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ScienceDirect

Neuromuscular Disorders 25 (2015) 388–391

www.elsevier.com/locate/nmd

Zebra body myopathy is caused by a mutation in the skeletal muscle actin gene (*ACTA1*)

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Received 28 November 2014; received in revised form 27 January 2015; accepted 9 February 2015

Abstract

We present follow up data on the original case of 'zebra body myopathy' published by Lake and Wilson in 1975. Pathological features in a second biopsy performed at the age of 29 years included a wide variation in fibre size, multiple split fibres, excess internal nuclei and endomysial connective tissue, rimmed vacuoles, accumulation of myofibrillar material and large 'wiped out' areas lacking stain for oxidative enzymes. The presence of nemaline rods and actin-like filaments in addition to small zebra bodies suggested *ACTA1* as a candidate gene. This has been confirmed by the identification of a novel c.1043T.p.Leu348Gln mutation, which probably occurred *de novo*. This case illustrates that the myopathy associated with zebra bodies is part of the spectrum of myopathies associated with the *ACTA1* gene. It also highlights that accumulation of actin filaments is not confined to severe neonatal *ACTA1* cases and that progression of weakness can occur in congenital myopathies, as the patient is now wheelchair bound and can only stand with the aid of a walking frame.

Keywords: Nemaline myopathy; Nemaline rods; Zebra bodies; Congenital myopathy; ACTA1

1. Introduction

In 1975 Lake and Wilson published a case report on a 15 year old boy who had presented with a history compatible with a congenital myopathy. Ultrastructural studies of the biopsy revealed several zebra bodies and the authors cautiously suggested the myopathy be named 'zebra body myopathy' [1]. Zebra bodies are small, striped filamentous structures. A second case published in 1987 by Reyes et al. suggested that 'zebra body myopathy' was a distinct entity [2]. Biopsies of both cases also showed nemaline rods. We had previously shown that patients homozygous for null *ACTA1* mutations revealed more zebra body structures than usually encountered in human neuromuscular disorders, and Drosophila *ACTA1* mutant flies often show structures that resemble zebra bodies [3–5] . We therefore proposed that *ACTA1* could be a candidate gene for 'zebra body myopathy'. Subsequent DNA analysis of the Lake and Wilson case, who is now 55 years old, revealed a novel *ACTA1* mutation, c.1043T>p.Leu348Gln. We present here retrospective assessment of a second biopsy performed on this patient at the age of 29 years, and his current clinical status.

2. Case report

Lake and Wilson reported in 1975 that the male patient was hypotonic at birth and fetal movements were weak [1]. He sat unsupported at 6 months of age if placed in position and walked at 15 months, if helped to stand. He was unable to pull himself up to stand until 2.5 years of age. He was never able to run, hop or climb stairs. He was first investigated at Great Ormond Street Hospital for Children when approximately 6 years of age and at that time was said to 'walk reasonably and had a weak grip'. Serum creatine kinase activity was normal. He remained relatively stable until 14 years of age but he had 2 episodes of painful torticollis at this age. He had mild facial and neck muscle weakness, a waddling gait, and rose from the floor with a Gowers' manoeuvre. Motor nerve conduction velocity was normal and electromyography showed a myopathic pattern. Serum creatine kinase at that time was raised at twice the normal level.

The sample, slides and electron micrographs of the quadriceps biopsy reported by Lake and Wilson in 1975 [1] cannot be traced but the publication shows that the features

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http://dx.doi.org/10.1016/j.nmd.2015.02.003
0960-8966/

included a wide variation in fibre size, with hypertrophic fibres and small fibres, some of which were in clusters surrounded by larger fibres. Some larger fibres with increased acid phosphatase showed vacuoles that were generally unstained but some contained calcium. Endomysial connective tissue, fat and the number of internal nuclei were increased and there was necrosis in one area. Histochemical stains showed a predominance of type 1 fibres but fibre type disproportion was not apparent in the published images. Electron microscopy revealed myofibrillar disorganisation, the presence of nemaline rods (not mentioned as being visible with Gomori trichrome staining) and myelin-like whorls in vacuolated fibres. Zebra bodies were numerous and present in disorganised fibres as well as in relatively normal appearing fibres. No large areas of actin-like filaments were mentioned or illustrated.

The slides and electron microscopy photographs of a second muscle biopsy taken at the age of 29 years were traced in the archives at the Institute of Neurology, Queen Square, London and examined. These showed some similarities to the first biopsy with a wide variation in fibre size, fibrosis, multiple split fibres, excess internal nuclei, ring fibres, nemaline rods, cytoplasmic bodies, rimmed vacuoles, accumulation of dark green myofibrillar material with the Gomori trichrome stain and wiped out areas in the oxidative enzyme preparations. (Fig. 1A–D). No fibre type disproportion was apparent. Red stained, rod-like structures (the presence of which was confirmed with electron microscopy) were present in multiple split fibres; Fig. 1B). In addition to nemaline rods electron microscopy revealed only a few zebra bodies, often at the periphery of fibres, and accumulation of actin-like thin filaments (Fig. 2A–C). Electron microscopy also confirmed the presence of autophagic vacuoles (Fig. 2D). One micrograph also showed a structure that was probably a nuclear rod (not shown). The micrographs did not reveal any areas of marked myofibrillar disruption that might have correlated with the 'wiped out' areas seen with staining for oxidative enzymes.

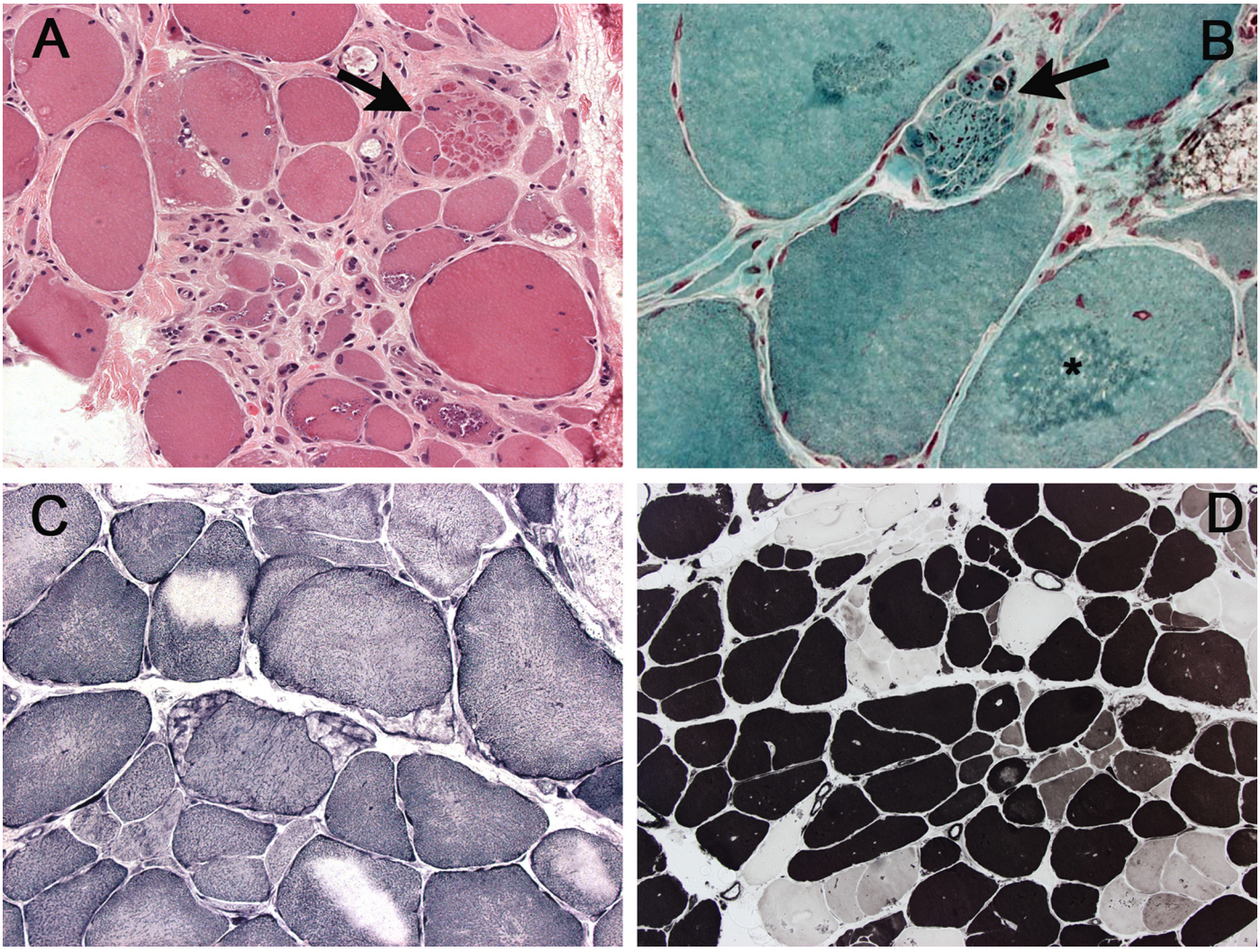

Fig. 1. Biceps biopsy from the patient aged 29 years stained with (A) haematoxylin and eosin showing a wide abnormal variation in fibre size, excess connective tissue, multiple split fibres (arrow), rimmed vacuoles and an increase in internal nuclei; (B) staining with Gomori trichrome showing dark stained myofibrillar material (*) and rods in some fibres (arrow); (C) staining for NADH-TR showing large areas with absent stain in some fibres and (D) staining for myosin ATPase pH 4.4 showing a predominance of darkly stained type 1 fibres.