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A canine *BCAN* microdeletion associated with episodic falling syndrome

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ABSTRACT

Episodic falling syndrome (EFS) is a canine paroxysmal hypertonicity disorder found in Cavalier King Charles spaniels. Episodes are triggered by exercise, stress or excitement and characterized by progressive hypertonicity throughout the thoracic and pelvic limbs, resulting in a characteristic 'deer-stalking' position and/or collapse. We used a genome-wide association strategy to map the EFS locus to a 3.48 Mb critical interval on canine chromosome 7. By prioritizing candidate genes on the basis of biological plausibility, we found that a 15.7 kb deletion in *BCAN*, encoding the brain-specific extracellular matrix proteoglycan brevican, is associated with EFS. This represents a compelling causal mutation for EFS, since brevican has an essential role in the formation of perineuronal nets governing synapse stability and nerve conduction velocity. Mapping of the deletion breakpoint enabled the development of Multiplex PCR and Multiplex Ligation-dependent Probe Amplification (MLPA) genotyping tests that can accurately distinguish normal, carrier and affected animals. Wider testing of a larger population of CKCS dogs without a history of EFS from the USA revealed that carriers are extremely common (12.9%). The development of molecular genetic tests for the EFS microdeletion will allow the implementation of directed breeding programs aimed at minimizing the number of animals with EFS and enable confirmatory diagnosis and pharmacotherapy of affected dogs.

Introduction

Episodic falling syndrome (EFS) is a well-recognized paroxysmal disorder found in Cavalier King Charles spaniels (CKCS). Episodes begin between fourteen weeks and four years of age and are triggered by exercise, stress, apprehension or excitement (Herrtage and Palmer, 1983). Episodes are of variable frequency and severity but are characterized by progressive hypertonicity involving thoracic and pelvic limbs (Fig. 1a) until the dogs are ultimately immobilized in a characteristic 'deer-stalking' or 'praying' position (Fig. 1b). Stiffening of all four limbs during exercise can cause falling (Fig. 1c), although there is no loss of consciousness or cyanosis. Other clinical signs may include facial muscle stiffness, stumbling, a 'bunny-hopping' gait, arching of the back or vocalization. Curiously, between episodes, dogs appear to be completely normal neurologically. Spontaneous activity was not observed in muscle electrodiagnostic testing, ruling out myotonia congenita (Wright et al., 1986, 1987). Muscle biopsies are typically normal at the light microscopic level, excluding many congenital myopathies. However, EFS has been linked to ultrastructural defects in skeletal muscle including dilatation and proliferation of the sarcoplasmic reticulum, mitochondrial swelling and degeneration (Wright et al., 1986, 1987). EFS has also been compared (Rusbridge, 2005) with startle disease/hyperekplexia, typically characterized by noise- or touch-evoked neonatal hypertonicity due to defects in inhibitory glycine receptor (*GLRA1*, *GLRB*; Shiang et al., 1993; Rees et al., 2002) or glycine transporter GlyT2 (*SLC6A5*) genes (Rees et al., 2006; Harvey et al., 2008). However, a microdeletion in the GlyT2 gene in Irish Wolfhounds results in severe neonatal muscle stiffness and tremor in response to handling (Gill et al., 2011), which is inconsistent with the observed clinical signs of EFS. Comparisons with startle disease may have been made because affected dogs often respond well to the benzodiazepine clonazepam (Garosi et al., 2002), an effective anticonvulsant, anxiolytic and muscle relaxant that is the most effective known treatment for human hyperekplexia (Thomas et al., 2010). However, the carbonic anhydrase inhibitor acetazolamide, used to treat certain types of human episodic ataxia (Tomlinson et al., 2009) and hyperkalemic periodic paralysis (Matthews and Hanna,

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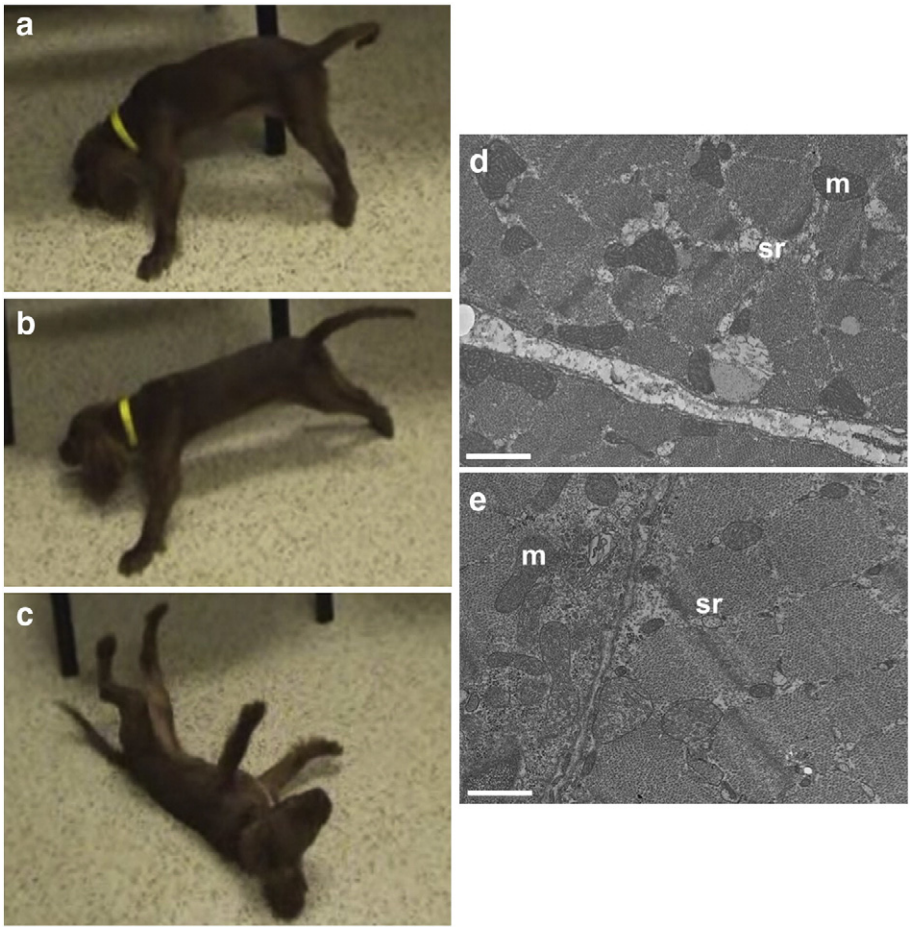

Fig. 1. Clinical signs of episodic falling syndrome and muscle pathology. A 5-month-old female Cavalier King Charles spaniel presented with typical episodes of excitement or exercise-induced muscle stiffness (a, hypertonicity) that would involve all four limbs and progress to an usual 'deer-stalking' or 'praying' posture (b), eventually resulting in falling (c). While EFS muscle was normal histologically by light microscopy, electron microscopy (d) revealed that the sarcoplasmic reticulum (sr) appeared dilated and contained finely granular material compared to control muscle (e). Mitochondria (m) and myofibrils were normal in appearance in both tissues. Scale bars = 0.31 μm.

2010), also appears to have therapeutic value in the treatment of EFS (http://www.cavalierhealth.org/episodic_falling).

Since a ten-year breeder-led investigation into the inheritance of EFS suggested an autosomal recessive mode of inheritance (http://cavalierepisodicfalling.com/), we used a genome-wide association strategy (Karlsson et al., 2007) to map the EFS locus to a defined region of canine chromosome 7. Candidate gene analysis enabled us to identify a microdeletion affecting the brevican gene (*BCAN*), confirm the deletion breakpoint and develop rapid genotyping tests for EFS.

Materials and methods

Light and electron microscopy

For light microscopy, unfixed biopsies from the biceps femoris, vastus lateralis and triceps brachii muscles were collected from five affected CKCS dogs under general anesthesia and frozen in isopentane pre-cooled in liquid nitrogen. Cryosections were cut (8 μm) and the following histochemical stains and reactions performed: hematoxylin and eosin, modified Gomori trichrome, periodic acid Schiff, phosphorylase, esterase, ATPase reactions at pH of 9.8 and 4.3, nicotinamide adenine dinucleotide-tetrazolium reductase, succinic dehydrogenase, acid phosphatase, alkaline phosphatase and oil red O. For electron microscopy, glutaraldehyde-fixed muscle specimens were post-fixed in osmium tetroxide, and dehydrated in serial alcohol solutions and propylene oxide prior to embedding in araldite resin. Thick sections (1 μm) were stained with toludine blue for light microscopy and ultrathin sections (60–90 nm) were stained with uranyl acetate and lead citrate for electron microscopy.

Study cohort and DNA preparation

Our study cohort comprised: EFS affected—10 animals (6 from the USA, 2 from New Zealand and 2 from the UK); Obligate EFS carriers—8 animals (2 from the USA, 6 from New Zealand); Animals related to carriers or affected dogs—21 animals (7 from the USA, 14 from New Zealand); Controls—CKCS with no EFS history—14 animals (all from the USA). Genomic DNA was isolated from whole blood or buccal cells using the Gentra Puregene Blood Kit (QIAGEN, Valencia, USA). Additional DNA samples from 155 CKCS with no clinical history of EFS and other pure bred-dogs were available from unrelated studies and other sources (e.g. Cornell Medical Genetic Archive: http://www.vet.cornell.edu/research/dnabank/).

Genome-wide association mapping

Thirteen CKCS genomic DNA samples isolated from blood (five cases, one obligate carrier and seven controls from the USA) were genotyped for 127,000 SNPs on the Affymetrix Canine SNP Array version 2 (http://www.broadinstitute.org/mammals/dog/caninearrayfaq.html). The two main drivers for sample selection were: i) lack of relatedness—i.e., that the animals used for case-control analysis should not share a common ancestor within at least three generations and ii) the quality and quantity of genomic DNA available. Arrays were processed at the