' biopsies may be a result of sampling or timing of the muscle biopsy. Nemaline rods may develop over time with disease progression, as illustrated by a patient in whom nemaline rods were absent on the first biopsy but present in a subsequent, second biopsy of the same muscle at a later time [13].

It is well established that the number of nemaline rods present in patients' biopsies does not correlate with disease severity [14–16]. This is confirmed in our patients who are on the severe end of the disease spectrum of *ACTA1*-related myopathy but do not have typical nemaline pathology. However, some preliminary association between genotype and histologic phenotype has been proposed, specifically for the *ACTA1* missense mutation at p.Val165 (p.Val165Met and p.Val165Leu) and their association with intranuclear rod pathology and severe weakness [2]. Interestingly, both families reported here have identical *ACTA1* missense mutations (p. Asn94Lys) and cytoplasmic bodies on muscle pathology. Similar to the p.Val165 mutations, it can be hypothesized that the p.Asn94 Lys mutation is associated with clinically severe myopathy. The p.Asn94Lys mutation has been reported previously in two patients with nemaline myopathy, one with severe disease and the other of

unknown severity. Detailed information regarding phenotype and pathophysiologic features were not provided [1]. Our cases suggest that cytoplasmic body formation may be a possible histological expression of this mutation.

Ilkovski et al. described a patient with a heterozygous *de novo* p.Glu72Leu *ACTA1* mutation with severe arthrogryposis and hypotonia who passed away two hours after birth from respiratory failure [17]. Histology of the quadriceps muscle at autopsy revealed numerous nemaline rods, moderate variation in fiber size and predominance of very small fibers. EM revealed severe sarcomeric disorganization and the presence of both nemaline rods and cytoplasmic bodies. In our *ACTA1* patients, we did not identify clear nemaline rods; however, nemaline rod pathology in *ACTA1*-myopathy may develop over time as the disease progresses. This was previously observed in a boy with severe p.Asn111Ser *ACTA1*-myopathy in whom cytoplasmic bodies were the main pathological feature on muscle biopsy performed at age 7 weeks of age, and clear nemaline rods were not seen until a repeat muscle biopsy was performed at age 15 months [18]. Perhaps not surprisingly, the co-occurrence of nemaline rods and cytoplasmic bodies has also been reported in a boy with severe nemaline myopathy of unknown genetic etiology [19], suggesting that a common pathophysiologic mechanism may underlie both morphologic findings. It thus cannot be excluded that the histological manifestations in our cases may also include nemaline rod formation, if for example a different muscle was sampled at a different time point. Additional cases are necessary to more firmly establish associations between *ACTA1* genotypes, phenotypes and histotypes – inclusive of clinical and muscle pathological findings.

Pathologically, both nemaline rods and cytoplasmic bodies are likely derived from Z-disk material and share certain characteristics. They are pathologically distinguished based on aggregation pattern and their specific appearance on EM. The mechanism by which *ACTA1* mutations cause a spectrum of muscle pathology is not clearly understood. *ACTA1* is very highly conserved and is known to interact with more than 100 proteins, so that different molecular consequences may arise from mutations in different residues of the protein [20]. The pathophysiologic pathway of nemaline rod formation is shared by mutations in genes encoding other thin filament proteins, and their maintenance, such as nebulin and the tropomyosins. In nemaline myopathy, thin filament mutations may destabilize the sarcomeric structure, which anchors directly to the Z-line, resulting in the formation of nemaline rods which often contain Z-line proteins [2]. The origin of cytoplasmic bodies is less certain; however, they are also composed of a particular arrangement of intermediate and thin filaments in association with an electron-dense center containing Z-line material [3].

Recessive inheritance was initially suspected in family 2, since the affected fraternal twin boys were born to apparently unaffected parents. Genetic testing revealed the same heterozygous *ACTA1* mutation in each boy, while parental segregation testing did not reveal the presence of this mutation. We suspect that one of the parents carries the *ACTA1* mutation in a mosaic state, which resulted in subsequent full inheritance in the fraternal twin boys. This phenomenon of *ACTA1* parental mosaicism has been previously reported [1,21]. The chromatograms for the parental *ACTA1* sequencing were not suggestive of somatic

mosaicism, as the sequence only showed the typical homozygous wild-type peak without any traces of the mutant. As we only analyzed gDNA extracted from peripheral blood from the parents, and did not evaluate other parental tissue such as skin or saliva for evidence of mosaicism, we are unable to establish whether or not the mosaicism was confined to the germline or present in a low ratio throughout somatic tissues in one of the parents. Since the type of mosaicism is unknown, genetic counseling and accurate recurrence risk assessment in this case is challenging. Previous data on families with parental mosaicism for osteogenesis imperfecta and retinoblastoma estimated a recurrence rate of 10% to 27%; however, in reality the risk for recurrence in future pregnancies in the setting of parental mosaicism may be as high as 50% [22,23].

These cases illustrate the possible advantage of “unbiased” whole exome sequencing in the diagnosis of patients with undiagnosed neuromuscular disease, as a dominant *ACTA1*-myopathy was not suspected based on either the inheritance patterns, the clinical presentations, or muscle pathology findings in these three patients. The widespread application of next generation sequencing in the undiagnosed neuromuscular disease population will continue to expand previously defined phenotypic and histopathological spectra associated with known disease genes, thus underscoring the importance of continued careful phenotype–histotype–genotype correlations [24].

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References

1. Laing NG, Dye DE, Wallgren-Pettersson C, et al. Mutations and polymorphisms of the skeletal muscle alpha-actin gene (ACTA1). *Hum Mutat.* 2009; 30(9):1267–77. [PubMed: 19562689]
2. Nowak KJ, Ravenscroft G, Laing NG. Skeletal muscle alpha-actin diseases (actinopathies): pathology and mechanisms. *Acta Neuropathol.* 2013; 125(1):19–32. [PubMed: 22825594]
3. Macdonald RD, Engel AG. The cytoplasmic body: another structural anomaly of the Z disk. *Acta Neuropathol.* 1969; 14(2):99–107. [PubMed: 4242247]
4. Vajsaar J, Balslev T, Ray PN, et al. Congenital cytoplasmic body myopathy with survival motor neuron gene deletion or Werdnig-Hoffmann disease. *Neurology.* 1998; 51(3):873–5. [PubMed: 9748045]
5. Schessl J, Columbus A, Hu Y, et al. Familial reducing body myopathy with cytoplasmic bodies and rigid spine revisited: identification of a second LIM domain mutation in FHL1. *Neuropediatrics.* 2010; 41(1):43–6. [PubMed: 20571991]
6. Osborn M, Goebel HH. The cytoplasmic bodies in a congenital myopathy can be stained with antibodies to desmin, the muscle-specific intermediate filament protein. *Acta Neuropathol.* 1983; 62(1–2):149–52. [PubMed: 6318501]
7. Goebel HH, Schloos H, Lenard HG. Congenital myopathy with cytoplasmic bodies. *Neuropediatrics.* 1981; 12(2):166–80. [PubMed: 6267501]
8. Engel WK. The essentiality of histo- and cytochemical studies of skeletal muscle in the investigation of neuromuscular disease. 1962. *Neurology.* 1998; 51(3):655–72. [PubMed: 9748002]

9. Teer JK, Green ED, Mullikin JC, et al. VarSifter: visualizing and analyzing exome-scale sequence variation data on a desktop computer. *Bioinformatics*. 2012; 28(4):599–600. [PubMed: 22210868]
10. Nakano S, Engel AG, Waclawik AJ, et al. Myofibrillar myopathy with abnormal foci of desmin positivity. I. Light and electron microscopy analysis of 10 cases. *J Neuropathol Exp Neurol*. 1996; 55(5):549–62. [PubMed: 8627346]
11. Ryan MM, Ilkovski B, Strickland CD, et al. Clinical course correlates poorly with muscle pathology in nemaline myopathy. *Neurology*. 2003; 60(4):665–73. [PubMed: 12601110]
12. North, K. Congenital myopathies. In: Franzini-Armstrong, C., Engel, AG., editors. *Myology*. New York: McGraw-Hill; 2004. p. 1473-533.
13. Wallgren-Pettersson C. Congenital nemaline myopathy. A clinical follow-up of twelve patients. *J Neurol Sci*. 1989; 89(1):1–14. [PubMed: 2926439]
14. Nonaka I, Tojo M, Sugita H. Fetal muscle characteristics in nemaline myopathy. *Neuropediatrics*. 1983; 14(1):47–52. [PubMed: 6682187]
15. Nienhuis AW, Coleman RF, Brown WJ, et al. Nemaline myopathy. A histopathologic and histochemical study. *Am J Clin Pathol*. 1967; 48(1):1–13. [PubMed: 4165764]
16. Kaimaktchiev V, Goebel H, Laing N, et al. Intranuclear nemaline rod myopathy. *Muscle Nerve*. 2006; 34(3):369–72. [PubMed: 16477620]
17. Ilkovski B, Nowak KJ, Domazetovska A, et al. Evidence for a dominant-negative effect in *ACTA1* nemaline myopathy caused by abnormal folding, aggregation and altered polymerization of mutant actin isoforms. *Hum Mol Genet*. 2004; 13(16):1727–43. [PubMed: 15198992]
18. Ladha S, Coons S, Johnsen S, et al. Histopathologic progression and a novel mutation in a child with nemaline myopathy. *J Child Neurol*. 2008; 23(7):813–17. [PubMed: 18487519]
19. Suwa K, Mizuguchi M, Momoi MY, et al. Co-existence of nemaline and cytoplasmic bodies in muscle of an infant with nemaline myopathy. *Neuropathology*. 2002; 22(4):294–8. [PubMed: 12564770]
20. dos Remedios CG, Chhabra D, Kekic M, et al. Actin binding proteins: regulation of cytoskeletal microfilaments. *Physiol Rev*. 2003; 83(2):433–73. [PubMed: 12663865]
21. Nowak KJ, Wattanasirichaigoon D, Goebel HH, et al. Mutations in the skeletal muscle alpha-actin gene in patients with actin myopathy and nemaline myopathy. *Nat Genet*. 1999; 23(2):208–12. [PubMed: 10508519]
22. Sippel KC, Fraioli RE, Smith GD, et al. Frequency of somatic and germ-line mosaicism in retinoblastoma: implications for genetic counseling. *Am J Hum Genet*. 1998; 62(3):610–19. [PubMed: 9497263]
23. Pyott SM, Pepin MG, Schwarze U, et al. Recurrence of perinatal lethal osteogenesis imperfecta in sibships: parsing the risk between parental mosaicism for dominant mutations and autosomal recessive inheritance. *Genet Med*. 2011; 13(2):125–30. [PubMed: 21239989]
24. Foley AR, Donkervoort S, Bonnemann CG. Next-generation sequencing still needs our generation's clinicians. *Neurol Genet*. 2015; 1(2):e13. [PubMed: 27066550]

Fig. 1.

Patient 1 (A) at age 4 years with profound generalized weakness and hypotonia. He has a myopathic-appearing face and is unable to close his mouth. He is on permanent mechanical ventilation. When sitting upright in his wheelchair, his head is supported with a cervical collar. He has suspended arm braces mounted on the sides of his wheelchair to allow for some movement in the plane of gravity. Patient 2 (B) and Patient 3 (C) at age 4.5 months with generalized hypotonia and profound weakness. Patient 2 has a myopathic-appearing face and 'frog-leg' posturing with minimal limb movements.

Fig. 2.

Pedigree of Family 2 (A) and sequence chromatograms of targeted Sanger sequencing validation of the *ACTA1* mutation identified on exome (B). Mutations were confirmed on gDNA extracted from blood. Sequencing results of Family 2 showing heterozygous c. 282C>A (p.Asn94Lys) *ACTA1* mutation in patients P2 and P3 (bottom), which was not identified in the father or mother (top).

Fig. 3.

Histology of quadriceps muscle in Patient 1 (A–C), Patient 2 (D–F) and Patient 3 (G–I). H&E images (40×) in Patient 1 (A) shows end stage muscle with extensive fatty infiltration and fibrosis. Atrophic and hypertrophic fibers remain. The hypertrophic fibers portrayed have eosinophilic material in a whorled pattern. EM of Patient 1 (B,C) shows cytoplasmic bodies with characteristic dense cores surrounded by radiating filaments (bar = 3 μm). H&E images (40×) in P2 (D) and P3 (G) show marked variation in fiber size, polygonal and rounded myofiber atrophy and some fibers with eosinophilic deposits. Gomori trichrome images (40×) in P2 (E) and P3 (H) illustrate scattered fibers with multiple darkly stained deposits located centrally or peripherally in the fiber (arrows). These deposits were only rarely found in aggregate form. EM of Patient 2 (F) shows a central density surrounded by less dense filaments, suggestive of a cytoplasmic body (bar = 1 μm). EM of Patient 3 (I) shows an area of increased density, possibly suggestive of a cytoplasmic body (bar = 2 μm).