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Intragenic duplication disrupting the reading frame of *MFSD8* in Small Swiss Hounds with neuronal ceroid lipofuscinosis

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Abstract in English

Vetsuisse Faculty, University of Bern 2024

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Intragenic duplication disrupting the reading frame of *MFSD8* in Small Swiss Hounds with neuronal ceroid lipofuscinosis

Neuronal ceroid lipofuscinosis (NCL) represents a heterogenous group of lysosomal storage disorders resulting in progressive neurodegeneration. We investigated two Small Swiss Hound littermates with neurological signs starting around the age of twelve months. Both dogs had to be euthanized a few months after the onset of the disease due to the severity of their clinical signs. Pathological investigation of one affected dog revealed cerebral and cerebellar atrophy with cytoplasmic accumulation of autofluorescent material in degenerating neurons. The clinical signs and characteristic histopathology led to a tentative diagnosis of NCL. In the genetic investigation a duplication of 18,819 bp within the MFSD8 gene was revealed. The duplication breakpoints were predicted to disrupt the reading frame of the gene. Both affected dogs carried the duplication in a homozygous state and there was perfect cosegregation of the genotypes with the phenotype in a large pedigree, consistent with autosomal recessive inheritance. MFSD8 loss-of-function variants are a known cause of NCL7 in human patients, dogs and other mammalian species. The existing knowledge on MFSD8 together with the experimental data strongly suggests that the identified intragenic MFSD8 duplication caused the disease in the Small Swiss Hounds. These results allow to refine their diagnosis to NCL7 and enable genetic testing in the breed to avoid further unintentional carrier x carrier matings.

Canis lupus familiaris; dog; neurology; whole genome sequencing; precision medicine

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Zusammenfassung auf Deutsch

Vetsuisse-Fakultät Universität Bern 2024

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Intragenic duplication disrupting the reading frame of *MFSD8* in Small Swiss Hounds with neuronal ceroid lipofuscinosis

Neuronale Ceroid-Lipofuszinosen (NCL) repräsentieren eine heterogene Gruppe von lysosomalen Speicherkrankheiten, die zu einer fortschreitenden Neurodegeneration führen. Wir untersuchten zwei Schweizer Niederlaufhunde mit neurologischen Symptomen ab dem Alter von zwölf Monaten. Beide Hunde mussten einige Monate nach Ausbruch der Krankheit eingeschläfert werden. Die pathologische Untersuchung eines Hundes ergab eine zerebrale und zerebelläre Atrophie mit zytoplasmatischer Anhäufung von autofluoreszierendem Material in degenerierenden Neuronen. Die klinischen Anzeichen und die charakteristische Histopathologie führten zur vorläufigen Diagnose von NCL. Bei der genetischen Untersuchung wurde eine Duplikation von 18 819 bp innerhalb des *MFSD8*-Gens festgestellt. Die Bruchpunkte der Duplikation unterbrechen das Leseraster des Gens. Beide betroffenen Hunde trugen die Duplikation in homozygotem Zustand und es gab eine passende Cosegregation der Genotypen mit dem Phänotyp in der Familie. *MFSD8*-Funktionsverlustvarianten sind eine bekannte Ursache von NCL7 bei menschlichen Patienten, Hunden und anderen Säugetierarten. Das vorhandene Wissen über *MFSD8* in Verbindung mit den experimentellen Daten deutet darauf hin, dass die identifizierte *MFSD8*-Duplikation NCL bei den Schweizer Niederlaufhunden verursacht. Diese Ergebnisse ermöglichen es, die Diagnose auf NCL7 zu verfeinern und genetische Tests in der Rasse durchzuführen, um unbeabsichtigte Verpaarungen von Trägern zu vermeiden.

Canis lupus familiaris; Hund; Neurologie; Vollständige Genomsequenzierung; Präzisionsmedizin

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RESEARCH ARTICLE

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Intragenic duplication disrupting the reading frame of *MFSD8* in Small Swiss Hounds with neuronal ceroid lipofuscinosis

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Abstract
Neuronal ceroid lipofuscinosis (NCL) represents a heterogenous group of lysosomal storage diseases resulting in progressive neurodegeneration. We investigated two Small Swiss Hound littermates that showed progressive ataxia and loss of cognitive functions and vision starting around the age of 12 months. Both dogs had to be euthanized a few months after the onset of disease owing to the severity of their clinical signs. Pathological investigation of one affected dog revealed cerebral and cerebellar atrophy with cytoplasmic accumulation of autofluorescent material in degenerating neurons. The clinical signs in combination with the characteristic histopathology led to a tentative diagnosis of NCL. In the subsequent genetic investigation, the genome of one affected dog was sequenced. This revealed a duplication of 18 819 bp within the *MFSD8* gene. The duplication breakpoints were located in intron 3 and exon 12 of the gene and were predicted to disrupt the reading frame. Both affected dogs carried the duplication in a homozygous state and there was perfect cosegregation of the genotypes with the phenotype in a large pedigree, consistent with autosomal recessive inheritance. *MFSD8* loss-of-function variants are a known cause of NCL7 in human patients, dogs and other mammalian species. The existing knowledge on *MFSD8* together with the experimental data strongly suggests that the identified intragenic *MFSD8* duplication caused the disease in the Small Swiss Hounds. These results allow their diagnosis to be refined to NCL7 and enable genetic testing in the breed to avoid further unintentional carrier × carrier matings.

KEYWORDS
Canis lupus familiaris, dog, neurology, precision medicine, whole genome sequencing

INTRODUCTION

Neuronal ceroid lipofuscinosis (NCL) comprises a heterogenous group of lysosomal storage diseases leading to progressive neurodegeneration and degradation of cognitive skills (Williams & Mole, 2012). Today 14 different types of NCL are known in humans. All but NCL9 have an identified monogenetic origin (Mole & Cotman, 2015). They are mostly inherited in an autosomal recessive manner, with only NCL4 following an autosomal dominant mode of inheritance (Gardner & Mole, 2021). The disease commonly begins during childhood, but juvenile and adult-onset forms are also known (Kohlschütter et al., 2019). Clinical signs comprise progressive mental and motor deterioration, visual impairment, epileptic seizures and premature death

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(Haltia, 2003). Typical pathological characteristics are the intracellular accumulation of autofluorescent lipopigment and a loss of neurons, most prominently in the cerebral and cerebellar cortices. Atrophy of the retina is commonly seen in some forms. The diagnosis is confirmed by either genetic analysis or morphological features, if no genetic analysis is available (Radke et al., 2015).

Pathogenic variants for eight of the 13 known human NCL genes have been identified in dogs (Table 1). The association of *ATP13A2* with NCL12 was initially established in Tibetan Terriers and only later confirmed in human patients (Bras et al., 2012; Farias et al., 2011; Wöhlke et al., 2011).

Similar to humans, typical features in dogs include visual, cerebral and cerebellar decline. For the presumptive diagnosis of a canine NCL, evidence of heritability, progressive neurological dysfunction and accumulation of autofluorescent storage material in neurons should be present (Katz et al., 2017).

In this study, we investigated two Small Swiss Hound littermates, both of whom started to show signs of progressive neurological decline around the age of 12 months. The aim of the study was to provide an initial characterization of the clinical and pathological phenotype of these two dogs and to identify the underlying genetic defect.

METHODS

Animal selection

This study was performed with 106 Small Swiss Hounds including the two NCL-affected siblings, their unaffected parents and two unaffected littermates. The male affected dog is referred to as case 1 while the affected female sibling is designated case 2.

Clinical and histopathological examinations

Cases 1 and 2 were clinically and neurologically examined. Magnetic resonance imaging was performed on case 2. Subsequent to euthanasia, a pathological examination of the brain of case 2 was also performed.

TABLE 1 Canine forms of neuronal ceroid lipofuscinosis (NCL) with known pathogenic variants.

Type of NCL	Gene	Affected breeds	Reference	OMIA
NCL1	*PPT1*	Dachshund, Italian Cane Corso	Kolicheski et al. (2017) and Sanders et al. (2010)	001504-9615
NCL2	*TPP1*	Dachshund	Awano, Katz, O'Brien, Sohar, et al. (2006) and Awano, Katz, O'Brien, Taylor, et al. (2006)	001472-9615
NCL5	*CLN5*	Australian Cattle Dog, Border Collie, Golden Retriever	Gilliam et al. (2015) and Melville et al. (2005)	001482-9615
NCL6	*CLN6*	Australian Shepherd	Katz et al. (2011)	001443-9615
NCL7	*MFSD8*	Chihuahua, Chinese Crested Dog	Ashwini et al. (2016), Faller et al. (2016), Guo et al. (2015) and Karli et al. (2016)	001962-9615
NCL8	*CLN8*	English Setter, Australian Shepherd, Alpine Dachsbracke, Saluki	Guo et al. (2014), Hirz et al. (2017), Katz et al. (2005) and Lingaas et al. (2018)	001506-9615
NCL10	*CTSD*	American Bulldog	Awano, Katz, O'Brien, Sohar, et al. (2006) and Awano, Katz, O'Brien, Taylor, et al. (2006)	001505-9615
NCL12	*ATP13A2*	Tibetan Terrier, Australian Cattle Dog	Farias et al. (2011), Schmutz et al. (2019) and Wöhlke et al. (2011)	001552-9615

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DNA extraction

EDTA-blood samples were obtained from all dogs in the study and genomic DNA was isolated using the Maxwell RSC Whole Blood Kit and a Maxwell RSC 48 instrument (Promega, Dübendorf, Switzerland). Extracted DNA samples were stored at −20°C.

Whole genome sequencing

A PCR-free DNA library with an insert size of approximately 350 bp was generated from genomic DNA of case 1. The library was sequenced using an Illumina NovaSeq 6000 instrument (Illumina, San Diego, CA, USA), achieving 23.3× coverage. The resulting 2 × 150 bp paired end reads were aligned to the UU_Cfam_GSD_1.0 reference genome assembly. The sequence data of case 1 are publicly available at the European Nucleotide Archive under accession number SAMEA114382048.

Variant calling and filtering

Single nucleotide and small indel variants of case 1 were called using the GATK Haplotype Caller in gVCF mode as previously described (Jagannathan et al., 2019; McKenna et al., 2010). NCBI annotation release 106 and the SnpEff software were used to predict the functional effects of the called variants (Cingolani et al., 2012). Variants with a SnpEff-predicted impact of 'high' or 'moderate' were considered protein changing. The whole genome sequencing data of case 1 were filtered against publicly available genome sequence data of 1530 control dogs from various breeds (Table S1).

SNV genotyping

The two affected Small Swiss Hounds, an unaffected sibling and their unaffected parents were genotyped using illumina_HD canine BeadChips containing 220 853 markers (Neogen, Lincoln, NE, USA).

Linkage analysis and autozygosity mapping

Linkage analysis was performed with SNV genotype data of one single family consisting of both parents and three offspring, cases 1 and 2, and an unaffected male sibling. The call rate for all dogs was over 98%. All markers that were non-informative, located on the sex chromosomes or missing in any dog, as well as markers with Mendel errors or a minor allele frequency <0.01, were removed using plink v1.9 (Purcell et al., 2007). The final pruned data set contained 95 596 markers. The merlin software was used to perform parametric linkage analysis (Abecasis et al., 2001). An autosomal recessive mode of inheritance with a disease allele frequency of 0.3 and full penetrance of the phenotype were assumed. Autozygosity mapping was performed with SNV genotype data of cases 1 and 2. Markers on the sex chromosomes were excluded. The --homozyg-group option from plink v1.9 was utilized to identify homozygous regions ≥1 Mb in both affected dogs and regions with shared homozygous haplotypes. Overlapping regions of linkage analysis and homozygosity mapping were visually identified in Excel spreadsheets (Table S3).

Coverage plot and WGS data visualization

For detection of structural variants, the coverage per base from the WGS data of case 1 was determined and plotted for all regions previously identified with linkage analysis and autozygosity mapping. This was accomplished using a bash script plotting 10 000 observations in each defined window. The output was visualized using the software r. Regions with noticeable differences were then visually inspected in the Integrative Genomics Viewer (Robinson et al., 2011). The script utilized is available on GitHub (https://github.com/ihaefliger/program_coveragePlot_region).

PCR amplification

The presence of the intragenic *MFSD8* duplication was confirmed by PCR amplification of a 275 bp fragment spanning the junction point of the duplicated sequences. A second control amplicon located in the *ATP2A2* gene was used to confirm successful PCR reaction. Primer sequences are given in Table 2. Amplicons were visualized using a 5200 Fragment Analyzer System (Agilent, Santa Clara, CA, USA).

Droplet digital PCR

Copy number at NC_049240.1:g.13811543_13830361dup was determined with droplet digital PCR (ddPCR) and a TaqMan assay. Two primer sets, each consisting of a fluorescently labeled probe and flanking PCR primers, were designed using the primer3plus software (Table 2). The FAM-labeled probe was designed to bind inside the duplicated *MFSD8* region, while the HEX-labeled probe was located in the *MC1R* gene and used as a single copy reference. The probes were modified with a black hole quencher (BHQ1) at the 3-prime-end to supress the fluorescence signal. Genomic DNA samples were first digested for 15 min at 37°C using BamHI to separate the binding sites in the duplicated region. The amplification reaction was prepared by combining the respective sets of primers and probes with the ddPCR

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TABLE 2 Primer sequences for PCR amplification and droplet digital PCR.

Name	Sequence	Product size	Function
PCR			
*MFSD8*_dog_F	5′-GATGGAAAAAGTGATTCTTGTGA-3′	275 bp	*MFSD8* junction
*MFSD8*_dog_R	5′-GGAAAAAATCCTTGGGAAACTT-3′		*MFSD8* junction
*ATP2A2*_dog_F	5′-CCTGAAGAAATCGGAGATCG-3′	382 bp	PCR control
*ATP2A2*_dog_R	5′-TCATTTCTATCTTTGAGAGCATCC-3′		PCR control
ddPCR			
*MFSD8*_ddPCR_F	5′-GGGACCCAATCTGGTCAGTA-3′	103 bp	*MFSD8* CNV
*MFSD8*_ddPCR_R	5′-TGCAATCTTTTGCACAGAGG-3′		*MFSD8* CNV
*MFSD8*_ddPCR_FAM	FAM-5′-ATGTCCTCCTTGGGGAGTGT-3′-BHQ1	–	*MFSD8* CNV
*MC1R*_ddPCR_F	5′-CCGAAAGACTCTCCAAGAGGT-3′	100 bp	Single copy reference
*MC1R*_ddPCR_R	5′-TCACATGGGTATCAATCACCAC-3′		Single copy reference
*MC1R*_ddPCR_HEX	HEX-5′-TATGTTCCTGGTGAGGCTGC-3′-BHQ1	–	Single copy reference

TABLE 3 Results of variant filtering in case 1 against 1530 controls.

Filtering step	Homozygous variants	Heterozygous variants
All variants in case 1	2 742 181	3 729 824
Private variants	668	5292
Private protein changing variants	1	45
Private protein changing variants in NCL candidate genes	0	0

Supermix for probes, no UTP (Bio-Rad Laboratories, Hercules, CA, USA). The digested genomic DNA was added accordingly. Droplet generation was accomplished with an automated droplet generator (Bio-Rad Laboratories, Hercules, CA, USA). Subsequent thermocycling was performed as follows: denaturation at 95°C for 10 min, followed by 40 cycles of 95°C for 30 s and 60°C for 1 min, and a final denaturation at 98°C for 10 min. Fluorescence in the droplets was detected on a QX200 Droplet Digital PCR system (Bio-Rad Laboratories, Hercules, CA, USA). The results were analyzed using the QUANTASOFT software. Determined copy numbers were within a maximum range of 0.35 above and below the respective integer.

RESULTS

Clinical and pathological examination

Case 1 started to bump into obstacles around the age of 12 months, seemed to be disorientated and had trouble walking up stairs. The dog was presented to a veterinarian at the age of 18 months and had to be euthanized within a few weeks after consultation. No pathological follow-up was performed.

Case 2 also started to show neurological signs at the age of 12 months, when the dog progressively lost cognitive function, became increasingly restless and showed signs of visual deficits. At the age of 20 months, the owner presented the dog and the neurological examination revealed generalized ataxia, absent menace response and reduced proprioception in all four limbs. Neuroanatomical involvement of forebrain and cerebellum was considered. Magnetic resonance imaging of the brain was indicative for a diffuse degenerative encephalopathy. The neurological signs progressed, and the dog was euthanised 1 week later. In the subsequent pathological examination, concentric atrophy of the cerebral and cerebellar cortices and nuclei as well as neuronal cytoplasmic storage of autofluorescent material were noted. Findings were most consistent with neuronal ceroid lipofuscinosis.

Genetic analysis

Comparison of the whole genome sequencing data of case 1 to 1530 controls yielded 5292 heterozygous and 668 homozygous private variants for the affected Small Swiss Hound (Table 3, Table S2). Only private and protein changing variants were further considered. However, none of these variants was located in an NCL candidate gene.

As the initial analysis did not yield any obvious candidate variants, we performed linkage analysis and autozygosity mapping to determine the most likely position of a hypothetical pathogenic variant in the genome. These analyses resulted in 12 genomic intervals comprising approximately 45 Mb and spread over 10

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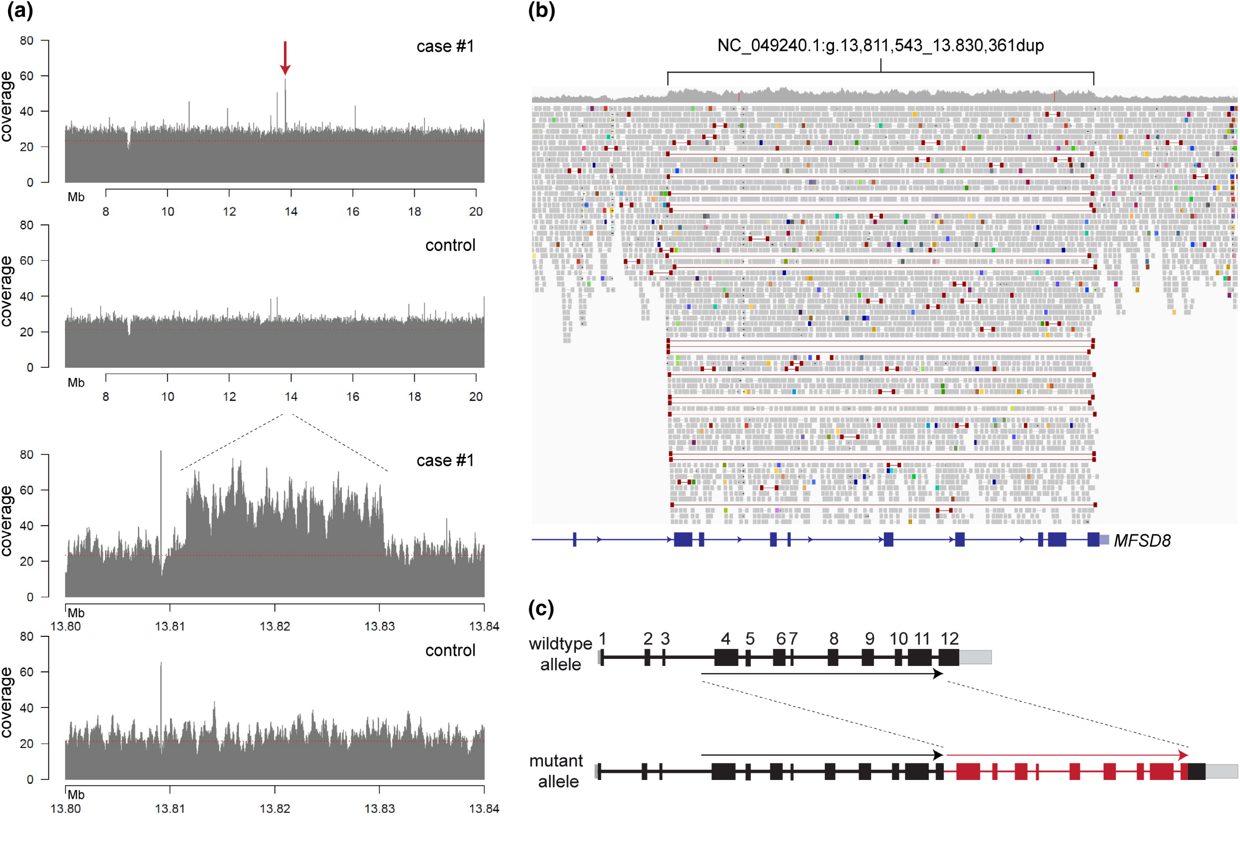


FIGURE 1 Details of the identified *MFSD8* variant. (a) Coverage plot of the identified region on chromosome 19 harboring the *MFSD8* duplication. Coordinates refer to the UU_Cfam_GSD_1.0 assembly. The coverage is approximately doubled at 13.81–13.83 Mb. (b) Integrative Genomics Viewer screenshot of the whole genome sequencing data of case 1 depicting the homozygous duplication. Each arrow resembles a read. An increase of reads as well as the outward facing red arrows are characteristic for the presence of a genomic duplication. The *MFSD8* gene is shown in blue with boxes indicating exons. (c) Schematic depiction of the genomic organization and duplicated region in the *MFSD8* gene. The breakpoints of the duplication are located in intron 3 and exon 12, disrupting the reading frame of the gene.

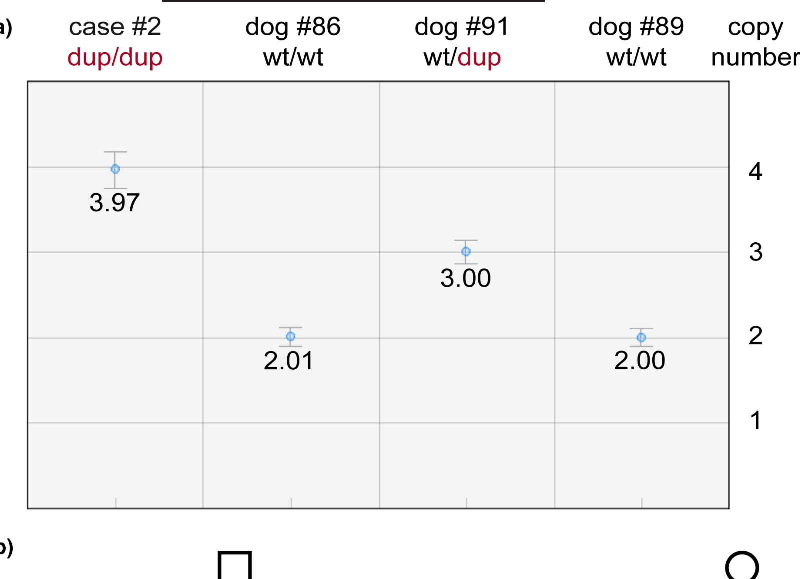
different chromosomes (Table S3). The sequence coverage of these intervals was extracted from the whole genome sequencing data of case 1 and compared with the coverage in an unaffected control (Figure 1a). Regions with a strong deviation were visually inspected using Integrative Genomics Viewer. This enabled the identification of a homozygous duplication spanning 18 819 bp within the *MFSD8* gene on chromosome 19. The variant can be designated as NC_049240.1:g.13811543_13830361 dup or XM_038564680.1:c.202-519_1365dup (Figure 1b). The sequence at the duplication breakpoints does not exhibit any special features. The breakpoints are located in single copy regions without interspersed repeats such as SINEs or LINEs.

The *MFSD8* duplication was analyzed in all 106 dogs of this study using a PCR amplifying the junction of the duplicated sequence copies. The experiment confirmed the presence of the duplication in either homozygous or heterozygous state in cases 1 and 2, and another eight unaffected Small Swiss Hounds. Copy number determination using ddPCR was performed with all 10 dogs carrying the duplication allele and a subset of 22 dogs without the duplication allele (Figure 2a). Cases 1 and 2 were both genotyped as homozygous for the duplication. The sire and dam of the affected dogs were genotyped as heterozygous as expected for obligate carriers of a recessive disease allele. Apart from the parents of the affected dogs, six additional heterozygous dogs were identified in our cohort, all of them related to the NCL affected cases 1 and 2. The genotypes in the available dogs from the pedigree were consistent with an autosomal recessive mode of inheritance and showed the expected cosegregation with the NCL phenotype (Figure 2b).

The *MFSD8* duplication allele occurred with a frequency of 5.7% in our study cohort comprising 106 Small Swiss Hounds and showed perfect genotype–phenotype association with the occurrence of NCL (Table 4). Genotyping results from all Small Swiss Hounds in this study can be found in Table S4.

DISCUSSION

We investigated two Small Swiss Hound siblings, both of which showed signs of progressive neurological degeneration. Owing to the pathological findings of ongoing



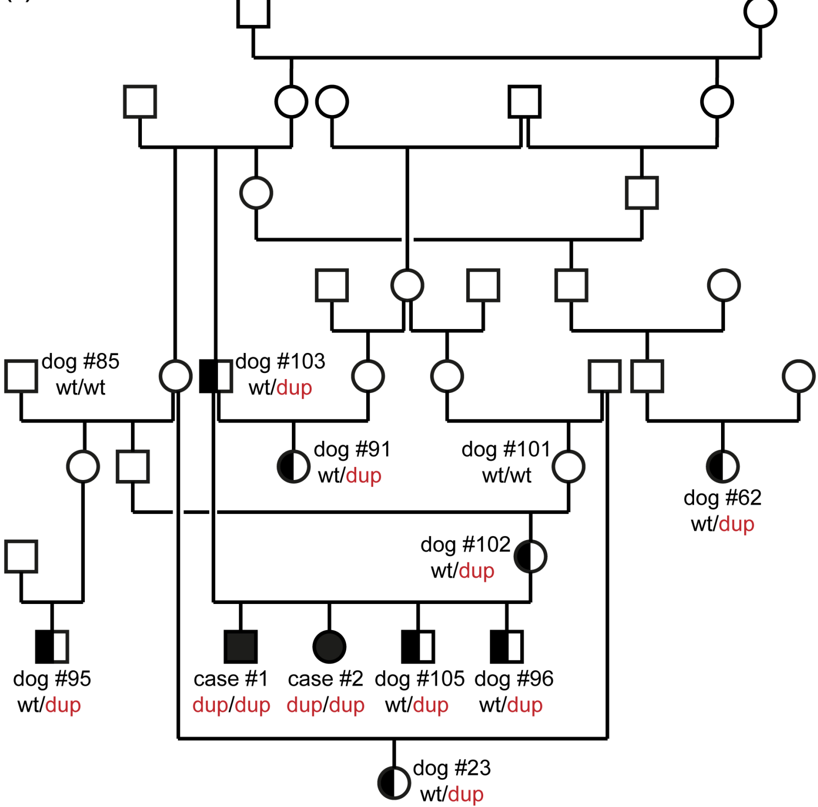


FIGURE 2 Genotyping of Small Swiss Hounds. (a) Selected results of copy number determination with ddPCR in four dogs. Homozygous dogs have four copies of the duplicated region, heterozygous dogs have three and dogs homozygous for the wildtype allele have two. (b) Pedigree of the investigated Small Swiss Hound family. The neuronal ceroid lipofuscinosis (NCL) affected animals are indicated by solid black symbols. Dogs that were genotyped as heterozygous are presumptive carriers and indicated with half-filled symbols. Genotypes are indicated for all dogs, from which a sample was available. The pedigree shows a level of inbreeding that is typical for many purebred dogs. The two dogs at the top are common ancestors of both cases and all presumptive carriers. However, this pedigree and the limited sample availability do not allow a reliable determination of the founder animal.

TABLE 4 Genotype–phenotype association of the *MFSD8* duplication with NCL.

Phenotype	Wt/wt	Wt/dup	Dup/dup
Affected by NCL (n=2)	–	–	2
Unaffected by NCL (n=104)	96	8	–

neurodegeneration and accumulation of autofluorescent material, neuronal ceroid lipofuscinosis was considered to be the most likely diagnosis. Utilizing whole genome sequencing and positional cloning methods we identified a homozygous duplication of 18 819 bp in both affected dogs. The duplication disrupts the reading frame of *MFSD8*, a known candidate gene for NCL7, encoding the major facilitator superfamily domain containing protein 8. The MFSD8 protein is highly conserved among mammals and pathogenic variants have been described in humans (Kousi et al., 2012), dogs (Ashwini et al., 2016; Guo et al., 2015; Karli et al., 2016), a cat (Guevar et al., 2020), Japanese Macaques (McBride et al., 2018) and a rabbit (Christen et al., 2024). MFSD8 is predominantly expressed in endosomes and lysosomes, spanning the membrane with 12 domains (Wang et al., 2021). The protein function remained unclear for a long time, even

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though the connection to NCL7 was established many years ago (Siintola et al., 2007). More recently, the murine Mfsd8 protein has been characterized as a chloride channel which regulates chloride homeostasis and thereby affects lysosomal membrane potential, pH and Ca^{2+} release, which are essential for lysosome formation and function (Wang et al., 2021). *Mfsd8*$^{-/-}$ knockout mice showed similar clinical and pathological signs to humans affected by NCL7 including the characteristic accumulation of autofluorescent lipofuscin (Wang et al., 2021).

Owing to a lack of suitable samples, we did not experimentally investigate the consequences of the genomic duplication for splicing and the mutant transcript(s). As one of the duplication breakpoints is located within exon 12 and as the duplication spans several internal exons, we consider it extremely unlikely that a functional transcript might be expressed from the mutant allele. We therefore assume that the duplication allele represents a true null allele and that homozygous mutant dogs are completely deficient in functional MFSD8 protein.

For assessment of pathogenicity, we applied the ACMG/AMP guidelines for human variants to the canine variant NC_049240.1:g.13811543_13830361dup (Richards et al., 2015). We considered three criteria as evidence for pathogenicity. The first is PVS1, a null variant in a gene where a loss of function is a known mechanism of disease. As described before, the mutant allele discovered in this study is unlikely to produce a functional transcript and loss of function is a known pathomechanism of *MFSD8* (Kousi et al., 2012). The second is PP1, cosegregation with disease in multiple affected family members in a gene definitely known to cause the disease. The cosegregation of the allele is in accordance with the occurrence of NCL7 in the investigated family of Small Swiss Hounds. Only the two affected dogs were homozygous for the duplication; both parents as well as both unaffected siblings were genotyped as heterozygous. The third is PP4, the patient's phenotype or family history is highly specific for a disease with a single genetic etiology. The progressive signs of neurodegeneration and the intracellular accumulation of autofluorescent material in both affected siblings are highly specific for NCL and thereby point to a monogenetic origin (Radke et al., 2015). The combination of one very strong and two supporting pieces of evidence for pathogenicity justifies the classification of the variant as pathogenic (Richards et al., 2015). In addition to these formal criteria, in a control cohort of 104 Small Swiss Hounds, none carried the duplication in a homozygous state, adding further support to the claimed pathogenicity.

To our knowledge we report the second *MFSD8* variant in dogs associated with NCL7. The discovery of this variant will enable genetic testing in the Small Swiss Hound breed, to monitor the population, implement genetic testing and a targeted breeding program, and thereby avoid further cases of this devastating disease. This study highlights the possibilities emerging from whole genome sequencing approaches for diagnostics in veterinary medicine.

AUTHOR CONTRIBUTIONS

Stefan J. Rietmann: Investigation; visualization; writing – original draft; writing – review and editing. **Shenja Loderstedt:** Conceptualization; investigation; writing – original draft; writing – review and editing. **Kaspar Matiasek:** Conceptualization; investigation; writing – original draft; writing – review and editing. **Ingmar Kiefer:** Investigation; writing – review and editing. **Vidhya Jagannathan:** Data curation; writing – review and editing. **Tosso Leeb:** Conceptualization; supervision; writing – original draft; writing – review and editing.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The genome sequence data were submitted to the European Nucleotide Archive. All accession numbers are listed in Table S1.

ETHICS STATEMENT

The affected dogs in this study were privately owned and blood samples for diagnostic purposes were collected with the consent of their owners. The collection of blood samples from healthy control dogs was approved by the 'Cantonal Committee for Animal Experiments' (Canton of Bern; permit BE94/2022).

CODE AVAILABILITY

The Bash script utilized for plotting of the WGS coverage is available from: https://github.com/ihaefliger/program_coveragePlot_region.

CONSENT TO PARTICIPATE

Consent of the dog owners was obtained for the use of samples and data in this study.

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SUPPORTING INFORMATION

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Eigenständigkeitserklärung

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