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SPONTANEOUSLY ARISING DISEASE

Muscular Dystrophy in a Dog Resembling Human Becker Muscular Dystrophy

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Summary

A 3-year-old, male Labrador retriever dog was presented with clinical signs of progressive exercise intolerance, bilateral elbow extension, rigidity of the forelimbs, hindlimb flexion and kyphosis. Microscopical examination of muscle tissue showed marked variability in myofibre size, replacement of muscle with mature adipose tissue and degeneration/regeneration of muscle fibres, consistent with muscular dystrophy. Immunohistochemical examination for dystrophin showed markedly reduced labelling with monoclonal antibodies specific for the rod domain and the carboxy-terminal of dystrophin, while expression of β-sarcoglycan, γ-sarcoglycan and β-dystroglycan was normal. Immunoblotting revealed a truncated dystrophin protein of approximately 135 kDa. These findings supported a diagnosis of congenital canine muscular dystrophy resembling Becker muscular dystrophy in man.

Keywords: dog; dystrophin; muscular dystrophy; myopathy

Muscular dystrophies are a group of hereditary disorders of skeletal muscle generally characterized by progressive muscle atrophy and weakness. In man, Duchenne muscular dystrophy (DMD) is a lethal X-linked disease caused by mutation in the *DMD* gene (Blake *et al*., 2002; Nowak and Davies, 2004). Males are particularly affected with a reported incidence of one case for every 3,500 people. The *DMD* gene is the largest gene in the human genome and it encodes the sarcolemmal protein dystrophin. Dystrophin consists of four main functional units: (1) the N-terminus, responsible for actin binding; (2) the central rod domain, which also contributes to actin binding; (3) the cysteine-rich domain, which is required for binding to β-dystroglycan; and (4) the C-terminal domain, which contains syntrophin and dystrobrevin binding sites (Ervasti, 2007; Le Rumeur *et al*., 2010). This protein is important for the maintenance of the structural integrity of muscle fibres during contraction. Mutations in this gene result in the loss or in the production of a non-functional form of dystrophin. This is accompanied by a defect of the dystrophin glycoprotein complex (DGC) in the sarcolemmal, leading to progressive muscle degeneration and, finally, necrosis (Hoffman *et al*., 1987; Campbell, 1995).

When a mutation in *DMD* disrupts the reading frame of amino acids (an out-of-frame mutation), the defect in dystrophin results in a severe phenotype of DMD. A mutation, which maintains the reading frame (an in-frame mutation) results, less frequently, in the production of truncated and only partly functional dystrophin. This mutation leads to the more benign phenotype (Monaco *et al*., 1988) known as Becker muscular dystrophy (BMD), which occurs in approximately three to six cases of DMD for every 100,000 males. The dystrophin-deficient skeletal

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muscle exhibits fibre necrosis and progressive replacement by fibrous or fatty tissue (Shelton *et al.*, 2001; Nakamura and Takeda, 2011).

A canine X-linked muscular dystrophy (CXMD) similar to DMD in man is reported in golden retrievers (Sharp *et al.*, 1992), Labrador retrievers (Bergman *et al.*, 2002), Samoyeds (Presthus and Nordstoga, 1993), Brittanys (Van Ham *et al.*, 1995), Irish terriers (Wentink *et al.*, 1972), Groenendaeler shepherds (Van Ham *et al.*, 1993), miniature schnauzers (Paola *et al.*, 1993), German shorthaired pointers (Schatzberg *et al.*, 1999), rat terriers (Wetterman *et al.*, 2000) and Cavalier King Charles spaniels (Walmsley *et al.*, 2010). Affected male dogs show severe clinical signs from about 8 weeks of age. Increased levels of serum creatine kinase (CK) can be detected in the first week of life. Canine muscular dystrophy with a molecular phenotype of BMD has been reported only in a family of Japanese Spitz dogs (Jones *et al.*, 2004; Atencia, 2013).

A 3-year-old, male Labrador retriever was presented with bilateral forelimb stiffness, weakness and progressive exercise intolerance. The owner had adopted the dog when it was 2 years old and at that time the dog had generalized muscle atrophy, gait abnormality and exercise intolerance. In the months prior to this presentation, the clinical signs had worsened and the dog could not walk for more than 5 min at a time.

On clinical examination the dog walked with bilateral elbow extension, rigidity of both forelimbs and flexion of both hindlimbs. There was also evidence of kyphosis (Fig. 1). The dog was in poor body condition and there was severe bilateral atrophy of the temporalis, supraspinatus, infraspinatus and biceps femoris muscles. During shoulder flexion the dog was in pain, but on neurological examination, the cranial and segmental spinal reflexes were normal. A primary myopathy was suspected.

Fig. 1. BMD in a Labrador retriever showing kyphosis, bilateral elbow extension, forelimb rigidity and generalized muscle atrophy.

Blood was taken for serum biochemistry and haematological examinations. Radiographs of the thorax and pelvis were taken. The complete blood count was within the normal range, serum CK activity was 96 U/l (reference range <378 U/l) and serum protein electrophoresis was normal. Faecal flotation was negative for parasites. Radiography did not reveal any diaphragmatic asymmetry, cardiomegaly or pelvic changes.

Muscle biopsy samples were taken from the supraspinatus and biceps femoris muscles and were snap frozen in isopentane pre-cooled in liquid nitrogen. Frozen muscle from the vastus lateralis muscle of a healthy dog was used as a control for immunohistochemical and immunoblotting studies. Frozen sections (10 μm) were subjected to a panel of histochemical stains (Paciello and Papparella, 2009) including haematoxylin and eosin (HE), Engel's modified Gomori trichrome, NADH-tetrazolium reductase (NADH-TR), succinate dehydrogenase (SDH), cytochrome oxidase (COX), ATPase at pH 9.4 and 4.3, periodic acid–Schiff (PAS) and oil red O. For immunohistochemistry (IHC), frozen sections (8 μm) were processed with the MACH1 Universal HPR-Polymer Detection Kit (Biocare Medical LLC, Concord, California, USA) using primary mouse monoclonal antibodies directed against dystrophin rod and c-terminal domains (clone Dy4/6D3 and clone Dy8/6C5, respectively; dilution 1 in 50), sarcoglycan (clone βSarc/5B1, dilution 1 in 100), γ-sarcoglycan (clone 35DAG/21B5, dilution 1in 100) and β-dystroglycan (clone 43DAG1/8D5, dilution 1 in 100). All antibodies were purchased from Novocastra Laboratories Ltd (Newcastle, UK). For western blotting, muscle tissue samples were processed as described by Paciello *et al.* (2006). The filters were probed with anti-dystrophin antibodies (dystrophin rod domain and c-terminal domains, clones Dy4/6D3 and Dy8/6C5, respectively; Novocastra Laboratories) overnight at 4°C and then incubated with horseradish peroxidase-conjugated anti-mouse IgG (Bio-Rad Laboratories, Milan, Italy) for 1 h at room temperature. After washing, bound antibody was visualized with an enhanced chemiluminescence system (Western Blotting Luminol Reagent, Santa Cruz Biotechnology, California, USA).

The supraspinatus and biceps femoris muscles showed microscopical changes of diffuse myopathy, but the forelimb muscle was more severely affected.