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Molecular and cellular insights into a distinct myopathy of Great Dane dogs

Kin-Chow Chang [a,*], Maj-Lis C. McCulloch [b], Thomas James Anderson [b]

[a] School of Veterinary Medicine and Science, University of Nottingham, Sutton Bonington Campus, Loughborough LE12 5RD, UK
[b] Institute of Comparative Medicine, University of Glasgow Veterinary School, Bearsden Road, Glasgow G61 1QH, UK

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ABSTRACT

A myopathy in the Great Dane dog with characteristic pathological and molecular features is reported. Young adults present with progressive weakness and generalised muscle atrophy. To better define this condition, an investigation using histopathology, confocal microscopy, biochemistry and microarray analysis was undertaken. The skeletal muscles of affected dogs exhibited increased oxidative fibre phenotype and core fibre lesions characterised by the disruption of the sarcomeric architecture and the accumulation of mitochondrial organelles. Affected muscles displayed co-ordinated expression of genes consistent with a slow-oxidative phenotype, which was possibly a compensatory response to chronic muscle damage. There was disruption of Z-lines in affected muscles which, at the molecular level, manifested as transcriptional dysregulation of several Z-line associated genes, including α-actinin, myotilin, desmin, vimentin and telethonin. The pathology of this canine myopathy is distinct from that of human central core myopathies that are characterised by cores devoid of mitochondria and by the presence of myofibrillar breakdown products.

Introduction

An unusual myopathy is reported in Great Dane dogs, characterised by disorganisation of centrally-located myofibrils within skeletal myofibres but without cardiac muscle involvement (Newsholme and Gaskell, 1987; Targett et al., 1994). Clinically, this disorder is associated with exercise intolerance, muscle wasting and weakness in growing male and female animals. The condition has been termed a 'central core' myopathy, based on the characteristic histopathologically abnormal centrally-located myofibrils, and the superficial similarity of the disease with the core myopathies in humans (Sewry et al., 2002). In man, such 'central core' disease is a well-defined clinical condition associated with mutations in the ryanodine receptor gene (RYR1), which leaves myofibre cores devoid of oxidative enzymatic activity (Monnier et al., 2001; Sewry et al., 2002; Davis et al., 2003).

In contrast, the abnormal central regions in affected Great Dane myofibres have increased oxidative activity (Targett et al., 1994) and this finding, along with ultrastructural differences, have led to suggestions that the canine myopathy is a distinct disease entity (Luján Feliu-Pascual et al., 2006). In the present study, techniques including histopathology, biochemistry and microarray gene expression analysis have been used to better define and characterise this breed-specific canine myopathy.

Material and methods

Muscle samples

Biopsies were taken from the quadriceps femoris of four clinically-affected dogs during clinical investigations, in compliance with the Veterinary Surgeons Act 1966 (Table 1). Four normal, age-matched controls samples were taken post mortem from dogs that were not Great Danes but were of similar size. These animals were considered clinically normal in terms of muscle function and development, and were used in accordance with local ethical review committee guidelines. Only the quadriceps femoris muscle was sampled because it is relatively large and accessible and in order to minimise the number of samples taken from live animals.

All four affected dogs presented for investigation of severe exercise intolerance, generalised muscle wasting and the development of muscle tremors on exercise. A diagnosis of Great Dane myopathy was based on the finding of typical pathological features (Newsholme and Gaskell, 1987; Targett et al., 1994; Luján Feliu-Pascual et al., 2006) and, in particular, on the presence of architectural abnormalities in the centres of myofibres.

Histopathology, immunohistochemistry and histochemistry

Muscle samples were snap frozen in liquid nitrogen and stored at −70 °C for biochemical analysis, or frozen in isopentane and chilled in dry ice for cryostat sectioning. Unfixed serial cryostat sections, 10 μm thick, were incubated with primary antibody solution overnight at 4 °C. Primary antibodies used were rabbit 'fast' monoclonal antibody M-32 (Sigma) which recognises all post-natal fast myosin heavy chains (MyHC) (2a, 2b and 2x) (da Costa et al., 2000, 2002; Sun et al., 2001), and human 'slow' monoclonal antibody NOQ7.5.4D (Sigma) which recognises only slow MyHCs (da Costa et al., 2002). Both were used at a 1:1000 dilution. Biotinylated rabbit anti-mouse antibody E0354 (Dako), was employed along with the avidin–biotin–peroxidase complex (ABC) immunocytochemical procedure to localise primary antibody binding (Dako). Routine haematoxylin and eosin (HE) staining,

* Corresponding author. Tel.: +44 115 951 6491; fax: +44 115 951 6440.
E-mail address: kin-chow.chang@nottingham.ac.uk (K.-C. Chang).

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Table 1
Animal details and serum creatine kinase concentrations of clinically-affected Great Dane dogs described in this study. Serum creatine kinase concentrations <150 IU/L are considered normal.

Dog number	Age at diagnosis (months)	Duration of clinical signs at presentation (months)	Sex	Serum creatine kinase concentration (IU/L)
1	12	6	M	406
2	12	6	F	866
3	9	1	F	3925
4	10	2	M	2252

succinate dehydrogenase (SDH) staining (for oxidative capacity), periodic acid-Schiff (PAS) staining (for glycogen) and myosin ATPase staining (pH 4.3) (for slow fibres) were also carried out on unfixed serial sections (Bancroft and Gamble, 2002).

Confocal microscopy

Cryostat sections were acetone fixed and incubated with primary antibody for 2 h followed by incubation with the secondary antibody for 45 min (both at 37 °C). The antibodies used were: monoclonal mouse anti-α-actinin EA-53 used at a 1:500 dilution (Sigma); monoclonal mouse anti-desmin DE-R-11 used at a 1:200 dilution (Dako); and sheep anti-mouse IgG-FITC used at a 1:200 dilution (Sigma). Images were captured as a stacked series (1 mm Z steps) using a confocal scanning microscope (Leica SP2 AOBS, Leica Microsystems Semiconductor).

Electron microscopy

Fixed muscle samples were embedded in Araldite (Huntsman advanced materials), stained with uranyl acetate (in 50% ethanol) and sections 70–80 nm thick were cut on an ultratome. Sections were examined and photographed on a transmission electron microscope (Joel CX-100).

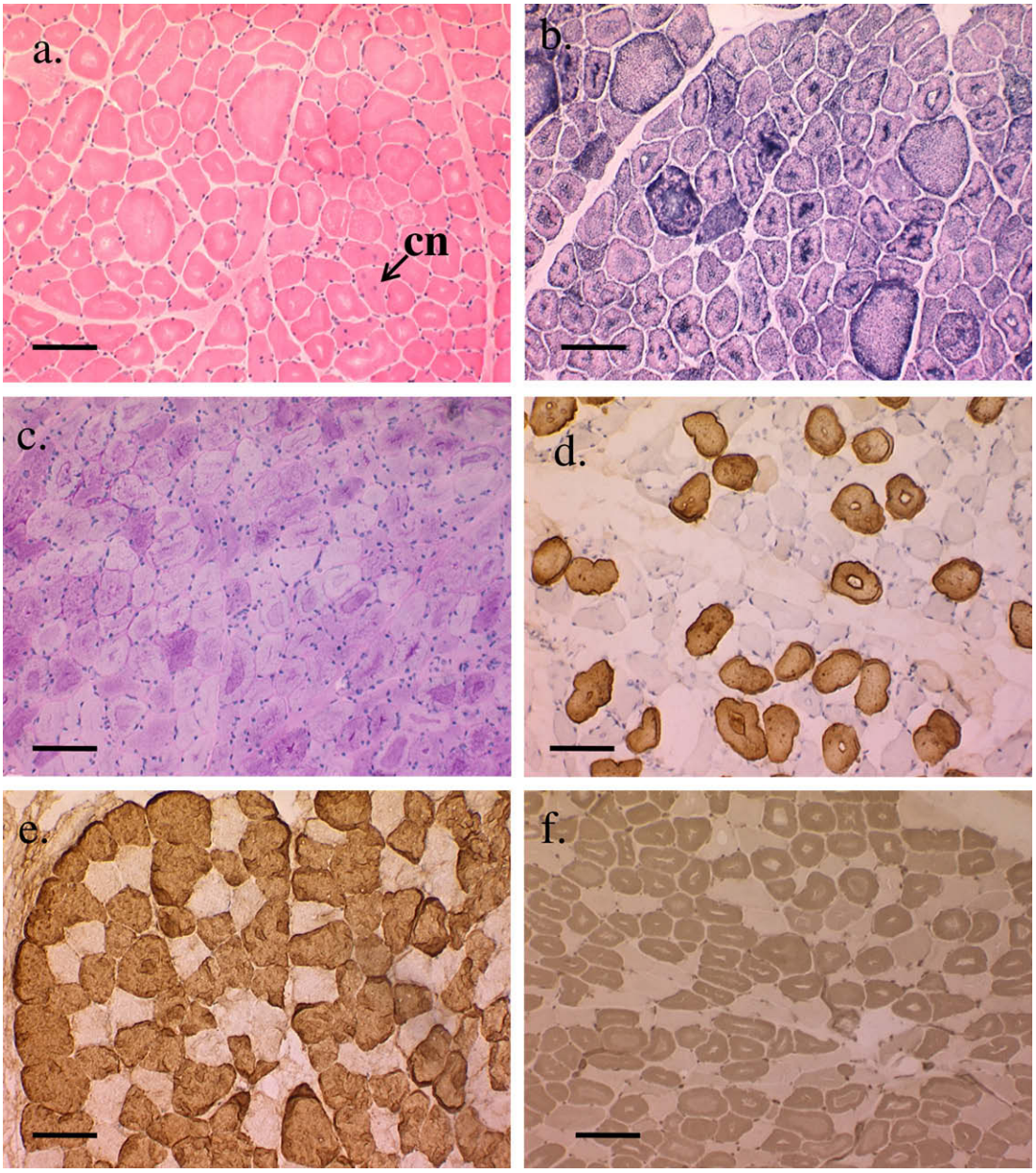

Fig. 1. Histopathological features of inherited myopathy of Great Dane dogs illustrating central or core myofibre lesions with disparity in fibre size and occasional central nuclei (cn) (a) (HE). These central lesions stain strongly for (b) succinate dehydrogenase (c) and glycogen (periodic acid-Schiff). Myofibre lesions are observed in fast (d) (immunocytochemical anti-fast MyHC stain) and slow (e and f) myofibres (e, immunocytochemical anti-slow MyHC stain; f, ATPase stain). Images shown are from four animals. Scale bar = 100 μm.