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Case report

A novel *ACTA1* mutation resulting in a severe congenital myopathy with nemaline bodies, intranuclear rods and type I fibre predominance

Gianina Ravenscroft [a,*], Jo M. Wilmshurst [b], Komala Pillay [c], Padma Sivadorai [d], William Wallefeld [a], Kristen J. Nowak [a], Nigel G. Laing [a]

[a] *Centre for Medical Research, The University of Western Australia, Western Australian Institute for Medical Research, Nedlands, Australia*
[b] *Paediatric Neurology and Neurophysiology, The University of Cape Town and the Red Cross Children's Hospital, Cape Town, South Africa*
[c] *National Health Laboratory Services, University of Cape Town and the Red Cross Children's Hospital, Cape Town, South Africa*
[d] *Neurogenetics, Royal Perth Hospital, Perth, Australia*

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Abstract

We describe a severe congenital myopathy patient of Xhosa native African origin with a novel *de novo* p.Gly152Ala skeletal muscle α-actin gene (*ACTA1*) mutation, who died at 6 months of age. The muscle pathology demonstrated abundant cytoplasmic and intranuclear rods, core-like areas and the unusual feature of larger type I than type II fibres. Our results further expand the phenotypes associated with *ACTA1* mutations and provide support for the hypothesis that the structural abnormalities seen are a pathological continuum dependent on the precise mutation and biopsy location. Our results also demonstrate the likely world-wide distribution of *de novo* mutations in this gene.

Keywords: Nemaline myopathy; Intranuclear rods; ACTA1

1. Introduction

To date, over 180 different skeletal muscle α-actin gene (*ACTA1*) mutations have been described [1], http://www.waimr.uwa.edu.au/research/lovd.html). The vast majority of the patients present at birth with severe hypotonia and respiratory insufficiency and die within the first year of life; however cases of childhood or adult onset with slow progression do occur [2,3]. The patients' muscles show a range of structural abnormalities. The abnormalities include actin accumulation [4], caps [5], cores [6], cores and rods [2], fibre type disproportion [7], intranuclear rods, nemaline [4] and zebra bodies [8]. The patients are classed as having a particular disease based on the findings in the muscle biopsy at the light and/or electron microscopy level. This classification then serves to streamline genetic analysis; however there is considerable heterogeneity, with overlap between histopathology and the underlying genetic cause, e.g. multiple genes can cause the same pathological features, as well as different mutations within the same gene causing different pathologies. There is a school of thought that the various structural abnormalities resulting from *ACTA1* mutations are a continuous spectrum rather than discrete pathological entities, with various parts of the spectrum in different patients, depending on the precise mutation. Here we describe an infant of Xhosa origin with a novel *de novo* c.455G>C, p.Gly152Ala, *ACTA1* mutation and a novel combination of abnormalities seen on biopsy.

* Corresponding author. Address: Centre for Medical Research, The University of Western Australia, Western Australian Institute for Medical Research, B Block, Hovea Lane, QE II Medical Centre, Nedlands 6009, Australia. Tel.: +61 8 9346 1981; fax: +61 8 9346 1818.
E-mail address: gina.ravenscroft@uwa.edu.au (G. Ravenscroft).

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2. Case report

This baby of indigenous African Xhosa origin was noted to have reduced *in utero* movements and was low toned from birth. She had contractures of her elbows and knees. Her expression was myopathic, with normal range of eye movements and no tongue fibrillations. Her deep tendon reflexes were absent. She appeared to be cognitively intact with a head circumference above the 75th percentile.

There was marginal improvement in her volitional mobility over the first few days, especially her distal movements, however she remained maximally weak proximally. From birth she had feeding difficulties and a weak cry. Though tachypnoeic she remained in room air. Management priorities concentrated on her respiratory function, feeding and mobility. She was confirmed to have marked gastro-oesophageal reflux with evidence of aspiration. She was maintained initially on naso-jejunal feeding.

Aged 3 months she underwent a Nissen's fundoplication with a per-enteral gastrostomy inserted. She remained stable and coped with feeding, gaining weight. She was discharged home aged almost 4 months.

She was readmitted a month later hypovolaemic with gastroenteritis and requiring ventilation support. She failed to extubate. A tracheostomy tube was inserted and over the next few weeks her ventilation requirements were weaned slowly. Her motor capacity slowly improved but she remained profoundly weak. Three weeks after readmission she deteriorated with an intercurrent chest infection. Despite active intervention she deteriorated developing abdominal distension and sepsis. She suffered a cardio-respiratory arrest from which she could not be resuscitated. She was aged almost 6 months old. Her results revealed an initial raised CK at 787 U/L 4 days after birth, with a repeat screen being 257 U/L. She had a normal head ultrasound.

2.1. Muscle pathology

Muscle biopsies were taken at 3 and 5 months of age when the patient underwent surgery for a per-enteral gastrostomy and a tracheostomy, respectively. In the quadriceps muscle biopsy conducted at the age of 3 months, from a site close to the myotendinous insertion, a marked variation in fibre diameters was observed, ranging between 3.5 and 38 μm (Fig. 1A). Gomori trichrome staining revealed the presence of red aggregates within myofibres indicative of nemaline bodies (Fig. 1B), and intranuclear rods were also observed in some myofibres both with haematoxylin and eosin and Gomori staining (insert, Fig. 1B). ATPase staining at pH 9.4 (Fig. 1C and D) indicates marked predominance of type I fibres, hypertrophy of type I fibres and scattered hypotrophic type II fibres. This large variation in fibre size (~3–35 μm) and type I fibre predominance persisted in a biopsy taken from the belly of the quadriceps muscle at 5 months of age (Fig. 1D). Large areas within the muscle fibres devoid of ATPase were also prominent.

Electron microscopy confirmed the presence of both cytoplasmic (Fig. 1E) and intranuclear (Fig. 1F) rods within the patient's muscle fibres. Numerous electron dense bodies could be seen to extend from the Z-disks. Core-like areas devoid of mitochondria and myofibrillar architecture (Fig. 1G) and occasional mini-cores (Fig. 1H) were also observed at the ultrastructural level. These features were observed in both muscle biopsies examined. These core-like regions and regions with almost complete loss of sarcomeric organisation are likely to correspond with regions that were negative by ATPase staining.

2.2. Genetic analysis

Bi-directional mutation analysis of the entire coding region of *ACTA1* revealed a novel heterozygous c.455G>C missense mutation in exon 4 (p.Gly152Ala, Fig. 2). This change was not present in genomic DNA from the peripheral blood of either parent, confirming a *de novo*, novel dominant mutation.

Patient DNA was also tested for the spinal muscular atrophy gene and was found not to be homozygous for the common deletion of exon 7 in the *SMN1* gene.

2.3. Cell culture studies

Transfection of C2C12 myoblasts with wild-type human *ACTA1* fused with the enhanced green fluorescent protein (EGFP) resulted in cytoplasmic localisation of the fusion protein and incorporation into stress fibres within 48 h (Fig. 3A), as previously described [9]. In contrast, transfection with *ACTA1*(G152A)-EGFP resulted in large, intense, most frequently perinuclear EGFP aggregates (Fig. 3B) and numerous EGFP-positive rods throughout the cytoplasm (Fig. 3C) and within nuclei (Fig. 3D). The mutant actin fusion protein was poorly incorporated into differentiating myotubes and many transfected myoblasts died prior to reaching maturity.

2.4. Modelling of ACTA1

Three-dimensional modelling of the actin monomer (http://polyview.cchmc.org/polyview3d.html; as per [1]) clearly indicates that the Gly152 residue (pink) lies within the intranuclear rod myopathy (IRM) hotspot (blue residues) and that this hotspot is closely associated with a putative nuclear export sequence (NES; cyan). Examination of the outer surface of the actin monomer with the amino acids associated with congenital fibre type disproportion (CFTD) shown in blue, indicate that the Gly152 residue (pink, not visible) does not lie on the same surface as the CFTD-causing residues (Fig. 3F).

3. Discussion

We describe a severe congenital myopathy patient with a novel *de novo* mutation in exon 4 of *ACTA1* resulting in