# Supporting Information

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## SI Methods

**Histopathology and Immunohistopathology.** For the stringency level 1 diagnosis of degenerative myelopathy (DM), thoracic spinal cords from dogs with clinical signs of DM were dissected and immersion fixed in neutral-buffered 10% formalin or in buffered 3.5% paraformaldehyde with 0.5% glutaraldehyde, paraffin embedded, and cut into 4-$\mu$m thick sections. These sections were stained with hematoxylin and eosin (H&E) and with luxol fast blue-periodic acid Schiff to detect myelin loss. Immunostaining for glial fibrillary acid protein to detect gliosis was performed on dewaxed, paraffin-embedded tissues by using rabbit anti-GFAP antibody (Dako Z0334; Dako). Antibody binding was done as described below, by using Nova Red chromogen (Vectorlabs). The diagnosis of DM was confirmed by demonstration of axonal degeneration, myelin loss, and gliosis.

To detect SOD1-containing cytoplasmic inclusions, spinal cord paraffin sections from DM-affected dogs and controls of similar age were prepared as previously described and kept overnight at 43 °C, then hydrated and steamed at 95 °C for 20 min in pH 6.0 citrate buffer. After the slides were cooled at room temperature for 20 min and rinsed, they were placed in Tris buffer for at least 5 min before immunostaining. Next, the slides were treated with 3% $H_20_2$ for 15 min, washed in buffer, submerged in Protein Blocking solution (Dako) for 5 min, and drained. They were then incubated in rabbit anti-SOD1 antibody, (Stressgen SOD100) at a 1:800 dilution for 60 min at room temperature. Negative controls were treated for 60 min with nonimmune rabbit IgG (Sigma) at a 1:1,000 dilution in place of the anti-SOD1 antibody. Antibody binding was visualized with the Rabbit Envision+ kit (Dako). The chromogen was Nova Red. Slides were then counterstained in Mayer's Hematoxylin (Newcomer's Supply) for 1 min, dehydrated, and coverslipped. Each immunohistochemical specimen was run with and without primary antibody to verify the specificity of staining. Muscle specimens were immersion-fixed in 10% neutral buffered formalin and evaluated in paraffin sections. Peripheral nerve specimens were immersion-fixed in 2.5% glutaraldehyde in 0.1M phosphate buffer, postfixed in 1% aqueous osmium tetroxide, and processed to araldite resin blocks; 1-$\mu$m thick sections were cut and stained with toluidine blue before light microscopic examination.

**Electrodiagnostic Testing.** Electrodiagnostic testing in some DM-affected dogs consisted of EMG and motor nerve conduction velocity studies. Dogs were premedicated with midazolam HCl (0.025 mg/kg s.c.) and hydromorphone (0.125 mg/kg s.c.). Anesthesia was induced with propofol (10 mg/kg i.v.) and maintained with 1.5–2.0% isoflurane. Electrophysiologic assessments were performed on the left side. Concentric needle EMG recorded intramuscular potentials were displayed in real time on a Cadwell Central Lab electrodiagnostic instrument. Spontaneous activity was identified according to the established parameters described by Kimura (1). Motor nerve conduction studies of the tibial or ulnar nerves were performed as previously described (2).

1. Kimura J (2001) *Electrodiagnosis in Diseases of Nerve and Muscle: Principles and Practice*, ed Kimura J (Oxford Univ Press, New York), pp 339–369.

2. Walker TL, Redding RW, Braund KG (1979) Motor nerve conduction velocity and latency in the dog. *Am J Vet Res* 40:1433–1439.

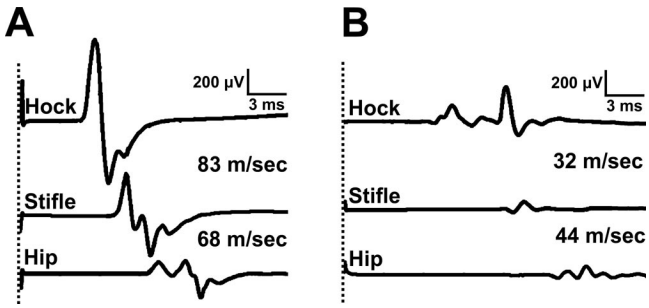



**Fig. S1.** Ischiatic/tibial M wave recordings, after stimulation at the hock, stifle, and hip, from a DM-affected 10-year-old Boxer during early and late disease stages. (*A*) At an early disease stage the motor nerve conduction velocities between proximal and distal stimulation sites were within or above the normal mean values for the tibial nerve (66.9 ± 2.4 m/s) (2), although the M wave amplitudes (6.0, 3.1 and 0.8 mV) were below the normal mean values of 22.2 ± 2.6 mV (2), and there was mild temporal dispersion. (*B*) M wave recordings at a late disease stage showed further decreases in amplitude (1.2, 0.6, and 0.4 mV) with marked temporal dispersion. Also, the proximal and distal motor nerve conduction velocities were decreased when compared with the normal reference range. These findings provide evidence of motor axonopathy and demyelination in the late disease stage of degenerative myelopathy.

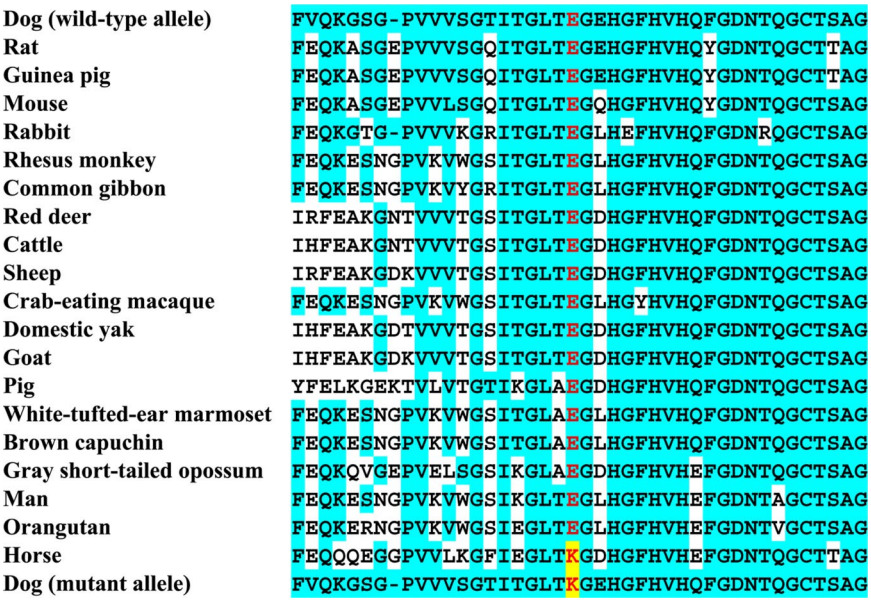
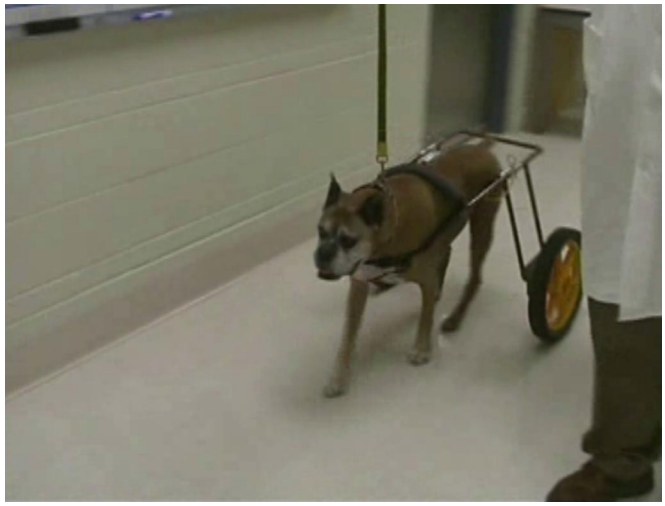



**Fig. S2.** Aligned predicted amino acid sequences for SOD1 from 20 mammalian species showing conservation of glutamic acid (in red) at the equivalent of canine position 40 in 19 species, and substitution by lysine (also in red) in the horse and in the mutant canine allele.



**Movie S1.** Dogs with degenerative myelopathy from early to latter disease stages: asymmetric spastic paraparesis and general proprioceptive ataxia; nonambulatory paraparesis to paraplegia; paraplegia to thoracic limb weakness; flaccid paralysis and hyporeflexia; and, flaccid tetraplegia. These clips demonstrate disease onset with upper motor neuron signs in the pelvic limbs that progresses to generalized lower motor neuron signs. The dog with the flaccid tetraplegia also had clinical signs of dysphagia and motor deficits in the tongue.

Movie S1 (WMV)

**Table S1. Intracytoplasmic staining characteristics produced by anti-SOD1 antibodies in spinal cord sections from aged DM-affected and normal dogs**

| Breed | Age, yr | Genotype | Staining characteristic | Disease status |
|---|---|---|---|---|
| Boxer | 10 | A/A | Well-defined dark-staining clumps | DM-affected |
| Boxer | 10 | A/A | Well-defined dark-staining clumps | DM-affected |
| Boxer | 8 | A/A | Well-defined dark-staining clumps | DM-affected |
| Chesapeake Bay retriever | 9 | A/A | Well-defined dark-staining clumps | DM-affected |
| Pembroke Welsh corgi | 13 | A/A | Well-defined dark-staining clumps | DM-affected |
| Pembroke Welsh corgi | 13 | A/A | Well-defined dark-staining clumps | DM-affected |
| Rhodesian ridgeback | 8 | A/A | Well-defined dark-staining clumps | DM-affected |
| German Shepherd dog | 8 | A/G | Well-defined light-staining clumps | Asymptomatic |
| Tibetan terrier | 14 | A/G | Poorly defined light staining regions | Asymptomatic |
| Rhodesian ridgeback | 15 | A/G | No staining or diffuse light staining | Asymptomatic |
| Australian Shepherd | 8 | A/G | No staining or diffuse light staining | Asymptomatic |
| Boxer | 8 | A/G | No staining or diffuse light staining | Asymptomatic |
| Mixed breed | 13 | G/G | No staining or diffuse light staining | Asymptomatic |
| Rhodesian ridgeback | 13 | G/G | No staining or diffuse light staining | Asymptomatic |
| Labrador retriever | 9 | G/G | No staining or diffuse light staining | Asymptomatic |
| Labrador retriever | 13 | G/G | No staining or diffuse light staining | Asymptomatic |
| Labrador retriever | 13 | G/G | No staining or diffuse light staining | Asymptomatic |

Genotype at SOD1:c.118G>A.

**Table S2. Primers for PCR and RT-PCR amplification**

| Target | Forward primer/reverse primer | Amplicon size, bp | Template | Method |
|---|---|---|---|---|
| *SOD1* exon 1 | GCTGCCCTCGGACTGCTG/TTGAGGATTTTCAATGTTTAGGAGTAGC | 196 | RNA | RT-PCR |
| *SOD1* exon 2 | AAGTCCATGTTCCTTCCACTTTCTTGTG/ TTGAGGATTTTCAATGTTTAGGAGTAGC | 294 | DNA | PCR |
| *SOD1* exon 3 | GTATTTGAGGTTTTAGCAATGGC/GCAAGGAATACACCCGTACTGA | 252 | DNA | PCR |
| *SOD1* exon 4 | CTCACAAACTAGCCTGAATCAGTCC/ GCCCTTCCTAATCTGAACTAAAG | 307 | DNA | PCR |
| *SOD1* exon 5a | GCGCTAAGTTATGTTAATGTTCTT/ AGTTCTCATTACAGGTACTTAAAGCAAT | 296 | DNA | PCR |
| *SOD1* exon 5b | AAACATTGTAACCTTAAAAGTGTAATTTG/ CGTTCAAGCTCTAATGTAGGAGTATGAAG | 324 | DNA | PCR |