

A frameshift-deletion mutation in Reelin causes cerebellar hypoplasia in White Swiss Shepherd dogs

Abstract

Cerebellar hypoplasia is a heterogeneous neurological condition in which the cerebellum is smaller than usual or not completely developed. The condition can have genetic origins, with Mendelian-effect mutations described in several mammalian species. Here, we describe a genetic investigation of cerebellar hypoplasia in White Swiss Shepherd dogs, where two affected puppies were identified from a litter with a recent common ancestor on both sides of their pedigree. Whole genome sequencing was conducted for 10 dogs in this family, and filtering of these data based on a recessive transmission hypothesis highlighted five protein-altering candidate variants – including a frameshift-deletion of the Reelin (*RELN*) gene (p.Val947*). Given the status of *RELN* as a gene responsible for cerebellar hypoplasia in humans, sheep and mice, these data strongly suggest the loss-of-function variant as underlying these effects. This variant has not been found in other dog breeds nor in a cohort of European White Swiss Shepherds, suggesting a recent mutation event. This finding will support the genotyping of a more diverse sample of dogs, and should aid future management of the harmful allele through optimised mating schemes.

Cerebellar hypoplasia (CH) is a feature of several neurological conditions wherein the cerebellum fails to fully develop. Genetic causes of CH have been demonstrated in a variety of mammalian species including humans (Ross et al., 2001), sheep (Suárez-Vega et al., 2013) and dogs (Gerber et al., 2015). We investigated two recent cases of CH found at autopsy in White Swiss Shepherd littermates. These puppies were born clinically normal and were part of a litter of nine (five males, four females) which included a stillborn animal. Both CH-affected puppies failed to gain weight and developed progressive ataxia from around 2 weeks of age. The puppies had difficulty standing, could not walk in a straight line, had a good suckle reflex but had difficulty latching on to the teat. The puppies had no spontaneous or positional

nystagmus, had a normal pupillary light reflex, lacked a menace reflex (normal for age) and segmental spinal reflexes were intact. A congenital brain defect, possibly cerebellar or vestibular, was considered likely, and the puppies were euthanised at 4 weeks of age on humanitarian grounds.

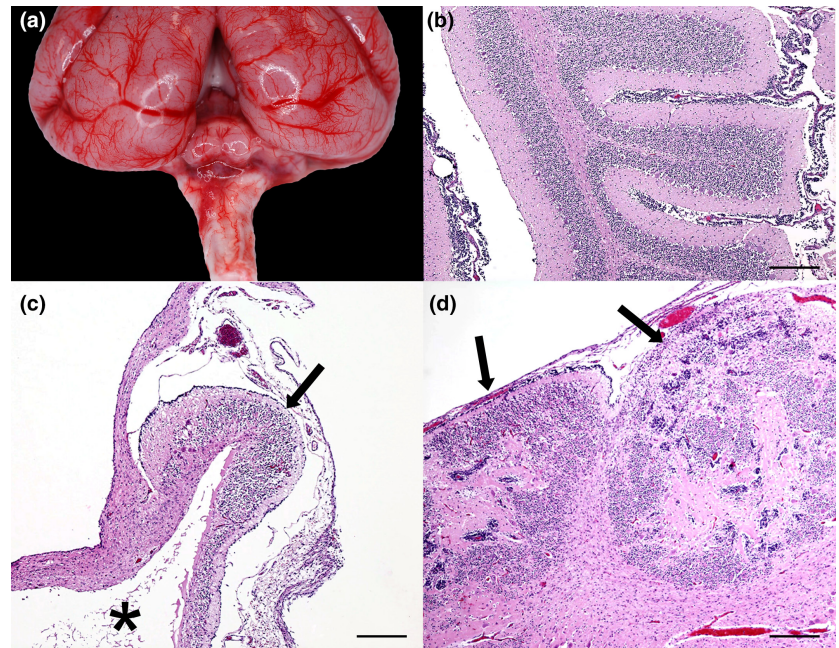
Autopsy revealed anatomical abnormalities in the brains of both affected puppies, with both animals showing severe CH with lissencephaly (Figure 1a) and moderate internal hydrocephalus with distended lateral and fourth ventricles. The cerebellum measured 25 mm in width × 10 mm in length × 5 mm in height in one puppy, and 25 × 8 × 5 mm in the other. In both puppies the cerebellum lacked cerebellar folia. Brain samples were processed for histology, embedded in paraffin wax, sectioned at 3–4 µm and stained with haematoxylin and eosin. Microscopically, the normal layered structure (example in Figure 1b) of the cerebellum was disorganised (Figure 1c,d) and the molecular and granular layers were thin, with the granular layer of irregular thickness (Figure 1c,d) and often forming islands of cells (Figure 1d). Purkinje cells were scattered throughout all layers (neuronal heterotopia; Figure 1d). Vascular structures were prominent. The cerebrum lacked sulci and gyri (agyria) and the white matter was thinned. The cerebral cortex was disorganised with increased thickness of the cortical laminae and neuronal cell bodies that were not vertically aligned.

Cerebellar hypoplasia in dogs may be caused by genetic factors or a teratogenic cause due to *in utero* or neonatal parvovirus infection. We considered the former explanation most likely, as parvovirus involvement in canine CH is rare (Wünschmann et al., 2020) and the dam was fully vaccinated against parvovirus (Vanguard 5; Zoetis NZ Ltd) and was healthy during the pregnancy. We also noted that parents of the affected litter shared the same paternal grandsire/maternal great grandsire. This observation suggested a possible recessive mode of inheritance and we therefore conducted whole genome sequencing of 10 animals from the pedigree to attempt to identify the causative variant (Figure S1). Blood or cheek cell samples were used to extract DNA using Qiagen MagAttract

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FIGURE 1 Pathological findings in 4-week-old White Swiss Shepherd puppies with cerebellar hypoplasia (CH). (a) Caudocranial view of the brain showing lissencephaly and cerebellar hypoplasia. (b) Cerebellum of a 4-week-old control dog (Huntaway crossbred unrelated to study, euthanised owing to carpal joint contracture). Haematoxylin and eosin (HE). Bar 500 μ m. (c) Cerebellum (arrow) and a dilated fourth ventricle (*). HE. Bar 500 μ m. (d) Cerebellum showing disorganisation of the cerebellar layers and absence of folia. Arrows point to areas of disorganised cerebellum. HE. Bar 500 μ m.



HMW DNA kits, and sequencing libraries were prepared using an Illumina DNA Prep Tagmentation kit. Paired-end sequencing (2×150 bp reads) was performed on the Illumina Novaseq 6000 instrument (GeneMark), and sequence read data were processed using TRIMMOMATIC v0.39 (Bolger et al., 2014). These data were then mapped to the German Shepherd-based CanFam4 genome assembly UU_Cfam_GSD_1.0 (Wang et al., 2021) using BWA-MEM2 v2.2.1 (Md et al., 2019), resulting in a $18.9\text{--}131.1\times$ mapped read depth per sample. Variants were called from sequence alignments using GATK HAPLOTYPECALLER v4.2.4.1 (Poplin et al., 2018), resulting in a total of 7 351 661 variants following generic quality filtering based on GATK guidelines (Caetano-Anolles, 2023).

We first inspected read alignments for the presence of the Very Low Density Lipoprotein Receptor gene (*VLDLR*) frameshift-deletion variant (chr1:91944760AC>A; CanFam4) that has been previously shown to cause CH in Eurasier dogs (Gerber et al., 2015). None of the sequenced individuals were found to carry that variant, so we next aimed to filter genome-wide variants based on an autosomal recessive hypothesis assuming the common ancestor as the origin of the mutant allele. Filters were applied to exclude variants below the nominal minor allele frequency afforded by this hypothesis ($\text{MAF} < 0.35$; seven alleles in 20), and with the following zygosity expectations: that variants should be homozygous non-reference in the two affected animals, heterozygous in the sire, dam and dam's sire, and heterozygous or homozygous reference for the five remaining animals (Figure S1). Variants were also filtered to remove moderately frequent variants ($\text{MAF} > 0.1$) catalogued from a publicly available multi-breed sequence dataset (Plassais et al., 2019), after these data were first 'lifted over' from the CanFam3 to CanFam4 assembly using LIFTOVERVCF

v4.2.4.0 (Picard; Broad Institute, 2022). Application of these filters yielded a total of 3813 segregating variants as candidates for the CH disorder. Functional prediction was then performed on these variants using SNPEFF v5.1 (Cingolani et al., 2012) in conjunction with RefSeq annotation release 106 to identify 12 protein-altering candidates genome-wide (i.e. those with a 'moderate' to 'high' SNPEff effect).

Visualisation of sequence alignments to manually curate the 12 candidates of primary interest showed that eight were various misrepresentations of a single structural variant overlapping the *LOC106560122* gene (the manually corrected allele is represented in Table 1). The other four candidates included a stop-lost variant in an olfactory receptor (Olfactory Receptor Family 10 Subfamily H Member 1; *OR10H1*), frameshift-deletions in the Phospholipid Phosphatase Related 2 (*PLPPR2*) and Reelin (*RELN*) genes, and a missense variant in RAD50 Interactor 1 (*RINT1*; Table 1). Of the five refined candidates, the most biologically plausible (if not obvious) causative variant was the 1 bp frameshift-deletion of *RELN* (chr18:16909942TG>T; XM_038562771.1). Null mutations in this gene have previously been shown to cause CH with lissencephaly in humans (Hong et al., 2000), sheep (Suárez-Vega et al., 2013) and mice (D'Arcangelo et al., 1995), with the White Swiss Shepherd variant predicted to cause premature termination at the codon immediately following the 1 bp deletion (p.Val1947*; predicted loss of 73% of the 3474aa wild-type protein XP_038418699.1). These observations, and the role of the Reelin protein in neuronal migration and development (Jossin, 2020), strongly support the p.Val1947* variant as responsible for the CH observed in these dogs. It is further noteworthy that Reelin may influence neuronal development through direct interaction with

TABLE 1 List of five co-segregating protein-altering candidate variants identified through whole-genome sequencing.

Chromosome	Position	Reference	Alt	Gene	Consequence	Accession ID
chr18	15 154 326	T	C	<i>RINT1</i>	p.Asn185Ser	XM_038562736.1
chr18	16 909 942	TG	T	<i>RELN</i>	p.Val1947fs	XM_038562771.1
chr20	32 645 759 ^a	^a	^a	<i>LOC106560122</i>	p.Arg54delinsLeuPro*	XM_038566048.1
chr20	46 971 084	A	G	<i>OR10H1</i>	p.Ter316Argext*	XM_038565308.1
chr20	50 356 072	CA	C	<i>PLPPR2</i>	p.Leu522fs	XM_038567212.1

Note: Positions are based on the CanFam4 assembly; consequence predicted from mutant translation.

Abbreviations: Alt, alternative base(s); Ref, reference base(s).

^aCompound indel derived from manual sequence annotation g.32645759_32645840delinsCCTCCCAGGCTCTGCCTCCCCGGGCGGGCCCGAGGC CCCTCGGTCTCAGGGGA.

VLDLR (Hiesberger et al., 1999), the same gene that has been suggested to underlie CH in Eurasier dogs (Gerber et al., 2015).

The cerebellar and cerebral lesions in the White Swiss Shepherd dogs appear similar to those described in Churra sheep with a 31 bp frameshifting *RELN* mutation (Pérez et al., 2013; Suafez-Vega et al., 2013). However, the cerebellar lesions in the dogs appear to be more severe, with a complete absence of folia and less readily identifiable cerebellar layers. Interestingly, affected sheep showed clinical signs of cerebellar dysfunction from birth, whereas the CH-affected puppies in this study did not show clinical signs until 2 weeks of age. Two to four weeks is a common age for dogs with congenital cerebellar lesions to develop clinical signs, as this is when they begin to walk (Thomas, 1999). The difference in age of presentation may be due to the differences in development and movement between precocial species such as sheep and altricial species such as dogs, where the former have more developed brains at birth (Kalusa et al., 2021; Muir, 2000).

Notably, none of the 722 animals in the publicly available sequence dataset used for population filtering carried the *RELN* frameshift deletion, although no White Swiss Shepherds were present in that dataset. The variant is also missing from the Dog Biomedical Variant Database Consortium variant catalogue (Jagannathan et al., 2019), and a large collection of genomes from the Dog10K project (unpublished data; Ostrander et al., 2019). To determine if the *RELN* variant was present in a sample more representative of the breed, 88 White Swiss Shepherds of European origin, stored in the VetGen Biobank at the University of Bern, were genotyped by PCR and Sanger sequencing on the ABI3730xl instrument (primers: forward, 5'-TGCTTTTCAGTTTCACAGGAGA-3'; reverse, 5'-CTCTTGACCAGGTGCCA-3'; 203 bp amplicon). None of the Swiss dogs were found to carry the *RELN* mutation, suggesting that it is unlikely to be ubiquitous in the White Swiss Shepherd breed. However, it should be noted that carriers in the pedigree included artificial insemination stud dogs with links to kennels in South Africa, Australia and New Zealand. These results may therefore be highly relevant

to breeding of animals from those regions, allowing future mating decisions to avoid crossing carriers. These data will also support testing efforts to determine the prevalence of the variant in the global White Swiss Shepherd population and in other breeds, ultimately helping to manage the undesired allele.

KEYWORDS

canine, cerebellar hypoplasia, dogs, mutation, neurological development, Reelin, *RELN*, whole genome sequencing

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.


DATA AVAILABILITY STATEMENT

All sequence data are available for download via the NCBI Sequence Read Archive SUB13063844/PRJNA957464.

ETHICS STATEMENT

All experiments were performed in strict accordance with the rules and guidelines outlined in the New Zealand Animal Welfare Act 1999. Affected animals were euthanised on humanitarian grounds prior to the onset of this study, with samples obtained retrospectively.

Prospectively gathered samples were obtained in accordance with protocols approved by the Massey University Animal Ethics Committee, Palmerston North, New Zealand (approval MUAEC 21/33). The control brain displayed in **Figure 1b** was sourced from a Massey University bank of archival samples, originally submitted for routine diagnostic necropsy outside the scope of activities requiring formal committee assessment and approval. No additional animals were sacrificed for this study. The dogs in this study from the VetGen Biobank at the University of Bern were privately owned and samples were collected with the consent of their owners. The collection of blood samples from control dogs was approved by the 'Cantonal Committee for Animal Experiments' (Canton of Bern; permit 71/19; approval date 9 September 2019).

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