The Journal of Veterinary Medical Science

NOTE

Internal Medicine

Long-term survival of a dog with Alexander disease

Yui KOBATAKE[1)]*, Nao NISHIMURA[1)], Hiroki SAKAI[1-3)], Syunsuke IWANA[4)], Osamu YAMATO[5)], Naohito NISHII[1,2)] and Hiroaki KAMISHINA[1–3)]

[1)]Faculty of Applied Biological Sciences, Gifu University, Gifu 501-1193, Japan
[2)]The United Graduate School of Veterinary Sciences, Gifu University, Gifu 501-1193, Japan
[3)]Center for Highly Advanced Integration of Nano and Life Sciences, Gifu University, Gifu 501-1193, Japan
[4)]Kohnan Animal Hospital, Aichi 483-8085, Japan
[5)]Joint Faculty of Veterinary Medicine, Kagoshima University, Kagoshima 890-0065, Japan

J. Vet. Med. Sci.
82(11): 1704–1707, 2020
doi: 10.1292/jvms.20-0133

Received: 9 March 2020
Accepted: 19 September 2020
Advanced Epub:
15 October 2020

ABSTRACT. A 1-year- and 11-month-old spayed female toy poodle had showed progressive ataxia and paresis in the hindlimbs since 11 months old. Magnetic resonance imaging revealed high signal intensity on T2-weighted and fluid-attenuated inversion recovery images at the thoracic and lumbar spinal cord. The dog's neurological condition slowly deteriorated and flaccid tetraparesis was exhibited. At 4 years and 11 months old, the dog died of respiratory failure. On postmortem examination, eosinophilic corkscrew bundles (Rosenthal fibers) were observed mainly in the thoracic and lumbar spinal cord. Histological features were comparable to previously reported cases with Alexander disease. This is a first case report to describe the clinical course and long-term prognosis of a dog with Alexander disease.

KEY WORDS: Alexander disease, astrocyte, canine, neurodegenerative disease, Rosenthal fibers

Alexander Disease (AxD) is a progressive, fatal neurodegenerative disorder that has been recognized in several species, such as humans, sheep and dogs [7, 10, 12, 14, 15]. The characteristic pathological feature of AxD is the presence of abnormal astrocytes that contain glial fibrillary acidic protein (GFAP) aggregates, termed Rosenthal fibers, distributed around the vessels in the white matter as well as subpial and subependymal areas [10, 20]. In total, 13 dogs have been clinically and histopathologically diagnosed with AxD [1, 5, 8, 9, 14, 18, 21–23]. Because of the poor prognosis, the owners of dogs with AxD tend to elect euthanasia. Accordingly, there are no case reports of long-term survival in dogs with AxD. We present the clinical course and long-term prognosis of a dog with AxD.

A 1-year- and 11-month-old spayed female toy poodle weighing 2.6 kg was referred with a history of progressive ataxia and paresis in the hindlimbs, which had been present since the animal was 11 months old. Although the dog was given a vitamin B group supplement for 1 week, no obvious therapeutic effect was observed. On neurological examination, the dog was alert and responsive; however, postural reactions and spinal reflexes were reduced in the hindlimbs. Cranial nerve examinations were unremarkable. Based on these findings, a focal lesion of the L4-S1 spinal cord segment or a multifocal lesion of the peripheral nervous system (PNS) and spinal cord was suspected. Complete blood count and serum chemistry profile findings were within normal limits.

We performed magnetic resonance imaging (MRI) (0.4-Tesla APERTO Eterna; Hitachi, Tokyo, Japan) in order to evaluate the central nervous system. Anesthesia was induced using propofol (propofol for animals; Mylan Inc., Canonsburg, PA, USA) and maintained with isoflurane (isoflurane for animals; Mylan Inc.). Pre- and post-contrast (following an intravenous injection of 0.1 mmol/kg of Gadodiamide hydrate) (Omniscan; Daiichisankyo, Tokyo, Japan) T1-weighted images (TR=400 msec and TE=13 msec), T2-weighted images (TR=2,500 msec and TE=120 msec), and fluid-attenuated inversion recovery (FLAIR) images (TR=9,000 msec and TE=100 msec) of the thoracic and lumbar spinal cord were obtained. MRI of the T9-T10 and L4 spinal cord segment showed high signal intensity on T2-weighted and FLAIR images and iso signal intensity on pre/post-contrast T1-weighted images (Fig. 1). In anticipation of a possible inflammatory disease of the nervous system, we treated the dog with prednisolone (0.5 mg/kg/day) for 1 week but no improvement in symptoms was observed. Given the ineffectiveness of prednisolone and other factors, such as the juvenile-onset and chronic progression of the disease, a tentative diagnosis of neurodegenerative disease was made. Differential diagnosis included lysosomal storage disease, cerebellar cortical abiotrophy, neuroaxonal dystrophy, and leukodystrophy myelopathy.

At 2 years and 4 months old, the dog presented with paresis not only in the hindlimbs but also in the forelimbs (Fig. 2). Follow-up MRI of the cervical and thoracolumbar regions showed no change from the previous examination. At 2 years and 7 months old,

*Correspondence to: Kobatake, Y.: kobatake@gifu-u.ac.jp

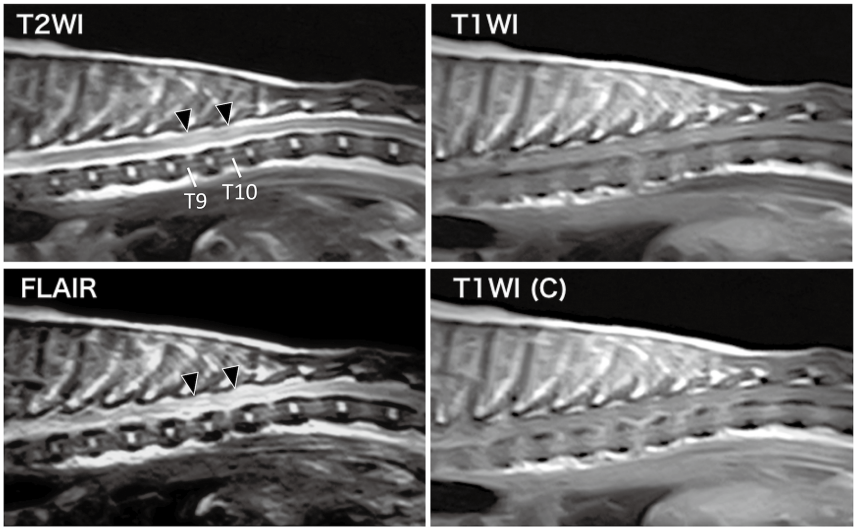

Fig. 1. Sagittal magnetic resonance images of the thoracic spinal cord. T2-weighted images (A), T1-weighted pre-contrast images (B), Fluid-attenuated inversion recovery images (C), T1-weighted post-contrast images (D). The T9–10 spinal cord segment showed slightly high signal intensity on T2WI and Fluid-attenuated inversion recovery images (black arrowheads).

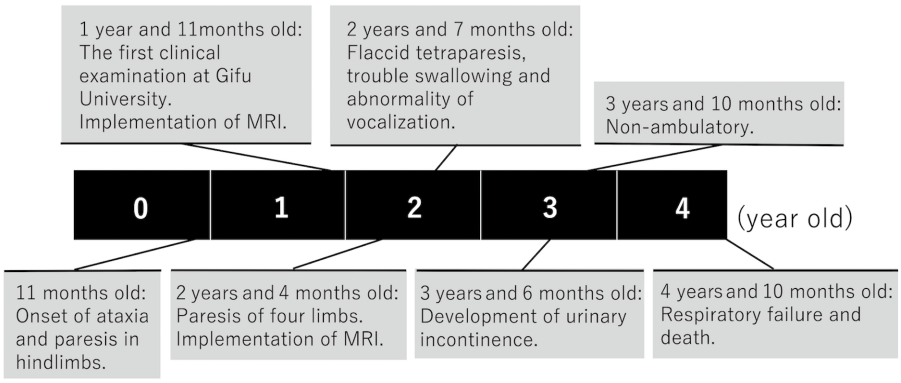

Fig. 2. The time course of clinical signs.

the dog exhibited flaccid tetraparesis. The dog also had difficulty swallowing hard food, and showed vocalization abnormalities. On neurological examination, postural reactions and spinal reflexes were absent in the hindlimbs, and reduced in the forelimbs. As a result, the dog showed exaggerated hopping on the bilateral forelimbs. On cranial nerve examination, the dog presented with reductions in bilateral palpebral reflex, corneal reflex and menace reaction. Based on neurological examination, a multifocal lesion of the spinal cord and PNS, including the facial and trigeminal nerve, was suspected. At 3 years and 1 month old, we initiated a supplement therapy containing the neuroprotective drug curcumin (Neuroact; Zenoaq, Fukushima, Japan), used for treating neurodegenerative disease [4], at a dosage of 1.0 m*l*/head/day until her death. However, there were no improvements in clinical manifestation. At 3 years and 6 months old, the dog developed urinary incontinence, which resulted in bacterial cystitis. At 3 years and 10 months old, postural reactions and spinal reflexes were absent and the dog was non-ambulatory. At 4 years and 10 months old, the dog showed deterioration of its general condition, which was diagnosed as pericardial effusion by a general practitioner. Diuretic drug therapy was effective. At 4 years and 11 months old, the dog died of respiratory failure, after which necropsy was performed with the owner's consent.

Postmortem macroscopic examination revealed diffuse atrophy of the spinal cord and brain. For light microscopy, tissue samples were fixed in 10% formalin, and paraffin-embedded sections were stained with hematoxylin, eosin, and Luxol fast blue. Immunostaining was performed with antibodies against GFAP (Rabbit polyclonal anti-GFAP antibody, Dako, Glostrup, Denmark). On microscopic examination, eosinophilic corkscrew bundles (Rosenthal fibers) were observed in the subpial parenchyma, perivascular parenchyma and periependymal cells in the white matter of the thoracic and lumbar spinal cord (Fig. 3A). Furthermore, many hypertrophic astrocytes and axonal spheroids were presented in the white matter of the spinal cord (Fig. 3B). Rosenthal fibers were stained dark brown in immunostaining, which indicated GFAP-positive (Fig. 3C). Similar changes were seen in the white matter of the cerebrum, cerebellum, molecular layer of the cerebellar cortex, and brainstem-which showed milder changes compared with the spinal cord (Fig. 3D). Rosenthal fibers have been described in reactive tissues (for example, in gliosis),

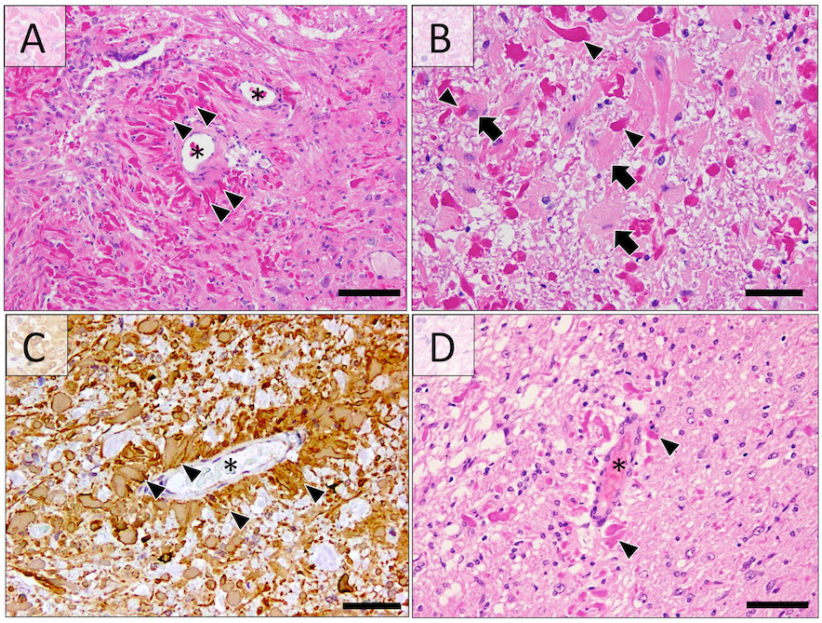

Fig. 3. Histopathological findings of the white matter of L4 spinal cord segment and cerebrum. A–C show the L4 spinal cord segment, and D shows the cerebrum. A, B and D were stained by hematoxylin and eosin, C was immunostained by anti- glial fibrillary acidic protein antibody. (A) The eosinophilic corkscrew bundles (Rosenthal fibers; black arrowheads, applied to A–D) were observed around blood vessels (asterisks, applied to A, C and D), (B) Many hypertrophic astrocytes (black arrows) were also observed, (C) Rosenthal fibers were immunostained dark brown by anti- glial fibrillary acidic protein antibody, (D) Although eosinophilic corkscrew bundles were observed in the cerebrum, severity was milder compared with the spinal cord. Scale bar=100 μm.

neoplasms (such as pilocytic astrocytoma), and the genetic disorder AxD. The fiber distribution in AxD is unique because fibers occur not only around blood vessels in the white matter, but also in subpial and subependymal areas [10]. The histopathology results we obtained, therefore, enabled us to make a postmortem diagnosis of AxD for the dog.

AxD in humans is a rare neurodegenerative disorder. It is commonly classified into infantile, juvenile and adult forms, depending on the age of onset [10]. More recently, a different classification system has been proposed, with only two categories, type I and type II, which are based on the distribution of lesions and clinical signs [17]. Type I is characterized by early onset, seizures, macrocephaly, motor delay, encephalopathy, failure to thrive and paroxysmal deterioration. Type II is characterized by later onset, autonomic dysfunction, ocular movement abnormalities and bulbar symptoms. Median survival time of type I patients was 14 years, and median survival time of type II patients was 25 years [17], indicating that the clinical symptoms of type II AxD progress more slowly than type I.

The present dog developed AxD at 11 months old, equivalent to the juvenile form in humans. Furthermore, its clinical signs (dysfunction of spinal cord and brain stem) were comparable to the juvenile and type II forms of AxD in humans. To date, neurodegenerative diseases resembling AxD have been reported in 13 dogs: 5 Bernese mountain dogs, 3 Labrador retrievers, 1 Bernese mountain crossbred dog, 1 Chihuahua, 1 French bulldog, 1 Miniature poodle and 1 Scottish terrier [1, 5, 8, 9, 14, 18, 21–23]. Similar to our case, most of these dogs showed symptoms similar to the juvenile forms of AxD. However, there are no reports of the long-term clinical course because the majority of owners elected euthanasia. This is the first report of long-term survival in a dog with AxD resembling the juvenile/type II form. As the disease progressed, the dog exhibited bulbar symptoms, such as dysphonia, dysphagia, facial and trigeminal nerve paralysis, similar to humans with type II AxD [17]. The results suggested that the prognosis of dogs with AxD was similar to that of humans with AxD and could offer a promising animal clinical model for AxD. The exaggerated hopping reaction of the dog seen in our case was unusual and unexpected for AxD, given the reduction of the spinal reflex. One explanation could be an additional cerebellar disorder. However, no other signs of cerebellar disorder were detected that supported such a diagnosis.

A previous study using an *in vitro* model of AxD suggested that curcumin reduces the expression of endogenous GFAP resulting in beneficial effects [2]. In our case, no obvious therapeutic effect of a one-year course of curcumin treatment was observed, with ataxia and paresis in the forelimbs gradually progressing over the year until death. On the day of her death, the dog displayed shortness of breath, which we attributed to aspiration pneumonia, although further clinical examination had not been performed. Aspiration pneumonia would be an expected consequence of the progressive swallowing disorder the dog exhibited for the previous 1 year and 8 months. In humans with type II AxD, death is often due to bulbar disorder (including respiratory insufficiency and dysphagia leading to aspiration pneumonia) [17]. Although the direct cause of death was not determined here, this case demonstrates the importance of care for respiratory insufficiency and long-term survival.

Our MRI of the T9-T10 and L4 spinal cord segments of the dog displayed a high signal intensity on T2-weighted images. In

humans with type II AxD, a failure of normal myelination is most likely to be responsible for at least part of the abnormal white matter signal on MR images [15]. However, histopathological examination of our case was performed only on T9-12 and L4-L7, and it was, therefore, not possible to use the pathological findings to characterize and distinguish the T2-weighted image of a hyperintense lesion from that of other isointense lesions.

The case exhibited lower motor neuron symptoms on four limbs, despite no abnormality of the cell body of the peripheral motor neurons in the ventral horn of the spinal cord. Unfortunately, we did not perform histopathological examination of the axonal fiber of the peripheral nervous system, of which possible myelin sheath dysfunction was indicated in a previous case report of a dog with AxD [23]. We cannot exclude the possibility that our dog had been suffering from such dysfunction. The lesions of the cerebrum and brainstem were milder compared with the spinal cord, which most likely resulted in survival for a longer period of time.

Both the infantile and juvenile forms of human AxD generally appear to be sporadic [11], but the rarity of cases in siblings suggests the possibility of autosomal inheritance [6, 16]. In contrast, the adult form is often familial [3, 13]. It is of interest that a male littermate of the present dog showed neurological symptoms, such as ataxia and megaesophagus, which had been present since the animal was 11 months old. The littermate died by aspiration pneumonia at 2 years and 11 months old. Unfortunately, the littermate was not pathologically examined. Although the pathogenesis of canine AxD is not currently established, observations suggest that the process could be genetic or congenital [19, 21].

ACKNOWLEDGMENTS. The authors would like to thank Dr. Daisuke Hasegawa for his contribution to the manuscript by providing information about a male littermate of the present dog. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

REFERENCES

1. Alemañ, N., Marcaccini, A., Espino, L., Bermúdez, R., Nieto, J. M. and López-Peña, M. 2006. Rosenthal fiber encephalopathy in a dog resembling Alexander disease in humans. *Vet. Pathol.* **43**: 1025–1028. [Medline] [CrossRef]
2. Bachetti, T., Di Zanni, E., Balbi, P., Ravazzolo, R., Sechi, G. and Ceccherini, I. 2012. Beneficial effects of curcumin on GFAP filament organization and down-regulation of GFAP expression in an in vitro model of Alexander disease. *Exp. Cell Res.* **318**: 1844–1854. [Medline] [CrossRef]
3. Balbi, P., Seri, M., Ceccherini, I., Uggetti, C., Casale, R., Fundarò, C., Caroli, F. and Santoro, L. 2008. Adult-onset Alexander disease: report on a family. *J. Neurol.* **255**: 24–30. [Medline] [CrossRef]
4. Cole, G. M., Teter, B. and Frautschy, S. A. 2007. Neuroprotective effects of curcumin. *Adv. Exp. Med. Biol.* **595**: 197–212. [Medline] [CrossRef]
5. Cox, N. R., Kwapien, R. P., Sorjonen, D. C. and Braund, K. G. 1986. Myeloencephalopathy resembling Alexander's disease in a Scottish terrier dog. *Acta Neuropathol.* **71**: 163–166. [Medline] [CrossRef]
6. Duckett, S., Schwartzman, R. J., Osterholm, J., Rorke, L. B., Friedman, D. and McLellan, T. L. 1992. Biopsy diagnosis of familial Alexander's disease. *Pediatr. Neurosurg.* **18**: 134–138. [Medline] [CrossRef]
7. Fankhauser, R., Fatzer, R., Bestetti, G., Deruaz, J. P. and Perentes, E. 1980. Encephalopathy with Rosenthal fibre formation in a sheep. *Acta Neuropathol.* **50**: 57–60. [Medline] [CrossRef]
8. Gruber, A., Pakozdy, A., Leschnik, M., Mai, S. and Weissenböck, H. 2010. Morbus Alexander-4 Fälle bei Hunden in Österreich. *Wien. Tierärztl. Mschr* **97**: 298.
9. Ito, T., Uchida, K., Nakamura, M., Nakashima, K., Suzuki, K. and Nakayama, H. 2010. Fibrinoid leukodystrophy (Alexander's disease-like disorder) in a young adult French bulldog. *J. Vet. Med. Sci.* **72**: 1387–1390. [Medline] [CrossRef]
10. Johnson, A. B. 2002. Alexander disease: a review and the gene. *Int. J. Dev. Neurosci.* **20**: 391–394. [Medline] [CrossRef]
11. Johnson, A. B. 2004. Alexander disease: a leukodystrophy caused by a mutation in GFAP. *Neurochem. Res.* **29**: 961–964. [Medline] [CrossRef]
12. Kessell, A. E., Finnie, J. W., Manavis, J., Cheetham, G. D. and Blumbergs, P. C. 2012. A Rosenthal fiber encephalomyelopathy resembling Alexander's disease in 3 sheep. *Vet. Pathol.* **49**: 248–254. [Medline] [CrossRef]
13. Li, R., Johnson, A. B., Salomons, G. S., van der Knaap, M. S., Rodriguez, D., Boespflug-Tanguy, O., Gorospe, J. R., Goldman, J. E., Messing, A. and Brenner, M. 2006. Propensity for paternal inheritance of de novo mutations in Alexander disease. *Hum. Genet.* **119**: 137–144. [Medline] [CrossRef]
14. McGrath, J. T. 1980. Fibrinoid leukodystrophy (Alexander's disease). pp. 147–148. *In*: Spontaneous Animal Models of Human Disease, vol. 2. (Andrews, E.W., Ward, B.C. and Altman, N. H. eds.), Academic Press, New York.
15. Messing, A., Brenner, M., Feany, M. B., Nedergaard, M. and Goldman, J. E. 2012. Alexander disease. *J. Neurosci.* **32**: 5017–5023. [Medline] [CrossRef]
16. Messing, A., Li, R., Naidu, S., Taylor, J. P., Silverman, L., Flint, D., van der Knaap, M. S. and Brenner, M. 2012. Archetypal and new families with Alexander disease and novel mutations in GFAP. *Arch. Neurol.* **69**: 208–214. [Medline] [CrossRef]
17. Prust, M., Wang, J., Morizono, H., Messing, A., Brenner, M., Gordon, E., Hartka, T., Sokohl, A., Schiffmann, R., Gordish-Dressman, H., Albin, R., Amartino, H., Brockman, K., Dinopoulos, A., Dotti, M. T., Fain, D., Fernandez, R., Ferreira, J., Fleming, J., Gill, D., Griebel, M., Heilstedt, H., Kaplan, P., Lewis, D., Nakagawa, M., Pedersen, R., Reddy, A., Sawaishi, Y., Schneider, M., Sherr, E., Takiyama, Y., Wakabayashi, K., Gorospe, J. R. and Vanderver, A. 2011. GFAP mutations, age at onset, and clinical subtypes in Alexander disease. *Neurology* **77**: 1287–1294. [Medline] [CrossRef]
18. Richardson, J. A., Tang, K. and Burns, D. K. 1991. Myeloencephalopathy with Rosenthal fiber formation in a miniature poodle. *Vet. Pathol.* **28**: 536–538. [Medline] [CrossRef]
19. Sorjonen, D. C., Cox, N. R. and Kwapien, R. P. 1987. Myeloencephalopathy with eosinophilic refractile bodies (Rosenthal fibers) in a Scottish terrier. *J. Am. Vet. Med. Assoc.* **190**: 1004–1006. [Medline]
20. Sosunov, A., Olabarria, M. and Goldman, J. E. 2018. Alexander disease: an astrocytopathy that produces a leukodystrophy. *Brain Pathol.* **28**: 388–398. [Medline] [CrossRef]
21. Van Poucke, M., Martlé, V., Van Brantegem, L., Ducatelle, R., Van Ham, L., Bhatti, S. and Peelman, L. J. 2016. A canine orthologue of the human GFAP c.716G>A (p.Arg239His) variant causes Alexander disease in a Labrador retriever. *Eur. J. Hum. Genet.* **24**: 852–856. [Medline] [CrossRef]
22. Weissenböck, H., Obermaier, G. and Dahme, E. 1996. Alexander's disease in a Bernese mountain dog. *Acta Neuropathol.* **91**: 200–204. [Medline] [CrossRef]
23. Wrzosek, M., Giza, E., Płonek, M., Podgórski, P. and Vandevelde, M. 2015. Alexander disease in a dog: case presentation of electrodiagnostic, magnetic resonance imaging and histopathologic findings with review of literature. *BMC Vet. Res.* **11**: 115. [Medline] [CrossRef]