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SHORT COMMUNICATION

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# A *PNPLA8* frameshift variant in Australian shepherd dogs with hereditary ataxia

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**Abstract**

Hereditary ataxias are common among canine breeds with various molecular etiology. We identified a hereditary ataxia in young-adult Australian Shepherd dogs characterized by uncoordinated movements and spasticity, worsening progressively and leading to inability to walk. Pedigree analysis suggested an autosomal recessive transmission. By whole genome sequencing and variant filtering of an affected dog we identified a PNPLA8:c.1169_1170dupTT variant. This variant, located in *PNPLA8* (*Patatin Like Phospholipase Domain Containing 8*), was predicted to induce a PNPLA8:p.(His391PhefsTer394) frameshift, leading to a premature stop codon in the protein. The truncated protein was predicted to lack the functional patatin catalytic domain of PNPLA8, a calcium-independent phospholipase. PNPLA8 is known to be essential for maintaining mitochondrial energy production through tailoring mitochondrial membrane lipid metabolism and composition. The Australian Shepherd ataxia shares molecular and clinical features with Weaver syndrome in cattle and the mitochondrial-related neurodegeneration associated with *PNPLA8* loss-of-function variants in humans. By genotyping a cohort of 85 control Australian Shepherd dogs sampled in France, we found a 4.7% carrier frequency. The PNPLA8:c.[1169_1170dupTT] allele is easily detectable with a genetic test to avoid at-risk matings.

**KEYWORDS**
canine, cattle, mitochondria, neurodegeneration, patatin domain, phospholipase, Weaver syndrome

Hereditary ataxias are common in dogs, but although they share phenotypic similarities characterized by uncoordinated and inaccurate movements and position, the genetics and pathophysiology of most of them remain undeciphered (Urkasemsin & Olby, 2014). To date, 14 genes responsible for canine ataxias owing to cerebellar cortical or spinocerebellar neurodegeneration have been identified (Urkasemsin & Olby, 2014;

omia.org/home/). In 2016, a purebred 21-month-old male proband Australian Shepherd showing ataxia in the four limbs and intention tremors was presented at the neurological clinic of VetAgro Sup (Lyon School of Veterinary Medicine, France). A female littermate of the proband was presented with similar clinical signs a few months later. Three additional cases, two males and one female Australian shepherd dogs, were recruited. The owners noticed the first signs between 4 and 19 months (Table S1). They described hypermetria, bunny-hopping (Figure 1a,b), wobbly and stiff gait on the pelvic limbs, and difficulties in walking up or down the stairs and in getting up. The initial neurological examination revealed moderate ataxia, more obvious on the pelvic limbs, with slight hypermetria and slight to no proprioceptive deficits on the pelvic limbs. Two of the five dogs showed discrete intention tremors (Table S1). These signs suggested symmetrical cerebellar involvement. Motor deficits progressed toward the inability to walk without help from the age of 30 to 44 months (Table S1). Neurological examination at this stage revealed non-ambulatory tetraparesis or tetraplegia. Severe spasticity of the hind limbs and proprioceptive deficits on all four limbs were present

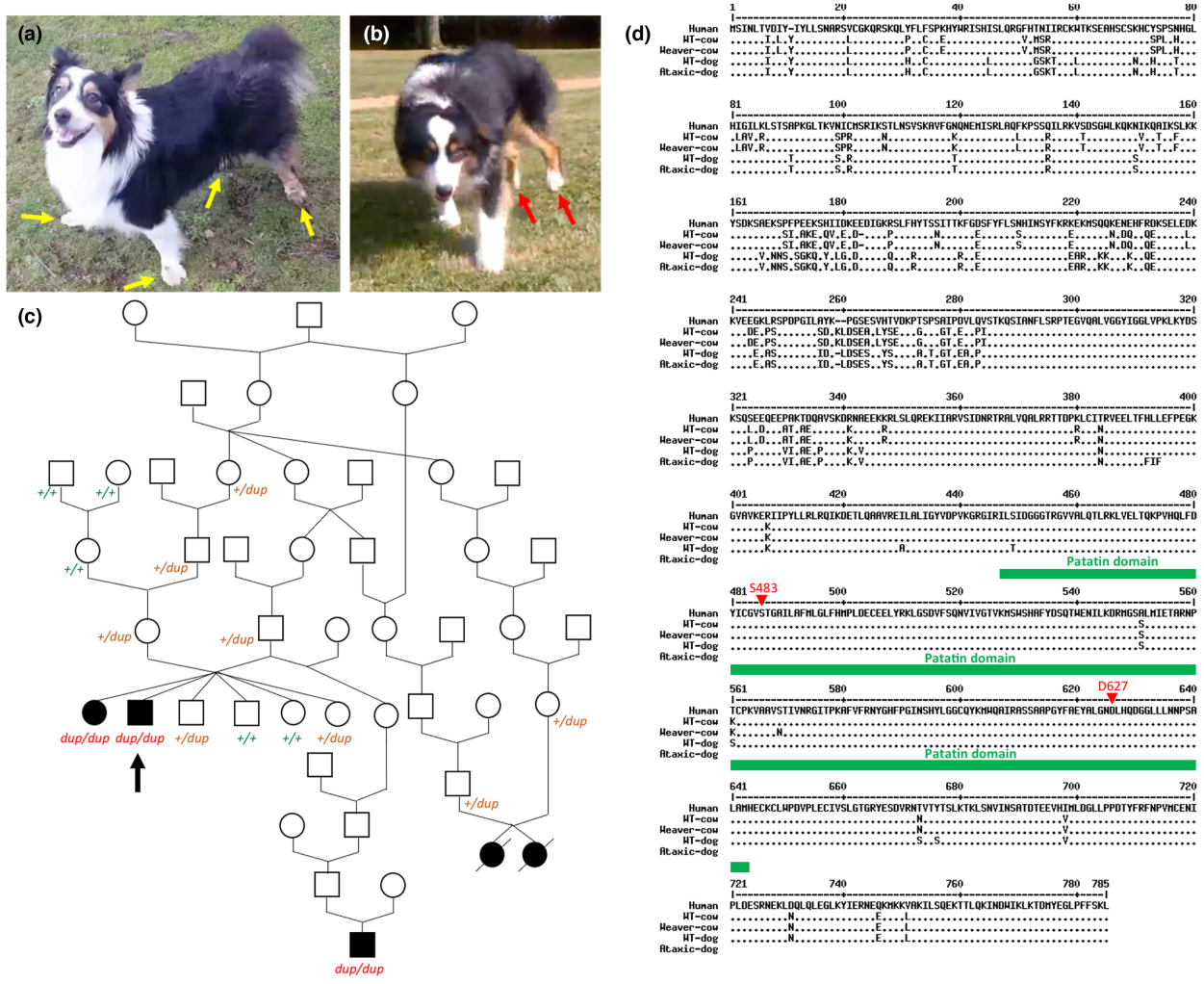


**FIGURE 1** Hereditary ataxia in a young-adult Australian shepherd is governed by a recessive PNPLA8:C.[1169_1170dupTT] frameshift allele. (a, b) ataxia in two affected dogs. (a) Picture of an affected Australian shepherd dog showing wide-based stance (static ataxia, yellow arrows). (b) Picture of the proband dog showing bunny-hopping (dynamic ataxia, red arrows). (c) Autosomal recessive inheritance pattern of the disease. Partial pedigree tree of the proband family. Circles represent females and squares represent males. Affected dogs are depicted with fully filled symbols and the proband is shown with an arrow. When available, the result of the genotyping assay for the PNPLA8:C.1169_1170dupTT variant is mentioned: +, wild-type allele; dup, mutant allele. Two affected female littermates were euthanized and no DNA samples were available (barred symbols). (d) The canine frameshift variant is predicted to produce a truncated PNPLA8 protein. Alignment of protein sequences of PNPLA8 translated from the wild-type (WT) alleles reported in human, cow and dog and from mutant alleles of Weaver cow and ataxic Australian shepherd dog. Residues that have been evolutionarily conserved are represented by dots in the animal sequences, compared with the reference human sequence. Non-conserved residues are represented by letters in animal sequences, while dashes represent deletions. The functional patatin domain of PNPLA8 is depicted in green. It starts with amino acid number 445 and ends with amino acid number 640 in human PNPLA8. Red arrow heads point out the two conserved serin 484 (S484) and aspartic acid 627 (D627) residues of the phospholipase catalytic dyad (Appendix S1)

in all affected dogs. An absent menace-response was observed in two dogs (Table S1). Neuroanatomical diagnosis was therefore suggestive of multifocal central nervous system damage. This led to consideration of spinocerebellar ataxia owing to cerebellar, extracerebellar brain and spinal cord involvement (Escriou C. et al., in preparation). The MRI (brain and cervical spinal cord) was unremarkable at 21 months but revealed cerebellar atrophy at 72 months in the last living dog. Four of the five affected dogs were euthanized between 24 and 39 months of age (Table S1). Preliminary brain histology results revealed diffuse demyelination and oligodendrogliosis (Escriou C. et al., in preparation).

Pedigree data supported an autosomal recessive inheritance pattern for the disease (Figure 1c).

We analyzed the whole genome sequence obtained with a mean coverage of 20× from the proband dog (ENA project accession no. PRJEB16012; sample accession no. SAMEA6862929). SNPs and indels were called against the CanFam 3.1 reference genome. We searched for private homozygous variants in the proband genome using 795 canine control genomes (Jagannathan et al., 2019; Appendix S1, Table S2). We identified two homozygous private variants with a high predicted impact and eight homozygous private missense variants with a moderate predicted impact (Table S3). The eight missense variants were predicted by PROVEAN to be neutral variants (Appendix S1, Table S3). A PNPLA8:c.1169_1170dupTT frameshift variant located on chromosome 18 in the third exon of *PNPLA8* (*Patatin Like Phospholipase Domain Containing 8*) drew our attention. Variants in *PNPLA8* had previously been reported to cause ataxia and neurodegenerative disorders in humans, cows and mice (Kunz et al., 2016; Masih et al., 2021; Saunders et al., 2015; Shukla et al., 2018).

We genotyped a total of 109 Australian Shepherd dogs including 24 dogs from the proband family for the PNPLA8:c.1169_1170dupTT variant. The five affected dogs were homozygous for the mutant allele. All four obligate carriers were heterozygous (Figure 1c, Table 1). We assessed the percentage of dogs carrying the PNPLA8:c.[1169_1170dupTT] variant allele in a subpanel of 85 control Australian Shepherd dogs excluding first-degree relatives and found it to be 4.7%.

The PNPLA8:c.1169_1170dupTT variant was predicted to induce a PNPLA8:p.(His391PhefsTer394) frameshift leading to a premature stop codon at position 394 in the protein. To evaluate the putative functional impairment of this duplication on PNPLA8, we aligned the truncated PNPLA8 protein sequence with those of wild-type PNPLA8 proteins in humans, dogs and cows (Figure 1d, Appendix S1). The global alignment (Figure 1d) showed that canine and human wild-type proteins displayed 90% identity. The predicted truncated PNPLA8 protein lacked the last 390/784 amino acids, including the functional patatin domain containing the catalytic site (Figure 1d, Hara et al., 2019).

**TABLE 1** Genotypes for the PNPLA8:C.1169_1170dupTT variant

| | *+/+* | *+/dup* | *Dup/dup* | **Total** |
|---|---|---|---|---|
| Ataxic Australian Shepherd dogs | 0 | 0 | 3 | 3 |
| Unregistered[a] ataxic Australian Shepherd dogs | 0 | 0 | 2 | 2 |
| Obligate-carrier Australian Shepherd dogs | 0 | 4 | 0 | 4 |
| Proband-related Australian Shepherd dogs | 9 | 6 | 0 | 15 |
| Control Australian Shepherd dogs | 81 | 4 | 0 | 85 |
| Total | | | | 109 |

*Note*: +, PNPLA8:c.[1169_1170dupTT] wild-type allele; *dup*, PNPLA8:c.[1169_1170dupTT] mutant allele.

[a]Australian Shepherd dogs without registration at the French Kennel Club (pedigree data were unavailable).

PNPLA8 was described as a calcium-independent patatin-like containing domain phospholipase contributing to mitochondrial function through tailoring mitochondrial membrane lipid metabolism and composition (Liu et al., 2017). *Pnpla8* null mice exhibit neurodegeneration characterized by degenerating mitochondria and autophagy (Mancuso et al., 2009). Recessive *PNPLA8* variants were identified in human patients with severe prenatal neurodegeneration (Masih et al., 2021), developmental delay, microcephaly, spasticity and cerebellar atrophy (Shukla et al., 2018) or a mitochondrial myopathy mainly characterized by muscle weakness, hypotonia, seizures and lactic acidosis (Saunders et al., 2015). Finally, a degenerative myeloencephalopathy characterized by pelvic limb weakness and ataxia has been described in cattle. The disease, known as Weaver syndrome, has been associated with a *PNPLA8* missense variant (Figure 1e; Kunz et al., 2016).

The identified canine variant is predicted to produce a protein lacking its catalytic site and therefore will probably lead to a complete loss of *PNPLA8* function. Taken together with the co-segregation of genotypes in the family, the perfect genotypic association in a fairly large cohort of Australian Shepherds and knowledge of the functional effects of *PNPLA8* variants in other species, this strongly suggests that the PNPLA8:c.1169_1170dupTT variant causes the phenotype.

In conclusion, we identified a frameshift variant associated with a hereditary ataxia in Australian Shepherd dogs which is easily detectable with a genetic test to avoid at-risk matings. Further studies are ongoing to characterize the pathophysiology of this canine neurodegenerative disease sharing molecular and clinical features with Weaver syndrome in cattle and the mitochondrial-related neurodegeneration associated with *PNPLA8* loss-of-function variants in humans.

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## CONFLICT OF INTEREST

Caroline Dufaure de Citres is an employee of Antagene, a company selling DNA tests for animals.

## DATA AVAILABILITY STATEMENT

Accessions of the whole-genome sequence data are listed in Table S2. Genomic sequences of *PNPLA8* exon 3 from wild-type and ataxic dogs (*Canis lupus familiaris*) were submitted to GenBank. The accession numbers are GenBank ID: ON411605 for the wild-type allele and GenBank ID: ON411606 for the PNPLA8:c.[1169_1170dupTT] variant allele.

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## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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