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Canine *RNF170* Single Base Deletion in a Naturally Occurring Model for Human Neuroaxonal Dystrophy

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Inhaltsverzeichnis

Abstract in Englisch ____ **3**

Zusammenfassung in Deutsch ____ **4**

Publikationsmanuskript ____ **5**

Danksagung ____ **15**

Eigenständigkeitserklärung ____ **16**

Abstract in Englisch

Vetsuisse Faculty, University of Bern 2024

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Canine *RNF170* Single Base Deletion in a Naturally Occurring Model for Human Neuroaxonal Dystrophy

Neuroaxonal dystrophy (NAD) is a group of inherited neurodegenerative disorders characterized primarily by the presence of spheroids (swollen axons) throughout the central nervous system. In humans, NAD is heterogeneous, both clinically and genetically. NAD has also been reported to naturally occur in large animal models, such as dogs. This study describes a newly recognized disorder in Miniature American Shepherd dogs (MAS), consisting of slowly progressive neurodegenerative signs, that was diagnosed as NAD via histopathology. Affected dogs were typically young adults and displayed an abnormal gait characterized by pelvic limb weakness and ataxia. A genome-wide association study and autozygosity mapping approach, followed by whole-genome sequencing, identified a 1-base-pair deletion in *RNF170* as the underlying genetic cause. This deletion is predicted to create a frameshift (XM_038559916.1:c.367delG) and early truncation of the RNF170 protein (XP_038415844.1:(p.Ala123Glnfs*11)) and perfectly segregates in an autosomal recessive pattern. *RNF170* variants were previously identified in human patients with autosomal recessive spastic paraplegia-85; this clinical phenotype shows similarities to the dogs described herein. We therefore propose that this novel MAS NAD could serve as an excellent large animal model for equivalent human diseases, particularly since affected dogs demonstrate a relatively long lifespan, which represents an opportunity for therapeutic trials.

animal model, hereditary spastic paraplegia, hereditary sensory ataxia 1, Miniature American Shepherd, spheroids

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Zusammenfassung in Deutsch

Vetsuisse-Fakultät Universität Bern 2024

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Kanine *RNF170* Einzelbasen-Deletion in einem natürlichen Modell für humane Neuroaxonale Dystrophie

Neuroaxonale Dystrophie (NAD) ist eine Gruppe erblicher neurodegenerativer Erkrankungen, die vor allem durch das Vorhandensein von Sphäroiden (geschwollene Axone) im zentralen Nervensystem gekennzeichnet ist. Beim Menschen ist NAD klinisch und genetisch heterogen. NAD wurde auch in Tiermodellen wie Hunden beobachtet. Diese Studie beschreibt eine neu erkannte Erkrankung in Miniature American Shepherds (MAS), die durch langsam fortschreitende neurodegenerative Anzeichen charakterisiert ist und histopathologisch als NAD diagnostiziert wurde. Betroffene Hunde waren meist junge Erwachsene und zeigten einen auffälligen Gang mit Schwäche und Ataxie der Hinterbeine. Eine genomweite Assoziationsstudie und Autozygotiekartierung, gefolgt von einer Sequenzierung des gesamten Genoms, identifizierte eine 1-Basenpaar-Deletion im *RNF170*-Gen als die genetische Ursache. Diese Deletion führt voraussichtlich zu einer Leserahmenverschiebung (XM_038559916.1:c.367delG) und vorzeitigen Verkürzung des RNF170-Proteins (XP_038415844.1:(p.Ala123Glnfs*11)) und folgt einem autosomal-rezessiven Erbgang. *RNF170*-Varianten wurden bereits bei Menschen mit autosomal-rezessiver spastischer Paraplegie-85 identifiziert, deren klinisches Bild den hier beschriebenen Hunden ähnelt. Daraus folgend stellt diese neuartige NAD bei MAS, insbesondere durch die lange Lebensspanne betroffener Hunde, ein exzellentes Tiermodell für vergleichbare menschliche Erkrankungen und die Möglichkeit für therapeutische Studien dar.

Tiermodell, hereditäre spastische Paraplegie, hereditäre sensorische Ataxie 1, Miniature American Shepherd, Sphäroide

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RESEARCH ARTICLE

Canine *RNF170* Single Base Deletion in a Naturally Occurring Model for Human Neuroaxonal Dystrophy

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ABSTRACT: Background: Neuroaxonal dystrophy (NAD) is a group of inherited neurodegenerative disorders characterized primarily by the presence of spheroids (swollen axons) throughout the central nervous system. In humans, NAD is heterogeneous, both clinically and genetically. NAD has also been described to naturally occur in large animal models, such as dogs. A newly recognized disorder in Miniature American Shepherd dogs (MAS), consisting of a slowly progressive neurodegenerative syndrome, was diagnosed as NAD via histopathology.
Objectives: To describe the clinical and pathological phenotype together with the identification of the underlying genetic cause.
Methods: Clinical and postmortem evaluations, together with a genome-wide association study and autozygosity mapping approach, followed by whole-genome sequencing.
Results: Affected dogs were typically young adults and displayed an abnormal gait characterized by pelvic limb weakness and ataxia. The underlying genetic cause was identified as a 1-bp (base pair) deletion in *RNF170* encoding ring finger protein 170, which perfectly segregates in an autosomal recessive pattern. This deletion is predicted to create a frameshift (XM_038559916.1:c.367delG) and early truncation of the RNF170 protein (XP_038415844.1:(p.Ala123Glnfs*11)). The age of this canine *RNF170* variant was estimated at ~30 years, before the reproductive isolation of the MAS breed.
Conclusions: *RNF170* variants were previously identified in human patients with autosomal recessive spastic paraplegia-85 (SPG85); this clinical phenotype shows similarities to the dogs described herein. We therefore propose that this novel MAS NAD could serve as an excellent large animal model for equivalent human

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Shawna R. Cook and Cleo Schwarz contributed equally to this work (shared first authorship).

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diseases, particularly since affected dogs demonstrate a relatively long lifespan, which represents an opportunity for therapeutic trials. © 2024 The Author(s). *Movement Disorders* published by Wiley Periodicals LLC on behalf of International Parkinson and Movement Disorder Society.

Key Words: animal model; hereditary spastic paraplegia; hereditary sensory ataxia 1; Miniature American Shepherd; spheroids

Neuroaxonal dystrophy (NAD) is a group of rare, inherited neurodegenerative diseases characterized by the presence of swollen axons (spheroids) throughout the central, and rarely peripheral, nervous system.[1]NAD has been described in humans[2] and spontaneously in domestic mammalian species, including dogs,[3] cats,[4] horses,[5] cattle,[6] and mice.[7] While all types of NAD share the histopathological hallmark of axonal spheroids and neuronal degeneration, there is a great clinical and genetic heterogeneity within species.[8,9]

In humans, multiple types of NAD are differentiated based on the clinical and morphological findings and underlying genetic defects. Some types of NAD are associated with increased iron accumulation in the basal ganglia (globus pallidus and substantia nigra) and are also classified as neurodegeneration with brain iron accumulation (NBIA).[10] The most common types of human NAD that also fall under the NBIA classification include pantothenate kinase-associated neurodegeneration (PKAN or NBIA1; OMIM 234200), caused by variants in the *PANK2* gene; early-onset infantile neuroaxonal dystrophy (INAD; OMIM 256600) or PLAN/NBI2A, caused by variants in *PLA2G6*; mitochondrial kinase-associated neurodegeneration (MPAN or NCBIA4; OMIM 614298), due to variants in *C19orf12*; and β-propeller protein-associated neurodegeneration (BPAN or NBIA5; OMIM 300894) with variants in *WDR45*.[10]

Canine NAD has previously been reported in both mixed breed and purebred dogs.[8,11–18] To date, four breed-specific forms have the underlying genetic cause identified. NAD in Papillons, which has a very early age-of-onset, is caused by a missense variant in *PLA2G6*. The homolog of this gene has been associated with human infantile neuroaxonal dystrophy and the affected Papillons were proposed as a valuable animal model for human NAD, especially since these patients demonstrate clinical signs within the first 4 months of life.[18] The other known causal variants for canine NAD comprise variants in *tectonin beta-propeller repeat-containing 2* (*TECPR2*) in Spanish Water dogs,[8] *vacuolar protein sorting 11* (*VPS11*) in Rottweilers,[11] and *mitofusin 2* (*MFN2*) in a colony of Schnauzer-Beagle crosses.[16] The existence of NAD in various dog breeds presents a unique opportunity to uncover novel genes linked to the disease, which could have implications not just for dogs, but also as potential candidates for understanding unexplained human cases.

Here, we report on Miniature American Shepherds (MAS) with a slowly progressive neurodegenerative disorder affecting the gait in young adult dogs primarily characterized by pelvic limb weakness and ataxia. Histopathologically, the syndrome is characterized by the presence of axonal spheroids and tissue changes indicative of neuronal degeneration and secondary gliosis. The aim of this study was to describe the clinical and pathological phenotype together with the identification of the underlying genetic cause. Ultimately, a 1-bp (base pair) deletion in *RNF170* was identified, with homozygous mutant dogs demonstrating clinical and pathological phenotypes strikingly similar to autosomal recessive spastic paraplegia-85 (SPG85) in RNF170-deficient human patients.

Methods

Ethics Statement

All examinations and animal experiments were carried out after obtaining informed written consent for participation by the owner and in accordance with local laws, regulations, and ethical guidelines. Sample collection was ethically approved by Purdue University's Institutional Animal Care and Use Committee (IACUC) (#1901001840) and the Cantonal Committee for Animal Experiments (Canton of Bern; permit BE 71/19). All dogs in this study were privately owned pet dogs.

Animal Selection, and Definition of Breed and Phenotype

This study was performed with DNA samples from a total of 937 dogs (485 MAS, 321 Australian Shepherds, 127 MAS, and 4 Toy Australian Shepherds; Table S1) originating either from the College of Veterinary Medicine, Purdue University (n = 151) or the Vetsuisse Biobank at the University of Bern (n = 786).

Each dog was assigned to one of three phenotypic categories. The neurological phenotypes were classified by a board-certified neurologist based on neurological examination, clinical reports, or videos of the dogs submitted by the owners. Twenty-five dogs were classified

as "neurological, clinical signs compatible with NAD" by the presence of hind limb weaknesses with or without ataxia, abnormal gait, scuffing of paws/dragging of digits, and kyphosis. All affected dogs exhibited a pacing/ambling gait (atypical ipsilateral movement of limbs) when gait could be adequately visualized. Six neurological dogs with signs that differed from the above mentioned were classified as "neurological, other." The remaining 906 dogs were used as controls. More detailed information on the 937 dogs is compiled in Table S1.

Clinical Examinations

A proportion of affected dogs were evaluated clinically by board-certified veterinary neurologists (n = 10 of 23 cases that ultimately were homozygous *del/del* at the discovered deletion variant, *RNF170*: XM_038559916.1:c.367delG; Table S1), with a complete neurological evaluation performed. The remaining 13 cases were variably under the care of general practice veterinarians. Following a call for screening of NAD in the breed, additional affected dogs were submitted to the study but not evaluated in person by a veterinary clinician. In this scenario, owners typically submitted videos of the dogs' gait, and these videos were reviewed by a board-certified neurologist (n = 9 of 23 *del/del* cases; Table S1). The remaining four *del/del* cases were submitted with clinical descriptions only provided by the owners.

Pathological Examinations

Necropsy reports were available for two neurologically affected MAS dogs (Case #3/Dog #79 and Case #4/Dog #139, Table S1), which were euthanized at the owner's discretion under the care of their general practitioner veterinarian, due to severely diminished quality of life.

A complete necropsy with harvesting and evaluation of all the major organs, the brain, and the spinal cord was performed on both cases. The tissues were fixed for 48–72 hours in 10% neutral buffered formalin and routinely trimmed and processed. Paraffin-embedded tissues were sectioned at 4–5 microns and stained with hematoxylin and eosin.

Additional sections of the central nervous system were mounted on charged slides (ProbeOn™ Thermo Fisher Scientific) and were used for immunohistochemistry staining for glial cells, including glial fibrillary acidic protein (GFAP) for astrocytes, and ionized calcium-binding adapter molecule 1 (Iba1) for microglia/macrophages (see Supplemental Methods in Data S1). Normal canine brain and spinal cord sections from a young, unaffected, mixed-breed dog were used as positive controls.

DNA Extraction and Exclusion of PNPLA8

Genomic DNA was isolated from the EDTA blood samples or buccal swabs.

Due to the close relatedness of MAS to Australian Shepherds and the overlap of clinical signs in affected dogs, the previously-published *PNPLA8*:XM_005630935.2:c.1169_1170dup variant, discovered in Australian Shepherds with hereditary ataxia, OMIA variant ID1470[19] was first tested and excluded in a subset (n = 16) of the MAS dogs.

SNV Genotyping

Genomic DNA from a total of 54 dogs was genotyped on the Illumina CanineHD BeadChips containing 220,853 markers (Neogen, Lincoln, NE, USA) (as designated in Table S1). All SNV positions reported herein correspond to the UU_Cfam_GSD_1.0/CanFam4 assembly.

GWAS

GWAS was performed with 54 samples (24 cases and 30 controls). Quality control of the SNV genotype data was performed using PLINK v.1.9.[20] After pruning, 54 dogs and 156,213 markers remained in the analysis and were used for the GWAS using the linear mixed model implemented in the GEMMA software (v0.94.1). Bonferroni correction was used to estimate the genome-wide significance threshold at $P = 0.05/156{,}213 = 3.2 \times 10^{-7}$. Manhattan- and QQ-plots were created using the qqman package in R.[21,22] The raw SNV genotype data are available in Supplementary File S1.

Autozygosity Mapping

The genotype data of 22 dogs that were homozygous for the disease-associated allele at the best associated marker were used for autozygosity mapping. Additionally, a tped file with all markers from chromosome 16 was created. Visual inspection of this file in an Excel spreadsheet was performed to exactly determine the shared homozygous haplotype in the 22 cases.

Whole-Genome Sequencing, Variant Filtering, and Copy Number Variant Analysis

A polymerase chain reaction (PCR)-free genomic DNA library was prepared from Case #9/Dog #161 and whole-genome sequencing at 24.8× coverage was performed on an Illumina Novaseq 6000 instrument (Illumina, Zurich, Switzerland). Reads were mapped to the UU_Cfam_GSD_1.0 reference genome assembly and variant calling was performed as described in Jagannathan et al. (2019).[23] SnpEff software (Cingolani et al., 2012)[24] together with National Center for Biotechnology Information (NCBI) annotation release

106 for the UU_Cfam_GSD_1.0 genome reference assembly was used to predict the functional effects of the called variants. The sequencing data of this single affected dog was compared against 960 control genomes of different breeds to filter for private variants (Table S2). Copy number variants within the mapped region were analyzed using Delly2[25] software. The output was filtered, keeping variants marked "PASS," and compared against the control genomes.

PCR and Sanger Sequencing

The candidate variant *RNF170*:XM_038559916.1:c.367delG was genotyped by direct Sanger sequencing of PCR amplicons. The resulting Sanger sequences were analyzed using the Sequencher 5.1 software (GeneCodes, Ann Arbor, MI, USA).

Post-hoc Linkage Mapping

Affected dogs from both Europe and North America were connected via one large pedigree (Fig. S1). A post-hoc logarithm of the odds (LOD) score was calculated in LAMP software.[26] The recessive genetic model option was used, and disease prevalence was set at 2%.

Estimation of Age of Mutation

Runs of homozygosity encompassing the identified variant in *RNF170* were identified and linkage map positions across the region were approximated.[27] The distance from the presumed causal variant and decay of homozygosity in either direction was calculated. The variant was dated using a previously described methodology[28] specifically designed for SNV array data from small datasets.

Consent to Participate

All dog owners provided written consent when donating samples from their dog(s).

Consent for Publication

Written consent for publication was obtained and is on record from the owner of the dog shown in the Supplemental Video.

Results

Clinical Phenotype

Clinical history of the first proband entailed a 2-year-old female intact MAS with slowly progressive hind limb weakness and incoordination (Case #9/Dog #161, Table S1). Initial signs of weakness were reported to have occurred at around 2 years of age. Poor performance during dog agility events also developed over time. Neurological evaluation identified normal mentation and cranial nerve responses. Behavior was abnormal with excessive anxiety and fear of strangers. Ambulatory paraparesis with hindlimb ataxia was evident. Segmental spinal reflexes were normal. Mild hypermetria (with hyperextension) in the thoracic limbs was also observed. There was no evidence of pain. The integrity of the sensory innervation was difficult to ascertain in light of the dog's fearful behavior. The neuroanatomical localization was to the T3-L3 spinal cord segments. A multifocal neuroanatomical localization including the T3-L3 spinal cord segments, C1-C5 spinal cord segments (thoracic limbs hypermetria), and forebrain (excessive anxiety) were not excluded. Magnetic resonance imaging (MRI) of the brain, spinal cord and cerebrospinal fluid (CSF) analysis only identified a mild coning of the cerebellum in the foramen magnum.

This dog's sibling also demonstrated ambulatory paraparesis with hindlimb ataxia and was evaluated by the same neurologist (Case #11/Dog #183, Table S1). For this dog, neither anxiety nor thoracic limb hypermetria was evident. Neuroanatomical localization was to the T3-L3 spinal cord segments. The clinical presentation started at a similar age as in the previously examined sibling and was similarly progressive. No MRI was performed on the second dog.

Review of the videos (see Video S1 for an example) of other affected dogs demonstrated that a majority had varying degrees of hind limb weakness and ataxia, together with scuffing of the nails/dragging of digits. Kyphosis and a pacing/ambling gait were commonly reported. A cerebellar gait was also noted in several dogs. A few dogs had a reported change in behavior. Seizures were only reported in one dog, albeit no video footage of the event was available.

Overall, in the present cohort of MAS dogs from a wide international genetic pool, the onset of clinical signs was typically around the second year of life, although this varied somewhat between dogs; this variability, to an extent, depended on the astuteness of the owner's observations. Slowly progressive T3-L3 myelopathy signs were observed as the most common clinical presentation, with possible cervical, cerebellar, or forebrain signs also developing. Neither pain nor vestibular signs were reported in affected dogs. Gait abnormalities were always more obvious during the walk compared to faster gaits.

Histopathological Phenotype

In both necropsied cases (Case #3/Dog #79 and Case #4/Dog #139, Table S1) the evaluation of the brain and spinal cord showed widespread and bilateral neuroaxonal degeneration throughout the gray and white matter with the lateral cuneate nuclei in the brainstem being most severely affected (Fig. 1A). The neuroaxonal degeneration consisted of variable numbers of large, swollen, and hypereosinophilic axons (spheroids),

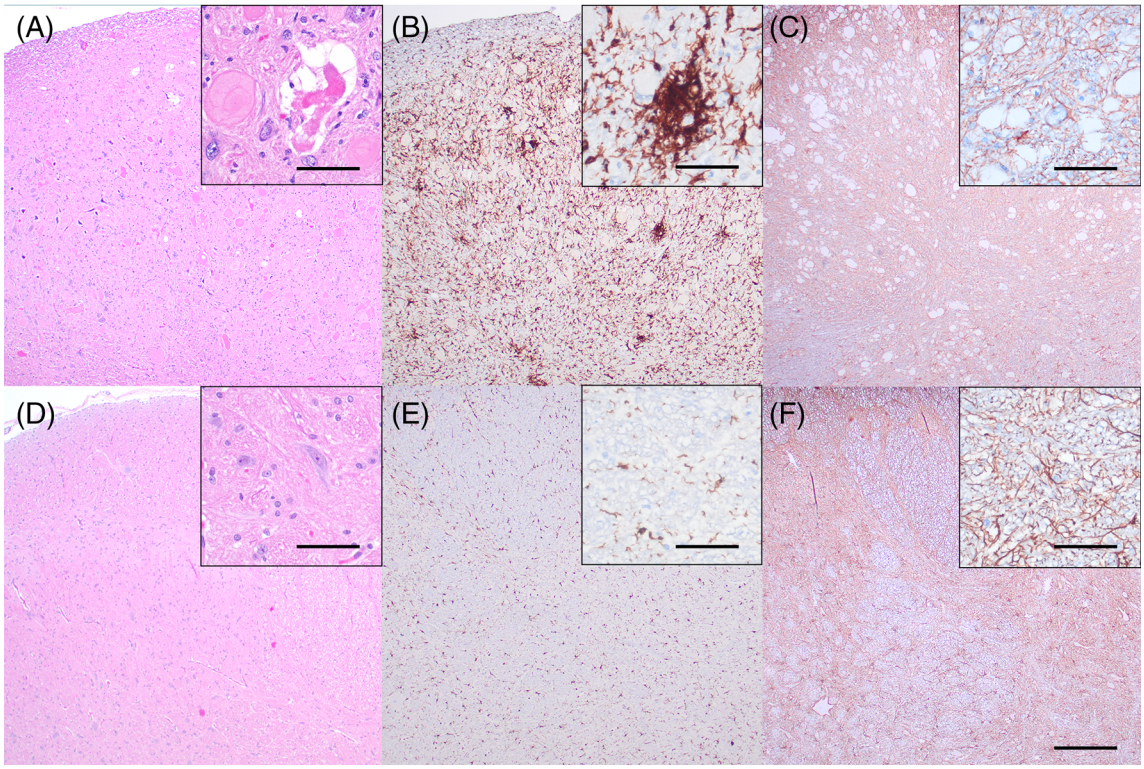

FIG. 1. Central nervous system pathology of affected dogs. Widespread neuroaxonal degeneration and gliosis are observed. (A) Brainstem, at the level of the lateral cuneate nucleus. The neuroparenchyma demonstrates high numbers of degenerated axons and spheroids along with increased glial cells and dead neurons (inset). Hematoxylin and eosin. (B) and (C) Immunohistochemistry for IBA-1 (B) confirms the marked increase in density of microglial cells in the affected regions with formation of Iba-1-positive cell aggregates (B, inset). Immunohistochemistry for glial fibrillary acidic protein (GFAP) (C) only shows a modest increase in astrocytes. Controls (D–F) obtained from a young, unaffected, intact male mixed-breed dog. 4× scale bar: 150 μm; insets: 40×, scale bar: 60 μm. [Color figure can be viewed at wileyonlinelibrary.com]

dilated myelin sheaths, degenerated or dead neurons, and mixed gliosis (Fig. 1A, inset). The gliosis was further highlighted by GFAP and Iba1 immunohistochemistry stains (Fig. 1B,C), which showed increased density of glial cells in the affected regions and occasional formation of glial cell aggregates. No other significant changes were seen in any of the organs evaluated.

Genetic Investigation

As the clinical and pathological examinations were highly suggestive of an inherited disease, we compiled pedigree information on the available cases, ultimately generating a pedigree spanning both European and North American cases (Fig. S1). The pedigree was compatible with a monogenic autosomal recessive mode of inheritance. For the identification of the causal genetic variant, we initially performed a GWAS with 24 dogs classified as "neurological, clinical signs compatible with NAD" and 30 unaffected controls. This analysis yielded a clear association signal on chromosome 16 exceeding the Bonferroni significance threshold ($P = 3.25 \times 10^{-8}$; Fig. 2A). Autozygosity mapping revealed that 22 of the GWAS-cases shared an identical ~2.69 Mb homozygous haplotype. The first heterozygous markers on either side of the homozygous region defined an exact critical interval for the NAD variant, Chr16:21,289,584–23,975,245 (all positions reported in UU_Cfam_GSD_1.0/CanFam4) (Fig. 2B, Table S3).

We sequenced the genome of one case (Case #9/Dog #161, Table S1) and compared the data to 960 control genomes (Table 1, Tables S2 and S4). Variant filtering revealed only two private homozygous variants in the critical interval. One was a deep intronic SNV in *SH2D4A* that was not further investigated, as it was not predicted to have any deleterious effects (Table S4). The remaining variant was a 1-bp coding deletion in the *RNF170* (*ring finger protein 170*) gene and can be designated as Chr16:23,653,872delG (UU_Cfam_GSD_1.0 assembly), located in the critical interval (Fig. 2C, Table S4). This variant, XM_038559916.1:c.367delG, is predicted to lead to a frameshift and truncation of 48% of the wild-type open reading frame, XP_038415844.1:(p.Ala123Glnfs*11) (Fig. S2). The Chr16:23,653,872delG variant is also absent from the most recently published Dog10K dataset comprising 2075 genetically diverse canids.[29] Copy number variants within the mapped region were excluded via Delly2.[25]

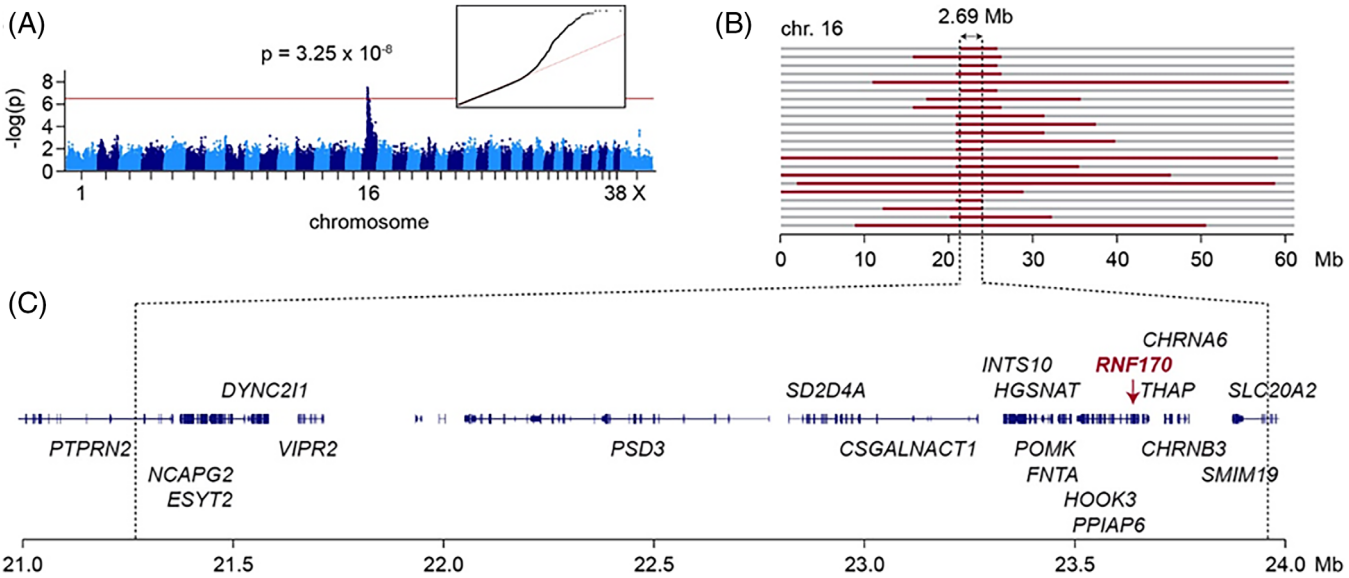

FIG. 2. Mapping of the Miniature Australian Shepherd dog (MAS) neuroaxonal dystrophy (NAD) locus. (A) Manhattan plot of a genome-wide association study (GWAS) using 24 neurological dogs and 30 controls showed a clear association signal on chromosome 16 with the best associated marker at 21,740,477 bp (base pairs). The inset shows the QQ-plot. (B) Autozygosity mapping in 22 NAD cases. Each horizontal line indicates the genotypes on chromosome 16 for one dog. Identical homozygous haplotypes are indicated in red. The shared segment defines the critical interval. (C) National Center for Biotechnology Information (NCBI) annotation release 106 lists 20 genes in the critical interval. Additionally annotated LOCs are not shown. The *RNF170* gene is highlighted in red. [Color figure can be viewed at wileyonlinelibrary.com]

We next genotyped a large cohort of 937 dogs, including the original GWAS cases, for the *RNF170* single base deletion. This revealed 27 dogs as homozygous for the deletion, 98 heterozygous carriers, and 813 dogs homozygous for the wild-type allele. Some 23 of the 27 homozygous mutant dogs showed clinical signs compatible with NAD (~85%). The remaining four dogs did not show any signs of NAD. However, these four discordant dogs were relatively young (6, 19, 27, and 43 months of age, respectively). The cohort also comprised six dogs with neurological signs differing from the NAD phenotype. These dogs were either homozygous (n = 5) or heterozygous (n = 1) for the wild-type allele. Two additional neurological dogs whose clinical signs were indistinguishable from the other NAD cases also did not carry the mutant *RNF170* allele (Table 2).

Post-hoc linkage mapping calculated a LOD score of 9.70 for the identified *RNF170* deletion in an extended pedigree (Fig. S1), demonstrating significant linkage between the genotype and NAD phenotype.

Estimation of the age of the mutation indicated that the variant arose approximately 13.7 generations ago (95% CI 2.9–25.1), under a "correlated genealogy" model (a model allowing for more than one common/shared ancestor, which is nearly always the case in

TABLE 1 *Results of variant filtering in a neuroaxonal dystrophy (NAD)-affected Miniature Australian Shepherd (MAS) dog against 960 control genomes*

Filtering step	Homozygous variants
All variants in the affected MAS dog	2,428,434
Private variants in whole genome	434
Protein-changing private variants in whole genome	2
Private variants in the critical interval	2
Protein-changing private variants in the critical interval	1
Located in functional candidate genes for similar phenotypes in other species	1

TABLE 2 *Association of the genotypes at* RNF170*:c.367delG variant with phenotype in 937 dogs*

Phenotype group	G/G	G/del	del/del
Neurological, clinical signs compatible with neuroaxonal dystrophy (n = 25)	2	–	23
Neurological, other (n = 6)	5	1	–
Controls, Miniature/Toy American/Australian Shepherd dogs (n = 586)	485	97	4[a]
Controls, Australian Shepherd dogs (n = 320)	320	–	–

[a]These four dogs were all younger than 43 months at the time of writing.

purebred dogs). Given a generation interval of 2 years, the mutation event is predicted to have occurred ~27.4 years ago.

Discussion

In a highly unique situation, three different research laboratories (Purdue University, University of Bern, and University of Pennsylvania) all independently identified the *RNF170* variant in NAD-affected dogs using slightly different approaches. After realizing that all three groups worked on the same disease and a related set of dogs, we combined the data to produce the present comprehensive report characterizing neuroaxonal dystrophy in the MAS, a new autosomal recessively inherited canine neurologic disease.

Histopathological examination of affected dogs demonstrated abundant large, irregularly-shaped spheroids, particularly in the brainstem, which is consistent with neuroaxonal dystrophies.[30] Although these spheroids can be found throughout the brain, they are usually found in the gray matter of brainstem nuclei and spinal cord (dorsal column nuclei), which is considered the characteristic distribution for NAD.[6,30] The neurohistopathological diagnosis of NAD corroborates the genetic findings in these dogs.

A hypothesis-free genetic analysis identified a deletion predicted to cause a frameshift in the *RNF170* gene as the most likely causal variant. *RNF170* encodes an E3 ubiquitin ligase located in the endoplasmic reticulum membrane (ER) that mediates ubiquitination-dependent degradation of inositol 1,4,5-triphosphate receptors (IP3Rs) via the ER-associated protein degradation (ERAD) pathway.[31,32] Activation of IP3Rs leads to Ca^{2+} efflux from the ER into the cytoplasm. After activation, RNF170 is recruited by the ERLIN1/ERLIN2 complex and enables the proteasomal degradation of IP3 receptors, thus having an impact on Ca^{2+} homeostasis.[32,33] Dysregulation of Ca^{2+} homeostasis and signaling have been implicated in various neurodegenerative diseases such as Alzheimer's and Huntington's disease.[34,35] Variants in key genes encoding components of the ERAD pathway, such as in *ERLIN1*, *ERLIN2*, or *RNF170*, have been reported to cause hereditary spastic paraplegia (HSP) in humans (MIM #615681, MIM #611225, MIM #619686).[36–39] Human *RNF170* variants are further associated with autosomal dominant sensory ataxia-1 (SNAX1) (MIM #608984).[40,41]

HSP comprises a genetically and clinically heterogenous group of rare, neurodegenerative, motor neuron disorders.[42] More than 80 genes have been associated with HSP,[43] and the clinical and genetic heterogeneity can make the diagnosis challenging.[44] To date, seven different variants in *RNF170* were identified in human patients with early to adolescent-onset HSP.[39,44–46] Even among patients with known *RNF170* variants underlying their HSP, the clinical picture can vary.[39]

SNAX1 in humans is caused by a heterozygous *RNF170* variant (R199C), and affected individuals experience adult-onset, slowly-progressive gait ataxia and clumsiness, together with distal sensory loss.[40,47] Interestingly, however, the SNAX1 *RNF170*:p.R199C variant does not affect the ubiquitination of activated IP3R.[48] Given this, and the fact that heterozygous carriers of nonsense variants in *RNF170* have been shown to be unaffected,[39,48,49] it has been hypothesized that the age-dependent gait problems of human SNAX1 patients might be due to toxic gain-of-function of mutant RNF170 proteins, independent from endogenous RNF170 function.[47]

A murine model ($Rnf170^{-/-}$) also demonstrated later onset (~12 months) and progression of clinical signs over time.[49] In mice, the $Rnf170^{-/-}$ gait abnormality was suggested to be specifically associated with inter-limb coupling and step sequence mechanisms, rather than a secondary effect.

The naturally occurring MAS canine model of NAD in the present study clearly demonstrates autosomal recessive inheritance, together with a predicted loss-of-function variant, thus mimicking human *RNF170*-related HSP conditions. However, clinically, the affected dogs may share more similarities with the typical presentation of *RNF170* SNAX1 in humans, including post-adolescent age of onset. Although not fatal per se, affected MAS dogs eventually develop more severe disabilities, leading to poor quality of life and frequent euthanasia. HSPs in people have historically been classified as "pure" and "complex/complicated" forms, with the former characterized primarily by spasticity without other significant findings and the latter being associated with additional features, such as seizures, neuropathies, short stature, visual abnormalities, and others.[50] Today, such clinical classifications have been largely superseded by genetic classifications.[50]

Long-term outcome information for NAD-affected MAS is currently limited, as many of these dogs are still alive. The T3-L3 myelopathy was the most evident finding; whether or not the thoracic limb hypermetria and behavioral changes (and reported seizures in a single case) are NAD-related is unclear but could suggest these dogs experience a more "complex/complicated" phenotype rather than a "pure" form of the disease. This highlights the need for further studies over longer time frames. Crucially, the lifespan of NAD-affected MAS is not markedly decreased, representing an opportunity for therapeutic trials in this large animal model. There is currently no treatment for NAD in affected

dogs; the findings in the present study provide a foundation for potential future studies investigating treatment options.

One limitation to this study was our inability to carry out either mRNA or protein investigations for confirming the predicted loss-of-function effect of the identified *RNF170* variant; such work was precluded by the lack of sample availability (affected dogs still alive and living as pets in individual homes) and may become possible with future postmortem samples. Second, while the association of the genotypes at the *RNF170*:c.367delG variant with the NAD phenotype was very strong, it was not perfect. We observed a total of six discordant dogs in a cohort of 937 dogs. Four dogs that were homozygous for the deletion did not show clinical signs. Two of them were younger than 2 years of age and thus below the typical age of onset for NAD in our cohort. The other two were 27 and 43 months old and thus of an age when clinical signs were noticeable in most of the other cases. We speculate that the NAD phenotype in these two dogs was particularly mild or had a delayed age of onset. This underscores the importance of genetic testing in order to stop further propagation of the disease. We also cannot exclude the possibility of incomplete penetrance. Two other dogs showed clinical signs indistinguishable from NAD but had a wild-type genotype. This most likely points to genetic heterogeneity and the potential existence of additional inherited, degenerative neurological diseases in the MAS.

We observed the mutant allele only in MAS and related breeds, but not in standard Australian Shepherds. This may indicate that the deleterious allele arose only recently after the separation of the populations. Estimation of the age of the deletion variant predicts the mutation event occurred approximately 27 years ago, or around the early- to mid-1990s. The first parent breed club and registry for North American MAS was formed in 1990 (https://mascusa.org/breed/history, accessed January 20, 2024). Therefore, we cannot definitively rule out the presence/absence of this deletion in the Australian Shepherd breed; instead, we recommend confirmation by testing additional representative standard Australian Shepherd dogs from different countries before a general all-clear signal is given to this breed.

Importantly, the disease allele frequency specifically in the MAS control cohort was found at 8.3% (this does not include the standard Australian Shepherds), which corresponds to a carrier frequency of 16.6%. While these numbers may overestimate the true frequencies due to biased sampling for our study, they clearly warrant genetic testing and a targeted breeding program to avoid future carrier x carrier matings for these dogs.

In conclusion, the clinical and pathologic picture presented by MAS affected with *RNF170*-related NAD is strikingly similar to the phenotypic spectrum (HSP, SNAX1) seen in human patients with *RNF170* variants. Thus, NAD in the MAS represents an excellent, spontaneously occurring large animal model for *RNF170* conditions in people.

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Data Availability Statement

Dog single nucleotide variant (SNV) array data are given in File S1. Whole-genome sequence from Case #9/Dog #161 is publicly available under study accession number PRJEB16012 and sample accession number SAMEA112638856. All other accessions are given in Table S2.

References

1. Nardocci N, Zorzi G. Axonal dystrophies. Handb Clin Neurol 2013;113:1919–1924. https://doi.org/10.1016/B978-0-444-59565-2.00062-9
2. Cowen D, Olmstead EV. Infantile neuroaxonal dystrophy. J Neuropathol Exp Neurol 1963;22(2):175–236.
3. Cork LC, Troncoso JC, Price DL, Stanley EF, Griffin JW. Canine neuroaxonal dystrophy. J Neuropathol Exp Neurol 1983;42(3):286–296.
4. Carmichael KP, Howerth EW, Oliver JE, Klappenbach K. Neuroaxonal dystrophy in a group of related cats. J Vet Diagnostic Investig 1993;5(4):585–590.
5. Adams AP, Collatos C, Fuentealba C, Illanes O, Blanchard R. Neuroaxonal dystrophy in a two-year-old quarter horse filly. Can Vet J 1996;37(1):43–44.
6. Hanshaw DM, Finnie JW, Manavis J, Kessell AE. Axonal spheroid accumulation in the brainstem and spinal cord of a young Angus cow with ataxia. Aust Vet J 2015;93(8):283–286.
7. Bouley DM, McIntire JJ, Harris BT, Tolwani RJ, Otto GM, DeKruyff RH, et al. Spontaneous murine neuroaxonal dystrophy: a model of infantile neuroaxonal dystrophy. J Comp Pathol 2006;134(2–3):161–170.
8. Hahn K, Rohdin C, Jagannathan V, Wohlsein P, Baumgärtner W, Seehusen F, et al. TECPR2 associated neuroaxonal dystrophy in Spanish Water dogs. PLoS One 2015;10(11):1–18. https://doi.org/10.1371/journal.pone.0141824
9. Kruer MC. The neuropathology of neurodegeneration with brain iron accumulation. Int Rev Neurobiol 2013;110:165–194. https://doi.org/10.1016/B978-0-12-410502-7.00009-0
10. Hayflick SJ, Kurian MA, Hogarth P. Neurodegeneration with brain iron accumulation. Handb Clin Neurol 2018;147:293–305.
11. Lucot KL, Dickinson PJ, Finno CJ, Mansour TA, Letko A, Minor KM, et al. A missense mutation in the vacuolar protein sorting 11 (VPS11) gene is associated with neuroaxonal dystrophy in Rottweiler dogs. G3 Genes, Genomes, Genet 2018;8(8):2773–2780.
12. Clark RG, Hartley WJ, Burgess GS, Cameron JS, Mitchell G. Suspected inherited cerebellar neuroaxonal dystrophy in collie sheep dogs. N Z Vet J 1982;30(7):102–103.
13. Degl'Innocenti S, Asiag N, Zeira O, Falzone C, Cantile C. Neuroaxonal dystrophy and cavitating leukoencephalopathy of

Chihuahua dogs. Vet Pathol 2017;54(5):832–837. https://doi.org/10.1177/0300985817712557

14. Sacre B, Cummings J, Delahunta A. Neuroaxonal dystrophy in a Jack Russell terrier pup resembling human infantile neuroaxonal dystrophy. Cornell Vet 1993;83(2):133–142.
15. Pintus D, Cancedda MG, Macciocu S, Contu C, Ligios C. Pathological findings in a Dachshund-cross dog with neuroaxonal dystrophy. Acta Vet Scand 2016;58(1):1–7.
16. Fyfe JC, Al-Tamimi RA, Liu J, Schäffer AA, Agarwala R, Henthorn PS. A novel mitofusin 2 mutation causes canine fetal-onset neuroaxonal dystrophy. Neurogenetics 2011;12(3):223–232.
17. Tanaka M, Yamaguchi S, Akiyoshi H, Tsuboi M, Uchida K, Izawa T, et al. Ultrastructural features of canine neuroaxonal dystrophy in a Papillon dog. J Vet Med Sci 2017;79(12):1927–1930.
18. Tsuboi M, Watanabe M, Nibe K, Yoshimi N, Kato A, Sakaguchi M, et al. Identification of the PLA2G6 c.1579G>a missense mutation in Papillon dog neuroaxonal dystrophy using whole exome sequencing analysis. PLoS One 2017;12(1):1–17. https://doi.org/10.1371/journal.pone.0169002
19. Abitbol M, Jagannathan V, Laurent N, Noblet E, Dutil GF, Troupel T, et al. A PNPLA8 frameshift variant in Australian Shepherd dogs with hereditary ataxia. Anim Genet 2022;53(5):709–712.
20. Chang CC, Chow CC, Tellier LCAM, Vattikuti S, Purcell SM, Lee JJ. Second-generation PLINK: rising to the challenge of larger and richer datasets. Gigascience 2015;4(1):1–16.
21. Turner SD. Qqman: an R package for visualizing GWAS results using Q-Q and manhattan plots. bioRxiv 2014;81:559–575.
22. R Core Team. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing. Vienna, Austria; 2019. https://www.R-project.org/
23. Jagannathan V, Drögemüller C, Leeb T. Dog Biomedical Variant Database Consortium (DBVDC). A comprehensive biomedical variant catalogue based on whole genome sequences of 582 dogs and eight wolves. Anim Genet 2019;50(6):695–704. https://doi.org/10.1111/age.12834
24. Cingolani P, Platts A, Wang le L, Coon M, Nguyen T, Wang le L., et al. A program for annotating and predicting the effects of single nucleotide polymorphisms, SnpEff: SNPs in the genome of Drosophila melanogaster strain w1118; iso-2; iso-3. Fly (Austin) 2016;6(2):80–92. https://doi.org/10.4161/fly.19695
25. Rausch T, Zichner T, Schlattl A, Stütz AM, Benes V, Korbel JO. DELLY: structural variant discovery by integrated paired-end and split-read analysis. Bioinformatics 2012;28(18):333–339.
26. Li M, Boehnke M, Abecasis GR. Efficient study designs for test of genetic association using sibship data and unrelated cases and controls. Am J Hum Genet 2006;78(5):778–792.
27. Wong AK, Ruhe AL, Dumont BL, Robertson KR, Guerrero G, Shull SM, et al. A comprehensive linkage map of the dog genome. Genetics 2010;184(2):595–605.
28. Gandolfo LC, Bahlo M, Speed TP. Dating rare mutations from small samples with dense marker data. Genetics 2014;197(4):1315–1327.
29. Meadows JRS, Kidd JM, Wang GD, Parker HG, Schall PZ, Bianchi M, et al. Genome sequencing of 2000 canids by the Dog10K consortium advances the understanding of demography, genome function and architecture. Genome Biol 2023;24(1):187. https://doi.org/10.1186/s13059-023-03023-7
30. Sisó S, Hanzlíček D, Fluehmann G, Kathmann I, Tomek A, Papa V, et al. Neurodegenerative diseases in domestic animals: a comparative review. Vet J 2006;171(1):20–38.
31. Vembar SS, Brodsky JL. One step at a time: endoplasmic reticulum-associated degradation. Nat Rev Mol Cell Biol 2008;9(12):944–957.
32. Lu JP, Wang Y, Sliter DA, Pearce MMP, Wojcikiewicz RJH. RNF170 protein, an endoplasmic reticulum membrane ubiquitin ligase, mediates inositol 1,4,5-trisphosphate receptor ubiquitination and degradation. J Biol Chem 2011;286(27):24426–24433.
33. Gao X, Wojcikiewicz RJH. The emerging link between IP3 receptor turnover and hereditary spastic paraplegia. Cell Calcium 2020;86:2019–2020.
34. Tong BCK, Wu AJ, Li M, Cheung KH. Calcium signaling in Alzheimer's disease & therapies. Biochim Biophys Acta Mol Cell Res 2018;1865(11):1745–1760.
35. Czeredys M. Dysregulation of neuronal calcium signaling via store-operated channels in Huntington's disease. Front Cell. Dev Biol 2020;8:611735.
36. Novarino G, Fenstermaker AG, Zaki MS, Hofree M, Silhavy JL, Heiberg AD, et al. Exome sequencing links corticospinal motor neuron disease to common neurodegenerative disorders. Science 2014;343(6170):506–511.
37. Wakil S, Bohlega S, Hagos S, Baz B, Al Dossari H, Ramzan K, et al. A novel splice site mutation in ERLIN2 causes hereditary spastic paraplegia in a Saudi family. Eur J Med Genet 2013;56(1):43–45.
38. Rydning SL, Dudesek A, Rimmele F, Funke C, Krüger S, Biskup S, et al. A novel heterozygous variant in ERLIN2 causes autosomal dominant pure hereditary spastic paraplegia. Eur J Neurol 2018;25(7):943–948.
39. Wagner M, Osborn DPS, Gehweiler I, Nagel M, Ulmer U, Bakhtiari S, et al. Bi-allelic variants in RNF170 are associated with hereditary spastic paraplegia. Nat Commun 2019;10(1):1–13.
40. Valdmanis PN, Dupré N, Lachance M, Stochmanski SJ, Belzil VV, Dion PA, et al. A mutation in the RNF170 gene causes autosomal dominant sensory ataxia. Brain 2011;134(2):602–607.
41. Moeller JJ, Macaulay RJB, Valdmanis PN, Weston LE, Rouleau GA, Dupré N. Autosomal dominant sensory ataxia: a neuroaxonal dystrophy. Acta Neuropathol 2008;116(3):331–336.
42. Fink JK. Hereditary spastic paraplegia: clinico-pathologic features and emerging molecular mechanisms. Acta Neuropathol 2013;126(3):307–328.
43. Elsayed LEO, Eltazi IZ, Ahmed AE, Stevanin G. Insights into clinical, genetic, and pathological aspects of hereditary spastic paraplegias: a comprehensive overview. Front Mol Biosci 2021;8:690899.
44. Chouery E, Mehawej C, Megarbane A. A novel homozygous variant in RNF170 causes hereditary spastic paraplegia: a case report and review of the literature. Neurogenetics 2022;23(2):85–90. https://doi.org/10.1007/s10048-022-00685-6
45. de Sainte Agathe JM, Mercier S, Mahé JY, Péréon Y, Buratti J. RNF170-related hereditary spastic paraplegia: confirmation by a novel mutation. Mov Disord 2021;36(3):771–774.
46. Fu JX, Wei Q, Chen YL, Li HF. Novel stop-gain RNF170 variation detected in a Chinese family with adolescent-onset hereditary spastic paraplegia. Clin Genet 2023;103(1):87–92.
47. Cortese A, Callegari I, Curro R, Vegezzi E, Colnaghi S, Versino M, et al. Mutation in RNF170 causes sensory ataxic neuropathy with vestibular areflexia: a CANVAS mimic. J Neurol Neurosurg Psychiatry 2020;91(11):1237–1238.
48. Wright FA, Lu JP, Sliter DA, Dupré N, Rouleau GA, Wojcikiewicz RJH. A point mutation in the ubiquitin ligase RNF170 that causes autosomal dominant sensory ataxia destabilizes the protein and impairs inositol 1,4,5-trisphosphate receptor-mediated Ca2+ signaling. J Biol Chem 2015;290(22):13948–13957.
49. Kim Y, Kim SH, Kim KH, Chae S, Kim C, Kim J, et al. Age-dependent gait abnormalities in mice lacking the Rnf1 70 gene linked to human autosomal-dominant sensory ataxia. Hum Mol Genet 2015;24(25):7196–7206.
50. Blackstone C. Hereditary spastic paraplegia. Handb Clin Neurol 2018;148:633–652. https://doi.org/10.1016/B978-0-444-64076-5.00041-7

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