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CASE REPORT

Long-term follow-up of laminin $\alpha 2$ (merosin)-deficient muscular dystrophy in a cat

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Progressive muscle weakness beginning at 6 months of age was observed in a male Persian-mix cat. Muscle atrophy and joint contracture progressed over the next 3 years. The cat had developed gait difficulty at 8 months of age. The cat died at age of 5 years and 3 months due to an acute respiratory disorder. The clinical, laboratory, necropsy and histopathological findings of the cat were consistent with those of muscular dystrophy. The cat was diagnosed as having laminin $\alpha 2$ (merosin)-deficient muscular dystrophy on the basis of immunohistochemical findings. The cat was born in an inbred colony, and another related cat exhibited similar clinical signs. Few cases of laminin $\alpha 2$ -deficient muscular dystrophy have been reported in cats, and this report provides additional information about the disease.

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A 6-month-old castrated male Persian-mix cat was presented with a complaint of weakness when jumping. General physical examination revealed no remarkable findings. At 7 months of age, the cat showed dropped hocks in the pelvic limbs and mild muscle atrophy in the thighs. At 8 months of age, notable muscle atrophy in all four limbs and gait difficulty were evident. The cat was able to move by turning its body axis, but could not stand. Mental status, behaviour, proprioception, spinal reflexes, cranial nerves and sensation were all normal. At 1 year of age, joint contracture and marked muscle atrophy were observed in all four limbs. The dorsolumbar area also showed marked muscle atrophy. The cat was recumbent and was unable to move its body, but head and neck motion was relatively well preserved, despite limited movement of the temporomandibular joint. Food often spilled from the

cat's mouth, and drinking of water was difficult. However, neither intravenous fluid administration nor force-feeding was required, as the cat managed to feed itself adequately, and dehydration was avoided by the addition of water to its food. As the cat tended to retain urine, manual bladder expression was required. Tension and contraction of the bladder appeared normal on palpation. After about 1 year of age, the rate of deterioration of the cat's condition slowed (Fig 1). The cat's voice faded and was lost at about 3 years of age. Thereafter, there was little further deterioration until the cat died due to acute respiratory insufficiency at 5 years and 3 months of age. The cat ate well and maintained a normal weight and mentation throughout its life, with no clinical or observed evidence of pain.

Complete blood counts at 6, 8, 9 and 10 months of age were unremarkable. Blood chemistry studies (using FDC5500V, Fujifilm, Tokyo, Japan) showed that the serum creatine kinase (CK) level was moderately elevated at 6 and

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Fig 1. A castrated male Persian-mix cat at 2 years and 2 months of age. The cat is recumbent, and flexed contracture and muscle atrophy in the limbs are evident. Head and neck motion is relatively well preserved.

8 months of age (436 and 2000 U/l, respectively; reference range 87–309 U/l), but thereafter it remained within the reference range. Serum alanine aminotransferase (ALT) activity was slightly elevated at 8 and 11 months of age (113 and 122 U/l, respectively; reference range 22–84 U/l). Glucose, electrolytes and other parameters were all within the reference ranges. Serological testing (Idexx, Tokyo, Japan) showed that the cat was negative for feline leukaemia virus (FeLV) antigen, and also for antibodies against feline immunodeficiency virus (FIV), feline coronavirus (FCoV) and *Toxoplasma gondii*. Findings of urinalysis at 6 and 8 months and 2 years of age were unremarkable.

Echocardiography at 7 months of age demonstrated slight enlargement of the left ventricular free wall (diastolic phase 9.4 mm; reference range 2.5–5.0 mm) and ventricular septum (diastolic phase 5.4 mm; reference range 2.5–5.0 mm). Electrocardiography (ECG) at 8 months of age demonstrated a high R wave in lead II (2.0 mV; upper reference value 0.9 mV).

Biopsy of the thigh muscles was performed at 2 years and 3 months of age. Grossly, the muscle tissue was pale and resembled chicken meat. Tissue samples of the sartorius and biceps muscles of the thigh were fixed in 10% formalin and processed routinely for paraffin sections. Histopathological examination of the specimens revealed severe muscle atrophy and diffuse perimysial infiltration by fatty tissue. The atrophic changes showed a diffuse distribution within the examined biopsy tissues, and the atrophic muscle fibres were angular in shape. The muscle fibres

were markedly reduced in number with mild infiltration by mononuclear cells, which appeared to be macrophages or histiocytes. At 2 years and 8 months of age, a second muscle biopsy was performed to search for dystrophin. Fresh tissue samples of the sartorius and biceps muscles of the thigh were frozen in dry ice, and cryostat sections were made. However, the results of immunohistochemical staining with monoclonal anti-human dystrophin antibody (Novocastra, Newcastle, UK) were normal.

On post-mortem examination, various degrees of muscle atrophy were observed in the general skeletal muscles, and most prominently in the pelvic limbs. The affected muscles were pale to white and resembled fatty tissue, the changes being quite severe. Similar severe to moderate atrophic muscle changes were also observed in the thoracic limbs, abdomen, lumbar paraspinal muscles and tail. The muscles in the head and neck and thoracic paraspinal muscles were relatively well preserved. The myocardium in the area of the ventricular septum and the left ventricular free wall was slightly enlarged. Diffuse haemorrhage and mild exudation were found in the lungs. No gross lesions were noted in the brain, spinal cord, peripheral nerves, tongue, oesophagus, diaphragm, urinary bladder or other viscera.

Post-mortem histopathological examination was performed. The tissue samples were fixed in 10% formalin and processed routinely for paraffin sections. Severe muscle atrophy characterised by marked reduction in both the size and number of the muscle fibres and diffuse infiltration by fatty tissue, especially in the limbs, was observed. Within the muscle lesions, the intramuscular nerve branches and muscle spindles were well preserved (Fig 2). The remaining muscle fibres varied in size and showed several types of degenerative change, including endomysial fibrosis and increased numbers of nuclei with occasional central nuclei. Similar changes were also observed in the longissimus and masseter muscles and the diaphragm, although these lesions were less prominent than those in the limbs. In the heart, changes were characterised by irregular arrangement, disarray and vacuolar changes of cardiac muscle fibres, and were consistent with hypertrophic cardiomyopathy. In the lungs, there was diffuse thickening of the alveolar walls, as well as focal crystalline deposits of cholesterol and accumulation of foamy macrophages and lymphocytes. These changes were consistent with lipid pneumonia. In the nervous

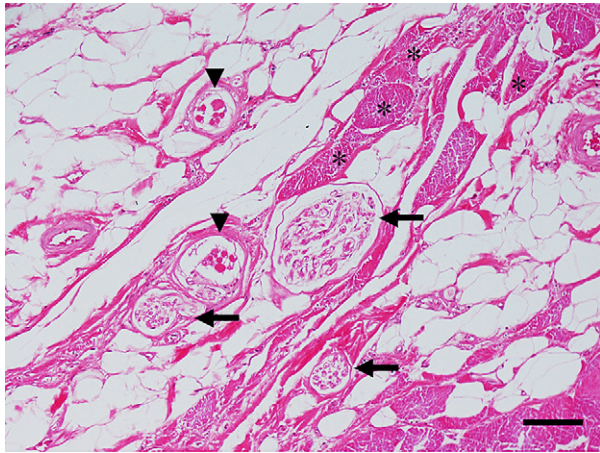


Fig 2. Histopathology of the biceps muscle of the thigh. There is marked atrophy and a decrease in the number of muscle fibres, with severe diffuse infiltration by fatty tissues. The intramuscular nerve branches (arrows) and muscle spindles (arrow heads) supplying the area of the lesion are well preserved. The asterisks represent remaining muscle fibres (haematoxylin and eosin stain; bar = 200 μ m).

tissues, including the brain and spinal cord, nerve roots, ganglia and peripheral nerves, no significant pathological changes were visualised in paraffin sections stained with haematoxylin and eosin and Luxol fast blue. In the urinary bladder and the other viscera, there were no remarkable histological lesions.

Immunohistochemical analysis for dystrophin, α - and β -dystroglycans, α -, β -, γ - and δ -sarcoglycans, emerin and laminin α 2 was performed using unfixed cryostat sections of necropsy specimens of muscles taken from this cat, and also from three cats without clinically evident neuromuscular disorders as controls. Monoclonal antibodies (1F9 and 5H2) (provided by Eva Engvall, Burnham Institute for Medical Research, La Jolla, CA, USA), which had been characterised previously, were used for laminin α 2-immunostaining (Leivo and Engvall 1988, O'Brien et al 2001). Commercially available antibodies were used to assess other muscle proteins. The results of immunohistochemistry with these antibodies, except laminin α 2, were equally positive in both this cat and the control cats. However, the muscle basement membrane from this cat was unreactive with both 1F9 and 5H2 antibodies against laminin α 2, whereas those of the control cats were intensely immunoreactive with both antibodies (Table 1, Fig 3). These results indicated an absence of laminin α 2 in the skeletal muscles of this cat.

The dam of this cat was of pedigree Persian breed. The sire was a Persian-mix cat, which

Table 1. Results of immunohistochemical staining

Antibody	Controls	Affected cat
Dystrophin (monoclonal anti-human)*	+	+
Dystrophin (monoclonal anti-human)†	+	+
α -Dystroglycan (monoclonal anti-rabbit)‡	+	+
β -Dystroglycan (monoclonal anti-human)§	+	+
α -Sarcoglycan (monoclonal anti-rabbit)	+	+
β -Sarcoglycan (monoclonal anti-human)	+	+
γ -Sarcoglycan (monoclonal anti-rabbit)	+	+
δ -Sarcoglycan (monoclonal anti-human)	+	+
δ -Sarcoglycan (polyclonal anti-human)¶	+	+
Emerin (monoclonal anti-human)§	+	+
Laminin α 2 1F9 (monoclonal anti-human)#	+	-
Laminin α 2 5H2 (monoclonal anti-human)#	+	-

+, Positive; -, negative.

*Chemicon International Inc, Temecula, CA, USA.

†Kamiya Biomedical Company, Seattle, WA, USA.

‡Upstate, Lake Placid, NY, USA.

§YLEM, Roma, Italy.

||Novocastra, Newcastle, UK.

¶Santa Cruz Biotechnology Inc, Santa Cruz, CA, USA.

#Provided by Eva Engvall, Burnham Institute for Medical Research, La Jolla, CA, USA.

was the offspring of the same dam, and, therefore, the proband cat was the product of dam and offspring inbreeding. Two other littermates of this cat were clinically normal. However, similar clinical signs were observed in another male cat in the next litter from the same dam and sire (Fig 4). Muscle weakness and atrophy and joint contracture progressed rapidly from 6 to 9 months of age in the affected sibling, and it died suddenly at 1 year and 11 months of age. At the owner's request, no further pathological examinations were performed.

The final diagnosis in the present cat was laminin α 2 (merosin)-deficient muscular dystrophy. The results of immunohistochemistry indicated a defect of laminin α 2 in the muscle basement membrane.

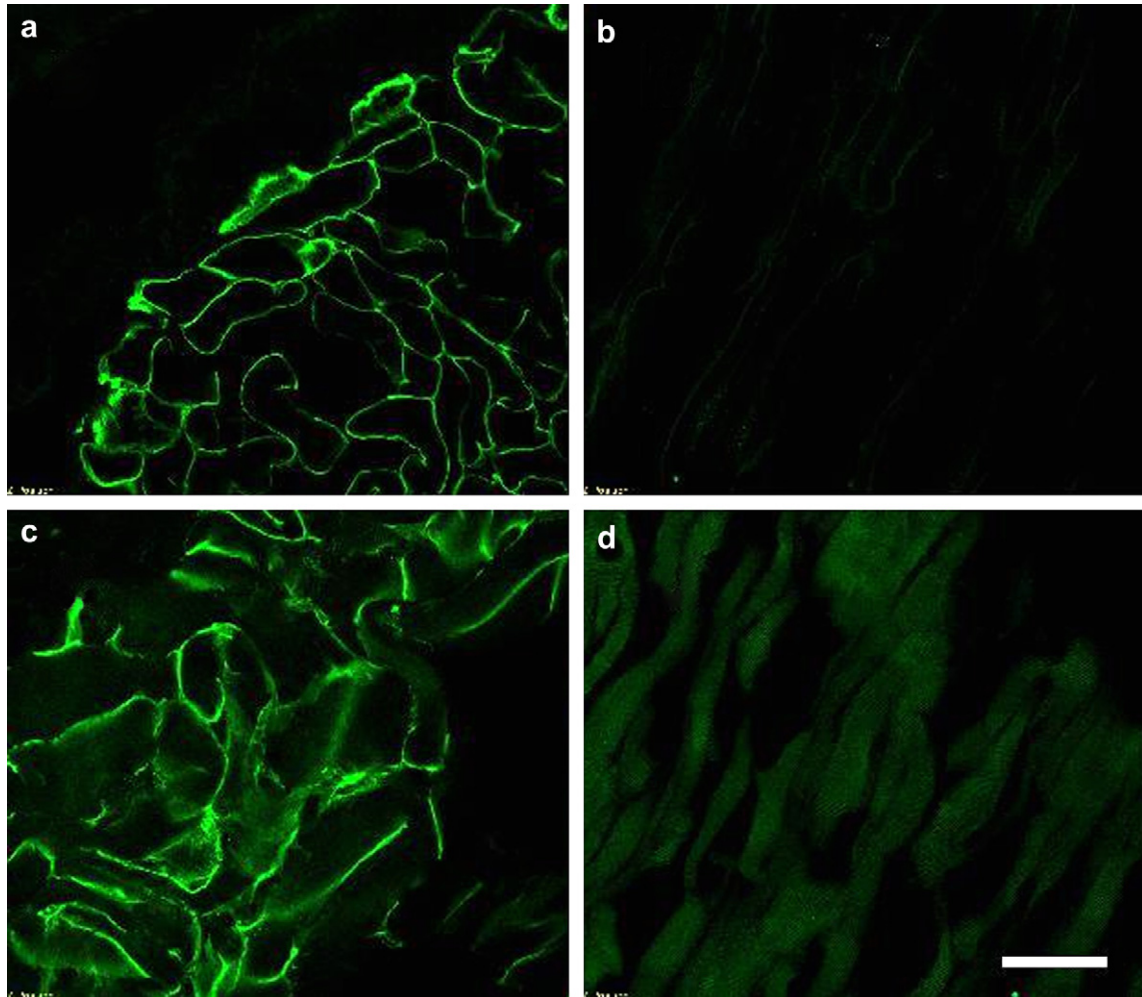


Fig 3. Immunofluorescence staining of muscles for laminin $\alpha 2$. (a) Control with 1F9 antibody, (b) affected muscle with 1F9 antibody, (c) control with 5H2 antibody and (d) affected muscle with 5H2 antibody. The green staining indicates positivity, and the lack of staining indicates negativity with the antibodies. The basement membranes of the affected muscles (b and d) are negative for the two monoclonal antibodies (1F9 and 5H2), whereas those of the controls (a and c) are intensely positive for both antibodies (bar = 100 μ m).

In human medicine, muscular dystrophies are described according to whether they are inherited conditions characterised by varying degrees of muscle weakness that are not neurogenic (Emery 2001a). Then whether they are associated with specific defects in proteins, which are normally expressed at least in the skeletal muscles (Emery 2001a). Finally, mitochondrial myopathies, as well as myopathies associated with characteristic pathological lesions such as central cores or nemaline rods, are excluded (Emery 2001a). Recently, muscular dystrophies have been classified into more than 30 different forms, according to whether they are due to defects in muscle proteins such as dystrophin, laminin $\alpha 2$, $\alpha 7$ integrin, dysferlin, caveolin-3, sarcoglycans, telethonin, myotilin, calpain-3, poly-A binding protein,

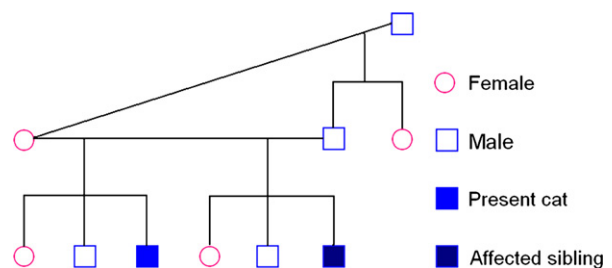


Fig 4. Family tree. The circles and boxes indicate females and males, respectively. The clear circles and boxes indicate clinically normal cats, and the coloured boxes indicate affected cats. The present cat and its siblings were inbred. There were two affected male cats (the present case and an affected sibling), two clinically normal male cats and two clinically normal female cats.

emerin and laminin A/C (Emery 2001a). Laminins are glycoproteins which are major components of the basement membrane and consist of α , β and γ subunits (Wewer and Engvall 1996, Hayashi and Arahata 1997). Laminin $\alpha 2$ is one of the five subclasses of laminin α chains (Wewer and Engvall 1996, Hayashi and Arahata 1997). Laminins which include the laminin $\alpha 2$ chain have been called merosin and are found in the basement membranes of skeletal and cardiac muscles, Schwann cells and the placenta (Leivo and Engvall 1988, Hayashi and Arahata 1997). Muscular dystrophy caused by laminin $\alpha 2$ deficiency is called merosin-deficient congenital muscular dystrophy, and shows autosomal recessive inheritance in humans (Tome et al 1994, Helbling-Leclerc et al 1995, Hayashi and Arahata 1997, Emery 2001b).

Several kinds of muscular dystrophies and dystrophy-like myopathies have been also described in companion animals (Shelton and Engvall 2002, Shelton 2004, Blot 2005, Gaschen and Jones 2005, Shelton and Engvall 2005). The muscular dystrophies whose causes have been identified are dystrophinopathy and laminin $\alpha 2$ deficiency in dogs and cats, and sarcoglycanopathies in dogs (Cooper et al 1988, Kornegay et al 1988, Valentine et al 1988, Carpenter et al 1989, Gaschen et al 1992, Gaschen and Burgunder 2001, O'Brien et al 2001, Shelton et al 2001, Poncelet et al 2003, Shelton and Engvall 2005). Muscular dystrophy with dystrophinopathy in cats, which is similar to Duchenne muscular dystrophy in humans, generally occurs in males as an X chromosome-linked disorder (Carpenter et al 1989, Gaschen et al 1992, Gaschen and Burgunder 2001). It is characterised by systemic muscle hypertrophy and is known as hypertrophic feline muscular dystrophy (Gaschen et al 1992, Gaschen and Burgunder 2001, Gaschen et al 2004). Muscular dystrophy with laminin $\alpha 2$ deficiency in cats has been reported in three females, and the disease was not familial (O'Brien et al 2001, Poncelet et al 2003).

In cats with laminin $\alpha 2$ -deficient muscular dystrophy, muscle atrophy and joint contracture have been described (O'Brien et al 2001, Poncelet et al 2003). These two signs were evident in the present cat. It is considered that muscle atrophy and joint contracture are characteristic clinical features of the disease, differing from those of hypertrophic feline muscular dystrophy. Although the present cat showed no evident neurological abnormality, peripheral nerve involvement has been described in three cats with laminin

$\alpha 2$ -deficient muscular dystrophy and also in merosin-deficient congenital muscular dystrophy in humans (Shorer et al 1995, O'Brien et al 2001, Poncelet et al 2003). In the present case pathological examinations of the nervous system were performed using only paraffin sections, and further specific investigations including ultrastructural studies would be needed to evaluate the morphological changes in the peripheral nerves. Cardiac changes similar to those of hypertrophic cardiomyopathy were recognised in the present cat. The relationship between the cardiac lesions and laminin $\alpha 2$ deficiency remains unclear, as the cardiac changes have not been described in previously reported cats with laminin $\alpha 2$ -deficient muscular dystrophy (O'Brien et al 2001, Poncelet et al 2003). Although the present cat showed a tendency for urine retention, no pathological changes which could have caused dysuria were identified.

In the present case, the cause of death was presumed to be aspiration pneumonia because of the clinical signs, and the findings of necropsy and pathological examination. The disease progression in cats with laminin $\alpha 2$ -deficient muscular dystrophy is not clear, as all three other reported cats were euthanased before 2 years of age (O'Brien et al 2001, Poncelet et al 2003). In humans with merosin-deficient congenital muscular dystrophy, the major cause of death is respiratory tract infection (Hayashi and Arahata 1997). As this cat was not euthanased the case history should provide additional information on the clinical course of this disease.

The present cat was the product of inbreeding, and had a sibling with similar signs as well as four normal siblings, which is consistent with recessive inheritance. As laminin $\alpha 2$ -deficient muscular dystrophy occurs in both male (the present case) and female (previously reported cases) cats, the disease is consistent with an autosomal recessive inheritance.

To the authors' knowledge, this is the first report of laminin $\alpha 2$ -deficient muscular dystrophy in a male cat, and the first to suggest a familial origin in this species. Most importantly, this is the first report to describe the clinical progression and course of this disease in a cat.

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