

Laminin $\alpha 2$ (merosin)-deficient muscular dystrophy and demyelinating neuropathy in two cats

Dennis P. O'Brien^{a,*}, Gayle C. Johnson^b, Ling A. Liu^c, Ling T. Guo^c, Eva Engvall^c,
Henry C. Powell^d, G. Diane Shelton^d

^a Department of Veterinary Medicine and Surgery, University of Missouri, 379 E. Campus Drive, Columbia, MO 65211, USA

^b Veterinary Diagnostic Laboratory University of Missouri, Columbia, MO, USA

^c The Burnham Institute, La Jolla, CA, USA

^d University of California, San Diego, La Jolla, CA, USA

Received 19 October 2000; received in revised form 16 March 2001; accepted 5 June 2001

Abstract

We report laminin $\alpha 2$ (merosin) deficiency associated with muscular dystrophy and demyelinating neuropathy in two cats. The cats developed progressive muscle weakness, and atrophy. Either hypotonia or contractures resulted in recumbency, necessitating euthanasia. Muscle biopsies showed dystrophic changes including marked endomysial fibrosis, myofiber necrosis, variability of fiber size, and perimysial lipid accumulation. Immunohistochemistry showed that laminin $\alpha 2$ chain was absent or reduced, while dystrophin and all the components of the dystrophin-associated glycoprotein complex were present and normal. One cat was examined in detail. Motor nerve conduction velocity (MNCV) was decreased, and ultrastructurally the peripheral nerves showed Schwann cell degeneration and demyelination. Brain imaging was not performed, but white matter changes were not apparent in the brain at necropsy. The disease in these cats is similar to primary or secondary merosin (laminin $\alpha 2$)-deficient congenital muscular dystrophy (CMD) in humans and to *dystrophia muscularis* in mice. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Laminin; Merosin; Congenital muscular dystrophy; Peripheral neuropathy; Demyelination; Feline; Animal model

1. Introduction

The most common muscular dystrophy in humans is caused by deficiency of dystrophin, and the best-characterized muscular dystrophies in domestic animals are also dystrophinopathies. Dogs and cats with dystrophin deficiency and mutations in the dystrophin gene have been described [1,2]. Muscular dystrophy has also been identified in sheep, but the molecular defect has not been identified. Deficiencies of dystrophin as well as dystrophin-associated proteins have been ruled out in that species [3,4].

Congenital muscular dystrophies (CMD) in humans are a heterogeneous group of autosomal recessive diseases manifest at birth or during infancy. They are characterized by muscle atrophy, hypotonia, weakness and contractures

[5–7]. About 40–50% of the “classic” CMD have a deficiency of laminin $\alpha 2$ expression in muscle. The remaining cases have normal expression of laminin $\alpha 2$ [7–9]. Laminins are large glycoproteins which contribute to the basement membrane in a variety of tissues. They are heterotrimers composed of α , β , and γ chains, and 11 laminin variants have been identified [10,11]. The primary variants in muscle are laminin 2 ($\alpha 2$ – $\beta 1$ – $\gamma 1$) and laminin 4 ($\alpha 2$ – $\beta 2$ – $\gamma 1$) (sometimes collectively referred to as merosin), which are connected to the sarcolemma via the dystrophin–glycoprotein complex [11,12] and integrins [13]. Laminin $\alpha 2$ is also found in the basement membrane of Schwann cells and in blood vessels within the brain [14,15] as well as in other tissues [11]. Most of the cases of laminin $\alpha 2$ -deficient CMD are associated with mutations in the laminin $\alpha 2$ gene (LAMA2) [6,7,9,16]. However, in the Japanese Fukuyama-type CMD and the Finnish muscle–eye–brain disease, the reduction in laminin $\alpha 2$ expression is secondary to a mutation in other genes [17,18]. Peripheral nervous system involvement in primary laminin $\alpha 2$ deficiency is suggested by reduced motor nerve con-

* Corresponding author. Tel.: +1-573-882-1922; fax: +1-573-884-5444.

E-mail address: OBrienD@missouri.edu (D.P. O'Brien).



Fig. 1. Affected DSH cat at 19 months of age. Muscle contracture caused rigidity and extension of the pelvic limbs and to a lesser extent the thoracic limbs. The tail and spine were also rigid. Generalized muscle atrophy was apparent.

duction velocity (MNCV) [19,20], while CNS involvement is suggested by changes in white matter on CT or MRI scans and slowed central conduction times [6,19,21–25].

The *muscularis dystrophica* mouse (*dy/dy*) was the first animal model of muscular dystrophy reported in 1955 [26], but it was not until almost 40 years later that a deficiency of laminin $\alpha 2$ was identified as the cause of the dystrophy and the mutation identified in the *dy2J/dy2J* mouse [27–29]. CMD has not been previously described in cats or dogs.

We report here the clinical and histological findings in two cats with muscular dystrophy and peripheral neuropathy associated with deficiency of laminin $\alpha 2$ in muscle and nerve. A preliminary report of these findings has been presented as an abstract [30].

2. Materials and methods

Two female cats, a Domestic Shorthair (DSH) and a Flame-point Siamese, were presented at 12 months of age for progressive weakness and muscle atrophy. Bulbar and spinal muscles were examined under general anesthesia by bipolar needle electromyography (EMG) in the DSH at 19 months of age. Motor nerve conduction velocity (MNCV) of the tibial nerve was also measured using standard techniques [31]. The clinical course of both cats was followed until euthanasia at 19 and 13 months of age, respectively. A complete necropsy was performed on the DSH.

Limb muscle biopsies were taken under general anesthesia from both cats at 12 months of age, and additional muscle samples were taken at necropsy in the DSH. Specimens were flash frozen in isopentane, precooled in liquid

nitrogen, and stored at -80°C until analyzed. Unfixed cryostat sections ($8\ \mu\text{m}$) were processed by a panel of standard histological and histochemical stains and enzyme reactions [32]. Immunofluorescence analysis was performed on cryostat sections of muscles from both cats. Sections of *vastus lateralis* muscle from a normal cat were similarly processed. The following monoclonal antibodies (dilution within parenthesis) were used: antibodies against the rod domain (1:25, NCL-DYS1) and carboxy terminus (1:50, NCL-DYS2) of dystrophin, antibody against α -sarcoglycan (1:50, NCL- α -SARC), β -sarcoglycan (1:50, NCL- β -SARC), γ -sarcoglycan (1:50, NCL- γ -SARC), all from Novocastra (Newcastle-upon-Tyne, UK), and antibody against α -dystroglycan (1:100, Upstate Biotechnology, Lake Placid, NY). The monoclonal antibodies against laminin $\alpha 2$ (5H2 and 1F9) and laminin $\gamma 1$ (2E8) and polyclonal antisera against laminin $\alpha 2$ and against laminin $\alpha 2$ - $\beta 1$ - $\gamma 1$ have been previously characterized [14]. A rabbit antiserum against the N-terminal domain VI of laminin $\alpha 2$ was generated by immunization with recombinant domain VI (Xu and Engvall, unpublished). Rabbit polyclonal antiserum against β -dystroglycan and α - and β -sarcoglycans were also used [33]. All dilutions of primary antibodies were made in 3% bovine serum albumin in phosphate-buffered saline, and incubations were for 1 h

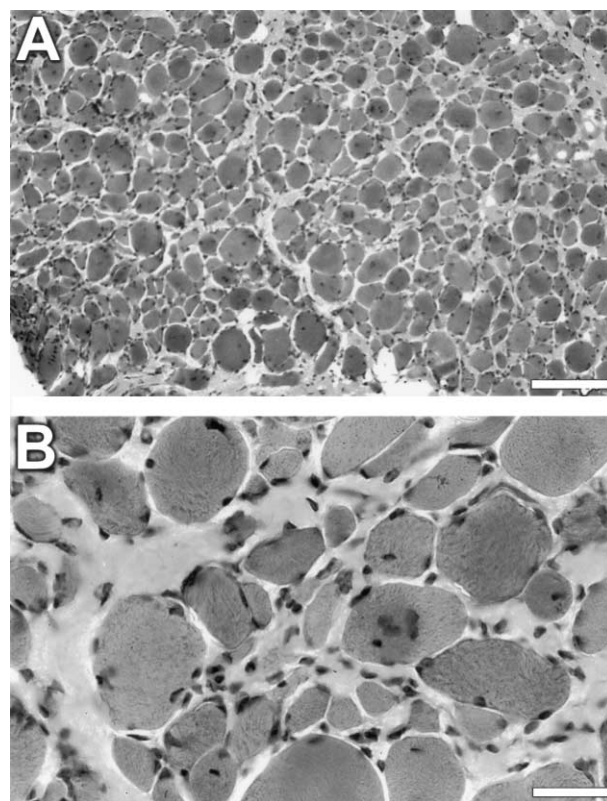


Fig. 2. Low (A: bar = $100\ \mu\text{m}$) and high (B: bar = $25\ \mu\text{m}$) power H and E stained sections of muscle from a DSH cat with absence of laminin $\alpha 2$. Note the marked endomysial fibrosis, myofiber necrosis, variability in fiber size, and perimysial lipid accumulation.

Table 1

Immunofluorescence detection of laminin $\alpha 2$ in muscle with different monoclonal and polyclonal antibodies

	DSH	Siamese	Control
<i>Monoclonal antibodies</i>			
5H2 (laminin $\alpha 2$)	–	–	+++
1F9 (laminin $\alpha 2$)	–	+/-	+++
2E8 (laminin $\gamma 1$)	+++	+++	+++
Dys 1	++	++	++
Dys 2	+++	+++	+++
α -Dystroglycan	+++	+++	+++
α -Sarcoglycan	+++	+++	+++
β -Sarcoglycan	+++	+++	+++
γ -Sarcoglycan	+++	+++	+++
<i>Polyclonal antibodies</i>			
Laminin $\alpha 2$ G domain	–	+/-	+++
Laminin $\alpha 2$ domain VI	–	+/-	+++
laminin $\alpha 1$ – $\beta 1$ – $\gamma 1$	+++	+++	+++
β -Dystroglycan	+++	+++	+++
α -Sarcoglycan	+++	+++	+++
β -Sarcoglycan	+++	+++	+++

–: No detectable staining; +/-: very weak staining. +++: strong staining.

at 37 °C. Secondary antibodies used were goat anti-mouse IgG-FITC (1:200, Cappel, West Chester, PA) and goat anti-rabbit IgG-FITC (1:200, Cappel) and incubation was for 1 h at room temperature.

Glutaraldehyde-fixed specimens from the radial and peroneal nerves taken at necropsy were postfixated in osmium tetroxide, and dehydrated in serial alcohol solutions and propylene oxide prior to embedding in araldite resin. Sections (1 μ m) were stained with toluidine blue-acid fuchsin for light microscopy and ultrathin sections stained with uranyl acetate and lead citrate for electron microscopy.

3. Results

3.1. Clinical examination and neurophysiologic study

Behavior and mental status of both cats appeared normal throughout the course of the disease and no seizures were reported. The DSH was reported to walk normally up until 6 months of age when pelvic limb weakness was noted. When examined at 12 months of age, she had atrophy and marked extensor contracture of the pelvic limbs. She could still walk if placed upright but would frequently fall and pull herself with the thoracic limbs. Her creatine kinase (CK) was elevated at 6742 IU/l (feline normal range: 0–820). At 19 months of age, she showed progression to involve the thoracic limbs and masticatory muscles. Pelvic limbs, spine and tail were rigid and only minimal distal limb movement apparent (Fig. 1). The thoracic limbs were weak, and the muscles were atrophic and mildly contracted. Moderate trismus was present, but she was still able to eat adequately. Cardiac ultrasound showed no evidence of myocardial dysfunction, and ophthalmic exam showed no abnormalities.

The EMG showed no abnormal spontaneous activity; individual motor unit action potentials and maximal contraction could not be examined under anesthesia. The tibial MNCV was slowed at 62.2 m/s in the proximal segment and 33.0 m/s in the distal segment (normal feline values 101.4 ± 12.9 and 97.4 ± 14.8 , respectively) [31]. Correcting for lower core body temperature under anesthesia (33 °C) would raise the conduction velocity by 8.5 m/s [34]. Limb temperature was not monitored, but even if the distal limb had cooled to room temperature, the corrected conduction velocity would only increase by 27.2 m/s; still well below normal for the cat. She was euthanized at 19 months of age and a complete necropsy performed.

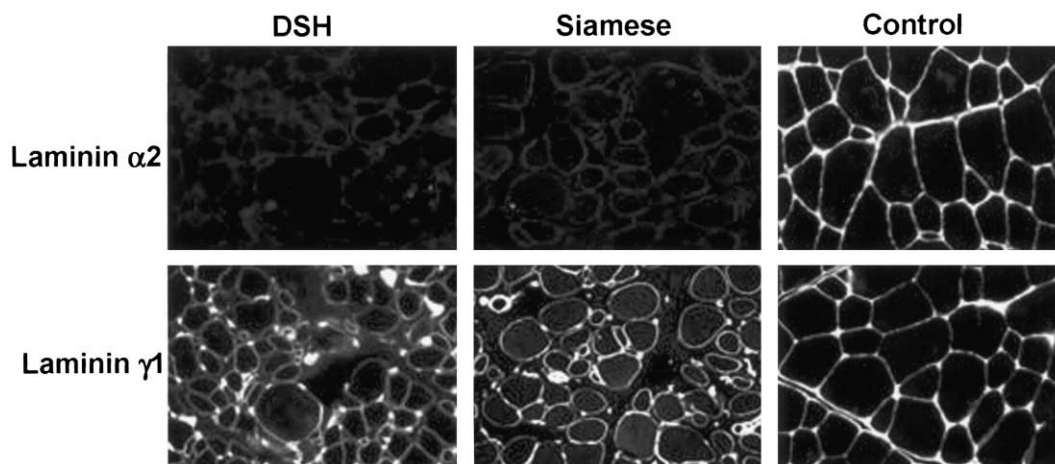


Fig. 3. Indirect immunofluorescent staining of muscle sections with antibodies against laminin $\alpha 2$ and laminin $\gamma 1$. The DSH and the Siamese had no detectable staining with the monoclonal laminin $\alpha 2$ antibody but had normal staining with the laminin $\gamma 1$ antibody.

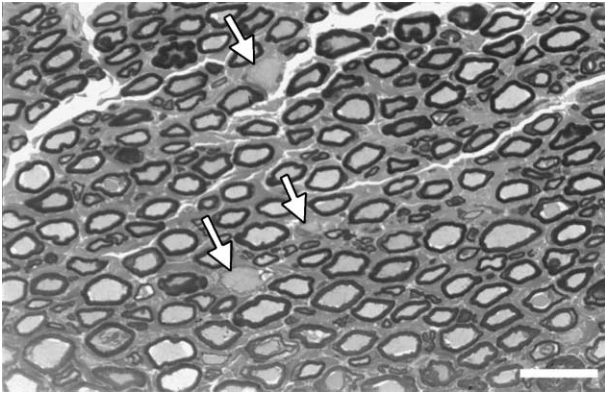


Fig. 4. Toluidine blue stained section of peroneal nerve from a DSH cat with absence of laminin $\alpha 2$ (bar = 100 μm). Large axons are devoid of myelin or poorly myelinated (arrows).

The Siamese cat was reported to be weak in the pelvic limbs when found at approximately 6 months of age. When examined at 12 months of age, she was non-ambulatory, was hypotonic and hyporeflexive in all four limbs, and had generalized muscle atrophy. Her CK was greater than 8000 IU/l. She developed respiratory distress and was euthanized at 13 months of age. The cause of the respiratory distress was not determined and no necropsy was performed.

3.2. Histopathology

Dystrophic changes were present in all skeletal muscles examined. Endomysial fibrosis was marked in both cats. In

addition, muscles from both cats showed myofiber necrosis, variability of fiber size and perimysial lipid accumulation (Fig. 2). Immunohistochemical labeling showed normal reactivity of the sarcolemmal membrane with antibodies to the rod domain and carboxy terminus of dystrophin; α -, β -, γ -, and δ -sarcoglycans; and α - and β -dystroglycans (Table 1). Antibodies against the C-terminal G domain and against the N-terminal domain VI of laminin $\alpha 2$ showed a complete absence (DSH) or drastic reduction (Siamese) in reactivity, while the antibodies reacted strongly with laminin $\alpha 2$ in normal cat muscle (Fig. 3; Table 1). Antibody to laminin $\gamma 1$ and an antiserum to laminin $\alpha 2$ - $\beta 1$ - $\gamma 1$ showed normal staining in all cats.

Abnormalities at necropsy in the DSH were limited to the peripheral nerve and muscle; no abnormalities were seen in the white matter of the brain or spinal cord. Pathological changes within all muscles were identical to those seen on biopsy. Light microscopic examination of 1 μm plastic embedded sections of the radial and peroneal nerves showed scattered thinly myelinated or demyelinated axons (Fig. 4). Ultrastructural examination of the peroneal nerve showed that the myelin changes were more severe than appreciated at the light level with generalized Schwann cell abnormalities and various stages of demyelination (Fig. 4). Marked periaxonal swelling was present consistent with the early phase of demyelination (Fig. 5A). Vacuoles were present in the Schwann cell cytoplasm. Detached and redundant myelin was present indicating a later stage of demyelination (Fig. 5B). The axoplasm appeared abnormally compacted with closely grouped neu-

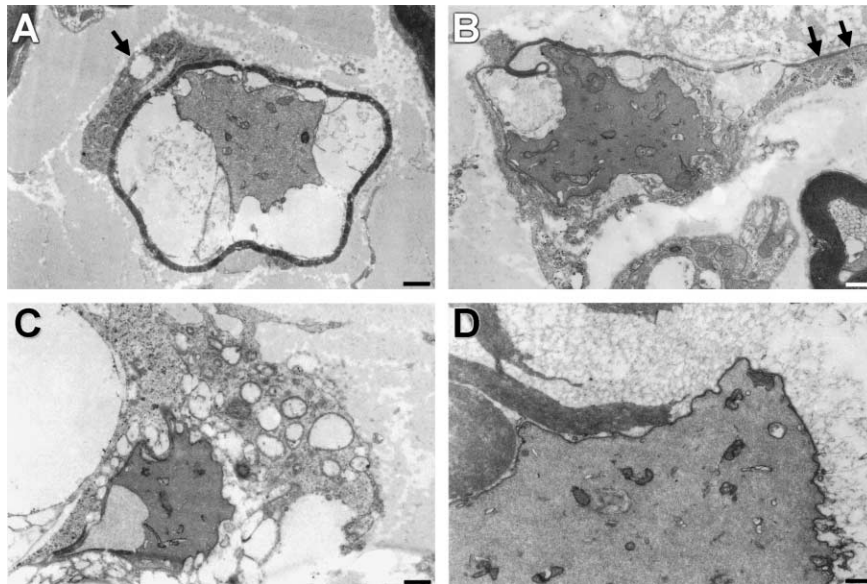


Fig. 5. Ultrastructural evaluation of the peroneal nerve from a DSH cat with the absence of laminin $\alpha 2$. (A) Marked periaxonal swelling in the early phase of demyelination. Note the vacuole in the Schwann cell cytoplasm (arrow). (B) Partially demyelinated axon with detached myelin in the right-hand third of the picture. Arrows point to redundant myelin draped over the Schwann cell cytoplasm. The axoplasm appears abnormally compacted with closely grouped neurofilaments and axoplasmic organelles. (C) Demyelinated axon showing periaxonal swelling consistent with Schwann cell disease. Note the disintegration of the Schwann cell with prominent vacuolar degeneration in the cytoplasm. (D) Higher power electron micrograph showing a completely demyelinated axon enveloped by the basal lamina which is closely applied to the axolemmal surface. Redundant myelin is seen adjacent to the axonal surface. (Bar = 1.0 μm for (A), (B) and (C), 0.05 μm for (D).)

rofilaments and axoplasmic organelles. Disintegration of the Schwann cell with prominent vacuolar cytoplasmic degeneration (Fig. 5C) and completely demyelinated axons (Fig. 5D) were present.

4. Discussion

The most common form of muscular dystrophy in humans, dogs, and cats is caused by mutations in the dystrophin gene and is characterized by absence or reduction in dystrophin protein at the sarcolemma. The dystrophin deficiency leads to a secondary reduction in the dystrophin-associated glycoproteins [12,35]. Previous reports of muscular dystrophy in cats have been limited to abnormalities in dystrophin [1,36]. Since the gene for dystrophin is located on the X chromosome, reported cases are males unless sired by an affected male with a carrier female. The cats in this report had normal dystrophin and dystrophin associated proteins, and they were females with no family history of disease.

The congenital muscular dystrophies (CMD) in humans are a heterogeneous group of autosomal recessive diseases that are not only characterized by muscular dystrophy but also varying degrees of nervous system involvement [5–7,37]. About half of the cases of classic CMD have decreased laminin $\alpha 2$ expression in muscle [7–9] associated with mutations in the laminin $\alpha 2$ gene (LAMA2) [9,16]. The *dy/dy* mouse also has an autosomal recessive muscular dystrophy [26] caused by a mutation in LAMA2 [29]. Spontaneous allelic variants have been described [26,38], and experimental null mutations generated [39,40].

The present cats showed undetectable or drastically reduced laminin $\alpha 2$, as judged by immunoreactivity with several antibodies directed against the carboxy-terminal G domain and the amino-terminal domain VI of the laminin $\alpha 2$. The most severely affected children with CMD also have a near absence of laminin $\alpha 2$ [41]. Complete deficiency of laminin $\alpha 2$ in CMD is usually associated with a mutation in the LAMA2 gene; the reduced expression seen in other forms of CMD is only partial [18]. The reduction of laminin $\alpha 2$ in the present cats is thus consistent with mutations in the LAMA2 gene being the cause of the disease although a secondary reduction cannot be ruled out.

The cats in this report demonstrated progressive weakness, muscle atrophy, elevated CK levels, and histologic changes on muscle biopsy typical of muscular dystrophy. Myopathic changes would be anticipated on EMG in CMD, but the EMG can be normal in up to 30% of cases of CMD [42]. The severity of muscle fiber loss and cooling of the limb under anesthesia may have contributed to the lack of EMG findings in the DSH. The Siamese cat was hypotonic while the severe contractures in the DSH lead to trismus and to rigidity of the limbs, spine, and tail. This mixture of hypotonia and contracture is similar to the signs reported

in children with CMD [5]. While the age of onset of signs is not known in the Siamese, the DSH developed normal ambulation and difficulties were not noted until 6 months of age. Signs of CMD in humans are apparent at birth or in early infancy and most affected children never walk [5,7,9]. A quadruped may compensate for deficits more readily than a biped, but it is unlikely that the gait would have been considered normal in the DSH if there were more than minor deficits. Cases of CMD in humans with later, even adult, onset of signs are reported, but most of these were cases with only partial laminin $\alpha 2$ deficiencies [8,24]. The cats in this study showed more drastic reduction. Signs progressed steadily in both cats necessitating euthanasia before 2 years of age. The *dy/dy* mouse develops clinical signs at a similar stage of development (3.5 weeks) with death before 6 months. As in these cats, signs in the mouse begin in the pelvic limbs and then progress to involve the axial and thoracic limb musculature. The paralysis in mice is described as either a flaccid paralysis [26] or contractures [39].

Dystrophin-deficient cats also begin showing clinical signs at 5–6 months of age, but develop hypertrophy of the appendicular and limb muscles as well as the tongue and diaphragm which results in feeding difficulties [1,36]. They also develop cardiomyopathy and sudden death [36,43]. No evidence of cardiomyopathy was seen on cardiac ultrasound, or at necropsy in the one cat examined in detail in this study.

Laminin $\alpha 2$ is found in Schwann cell basement membrane [14], and is thought to play a role in ensheathment and myelination in the peripheral nerve [44]. CMD patients and *dy/dy* mice have reduced MNCV [20,44,45]. Nerve histology in mice, showing demyelination most prominent in the proximal nerve [44–46], suggested a defect in the Schwann cell basement membrane [47]. MNCV was slowed in the one cat in this study and demyelination was evident histologically in the distal nerve. Difficulty maintaining normal body temperature during anesthesia in a cat with severe muscle atrophy complicated interpretation of the MNCV results. The degree of slowing could not be explained through temperature effects alone. Although demyelination was evident in only a minority of fibers on light microscopy, only distal nerves were examined. Even in these distal nerves, generalized Schwann cell changes were evident on electron microscopy. This supports a role for laminin $\alpha 2$ in myelination of peripheral nerve also in felines.

Seizures can occur and changes in brain MRI or CT scans can be detected in humans with laminin $\alpha 2$ -deficient CMD [9,19,21–25], but not in *dy/dy* mice [48]. Laminin $\alpha 2$ is highly expressed in vascular basement membrane in brain in humans [15] and mice, but is also expressed in other areas of the brain in some species [49,50]. There was no evidence of brain involvement in these cats either clinically or histologically, but brain scans were not performed.

In conclusion, feline laminin $\alpha 2$ deficiency is a new form of muscular dystrophy in this species. It is similar clinically, histologically and electrophysiologically to CMD, resulting from primary or secondary deficiency of laminin $\alpha 2$ and could serve as a complementary animal model of the disease.

Note in proof

Since submitting this manuscript, the authors have confirmed a third case of muscular dystrophy with deficient laminin alpha 2 immunostaining in a one year old, female, domestic shorthair cat with similar clinical signs.

Acknowledgements

Thanks to Dr. Apryl Steele for her assistance with the clinical evaluation of the Siamese cat, Dr. Virginia Louis Fuentes for cardiac ultrasonography, and Norma Prades for expert technical assistance. Portions of this study were funded by grants from the National Institutes of Health (EE) and the Muscular Dystrophy Association (EE).

References

- [1] Carpenter JL, Hoffman EP, Romanul FC, Kunkel LM, Rosales RK, Ma NS, et al. Feline muscular dystrophy with dystrophin deficiency. *Am J Pathol* 1989;135:909–19.
- [2] Cooper BJ, Winand NJ, Stedman H, Valentine BA, Hoffman EP, Kunkel LM, et al. The homologue of the Duchenne locus is defective in X-linked muscular dystrophy of dogs. *Nature* 1988;334:154–6.
- [3] Johnsen RD, Laing NG, Huxtable CR, Kakulas BA. Normal expression of adhalin and merosin in ovine congenital progressive muscular dystrophy. *Aust Vet J* 1997;75:215–6.
- [4] Nakada H, Jacobsen PF, Johnsen RD, Kakulas BA. Positive immunostaining of ovine congenital progressive muscular dystrophy with antibody against N- and C-terminal dystrophin. *Aust Vet J* 1990;67:271–2.
- [5] Brooke MH. A clinician's view of neuromuscular diseases. Baltimore: Williams & Wilkins, 1986.
- [6] Dubowitz V. 68th ENMC international workshop (5th international workshop): on congenital muscular dystrophy, 9–11 April 1999, Naarden, The Netherlands. *Neuromuscular Disord* 1999;9:446–54.
- [7] Tome FM, Evangelista T, Leclerc A, Sunada Y, Manole E, Estournet B, et al. Congenital muscular dystrophy with merosin deficiency. *C R Acad Sci, Ser III* 1994;317:351–7. *Sciences de la Vie*.
- [8] Dubowitz V. 41st ENMC International Workshop on Congenital Muscular Dystrophy 8–10 March 1996, Naarden, The Netherlands. *Neuromuscular Disord* 1996;6:295–306.
- [9] Pegoraro E, Marks H, Garcia CA, Crawford T, Mancias P, Connolly AM, et al. Laminin alpha2 muscular dystrophy: genotype/phenotype studies of 22 patients. *Neurology* 1998;51:101–10.
- [10] Aumailley M, Smyth N. The role of laminins in basement membrane function. *J Anat* 1998;193:1–21.
- [11] Wewer UM, Engvall E. Merosin/laminin-2 and muscular dystrophy. *Neuromuscular Disord* 1996;6:409–18.
- [12] Straub V, Campbell KP. Muscular dystrophies and the dystrophin-glycoprotein complex. *Curr Opin Neurol* 1997;10:168–75.
- [13] Mayer U, Safer G, Fassler R, Bornemann A, Echtermeyer F, von der Mark H, et al. Absence of integrin alpha 7 causes a novel form of muscular dystrophy. *Nat Genet* 1997;17:318–23.
- [14] Leivo I, Engvall E. Merosin, a protein specific for basement membranes of Schwann cells, striated muscle, and trophoblast, is expressed late in nerve and muscle development. *Proc Natl Acad Sci U S A* 1988;85:1544–8.
- [15] Villanova M, Malandrini A, Toti P, Salvestroni R, Six J, Martin JJ, et al. Localization of merosin in the normal human brain: implications for congenital muscular dystrophy with merosin deficiency. *J Submicrosc Cytol Pathol* 1996;28:1–4.
- [16] Helbling-Leclerc A, Zhang X, Topaloglu H, Cruaud C, Tesson F, Weissenbach J, et al. Mutations in the laminin alpha 2-chain gene (LAMA2) cause merosin-deficient congenital muscular dystrophy. *Nat Genet* 1995;11:216–8.
- [17] Cormand B, Avela K, Pihko H, Santavuori P, Talim B, Topaloglu H, et al. Assignment of the muscle–eye–brain disease gene to 1p32–p34 by linkage analysis and homozygosity mapping. *Am J Hum Genet* 1999;64:126–35.
- [18] Kobayashi K, Nakahori Y, Miyake M, Matsumura K, Kondo-Iida E, Nomura Y, et al. An ancient retrotransposal insertion causes Fukuyama-type congenital muscular dystrophy. *Nature* 1998;394:388–92.
- [19] Mercuri E, Muntoni F, Berardinelli A, Pennock J, Sewry C, Philpot J, et al. Somatosensory and visual evoked potentials in congenital muscular dystrophy: correlation with MRI changes and muscle merosin status. *Neuropediatrics* 1995;26:3–7.
- [20] Shorer Z, Philpot J, Muntoni F, Sewry C, Dubowitz V. Demyelinating peripheral neuropathy in merosin-deficient congenital muscular dystrophy. *J Child Neurol* 1995;10:472–5.
- [21] Philpot J, Topaloglu H, Pennock J, Dubowitz V. Familial concordance of brain magnetic resonance imaging changes in congenital muscular dystrophy. *Neuromuscular Disord* 1995;5:227–31.
- [22] Philpot J, Cowan F, Pennock J, Sewry C, Dubowitz V, Bydder G, et al. Merosin-deficient congenital muscular dystrophy: the spectrum of brain involvement on magnetic resonance imaging. *Neuromuscular Disord* 1999;9:81–5.
- [23] Sunada Y, Edgar TS, Lotz BP, Rust RS, Campbell KP. Merosin-negative congenital muscular dystrophy associated with extensive brain abnormalities. *Neurology* 1995;45:2084–9.
- [24] Tan E, Topaloglu H, Sewry C, Zorlu Y, Naom I, Erdem S, et al. Late onset muscular dystrophy with cerebral white matter changes due to partial merosin deficiency. *Neuromuscular Disord* 1997;7:85–9.
- [25] Vainzof M, Marie SK, Reed UC, Schwartzman JS, Pavanetto RC, Passos-Bueno MR, et al. Deficiency of merosin (laminin M or alpha 2) in congenital muscular dystrophy associated with cerebral white matter alterations. *Neuropediatrics* 1995;26:293–7.
- [26] Michelson AM, Russell ES, Harman PJ. Dystrophin muscularis: a hereditary primary myopathy in the house mouse. *Proc Natl Acad Sci U S A* 1955;41:1079–84.
- [27] Sunada Y, Bernier SM, Kozak CA, Yamada Y, Campbell KP. Deficiency of merosin in dystrophic dy mice and genetic linkage of laminin M chain gene to dy locus. *J Biol Chem* 1994;269:13729–32.
- [28] Xu H, Christmas P, Wu XR, Wewer UM, Engvall E. Defective muscle basement membrane and lack of M-laminin in the dystrophic *dy/dy* mouse. *Proc Natl Acad Sci U S A* 1994;91:5572–6.
- [29] Xu H, Wu XR, Wewer UM, Engvall E. Murine muscular dystrophy caused by a mutation in the laminin alpha 2 (Lama2) gene. *Nat Genet* 1994;8:297–302.
- [30] O'Brien DP, Johnson GC, Liu L, Engvall E, Shelton GD. Laminin alpha 2 deficient muscular dystrophy in two cats. *J Vet Intern Med* 2000;14:386.
- [31] Malik R, Ho S. Motor nerve conduction parameters in the cat. *J Small Anim Pract* 1989;30:396–400.

- [32] Dubowitz V. Muscle biopsy: a practical approach. Philadelphia: Bailliere Lindall, 1985.
- [33] Liu LA, Engvall E. Sarcoglycan isoforms in skeletal muscle. *J Biol Chem* 1999;274:38171–6.
- [34] Lee AF, Bowen JM. Effect of tissue temperature on ulnar nerve conduction velocity in the dog. *Am J Vet Res* 1975;36:1305–7.
- [35] Lim LE, Campbell KP. The sarcoglycan complex in limb-girdle muscular dystrophy. *Curr Opin Neurol* 1998;11:443–52.
- [36] Gaschen FP, Hoffman EP, Gorospe JR, Uhl EW, Senior DF, Cardinet III GH, et al. Dystrophin deficiency causes lethal muscle hypertrophy in cats. *J Neurol Sci* 1992;110:149–59.
- [37] Arahata K, Ishii H, Hayashi YK. Congenital muscular dystrophies. *Curr Opin Neurol* 1995;8:385–90.
- [38] Meier H, Southard JL. Muscular dystrophy in the mouse caused by an allele at the *dy*-locus. *Life Sci* 1970;9:137–44.
- [39] Kuang W, Xu H, Vachon PH, Liu L, Loechel F, Wewer UM, et al. Merosin-deficient congenital muscular dystrophy. Partial genetic correction in two mouse models. *J Clin Invest* 1998;102:844–52.
- [40] Miyagoe Y, Hanaoka K, Nonaka I, Hayasaka M, Nabeshima Y, Arahata K, et al. Laminin alpha2 chain-null mutant mice by targeted disruption of the *Lama2* gene: a new model of merosin (laminin 2)-deficient congenital muscular dystrophy. *FEBS Lett* 1997;415:33–9.
- [41] Cohn RD, Herrmann R, Sorokin L, Wewer UM, Voit T. Laminin alpha2 chain-deficient congenital muscular dystrophy: variable epitope expression in severe and mild cases. *Neurology* 1998;51:94–100.
- [42] Pegoraro E, Mancias P, Swerdlow SH, Raikow RB, Garcia C, Marks H, et al. Congenital muscular dystrophy with primary laminin alpha2 (merosin) deficiency presenting as inflammatory myopathy. *Ann Neurol* 1996;40:782–91.
- [43] Gaschen L, Lang J, Lin S, Ade-Damilano M, Busato A, Lombard CW, et al. Cardiomyopathy in dystrophin-deficient hypertrophic feline muscular dystrophy. *J Vet Intern Med* 1999;13:346–56.
- [44] Matsumura K, Yamada H, Saito F, Sunada Y, Shimizu T. Peripheral nerve involvement in merosin-deficient congenital muscular dystrophy and *dy* mouse. *Neuromuscular Disord* 1997;7:7–12.
- [45] Rasminsky M, Kearney RE, Aguayo AJ, Bray GM. Conduction of nervous impulses in spinal roots and peripheral nerves of dystrophic mice. *Brain Res* 1978;143:71–85.
- [46] Bradley WG, Jenkinson M. Abnormalities of peripheral nerves in murine muscular dystrophy. *J Neurol Sci* 1973;18:227–47.
- [47] Madrid RE, Jaros E, Cullen MJ, Bradley WG. Genetically determined defect of Schwann cell basement membrane in dystrophic mouse. *Nature* 1975;257:319–21.
- [48] Dubowitz DJ, Tyszka JM, Sewry CA, Moats RA, Scadeng M, Dubowitz V. High resolution magnetic resonance imaging of the brain in the *dy/dy* mouse with merosin-deficient congenital muscular dystrophy. *Neuromuscular Disord* 2000;10:292–8.
- [49] Hagg T, Portera-Cailliau C, Jucker M, Engvall E. Laminins of the adult mammalian CNS; laminin-alpha2 (merosin M-) chain immunoreactivity is associated with neuronal processes. *Brain Res* 1997;764:17–27.
- [50] Tian M, Hagg T, Denisova N, Knusel B, Engvall E, Jucker M. Laminin-alpha2 chain-like antigens in CNS dendritic spines. *Brain Res* 1997;764:28–38.