Case Report
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Degenerative Myelopathy in a Bernese Mountain Dog with a Novel *SOD1* Missense Mutation

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An 8-year-old female spayed Bernese Mountain Dog (BMD) presented to the Bush Veterinary Neurology Service (BVNS) for paraparesis. The dog had been seen at the BVNS 13 months earlier for evaluation of progressive pelvic limb weakness. At that time, the dog had a short-strided gait in the pelvic limbs, mild postural reaction deficits in the right pelvic limb, normal segmental reflexes, and laxity of the right coxofemoral joint. The dog was treated with meloxicam (0.15 mg/kg PO q24h) for 3 days, amantidine (2 mg/kg PO q24h) for 1 month, and doxycycline (6 mg/kg PO q12h) for 2 weeks with no improvement noted. The dog had a previous history of hypothyroidism that had been treated with 0.3 mg levothyroxine PO q12h.

Asymmetric general proprioceptive ataxia and spastic paraparesis were present on neurologic examination. Postural reactions were delayed in both pelvic limbs and segmental reflexes were normal. Initial diagnostic tests included a CBC, serum biochemistry, and thoracic and spinal radiographs. The results of all tests were within normal limits. A DNA test for the *SOD1:c.118G > A* mutation commonly associated with canine degenerative myelopathy (DM) was normal (homozygous for the G allele).[1] Physiotherapy was initiated.

Sixteen months after the initial presentation, signs had progressed to paraplegia with preservation of pain perception. Decreased patellar and withdrawal reflexes were present in both pelvic limbs and suggested neurodegeneration that had progressed to include the lower motor neurons associated with the femoral and sciatic nerves. Analysis of cerebrospinal fluid obtained by atlanto-occipital puncture revealed mild albuminocytologic dissociation with a protein concentration of 30 mg/dL and no nucleated cells. Magnetic resonance imaging of the spinal cord was performed 21 months after the initial presentation with a 1.5 T unit,[a] T2-weighted (T2W), T1-weighted (T1W), and half Fourier acquisition single-shot turbo spin echo (HASTE) sagittal sequences were acquired from the foramen magnum to the sacrum. Transverse T2W, T1W, and T1W postcontrast[b] weighted images were acquired from T12 to L4. Spondylosis and a mildly increased signal within the dorsal funiculus of the spinal cord were noted at the T12–13 disc space on T2W images. The T12–13 disc was well hydrated and similar in size to adjacent discs.

The dog was euthanized 21 months after the initial presentation because the disease had progressed to include fecal and urinary incontinence. Histopathologic evaluation of formalin-fixed spinal cord was performed at the University of Missouri Veterinary Medical Diagnostic Laboratory. Spinal cord cross sections were stained by standard methods with luxol fast blue (LFB), and periodic acid Schiff (PAS) as a counter stain. Immunostaining was performed to detect phosphorylated neurofilament and glial fibrillary acid protein (GFAP) as a measure of axonal loss and gliosis, respectively. Deparaffinized sections were treated with rabbit anti-GFAP antibody[c] without pretreatment, and binding was detected with horseradish-peroxidase-bound secondary antibodies and Romulin Red chromogen.[d] Phosphorylated neurofilament in axons was detected with a mouse monoclonal antibody,[e] an analogous mouse detection system and similar chromogen. Sections were lightly counterstained with hematoxylin; other sections were stained identically excluding the primary antibody.

In affected tissue, bilateral areas of sclerosis nearly devoid of myelin and with decreased axonal density were noted in the dorsal portion of the lateral funiculus, adjacent to the entry of the dorsal roots (Fig 1). Swollen axon sheaths with occasional digestion chambers were present in all white matter tracts. The GFAP staining was more homogenous and intense in these regions of myelin and axon loss, without an obvious increase in astrocytic nuclei. The associated spinal nerves appeared normal. Although the distribution of axonal dropout and gliosis was more diffuse and less concentrated in the dorsal funiculi than is typical of DM in *SOD1*:c.118A homozygous dogs, the overall pattern of histologic lesions, including the distribution of axon loss and demyelination in the spinal cord strongly supported a diagnosis of DM.

Resequencing all 5 *SOD1* exons in DNA from the affected BMD revealed a homozygous c.52A > T transversion, which predicts a p.T18S substitution. Nucleotide

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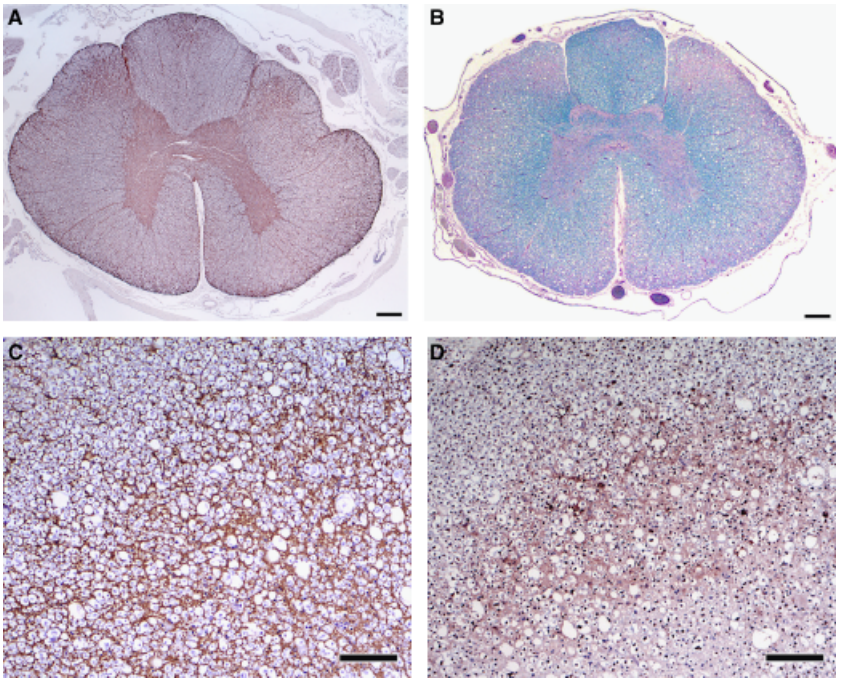

Fig 1. Microscopic appearance of thoracic spinal cord cross-sections. (**A**) Low magnification of the spinal cord highlights astrocyte processes by immunohistochemistry to detect glial fibrillary acidic protein reagent and Romulin Red chromogen. Poorly defined foci of fibrillary astrogliosis are situated lateral to the dorsal root entry zones, and localized staining of the fasciculus gracilis in the dorsal-most aspect of the section. Bar = 300 μm. (**B**) A similar section stained with Luxol fast blue demonstrates myelin loss in the areas of gliosis, which is also most severe in the dorsolateral funiculus. (**C**) Higher magnification on one of the dorsolateral lesions demonstrates GFAP positive astrocyte processes between remaining axons, next to more normal tissue in the upper left. Staining similar to (A), bar = 100 μm. (**D**) Demonstrates the same areas stained by immunohistochemistry to detect phosphorylated neurofilament M, found in axons. There is reduction in the density of axons in the center of the affected tissue, with occasional dilated axons and empty axons sheaths. Bar = 100 μm, All immunohistochemistries in this figure ultilize Romulin Red chromogen and hematoxylin counter stain.

sequence electrophorograms from the affected dog and from a normal dog are shown in Figure 2. Spinal cord motor neurons from dogs of various breeds that were confirmed to have suffered from DM associated with the *SOD1:c.118G > A* mutation exhibited accumulations of cytoplasmic aggregates that were immuno-positive for SOD1.[1] To determine whether the c.52A > T mutation was the cause of the neurodegeneration, sections of thoracic spinal cord were evaluated by immunohistochemistry for the presence of similar SOD1-containing cytoplasmic aggregates as described previously.[1] Spinal

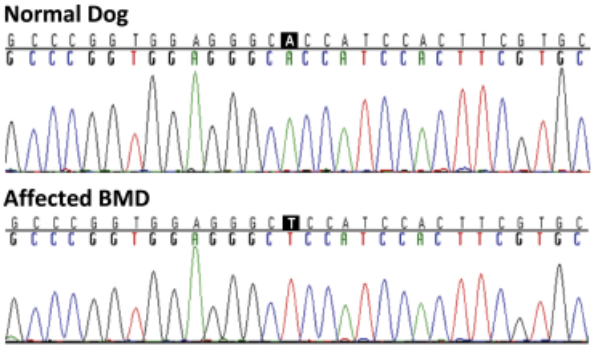

Fig 2. Nucleotide sequence electrophorograms from a normal dog (top) and the BMD with DM (bottom) showing the *c.A52 T* transversion.

cord sections from the dog, along with corresponding sections from 7 age-matched control dogs known to be homozygous wild-type *SOD1:c.118G* allele were immunostained simultaneously under identical conditions. Two of the negative controls had been presented for chronic thoracolumbar myelopathies, whereas the other 5 were euthanized for unrelated, nonneurologic disease. Each specimen was processed with and without primary antibody to verify the specificity of the staining. Motor neurons in the thoracic spinal cord of the ventral gray matter of the propositus contained cytoplasmic aggregates that stained darkly with the anti-SOD1 antibody (Fig 3). These aggregates appeared to be similar to those previously described in dogs with DM that were homozygous for the *SOD1:c.118A* allele.[1,2] No aggregates were detected in the 7 control dogs. Patches of SOD1 immunostaining more intense than the general background were observed in the neuropil of spinal cord sections from some but not all affected and control dogs (Fig 3B, C, E, F). Because this pattern of staining was present in both control and affected dogs and was seen inconsistently, it was most likely a postmortem artifact.

Canine DM is characterized by a slowly progressive, often asymmetric, general proprioceptive ataxia and upper motor neuron spastic paresis of the pelvic limbs beginning in late adulthood. Ultimately, paraplegia and thoracic limb involvement develop, necessitating euthanasia.[2,3] The overall prevalence of the disease among all

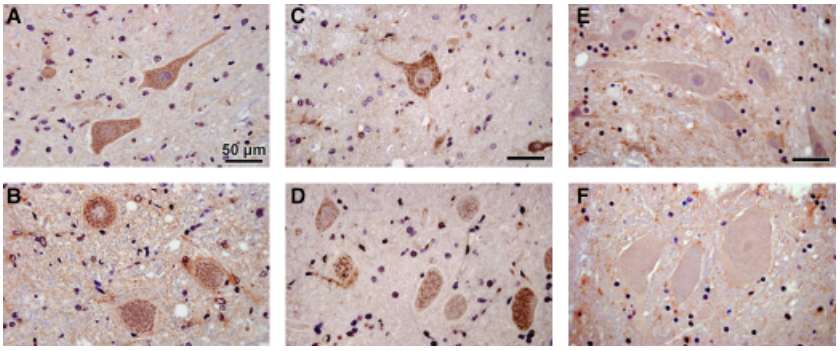

Fig 3. SOD1 immunohistochemistry of thoracic spinal cord motor neurons from a BMD homozygous for the novel missence mutation in SOD1 showing the cytoplasmic aggregates that stained darkly with an anti-SOD1 antibody (**A** and **B**). These aggregates are present in the thoracic spinal cord motor neurons from 2 dogs homozygous for the SOD1:c.118A mutation with clinical degenerative myelopathy (**C** and **D**). The aggregates are absent in 2 clinically normal dogs homozygous for the wild-type SOD1:c.118G allele (**E** and **F**).

dogs is estimated at 0.19%, but prevalence varies widely among breeds.[4] Earlier reports indicate that DM occurs in the BMD.[2,5] The pathogenesis appears to be complex, and immunologic, metabolic, nutritional, oxidative, excitotoxic, and genetic mechanisms have been proposed.[1,3,6–10]

Based on histopathology, DM can be best described as a multisystem central and peripheral axonopathy.[2] In general, the spinal cord pathology of DM is consistent with noninflammatory axonal degeneration. Dogs with DM have characteristic patterns of axon cylinder vacuolization and loss. Regional axonal loss is severe in many DM affected dogs with complete loss of axonal and myelin profiles and replacement by large areas of astrogliosis. Lesion distribution in the mid to caudal thoracic region varies among cases, but typically there are regions of increased lesion severity in the dorsal portion of the lateral funiculi.[3,11] The dog described here had a more diffuse distribution of lesions. A similar distribution has been noted in many other cases of DM,[6] and the axonal dropout and gliosis in this BMD were consistent with a diagnosis of DM.

Recently, we reported that dogs homozygous for a *SOD1:c.118G > A* missense mutation are at high risk of developing DM as they age.[1] Numerous mutations in human *SOD1* have been shown to underlie amyotrophic lateral sclerosis (ALS).[12] The clinical and genetic similarities between DM and ALS suggest that these diseases are analogous. Despite the similarities between DM and ALS, substantial pathological and clinical differences are observed. DM involves proprioceptive pathways as well as the upper motor neuron tracts in contrast to the predominantly motor disease of humans.[6,9] The neuronal cell body degeneration and loss in the ventral horn of the spinal cord of ALS patients is not a prominent histopathlogic finding in DM, although lower motor neuron signs develop late in the disease.[6,9]

Familial ALS (fALS) accounts for 5–10% of all ALS cases.[13,14] At least 9 genes and 6 additional mapped loci have been associated with fALS.[15] Since the initial discovery that mutations in *SOD1* can cause ALS,[16] more than 145 *SOD1* mutations have been identified in ALS patients (http://alsod.iop.kcl.ac.uk/). Thus, it seems likely that the previously described *SOD1:c.118G > A* mutation is not the only canine *SOD1* mutation that can cause DM.

The diagnosis of DM is based on the clinical characteristics of the disease and exclusion of other etiologies by normal spinal MRI and CSF. A definitive diagnosis requires histopathologic confirmation.[2] In this BMD, the age at onset and progression of clinical signs were similar to those in previously reported clinical descriptions of DM, although the neurologic signs progressed more slowly (21 months) than expected.[3,6,9] Different genotypic causes could underlie phenotypic variation as noted in humans with ALS.[17] A subtle intraparenchymal change was noted on MRI of the caudal thoracic segments. Intervertebral disc herniation was considered, but no specific focal pathologic changes were found at this site. Because of its small size and lack of histologic corroboration, the MRI finding was considered incidental.

The *SOD1:c.52A > T* missense mutation predicts the substitution of a serine for threonine at position 18 in the amino acid sequence of SOD1. Threonine occurs at position 18 in several other mammalian species, whereas isoleucine occurs in humans, the orangutan, the common gibbon, the tufted capuchin, and the guinea pig, and valine occurs in the rat and horse (Fig 4). Although this conservative amino acid substitution at a site harboring different amino acids across other mammalian species could be a neutral sequence variant unrelated to DM, it appears more likely to be the cause of DM in this BMD because motor neurons contained cytoplasmic aggregates that bound anti-SOD1 antibodies. These aggregates resemble those found in DM cases caused by homozygosity of the *SOD1:c.118A* allele, in human fALS cases associated with many different *SOD1* mutations, and in transgenic murine ALS models expressing mutant human *SOD1*.[18–20] The aggregates are thought to form because amino acid substitutions force SOD1 to assume an unstable conformation. It is unclear if the aggregates cause or contribute to the neurodegeneration or are a byproduct of other neurodegenerative processes. Aggregates binding anti-SOD1 antibodies have been consistently absent from spinal cords of healthy dogs lacking known *SOD1* mutations. On the other hand,

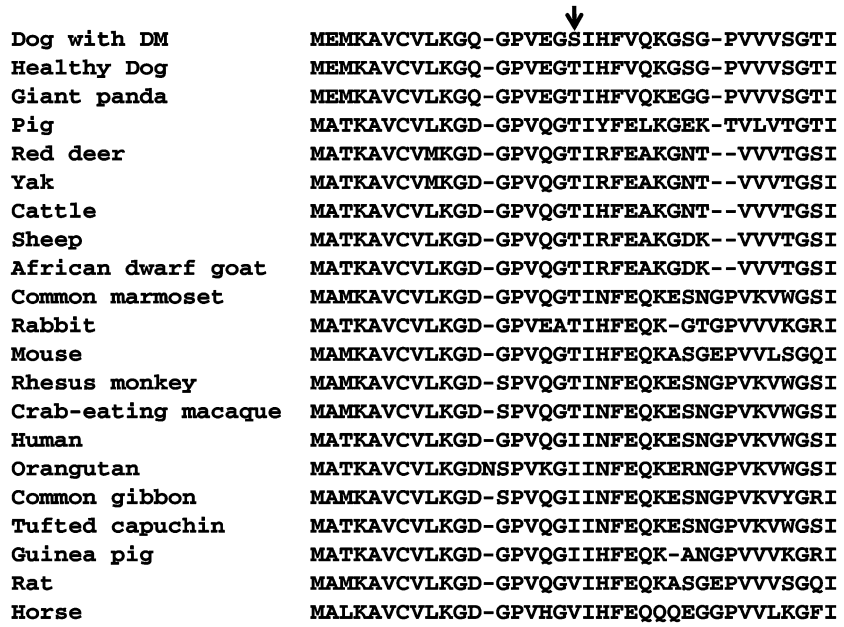

Fig 4. The N-terminal amino acid sequences for superoxide dismutase 1 from 20 mammalian species.

SOD1 antigen-containing aggregates have been detected in spinal cords from some clinically normal dogs heterozygous for *SOD1*:c.118, which may be indicative of preclinical or subclinical neurodegenerative processes.[1] Furthermore, antibodies that bind SOD1 with an aberrant conformation have been used to detect similar aggregates in patients that have sporadic ALS without *SOD1* mutations.[21] Thus, the occurrence of SOD1-containing aggregates in the BMD suggests but does not prove that the disease was caused by homozygosity for the *SOD1*:c.52 T allele.

In summary, we have described DM in a BMD that is typical of previously described DM except for a slower than average rate of disease progression and the DNA test result indicating homozygosity for the wild-type G allele at *SOD1:c.118*. This finding serves as a reminder that direct DNA tests indicate the presence or absence of disease-causing alleles but cannot be used to rule out a diagnosis because other sequence variants in the same gene or in a different gene might produce a similar disease phenotype. A definitive conclusion as to whether or not homozygosity of the *SOD1:c.52 T* allele was the cause of the DM in the BMD will require clinical and pathological evaluations of additional canine *SOD1:c.52 T* homozygotes.

Footnotes

[a] Signa, General Electric Healthcare, Milwaukee, WI
[b] Magnevist, Berlex Laboratories Inc, Cedar Knolls, NJ
[c] Z0334, Dako, Carpenteria, CA
[d] Biocare Medical, Concord, CA
[e] SMI 34, Covance Inc, Princeton, NJ

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