Degenerative myelopathy in a \textit{SOD1} compound heterozygous Bernese mountain dog

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Source/description: Canine degenerative myelopathy (DM) is a progressive neurodegenerative condition in dogs with clinical signs which do not manifest prior to an age of 8 years. A \textit{SOD1} missense mutation within exon 2 (c.118G>A; E40K) has been found strongly associated with DM.\textsuperscript{1} A single Bernese mountain dog (BMD) affected by DM was wild type for c.118G>A but had the homozygous missense mutation c.52A>T (T18S) in exon 1 of \textit{SOD1}.\textsuperscript{2} A definitive conclusion could not be drawn as to whether or not the c.52A>T mutation can cause DM.\textsuperscript{2} We have detected the c.52A>T mutation in 67/408 BMD. A dog heterozygous for both the exon 1 and exon 2 mutations was suspicious for DM. This dog subsequently was submitted for necropsy and genotyped on the canine Illumina high density (HD) beadchip (Illumina) to perform a haplotype analysis for dog chromosome (CFA) 31.

Samples/genotyping: We genotyped 408 BMD on the HD beadchip (Illumina) and for the mutations c.52A>T and c.118G>A within \textit{SOD1} using a PCR-RFLP and a Taqman assay respectively (Table S1).

Statistical analysis: Haplotypes blocks, haplotypes and their frequencies on CFA31 were determined using \textit{HAPLOVIEW} 4.0.\textsuperscript{3} We employed a total of 2238 SNPs including the two \textit{SOD1} mutations.

Comments: The c.118G>A mutation was heterozygous in 188 (46.1\%) and homozygous in 27 (6.6\%) BMD (Table S2). The c.52A>T mutation was heterozygous in 65 (15.9\%) and homozygous in two (0.5\%) dogs. Twenty-two of the animals had both \textit{SOD1} mutations heterozygous (5.4\%). The haplotype analysis for all BMD revealed no haplotype with both mutated alleles on a single chromosome (Figs S1 and S2). Therefore, these 22 dogs are likely to be compound heterozygous for the \textit{SOD1} haplotypes AA and TG.

Clinical signs of DM were reported for one of the compound heterozygotes. The dog suffered from a progressive caudal paresis from the age of 8 years. It displayed a complete caudal hemiparesis before dying at nearly 11 years of age when gross and histopathologic examinations were carried out. Severe bilateral atrophy of the hindlimb musculature was noted. In all segments of the spinal cord, multifocal dilated myelin sheaths and spheroid formation with multifocal evidence of myelinophagia were seen (Fig. S3). Within the filum terminale, single digestion chambers were present. These pathohistological findings are characteristic for DM.

Another four of the compound heterozygous dogs were 7.9–9.9 years old when they died, but without veterinary investigation of their DM status, presumably due to the fact that the breeders were unaware of DM at the time the animals died prior to 2010. For one dog, a tumor and for another dog debilitation due to the age of 9.9 years had been suspected by the respective owners. Further, six dogs died before having reached the earliest age of onset for DM. Eleven dogs are still alive, but all of them are below 6 years of age. Thus, there is no possibility for a diagnosis in these dogs.

We analyzed the pedigree of the DM-affected BMD. Genotype information was available for the dam and three (half-) siblings (Fig. S4). The dam had the haplotypes AG and AA. Because there is one half-sibling homozygous wild type for both mutations (haplotypes AG and AG), the sire is likely heterozygous for c.52A>T and homozygous wild type for c.118G>A (haplotypes AG and TG). We can infer from the pedigree that the compound heterozygous BMD received a maternal haplotype with the mutated c.118G>A allele and a paternal haplotype with the mutated c.52A>T allele. Both missense mutations are suspected to change the net negative charge of the protein, and due to reduced repulsive Coulombic forces or increased interaction with anionic membrane surfaces, it may be prone to aggregation.\textsuperscript{1,2,4-5} \textit{SOD1}-antigen-containing aggregates have been detected in c.118G>A heterozygous dogs.\textsuperscript{1} Assuming that \textit{SOD1} aggregates can be produced by both mutations in the heterozygous state, the compound heterozygosity may confer a similar risk to DM like the c.118G>A homozygous mutation. In summary, we propose that compound heterozygosity may increase risk to DM. Matings resulting in these genotypes should be avoided even if further cases have to be evaluated for a definitive conclusion.

References

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**Supporting information**

Additional supporting information may be found in the online version of this article.

**Table S1.** Genotyping of the previously reported mutations in SOD1.

**Table S2.** Joint distribution of genotypes of the 408 Bernese mountain dogs for the c.118G>A and c.52A>T polymorphisms in SOD1.

**Figure S1.** Haplotype blocks on dog chromosome 31 from 28.0 to 30.5 Mb (CanFam 2.0).

**Figure S2.** Haplotype frequencies of the 408 Bernese mountain dogs for the both DM-associated mutations (SNPs 58 and 59) containing haplotype block in SOD1 and the surrounding region at 28–30.5 Mb on dog chromosome 31 using Haploview 4.0.

**Figure S3.** Evidence of spheroids within dilated myelin sheaths and myelinophages in the cervical spinal cord white matter.

**Figure S4.** Pedigree of the Bernese mountain dog affected by degenerative myelopathy (DM).