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► To cite this version:

Anna Letko, Pascale Quignon, Maéva Quilleré, Jean-charles Husson, Caroline Dufaure de Citres, et al.. A RETREG1 variant is associated with hereditary sensory and autonomic neuropathy with acral self-mutilation in purebred German Spitz. *Animal Genetics*, 2024, 10.1111/age.13482 . hal-04767371

HAL Id: hal-04767371

<https://hal.science/hal-04767371v1>

Submitted on 5 Nov 2024

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RESEARCH ARTICLE

A *RETREG1* variant is associated with hereditary sensory and autonomic neuropathy with acral self-mutilation in purebred German Spitz

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Abstract

Hereditary sensory and autonomic neuropathies (HSAN) represent a group of genetic diseases affecting the peripheral nervous system. In humans, at least 16 loci have been associated with the disorder but do not explain the disease origin of all patients. In dogs, similar conditions have been documented for decades in various breeds with a severe impact on life quality and are often referred to as acral mutilation syndrome (AMS). Causal variants in three genes have been identified to date, suggesting larger genetic heterogeneity in the dog population. Our aim was to explain the genetic etiology of an early-onset HSAN/AMS in a purebred German Spitz. The affected dog showed progressive loss of pain sensation in the distal extremities, which led to intense licking, biting, and self-mutilation of digits and paw pads. Whole-genome sequencing identified a single candidate causal variant on chromosome 4 in the *RETREG1* gene (c.656C>T, p.Pro219Leu). This missense variant was previously recognized as deleterious in a mixed breed dog family with similar clinical signs. Haplotype analyses and targeted genotyping revealed a likely German Spitz ancestry of these mixed breed dogs. Further screening of an extensive cohort of ~900 000 dogs of various breeds hinted at the variant allele origin in the German Spitz breed. Disruption of *RETREG1* inhibits endoplasmic reticulum turnover and leads to neuron degeneration. Our findings provide evidence that this variant underlies the recessive form of HSAN/AMS in the German Spitz and support the use of whole-genome sequencing-based veterinary precision medicine for early diagnosis and prevention via a genetic test.

KEYWORDS

automutilation, *Canis familiaris*, hereditary sensory neuropathy, pain insensitivity, peripheral nervous system, whole-genome sequencing

Jocelyn Plassais and Catherine André shared senior authors.

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INTRODUCTION

Recurrent and poorly healing injuries are the main signs of genetic pain loss described in various species. This heterogeneous group of disorders includes hereditary sensory neuropathies, hereditary sensory and autonomic neuropathies (HSAN), and congenital insensitivity to pain (Lischka et al., 2022). In humans, at least 16 loci have been associated with HSAN to date (OMIM:PS162400). The affected individuals are insensitive to pain, which leads to a lack of nociceptive responses that would typically be elicited in response to harmful stimuli as a fundamental protective mechanism, serving to avoid tissue damage (Lischka et al., 2022). Genetic pain insensitivity therefore presents a unique opportunity for exploring the underlying genetic mechanisms of pain perception.

In dogs, this rare disease has a significant impact on life quality and is often referred to as acral mutilation syndrome (AMS). Canine AMS has been documented for decades as part of inherited sensory neuropathies (Coates & O'Brien, 2004; Granger, 2011). Progressive clinical signs include ataxia and decrease or loss of tactile and pain sensation in distal extremities, leading to skin ulceration and severe auto-amputation of digits. Although the disorder was reported in several dog breeds, causal variants in only three genes have been identified to date: *GDNF* (OMIA:001514–9615) in several hunting dog breeds (Plassais et al., 2016), *RETREG1*, formerly also known as *FAM134B* (OMIA:002032–9615) in Border Collies and mixed breed dogs (Forman et al., 2016; Gutierrez-Quintana et al., 2021), and *SCN9A* (OMIA:002616–9615) in mixed breed dogs (Gutierrez-Quintana et al., 2023).

Our study aimed to explain the genetic etiology of an early-onset progressive HSAN/AMS in a purebred German Spitz, exhibiting the typical clinical signs, through whole-genome sequencing (WGS). The results will support breed-relevant genetic testing to facilitate early diagnosis and aid breeding selection decisions as well as the relevance of affected dog breeds as natural models for specific human peripheral neuropathies (HSAN Type IIB, OMIM:613115) and pain genetic research.

MATERIALS AND METHODS

Animals and global cohorts

The work with dog samples was approved by the Ethics Committee (CREEA – #CE07-2020-11-CA). All samples were collected by veterinarians with the owners' consents in the course of the dogs' medical care and submitted to the Cani-DNA Biological Resource Center (BRC) at the University of Rennes (André et al., 2022). Histopathological examination through a skin biopsy was performed by the attending veterinarian for

diagnostic purposes. EDTA-stabilized blood samples were collected from the 7-month-old German Spitz diagnosed with HSAN/AMS, as well as both its unaffected parents, maternal granddam, and two unrelated dogs from the same breeder. The exact variety of German Spitz was reported as Medium (as classified by the Fédération Cynologique Internationale; DEUTSCHER SPITZ, 2023) by the breeder. The affected dog's health and welfare were monitored for 2.75 years after the first clinical diagnosis through its veterinarian and owner.

To assess the genotype–phenotype segregation of the detected *RETREG1* variant, 56 additional German Spitz dogs of different varieties (50 Keeshond, one Giant, three Pomeranian, and two of unknown type) from Cani-DNA BRC were genotyped by Sanger sequencing. As a result of the haplotype analysis described below, we also screened 63 Picardy sheepdogs, 94 German shepherds, and 69 Boxers available in the Cani-DNA BRC. DNA was extracted from blood samples using the NucleoSpin Blood Kit (Macherey-Nagel) according to the manufacturer's instructions.

To estimate the allele distribution in the population of French Spitz dogs, additional samples of German Spitz ($n=159$), Japanese Spitz ($n=22$), Italian Volpino ($n=4$), and Finnish Spitz ($n=1$) dogs were genotyped by the animal genetic testing company Antagene, France. These samples were voluntarily submitted by pet owners for genetic testing as a paid commercial service and the owners provided consent for the use of samples in scientific research at the time of submission.

Furthermore, 882159 dogs collected via the commercial panel genotyping platform by Wisdom Panel (Vancouver, WA, USA) were screened for the *RETREG1* missense variant, after the variant was included on the array in 2022. This test is run on a custom-developed Illumina genotyping microarray, the design and validation of which have been described in detail previously (Donner et al., 2016, 2018). Breed ancestry assignment in this cohort was ascertained using the BCSYS Local Ancestry Classifier algorithm (Garrigan et al., 2024). All dog owners provided consent for the use of their dog's DNA data in research through the client's agreement with the terms and conditions of DNA testing through Wisdom Panel.

Whole-genome sequencing and variant analyses

Whole genomes of the affected dog and its dam as well as two unaffected unrelated German Spitz dogs were sequenced using Illumina PCR-free paired-end reads library on NovaSeq 6000 by Integragen (Evry, France) at an average read depth of 43×. The whole-genome sequencing (WGS) data were submitted to the European Nucleotide Archive (Harrison et al., 2021) under study accession PRJNA1088627 (Table S1). Following the Genome Analysis Toolkit 4 (GATK) Best Practices

(Van der Auwera & O'Connor, 2020), raw fastq files were filtered using FASTP v0.23.1 (Chen et al., 2018) and subsequently aligned to the canine reference genome assembly UU_Cfam_GSD_1.0 (GCF_011100685.1) (Wang et al., 2021) using bwa-mem2 algorithm (Vasimuddin et al., 2019). Single nucleotide variants and small indels (SNVs) were called using GATK HaplotypeCaller (Poplin et al., 2018) as described before (Jagannathan et al., 2019). The functional impacts of the identified variants were predicted using SnpEff v5.0e (Cingolani, 2022).

SNVs were filtered based on an autosomal recessive mode of inheritance and further prioritized by the predicted impact on the encoded protein and localization in functional candidate genes previously associated with HSN/AMS in humans and dogs (OMIM:PS162400; OMIA:001514–9615, 002032–9615, 002616–9615). To evaluate the variant allele frequencies and identify SNVs present exclusively in the affected dog's genome, a Dog Biomedical Variant Database Consortium (DBVDC) cohort of publicly available 961 canine WGS was interrogated (Jagannathan et al., 2019). This cohort included 60 German Spitz individuals belonging to all five varieties of the breed (Keeshond, Giant, Medium, Miniature, and Pomeranian) as classified by the Fédération Cynologique Internationale (DEUTSCHER SPITZ, 2023). All accession numbers and breed designations of available WGS are listed in Table S1.

Candidate variant genotyping

RETREG1 candidate causal variant was genotyped using Sanger sequencing after a nested PCR with two primer pairs (pair1: 5'-ATCAAAGCGAAAACCCATTCT-3', 5'-GCAATAGTCCACCCCAAATAA-3' and pair2: 5'-CGGGAAAGCATGAAAGGGAG-3', 5'-AGGCTACATTACTTTCAGGGGA-3'). For PCR, we used the Type-it Microsatellite PCR kit (Qiagen) on C1000 and S1000 thermocyclers (Bio-Rad Laboratories). For Sanger sequencing, we used the BigDye Terminator v3.1 cycle sequencing kit (Thermo Fisher Scientific). Electrophoresis of the products was realized on an ABI 370 sequencer. Sequences were analyzed using the PhredPhrap and Consed software (Ewing et al., 1998; Ewing & Green, 1998; Gordon, 2003).

Haplotype analysis and mixed breed dogs' ancestry detection

To investigate the variant allele origin, we obtained Illumina Canine HD SNP array genotyping data (170 000 markers) of 1338 dogs available from Parker et al. (2017), representing 164 modern breeds (Table S2). The definition of breed clades and groups was kept as defined by Parker et al. (2017). To determine genome-wide haplotype sharing, PLINK v1.07 (Purcell et al., 2007) was used

to merge this public SNP array dataset with the subset of corresponding genotypes obtained from the herein produced WGS data for the four German Spitz dogs. Additionally, the publicly available WGS data of two previously reported HSN/AMS-affected mixed breed dogs with the *RETREG1*:c.656C>T variant (Gutierrez-Quintana et al., 2021) were re-aligned to the UU_Cfam_GSD_1.0 reference assembly (Wang et al., 2021) and processed as described above. The final dataset contained 120 662 SNPs and 1344 dogs.

Genome-wide haplotype sharing was assessed using Beagle v4.9 identity-by-descent sharing without imputation on 100 SNP windows with 25 SNP overlap and 10 SNP end trimming (Browning & Browning, 2013). Shared haplotypes were summed between all pairs of dogs that did not belong to the same breed. The distribution of haplotype sharing among dogs from breeds in the same clade versus dogs from breeds in different clades was assessed using a Kolmogorov–Smirnov test as implemented in R (R Core Team, 2019). Haplotype sharing across clades was determined to be significant between two breeds if greater than the 95th percentile boundary from all pairs of dogs from different clades. Boxplots of haplotype sharing distributions between a single breed and all other breeds were performed using R boxplot function (R Core Team, 2019).

To refine our analyses, we combined WGS data from the HSN/AMS-affected German Spitz and its dam, the 60 German Spitz from the DBVDC cohort of 961 canine WGS (Jagannathan et al., 2019), and the WGS of two previously reported HSN/AMS-affected mixed breed dogs (Gutierrez-Quintana et al., 2021). We then extracted all biallelic variants (SNV and small indels) to finally obtain a dataset containing 8 270 336 variants and 64 dogs.

We zoomed in on a 4-Mb sized locus on chromosome 4, centered on the *RETREG1* variant (chr4:85 000 000–89 000 000). Using PLINK v1.07 (Purcell et al., 2007) for SNP filtering (minor allele frequency < 0.01; call rate by marker and individual > 75%), we obtained a dataset of 901 SNPs to detect homozygous haplotypes in affected dogs following the hypothesis of a monogenic recessive disorder. Based on the three affected dogs, the limits of blocks were determined using the first recombination breakpoints (heterozygous markers) detected at each extremity of the homozygous block containing the *RETREG1* variant.

RESULTS

Clinical description

A 6-month-old German Spitz dog was diagnosed with HSN/AMS by an attending veterinarian in the course of the dog's medical care. Both the dam and the sire of the affected dog were reported unaffected by the veterinarian. The young dog was first seen by the veterinarian

in February 2021 due to its left hind paw being badly injured. Subsequently, histopathological diagnosis was established through a skin biopsy in March 2021 when the right hind paw began to be also affected. The dog exhibited licking and biting of its feet, with cutaneous lesions on both sides of the digit at the left hind paw (Figure 1a). The distal phalanx and its claw were missing, replaced by a large ulcerated, necrotic, fibrinous, hemorrhagic, suppurated crater at the bottom of which the distal diaphysis of the middle phalanx was exposed. The pathological report showed non-specific lesions, most probably secondary to self-induced trauma (distal phalanx avulsion, necrotic to suppurative tissular alterations, signs of follicular trauma, and thrombosed vascular profiles). The epidermis was parakeratotic and slightly crusted (Figure 1a).

On a clinical follow-up, the veterinarian noticed the absence of pain sensitivity in the lower extremities. The dog was treated with general antibiotics and anti-inflammatory drugs, as well as later on with gabapentin, which only showed a transient efficacy. At age 9 months, one digit had to be amputated (Figure 1b). The dog's self-mutilating behavior has since been successfully managed by the owner with the use of a protective collar and boots (Figure 1c), and further clinical follow-up at 1 year of age was encouraging. However, at the last known follow-up at age 3.4 years (November 2023), the dog showed proprioceptive deficits in all limbs and no toes were present on the dog's right hind paw (Figure 1d). The remaining stump was reported to be prone to bleeding and the

ligaments holding the heel were very loose. As a result, the affected dog had a tendency to walk with the heel hitting the ground first. The front paws were only slightly injured and well managed with only one boot.

WGS analyses

Whole genomes of four German Spitz dogs were sequenced as described above. A total of 6683122 variants were called in the WGS of the HSAN/AMS-affected German Spitz. Filtering for private variants with homozygous alternative allele in the affected dog and heterozygous in its dam, while homozygous reference or heterozygous in the two unrelated German Spitz controls yielded 398996 variants. Further prioritization of the detected variants was done by using the predicted protein impact and localization in functional candidate genes for sensory neuropathy (Table 1). Using this prioritization approach, together with the variant allele frequency of <1% in the DBVDC cohort, eight variants were left. Seven were present in genes unknown to be related to peripheral neuropathy (missense variants in *CCNYL1*, *GAK*, *PIGG*, *REEP6*, *RNF212*, *SHROOM4*, and a frameshift variant in *LORICRIN*), while the remaining candidate causal variant was present on chromosome 4 (CFA4) in the *reticulophagy regulator 1 (RETREG1)* gene that has been associated with HSAN type IIB in human (OMIM:613115) and sensory neuropathy in dog (OMIA:002032–9615).



FIGURE 1 Clinical presentation of the hereditary sensory and autonomic neuropathy with acral self-mutilation-affected dog at the time of (a) initial diagnosis (6 months) and during follow-up at (b) 8 months, (c) 9 months, and (d) 3.4 years.

Filtering step	Number of variants
Variants in the whole genome	2311 829
Variants heterozygous in the dam	751 137
Variants with protein-changing impact	1833
Variants with variant allele frequency <1% in DBVDC cohort	8
Variants in functional candidate gene	1

TABLE 1 Homozygous variants detected in the genome of the hereditary sensory and autonomic neuropathy/acral mutilation syndrome-affected German Spitz.

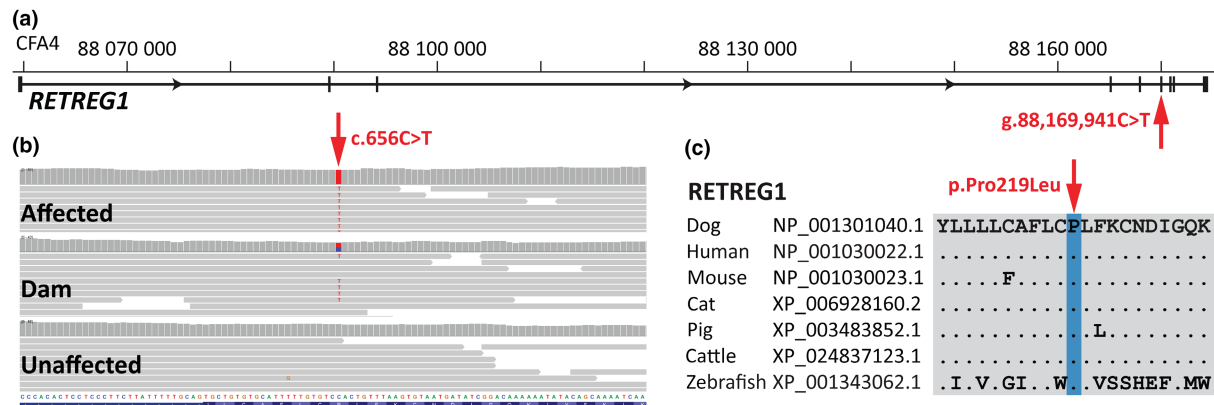


FIGURE 2 (a) Schematic representation of the *RETREG1* gene showing the whole-genome sequencing-detected variant location in exon 6 (NC_049225.1:G.88169941C>T). (b) Integrative Genomics Viewer (Thorvaldsdóttir et al., 2013) screenshot showing the short-read alignments of the acral self-mutilation-affected dog, its dam, and an unrelated unaffected German Spitz. (c) *RETREG1* amino acid multiple alignment indicating the residue (NP_001301040.1:P.Pro219Leu) is highly conserved across species.

The missense variant (c.656C>T, p.Pro219Leu) is located in exon 6 of the *RETREG1* gene and affects a highly conserved amino acid of the *RETREG1* protein (Figure 2). Only one other dog from the DBVDC cohort carried this variant in a heterozygous state. This dog's breed was reported as Pomeranian, one of the varieties of German Spitz (DEUTSCHER SPITZ, 2023). To further investigate the genomic relatedness between the 60 German Spitz dogs and the herein sequenced dogs ($n=4$), a principal component analysis plot was generated. This showed that the investigated German Spitz dogs clustered together with the Medium, Miniature, and Pomeranian individuals, while the Giant and Keeshond varieties of German Spitz formed separate groups (Figure S1).

Haplotype analysis and mixed breed dogs' ancestry detection

Strikingly, the exact same *RETREG1*:c.656C>T variant was identified in two previously reported HSAN/AMS-affected mixed breed dogs (Gutierrez-Quintana et al., 2021). While the pedigree information of these dogs was unknown, German Shepherd or Parson Russell Terrier ancestry was suspected (Gutierrez-Quintana et al., 2021). Therefore, we performed a shared haplotype analysis with the hypothesis of an unknown Spitz dog in their ancestry. First, the ~170000 Illumina Canine HD SNP array

positions were extracted from the WGS data of both mixed breed dogs and all German Spitz. This dataset was combined with 1338 genotyping data obtained from Parker et al (Parker et al., 2017), representing 164 modern breeds. Identity-by-descent haplotype sharing was calculated between all pairs of dogs to assess hybridization across the clades as described by Parker et al (Parker et al., 2017). Whole-genome haplotype comparison revealed that, as expected, the investigated German Spitz family clusters with other Spitz groups (Pomeranian, Italian Volpino), while the mixed breed dogs share haplotypes mainly with the New World and European Mastiff clades, represented herein by German Shepherd and Picardy Sheepdog, and Boxer, respectively (Figure 3, Table S2).

To refine the analyses, all biallelic variants from the WGS data were extracted in a 4-Mb locus on CFA4 centered on the *RETREG1* variant, and we searched for a common haplotype shared between the HSAN/AMS-affected German Spitz and the two affected mixed breed dogs. We thus identified a 3-Mb homozygous haplotype in the mixed breed dogs, for which 1 Mb was shared with the German Spitz case and contained the *RETREG1* variant (Figure 4). A heterozygous haplotype of the same size was found in the case's dam. Additionally, a ~500-kb heterozygous block was detected in the Pomeranian carrier of the *RETREG1* variant from the DBVDC cohort of 961 canine WGS (Jagannathan et al., 2019). Within the large cohort, this specific ~500-kb haplotype was only detected in the Spitz clade.

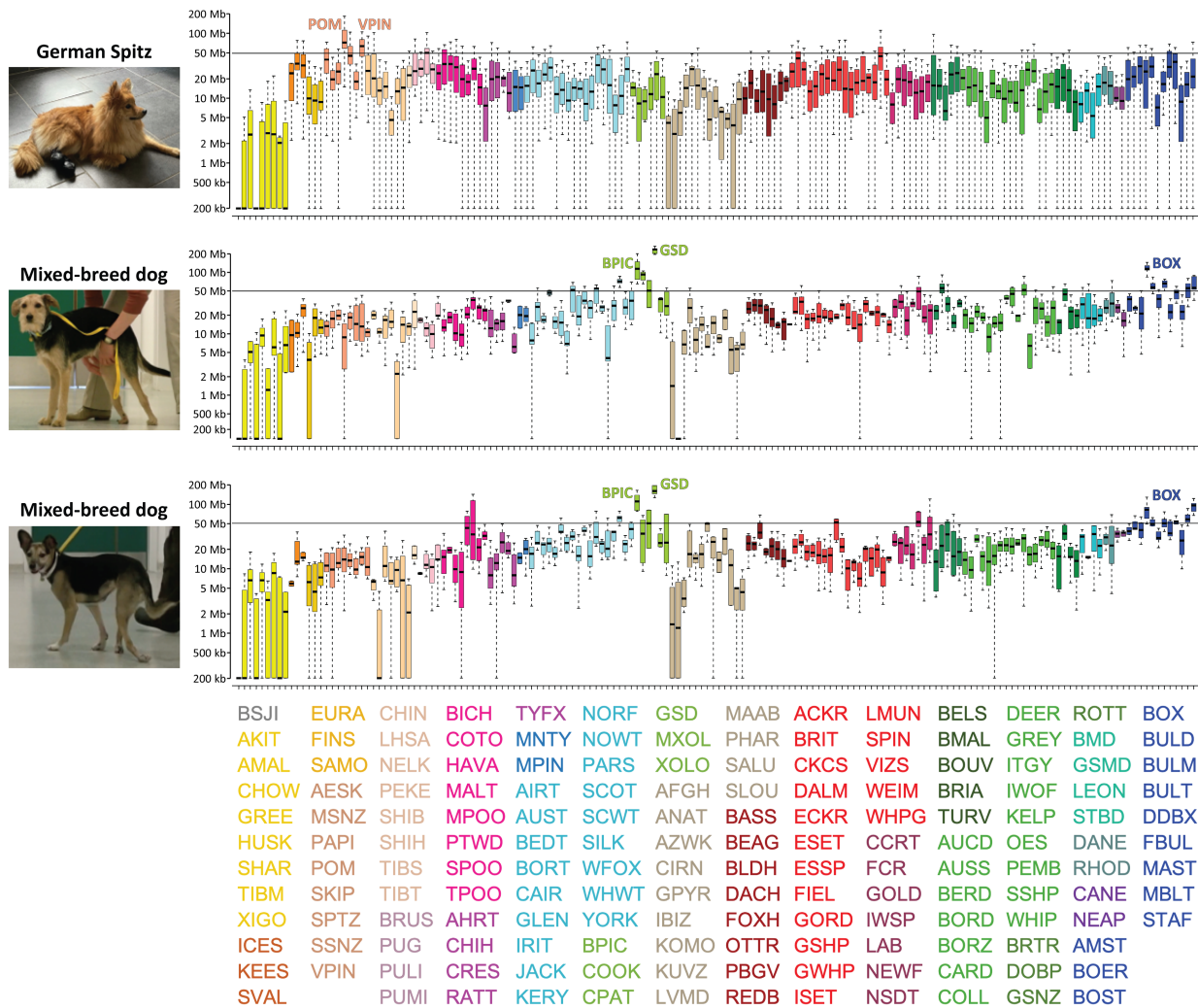


FIGURE 3 Whole-genome haplotype sharing between the German Spitz family, the two previously reported mixed breed dogs (Gutierrez-Quintana et al., 2021), and 164 breeds obtained from Parker et al. (2017). Combined haplotype length is displayed on the y-axis, and 164 breeds and populations are listed on the x-axis. Haplotype sharing of zero is set at 200 000 for graphing, a value just below what is detected in this analysis. The significance level of 95% is indicated by the horizontal line. Breeds and their abbreviations, detailed in Table S2, are colored by clade and listed as previously described in Parker et al. (2017). Mixed breed dogs' pictures were adapted from Gutierrez-Quintana et al. (2021).

Candidate variant genotyping

Targeted genotyping in a cohort of samples submitted to Cani-DNA BRC showed that the variant segregated perfectly in the studied German Spitz family and was absent in 56 unrelated unaffected German Spitz dogs (50 Keeshond, three Pomeranian, one Giant, and two unknown varieties). Only the initial AMS-affected dog was confirmed homozygous for the alternative allele and both its parents as well as a maternal granddam were heterozygous carriers (Table 2).

Additionally, 94 German Shepherd, 63 Picardy Sheepdogs, and 69 Boxers were screened for the variant allele due to the findings of haplotype sharing analyses and all were identified as homozygous for the reference allele (Table 2).

Furthermore, to assess the allele distribution in a larger Spitz population in France, the candidate variant

was genotyped by the Antagene company in 175 dogs of four Spitz breeds for which samples were available (Table 3). The variant was not detected in the limited number of samples from Japanese Spitz, Italian Volpino, and Finnish Spitz. The German Spitz group included 100 Pomeranian, 35 Miniature, 23 Keeshond, and one Medium individuals. Only 11 German Spitz dogs were found to have the alternative *RETREG1* T-allele (Table 3). Eight heterozygous and one homozygous genotype were detected in the Pomeranian variety and two heterozygous in the Miniature variety of German Spitz. The one homozygote was a 1-year-old female for which, after a follow-up with its owner, a veterinarian confirmed pain insensitivity in its hind legs using a needle test (Gutierrez-Quintana et al., 2021).

Considering all available German Spitz genotypes while excluding the initial family ($n = 277$), the *RETREG1* alternative T-allele frequency was estimated at 2.35%.

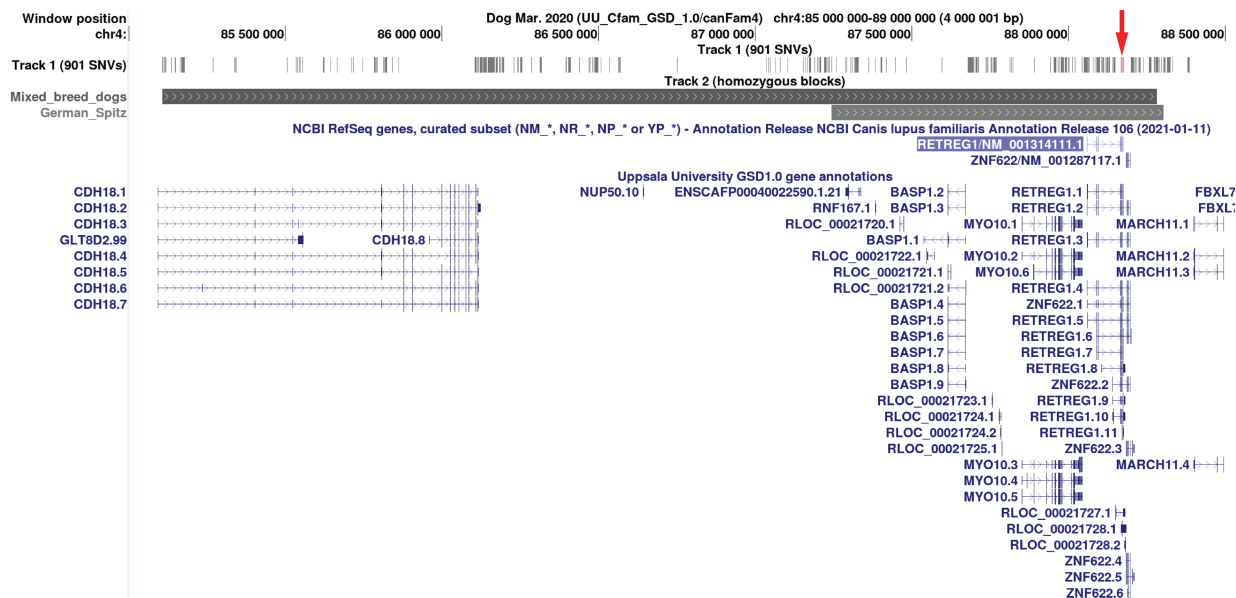


FIGURE 4 UCSC Genome Browser (<http://genome.ucsc.edu>) (Kent et al., 2002) screenshot of the 4-Mb critical region on chromosome 4 (85–89 Mb) shows the homozygous haplotype blocks including the candidate gene *RETREG1*. Track 1 shows the location of 901 variants used for the analysis. Track 2 shows two homozygous haplotype blocks found in the hereditary sensory and autonomic neuropathy with acral self-mutilation-affected mixed breed dogs as well as the German Spitz. The location of *RETREG1*:c.656C>T variant is represented as a vertical bar (red) and highlighted by a red arrow. Annotated genes are indicated as blue bars.

TABLE 2 Genotyping of *RETREG1*:c.656C>T variant in German Spitz and other relevant dog breeds.

Breed	ref/ref	ref/alt ^a	alt/alt ^a
German Spitz	56	3	1
German Shepherd	94	0	0
Picardy Sheepdog	63	0	0
Boxer	69	0	0

Abbreviations: Alt, alternative; Ref, reference.

^aThis includes the initial case (homozygous), its sire, dam, and maternal granddam (heterozygous).

TABLE 3 Genotyping of *RETREG1*:c.656C>T variant in four Spitz breeds in France.

Breed	ref/ref	ref/alt	alt/alt
German Spitz	148	10	1
Japanese Spitz	22	0	0
Italian Volpino	4	0	0
Finnish Spitz	1	0	0

Abbreviations: Alt, alternative; Ref, reference.

However, the heterozygous carrier frequency was particularly higher in the Pomeranian (7.38%, $n=123$) and Miniature varieties (4.17%, $n=48$) of German Spitz breed.

In addition, a global cohort of 882 159 dogs of various breeds was screened for the *RETREG1* variant through the Wisdom Panel, a commercial genotyping platform. In total, 614 dogs were found to be heterozygous and 22 dogs homozygous for the alternative allele (0.04% alternative T-allele frequency). Ancestry analysis of these

636 dogs revealed mostly small companion-type dogs. The breed ancestry of Pomeranian, German Spitz, and Keeshond was assigned to 93.87%, 5.97%, and 1.26% of the 636 dogs, respectively. Furthermore, all of the 22 homozygous dogs had a German Spitz dog in their ancestry (three purebred Pomeranians and 19 mixed breed dogs, of which 18 with Pomeranian and on with Keeshond ancestry, as detailed in Table S3).

Clinical records for nine of the 22 homozygous dogs were available for examination (Table S3). One dog (Dog9 in Table S3) showed diagnostic entries indicating a history of neuropathy (first reported at age ~1 year). The other eight homozygotes were aged between 4 months and 2.75 years at the time of their last health update that did not include any neurological diagnosis.

DISCUSSION

This study provides evidence that an autosomal recessive form of HSAN/AMS with pain insensitivity in German Spitz is caused by a pathogenic missense variant in *RETREG1* (OMIA Variant ID 1342) that is very rare in the general dog population. Targeted genotyping revealed considerable 2.35% allele frequency in the French population of Miniature and Pomeranian varieties of German Spitz. Clinical examination of the additional homozygous dog detected by Antagene company confirmed the causal effect of the variant in the German Spitz breed. Due to the limited number of dogs from other Spitz breeds available for genotyping, it was not feasible to exclude the possibility that the variant also segregates in one of these breeds.

The large publicly available canine WGS and SNP genotyping cohorts proved to be a valuable resource and demonstrate the benefits of FAIR open science principles in research (Rocca-Serra et al., 2023). The herein detected *RETREG1* variant was previously reported as causative in a family of mixed breed dogs affected by the same disease (Gutierrez-Quintana et al., 2021). Through haplotype analyses, we identified a unique 1-Mb homozygous region in all three HSN/AMS-affected dogs that could be further shortened to ~500 kb by using the single heterozygous unrelated German Spitz from the DBVDC cohort. Furthermore, we genotyped a number of dogs from the other detected ancestral breeds and did not find the alternative T-allele in any of them. Together, these results indicate that the HSN/AMS-affected mixed breed dogs (Gutierrez-Quintana et al., 2021) and the German Spitz case probably had a shared ancestor in their pedigree carrying the *RETREG1* variant.

Additional screening of almost 900 000 dogs of various breeds using the Wisdom Panel further confirmed the variant segregating in dogs of German Spitz ancestry. Unfortunately, most of the 22 additionally detected homozygous dogs did not have medical records available. Only one mixed breed dog's records showed a relevant neuropathy diagnosis, while the eight others with health records may have been too young to show signs at the time of their last recorded veterinary visit. It is also possible that the pain insensitivity may be underdiagnosed due to the fact that a dog may not show obvious signs until more serious wounds appear from self-mutilation. Although it is impossible to determine with absolute certainty, the herein described recessive form of canine HSN/AMS is most likely to be primarily associated with the German Spitz breed. Recent study on distribution and frequency of known genetic disorders in over one million dogs showed that the majority of disease-associated variants were encountered in both mixed breed and purebred dogs (Donner et al., 2023). Even though mixed breed dogs may show on average greater genetic diversity than purebred dogs, individual dogs can be strongly inbred due to undocumented pedigrees leading to a spread of disease-causing recessive alleles (Leeb et al., 2023). Therefore, genetic testing in German Spitz crosses but also in mixed breed dogs with unknown precise ancestry, which makes selection of breed-specific tests impossible, may be beneficial for precise diagnosis of the disease.

A different variant, a large inversion, disrupting the same gene was reported before in Border Collies (OMIA Variant ID 752) and their crosses diagnosed with a recessive form of sensory neuropathy with self-mutilation and loss of pain perception (Amengual-Batle et al., 2018; Forman et al., 2016). Notably, there are some differences in severity and progression of the clinical signs between the affected Border Collies and German Spitz. The herein described case showed comparably less severe form of HSN/AMS and no generalized muscle atrophy or autonomic signs. In this regard, the clinical signs of the

German Spitz case were more similar to the mixed breed dogs (Gutierrez-Quintana et al., 2021). Although long-term outcomes are difficult to evaluate due to frequent early euthanasia of the affected dogs, two of the mixed breed dogs were successfully managed with protective collars and bandages and one of them was reported to live to age 5 years when it showed marked proprioceptive ataxia and knuckling (Gutierrez-Quintana et al., 2021).

The *RETREG1* gene is highly conserved across species and encodes a Golgi transmembrane protein important for survival of nociceptive and autonomic ganglion neurons (Kurth et al., 2009). In human patients, homozygous mutations in this gene have been found to cause a rare autosomal recessive HSN type IIB (OMIM:613115) (Ilgaz Aydinlar et al., 2014; Kurth et al., 2009). The main reported clinical findings include sensory signs, recurrent painless injuries, ulcers, and poorly healing wounds, as well as various degrees of motor signs such as muscle weakness or spastic gait (Çakar et al., 2023). In addition to the predominant loss-of-function alleles, one missense variant (*RETREG1:c.646G>A*) was described in a HSN patient with less severe clinical phenotype and no motor involvement (Davidson et al., 2012). It was subsequently shown to cause gain-of-function defects that result in excessive degradation of endoplasmic reticulum by autophagy and sensory neuron death leading to progressive neurodegeneration (Jiang et al., 2020). The broad clinical spectrum and genetic heterogeneity of HSN complicate the interpretation of some genetic variants and result in large number of variants classified with uncertain significance. Consequently, the diagnostic yield in HSN patients is low and many cases remain unexplained (Lischka et al., 2022). The availability of spontaneous animal models such as the dog may benefit human medicine through a better understanding of the disease pathophysiology or therapeutic strategies.

It is important to note the limitation of our study as suitable tissue samples from the affected dog were not collected for functional analysis at the protein level. However, the functional effect of the described *RETREG1* variant was demonstrated previously by Gutierrez-Quintana et al. (2021). In their study, immunohistochemistry for RETREG1 showed no expression in the cerebellum of affected dogs and the variant was concluded to be probably pathogenic (Gutierrez-Quintana et al., 2021). Furthermore, it was not possible to obtain targeted health updates about all additional homozygous dogs. As reported previously, the insensitivity to pain alone is often undetected by the owners of affected dogs until there is an injury to the paw and the dog starts licking or biting it (Plassais et al., 2016). Nevertheless, the phenotype similarity, the unaffected parents, and the variant allele frequency in the global Spitz population highlight the *RETREG1:c.656C>T* variant as the most likely cause of the autosomal recessive HSN/AMS in German Spitz.

In conclusion, our findings provide evidence that the *RETREG1:c.656C>T* variant arose in a German Spitz ancestry and support the use of WGS-based veterinary precision medicine for early diagnosis and prevention via a genetic test. Testing this variant should be considered in breeding of German Spitz and their crosses because it holds implications for veterinary medicine and ethical treatment of animals as the affected dog's quality of life is diminished.

AUTHOR CONTRIBUTIONS

Anna Letko: Conceptualization; formal analysis; investigation; methodology; software; visualization; writing – original draft; writing – review and editing. **Pascale Quignon:** Investigation; project administration; writing – original draft; writing – review and editing. **Maéva Quilleré:** Investigation; methodology. **Jean-Charles Husson:** Investigation; resources. **Caroline Dufaure de Citres:** Resources; validation; writing – review and editing. **Jonas Donner:** Resources; validation; writing – review and editing. **Stéphane Dréano:** Investigation; methodology. **Jocelyn Plassais:** Formal analysis; investigation; methodology; visualization; writing – original draft; writing – review and editing. **Catherine André:** Conceptualization; funding acquisition; supervision; writing – original draft; writing – review and editing.

ACKNOWLEDGEMENTS

We are grateful to all owners and breeders who donated samples, information, photos, and follow-up of their dogs, as well as the veterinarians, especially Dr. Valérie Havet, who provided precise diagnosis. We thank Heidi G. Parker for guidance on haplotype analyses and Stephanie Mottier for the WGS data releasing process. The GenOuest Bioinformatics core facility (<https://www.genouest.org>) is acknowledged for providing the high-performance computational infrastructure and the Cani-DNA Biological Resource Center (<https://igdr.univ-rennes1.fr/crb-cani-dna>) for sample collection and storage. The authors acknowledge the financial support of CNRS and the University of Rennes through the Région Bretagne Stratégie d'Attractivité Durable (SAD) 'NeuroDog' project (SAD19053).

CONFLICT OF INTEREST STATEMENT

C.D.C. is an employee of Antagene, France, and J.D. is an employee of Wisdom Panel, Mars Petcare Science & Diagnostics, both companies offer canine genetic testing as a commercial service. The other authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

WGS data are publicly available from the European Nucleotide Archive (<https://www.ebi.ac.uk/ena>). All accession numbers and relevant metadata are listed in Table S1. The UCSC Genome Browser session of haplotype analysis can be accessed at https://genome-euro.ucsc.edu/s/LetkoA/RETREG1_HaplotypeAnalysis.

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How to cite this article: Letko, A., Quignon, P., Quilleré, M., Husson, J.-C., de Citres, C.D., Donner, J. et al. (2024) A *RETREG1* variant is associated with hereditary sensory and autonomic neuropathy with acral self-mutilation in purebred German Spitz. *Animal Genetics*, 55, 810–819. Available from: <https://doi.org/10.1111/age.13482>