ANIMAL GENETICS Immunogenetics, Molecular Genetics and Functional Genomics

doi: 10.1111/age.12629

Neuronal ceroid lipofuscinosis in Salukis is caused by a single base pair insertion in *CLN8*

F. Lingaas*, O-A. Guttersrud*, E. Arnet* and A. Espenes†

*Section of Genetics, Faculty of Veterinary Medicine, Norwegian University of Life Sciences, P.O. box 8146 Dep, 0033 Oslo, Norway.
†Section of Anatomy & Pathology, Faculty of Veterinary Medicine, Norwegian University of Life Sciences, P.O. box 8146 Dep, 0033 Oslo, Norway.

Summary

Neuronal ceroid lipofuscinoses (NCLs) are heterogenic inherited lysosomal storage diseases that have been described in a number of species including humans, sheep, cattle, cats and a number of different dog breeds, including Salukis. Here we present a novel genetic variant associated with the disease in this particular breed of dog. In a clinical case, a Saluki developed progressive neurological signs, including disorientation, anxiety, difficulties in eating, seizures and loss of vision, and for welfare reasons, was euthanized at 22 months of age. Microscopy showed aggregation of autofluorescent storage material in the neurons of several brain regions and also in the retina. The aggregates showed positive staining with Sudan black B and periodic acid Schiff, all features consistent with NCL. Whole genome sequencing of the case and both its parents, followed by variant calling in candidate genes, identified a new variant in the *CLN8* gene: a single bp insertion (c.349dupT) in exon 2, introducing an immediate stop codon (p.Glu117*). The case was homozygous for the insertion, and both parents were heterozygous. A retrospective study of a Saluki from Australia diagnosed with NCL identified this case as being homozygous for the same mutation. This is the fourth variant identified in *CLN8* that causes NCL in dogs.

Keywords dog, lysosomal storage disease, mutation, stop codon, whole genome sequencing

Introduction

Neuronal ceroid lipofuscinoses (NCLs) are heterogenic, autosomal recessive lysosomal storage diseases that have been described in a number of species including humans, sheep, goats, cattle, horses and cats, as well as many different breeds of dog. Disease onset in dogs varies from around 9 to 18 months of age. Dachshund and Cane Corso have a relatively early onset of disease, at around 9 months of age (Awano *et al.* 2006a; Sanders *et al.* 2010; Kolicheski *et al.* 2017). Time of onset in English Setters, Chihuahuas, American Bulldogs, Golden Retrievers, Border Collies and Australian Shepherds is around 12–18 months (Koppang 1992; Lingaas *et al.* 1998; Evans *et al.* 2005; Katz *et al.* 2005; Melville *et al.* 2005; O'Brien & Katz 2008; Gilliam *et al.* 2015; Faller *et al.* 2016). Late onset forms have been described in Tibetan Terriers and American Staffordshire Terriers with age of onset around 5–7 years (Wohlke *et al.* 2011; Nolte *et al.* 2016). In Salukis, NCL has been observed with progressive signs of lack of coordination from around 1 year of age (Appleby *et al.* 1982).

In humans with NCLs, high levels of allelic and locus heterogeneity have been observed (http://www.ucl.ac.uk/ncl/), and at least 14 genes are involved in different forms (Mole & Cotman 2015; Nita *et al.* 2016). Among these 14 forms (NCL1–NCL14), disease-associated variants have been identified in 13 (all except NCL9). The number of known mutations in each gene varies from one (*CLN12/14*) to at least 116 in *CLN2* (http://www.ucl.ac.uk/ncl/; May 2015); thus, many patients may be compound heterozygotes. Different forms of NCL also exist in a number of dog breeds, and mutations in at least eight genes—*CLN1* (Sanders *et al.* 2010), *CLN2* (also known as *TPP1*; Awano *et al.* 2006a), *CLN5* (Melville *et al.* 2005; Guo *et al.* 2014; Gilliam *et al.* 2015), *CLN6* (Katz *et al.* 2011), *CLN7* (also known as *MFSD8*; Guo *et al.* 2015; Faller *et al.* 2016), *CLN8* (Katz *et al.* 2005; Guo *et al.* 2014; Hirz *et al.* 2017), *CLN10* (also known as *CTSD;* Awano *et al.* 2006b) and *CLN12* (also known as *ATP13A2;* Farias *et al.* 2011; Wohlke *et al.* 2011)—have been associated with NCL in dogs to date. Within dog breeds, there is very limited allelic

Address for correspondence

F. Lingaas, Section of Genetics, Faculty of Veterinary Medicine-Norwegian University of Life Sciences, P.O. box 8146 Dep, 0033 Oslo, Norway.
E-mail: frode.lingaas@nmbu.no

Accepted for publication 17 November 2017

heterogeneity within the relevant genes, and thus, dog breeds could provide suitable large animal models for investigating treatment of these diseases. In this study, a case of a Saluki diagnosed with NCL is presented, and a novel mutation is described as the genetic basis for disease.

Material and methods

Clinical case description

A female, 22-month-old Norwegian Saluki had been exhibiting progressive changes in behaviour for a prolonged period, and the owner decided to have the dog euthanized. The first clinical signs that had been observed by the owner were anxiety and restlessness from around 10–12 months. At 15–16 months of age, the dog had problems when participating in lure coursing and developed signs of impaired vision and hearing. At around 18 months of age, the dog underwent a clinical eye examination, and the ophthalmologist noted that

> the dog appears confused, it collides with the door and seems to use its nose for orientation. It is hard to come into contact with the dog, it does not like being touched on the head, and it needs sedation for further investigation. The dog has open pupils and negative direct or indirect pupil-reflexes, and negative menace response. The retina is normal, with a normal distribution of blood vessels.

Central nervous system blindness was suspected. At around 20 months of age, the dog showed obvious vision problems when outside in the evenings. At this time, episodes of epileptic-like seizures and shivering occurred regularly and there was a loss of housetraining skills. From about 21–22 months of age, the dog had considerable difficulties when walking on a leash, developed problems swallowing and lost weight. The dog was euthanized at 22 months of age.

Samples

Salukis

With the owners' consent, blood samples (EDTA) were collected from the affected dog and both its parents, and DNA was extracted using standard commercial kits (E.Z.N.A; Blood DNA Mini Kit; Omega). At necropsy, samples from the brain and other tissues were collected and fixed in 4% buffered formalin. Brain samples were also snap-frozen in liquid nitrogen.

Through close collaboration with Saluki owners, we were also able to collect blood samples from related and unrelated Salukis in Norway, Sweden, Finland, UK, Australia and some other countries ($n = 99$). During this work, we were informed about two earlier cases of NCL from the same litter, born in 2011 in Australia. Two male siblings in a litter of eight puppies developed NCL and had been euthanized at around 2 years of age. DNA samples from one of these two Salukis, and also from several siblings from the same litter, had been stored and were available for analysis.

Other breeds

We also studied a cohort of 380 dogs of different breeds for which the owners had approved that samples from their dogs could be used in research projects. This sample included 242 samples from 142 different breeds, along with samples from 120 English Setters and 18 Gordon Setters that had previously been genotyped for the specific *CLN8* variant existing in those breeds.

Post-mortem morphological investigations

Tissue samples collected from several brain regions (cerebral cortex, midbrain, hippocampus and cerebellum) and the retina were fixed in 4% buffered formaldehyde and embedded in paraffin wax. Tissue sections were stained with haematoxylin and eosin (HE), periodic acid Schiff (PAS) and Sudan black B, and deparaffinized sections were examined for autofluorescence using a fluorescence microscope with a filter for green light (excitation filter BP 470/40; emission filter BP 525/50).

Whole genome sequence analysis

Full genome sequencing was conducted on the DNA obtained from the original case dog and both its parents. PCR-free libraries were constructed (TrueSeq PCR-free, insert size 350 bp). The samples were sequenced on an Illumina Hiseq X5 at the Norwegian Sequencing Centre. Two samples were run per lane and 125 bp paired-end sequencing was used. The coverage was, on average, around 28×. The sequences obtained were submitted to the European Nucleotide Archive (https://www.ebi.ac.uk/ena/; Accession nos. ERS1799414, ERS1799415, ERS1799416).

Candidate genes/variant calling

We followed the GATK (McKenna *et al.* 2010) best practice recommended workflows for sequence preprocessing and variant discovery. We focused on the 13 known candidate loci *CLN1–CLN8* and *CLN10–CLN14* (Nita *et al.* 2016). In addition to the 'official' 14 NCL-loci, we also included a variant in *CLCN6* (Poet *et al.* 2006), the *SGSH* gene (Sleat *et al.* 2009) and the *ARSG* gene, for which a variant has been observed in American Staffordshire Terriers (Abitbol *et al.* 2010). The sequence reads were aligned to Can Fam3.1 (Assembly: GCA_000002285.2, CanFam3.1) using BWA (Li & Durbin 2009) and GATK for sequence analysis (McKenna *et al.* 2010). We used SAMTOOLS (http://www.

htslib.org/doc/samtools.html) to export sequence regions from the affected dog and both parents from the 13 known human NCL genes and the three other potentially associated loci according to the chromosome-positions given in Table S1.

Results

Morphological verification of diagnosis

At autopsy, the brain and cerebellum were inspected and appeared normal without evidence of macroscopic changes. Extensive deposits characterized as ceroid lipofuscin were found mainly in neuronal cell bodies in the cerebrum, hippocampus, cerebellum and retina (Figs 1 & S1, S2). The abundant abnormal granular material in the cytoplasm was autofluorescent, PAS positive and demonstrated black granular staining with Sudan Black B. Some neuronal cells in the cerebral cortex showed signs of ongoing necrosis (Fig. 1b). These morphological findings, in conjunction with the clinical signs, provided supportive evidence that the dog had NCL.

Pedigree analysis

With help from owners/breeders of Salukis worldwide and the Saluki breeding archive (https://saluki.breedarchive.com/animal/), we studied an extended pedigree. Both parents of the Norwegian affected dog could be traced back to common ancestors about five to six generations back (Fig. S3).

Both cases reported from Australia, for which the clinical signs were reported as being similar to those of the Norwegian case, came from a litter that could be traced back to the same founders as the Norwegian proband, with the same number of generations. We also received unsubstantiated information about a Swedish litter with possible NCL, and that also could be traced back to the same founders (Fig. S3).

Molecular genetic findings

We performed variant calling on each of the 13 candidate genes and the three additional loci (Table S1) using GATK, filtering for variants for which both parents were heterozygous and the affected offspring was homozygous variant. We then inspected the short read alignments using the Integrated Genomics Viewer (IGV) (Thorvaldsdottir *et al.* 2013). We focused on coding regions and splice sites. In general, we observed very limited variation in the regions searched in the three dogs: the Norwegian euthanized case of NCL and the parent dogs. Only one variant was identified according to the criterion of both parents being heterozygous and the euthanized case homozygous. This was an insertion of 1 bp (T) in the *CLN8* gene (Fig. S2) in a short T repeat, thereby changing the stretch of 9 T's to a stretch consisting of 10 T's. The exact position of the inserted base could obviously not be verified, given that this was an insertion of a single base in a stretch of nine repeats of the same base (CFA37:g.30874628–30874636). According to the recommended nomenclature, the T insertion was noted as a T duplication in the T farthest in the 3′ direction (g.30874636dupT; c.349dupT). The sequence alignments in this gene region from the two parents and from the affected offspring are shown in Fig. S4.

The presence of the genomic insertion in the homozygous state was confirmed by PCR and Sanger sequencing (Fig. 2). The sequencing results in both sequencing directions in the heterozygous parents, in the euthanized dog diagnosed with NCL and in an unaffected and unrelated control (g.30,874,636dupT) are shown in Fig. 2a. In both heterozygous parents, the Sanger sequence became 'double'

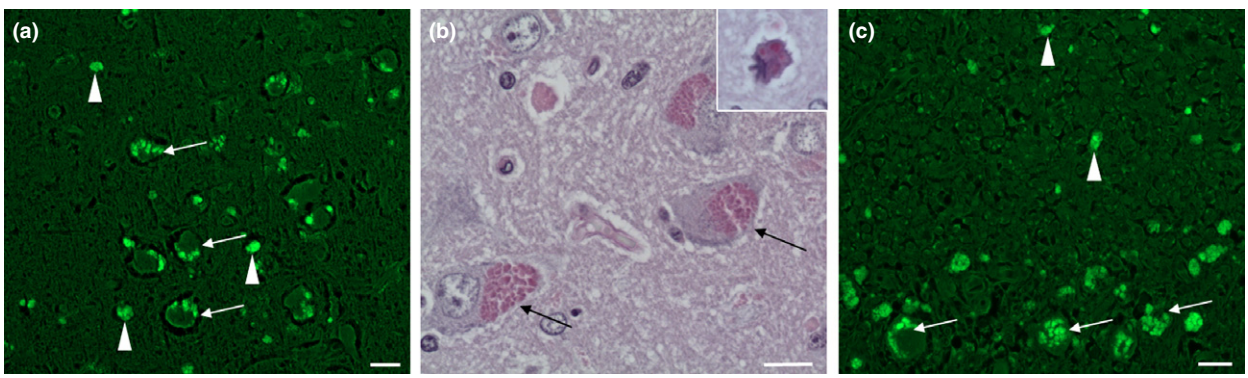

Figure 1 (a) The cytoplasm of cell bodies of large neurons (arrows) in the grey matter lamina of the cerebral cortex with autofluorescent, granular material. Similar material was also present in smaller cells, probably glia (arrowheads). (b) The granular material in the large neurons (arrows) and in small cells with dark, shrunken, irregular nuclei, probably necrotic neurons (inset), also stained positive with PAS. (c) Purkinje neurons (arrows) and small neurons in the granular layer (arrowheads) of the cerebellum had accumulated large amounts of fluorescent granular material. Bar: 20 μm.

after the T stretch, depending on sequence direction (Fig. 2a, yellow arrow), illustrating the 1-bp shift of the strand with the insertion compared with the other. The T insertion introduces a nonsense codon (TGA), changing a glutamic acid (Glu/E) to a stop (*) at amino acid 117 (of the predicted total 288 amino acids; p.Glu117*). The consequence of this mutation and comparison with other known variants in the canine *CLN8* gene are shown in Fig. 2b.

Diagnostic PCR was performed on genomic DNA from all available family members (parents and siblings) of the affected dog. Both parents were heterozygous, but none of the five healthy siblings were homozygous for this mutation.

After discovering the 1-bp insertion/nonsense variant in *CLN8* in the Norwegian litter, we genotyped all available individuals related to the two Australian dogs that had developed NCL. We discovered the same bp insertion in both these siblings, which had been euthanized, and also, as with the parents of the Norwegian dog, that both the parent dogs in Australia were heterozygous carriers. However, none of the other relatives (siblings/grandparents) were homozygous for the insertion, being either homozygous free or heterozygous carriers of the variant.

From samples provided by helpful Saluki owners, we typed a total of 99 Salukis, about half of which were samples from random dogs from the global Saluki population and about half of which were from siblings, other relatives and related breeding lines of the dogs known to have developed NCL. We found no homozygote T insertions

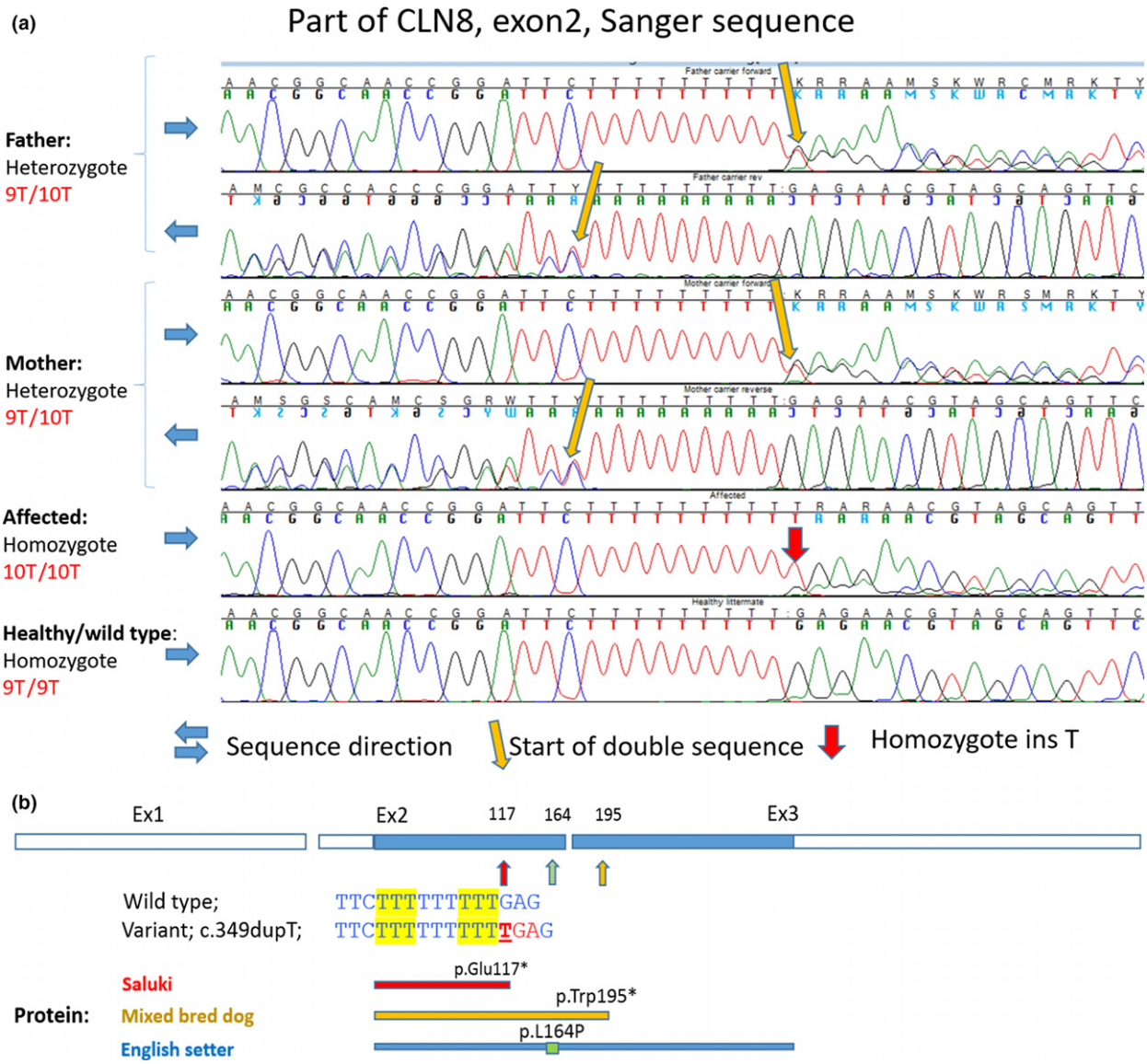

Figure 2 (a) Sanger sequencing of heterozygous parents, the affected dog and a homozygous normal dog. Note the double sequence after the T-stretch due to the 1-bp displacement in one strand. (b) Predicted consequence of the T insertion in Saluki *CLN8* (c.349dupT) and comparison with the known variants in English Setter and a mixed-bred dog (Katz *et al.* 2005; Guo *et al.* 2014).

in these dogs. Similarly, no heterozygous dogs were identified among the random dogs from the population. However, of the siblings and other dogs in close pedigrees of the NCL dogs, 14 were discovered to be carriers of the mutant allele. The pedigree and inheritance analysis support a recessive inheritance, which is common for lysosomal storage diseases.

Analysis of 380 dogs from different breeds did not reveal this variant occurring in any of these dogs.

Discussion

The clinical signs reported in the Norwegian clinical case Saluki, in combination with the morphological findings (abundant granular autofluorescent, PAS- and Sudan black B-positive material in neuronal cell bodies in a range of brain areas and the retina), support the diagnosis of NCL in the Norwegian proband. That a dog from an Australian litter diagnosed with NCL shares the homozygous T insertion and has common ancestors with the Norwegian proband, also supports the diagnosis.

More than 440 genetic variants have been reported in human NCLs, all available in the openly accessible NCL mutation database (www.ucl.ac.uk/ncl/). To date, all known human variants associated with NCL occur in one of 13 known loci. Similarly, most NCL-associated mutations in dogs have been identified in known CLN genes.

A high level of locus and allelic heterogeneity is observed in human NCLs (Mole & Cotman 2015). With respect to *CLN8* in humans, a phenotypic heterogeneity in patients with the same mutations is also seen (Mahajnah & Zelnik 2012). The most common variants are missense mutations (www.ucl.ac.uk/ncl/), but smaller and larger deletions are also common. So far, insertions in *CLN8* have not been reported in the database. In dogs, clinical NCL cases have been associated with a *CLN8* missense mutation in English Setters (Katz *et al.* 2005). A nonsense variant was found in a mixed breed dog with NCL (Guo *et al.* 2014) and NCL in two Alpenländische Dachsbracke dogs was suggested to be caused by deletion of the entire *CLN8* gene (Hirz *et al.* 2017). The present report adds a further novel defective allele variant to the list of possible mutations associated with NCL, due to a 1-bp insertion that introduces a stop codon in the second half of exon 2.

The extended pedigree of the Norwegian proband indicates a potential common ancestor for both parents of the affected litter that may have been the source of the mutation. The parents of the cases in Australia could also be traced back to these same dogs, and another suspected litter (in Sweden) also apparently had the same lineage. Thus, the pedigrees support the theory that a single variant has been spread to all the affected dogs. The youngest common ancestor of the affected dogs was born in 1972. Due to the frequent exchange of breeding dogs between countries, it seems likely that the same mutation might also have caused the disease in two Salukis in the UK, as described in 1982 (Appleby *et al.* 1982).

Our studies on a wider selection of dog breeds indicate that this genetic variant is specific for Salukis and may have originated in a litter from around 1972 (or earlier), thereafter spreading in Salukis due to breeding dogs being frequently used across national borders. Our data from non-related Salukis also suggest that the frequency of this allele is relatively low in Salukis; however, our random material was not sufficient to provide reliable estimates. Nevertheless, those Salukis that were identified as being heterozygous for the allele all fit into the extended pedigree and thus their variants could have been derived from the potential common ancestor.

The *CLN8* gene is postulated to function in lipid synthesis, transport or sensing. In humans, mutations in this gene are associated with progressive epilepsy with mental retardation, which is a subtype of NCL (Vantaggiato *et al.* 2009). The *CLN8* gene encodes a transmembrane protein that localizes to the endoplasmic reticulum (ER) and may recycle between the ER and ER–Golgi intermediate compartment. It contains a TLC (TRAM-LAG1-*CLN8*) domain of 200 amino acids (62–262 aa) found in a family of membrane-associated proteins that are proposed to have a role in sensing, biosynthesis and metabolism of lipids (Lonka *et al.* 2000; Winter & Ponting 2002). The missense variant in the English Setter model (p.Leu164Pro), the nonsense variant in the mixed breed dog (p.Trp195*) and the insertion/nonsense variant in Salukis presented here (p.Glu117*) are all in this region. The Saluki nonsense variant results in the loss of the last 172 of the 288 predicted amino acids, which is 78 more amino acids than observed in the mixed-bred NCL dog. The fact that the insertion/stop is in this region supports the suggestion that this mutation is the cause of NCL in Salukis. Despite the different types and sites of mutations in *CLN8* in dogs, the clinical signs and age of disease onset are relatively similar. Dogs in all breeds with NCL often present with impaired vision, increased sensitivity to noise, signs of anxiety, problems in walking on stairs, loss of housetraining skills and, in the later stages, with seizures (Koppang 1973; Guo *et al.* 2014). In humans, there is a phenotypic heterogeneity with two distinct phenotypes: progressive epilepsy with mental retardation with age of onset from 5 to 10 years and a late infantile variant (Kousi *et al.* 2012) with earlier onset (2–7 years), both characterized by frequent seizures. Epileptic seizures, a prominent feature of the human variants, do not seem to affect mice (Ranta *et al.* 1999), which suggests that the canine model may be more similar to the human forms in some aspects. Studies on the neurobiological function of *CLN8* (Vantaggiato *et al.* 2009) indicate that *CLN8* plays a role in cell proliferation during neuronal differentiation and in protection against cell death. Thus, the neuronal necrosis observed in the present study could be caused by the

accumulation of ceroid-lipofuscin, a defective CLN8-gene product or a combination of these putative causes.

In conclusion, in this work we identified a *CLN8* insertion that causes an immediate stop and was likely to be the cause of NCL in a Norwegian Saluki; the same genetic variant was also identified in distantly related cases of NCL from Australia. A gene test is available and can be used by breeders to minimise the risk of affected dogs being born in the future.

Acknowledgements

We acknowledge all dog owners and breed clubs, as well as contributing veterinarians, for providing us with blood samples. We also acknowledge Mari Katharina Aas Ådland for laboratory assistance and the Animal Health Trust for storing and providing several DNA-samples. The DNA sequence analyses/computations were performed on resources provided by UNINETT Sigma2 – the National Infrastructure for High Performance Computing and Data Storage in Norway. The Faculty of Veterinary Medicine in Oslo (NMBU), the Legacy of Veterinary Edvard Smidt and Agria Pet Insurance have co-funded this work. The authors declare that there are no conflicts of interest.

References

Abitbol M., Thibaud J.L., Olby N.J. *et al.* (2010) A canine *arylsulfatase G* (*ARSG*) mutation leading to a sulfatase deficiency is associated with neuronal ceroid lipofuscinosis. *Proceedings of the National Academy of Sciences of the United States of America* **107**, 14775–80.

Appleby E.C., Longstaffe J.A. & Bell F.R. (1982) Ceroid-lipofuscinosis in two Saluki dogs. *Journal of Comparative Pathology* **92**, 375–80.

Awano T., Katz M.L., O'Brien D.P., Sohar I., Lobel P., Coates J.R., Khan S., Johnson G.C., Giger U. & Johnson G.S. (2006a) A frame shift mutation in canine *TPP1* (the ortholog of human *CLN2*) in a juvenile Dachshund with neuronal ceroid lipofuscinosis. *Molecular Genetics and Metabolism* **89**, 254–60.

Awano T., Katz M.L., O'Brien D.P., Taylor J.F., Evans J., Khan S., Sohar I., Lobel P. & Johnson G.S. (2006b) A mutation in the *cathepsin D* gene (*CTSD*) in American Bulldogs with neuronal ceroid lipofuscinosis. *Molecular Genetics and Metabolism* **87**, 341–8.

Evans J., Katz M.L., Levesque D., Shelton G.D., de Lahunta A. & O'Brien D. (2005) A variant form of neuronal ceroid lipofuscinosis in American bulldogs. *Journal of Veterinary Internal Medicine* **19**, 44–51.

Faller K.M., Bras J., Sharpe S.J. *et al.* (2016) The Chihuahua dog: a new animal model for neuronal ceroid lipofuscinosis CLN7 disease? *Journal of Neuroscience Research* **94**, 339–47.

Farias F.H., Zeng R., Johnson G.S. *et al.* (2011) A truncating mutation in *ATP13A2* is responsible for adult-onset neuronal ceroid lipofuscinosis in Tibetan Terriers. *Neurobiology of Disease* **42**, 468–74.

Gilliam D., Kolicheski A., Johnson G.S., Mhlanga-Mutangadura T., Taylor J.F., Schnabel R.D. & Katz M.L. (2015) Golden Retriever dogs with neuronal ceroid lipofuscinosis have a two-base-pair deletion and frameshift in *CLN5*. *Molecular Genetics and Metabolism* **115**, 101–9.

Guo J., Johnson G.S., Brown H.A., Provencher M.L., da Costa R.C., Mhlanga-Mutangadura T., Taylor J.F., Schnabel R.D., O'Brien D.P. & Katz M.L. (2014) A *CLN8* nonsense mutation in the whole genome sequence of a mixed breed dog with neuronal ceroid lipofuscinosis and Australian Shepherd ancestry. *Molecular Genetics and Metabolism* **112**, 302–9.

Guo J., O'Brien D.P., Mhlanga-Mutangadura T., Olby N.J., Taylor J.F., Schnabel R.D., Katz M.L. & Johnson G.S. (2015) A rare homozygous *MFSD8* single-base-pair deletion and frameshift in the whole genome sequence of a Chinese Crested dog with neuronal ceroid lipofuscinosis. *BMC Veterinary Research* **10**, 960.

Hirz M., Drogemuller M., Schanzer A. *et al.* (2017) Neuronal ceroid lipofuscinosis (NCL) is caused by the entire deletion of *CLN8* in the Alpenlandische Dachsbracke dog. *Molecular Genetics and Metabolism* **120**, 269–77.

Katz M.L., Khan S., Awano T., Shahid S.A., Siakotos A.N. & Johnson G.S. (2005) A mutation in the *CLN8* gene in English Setter dogs with neuronal ceroid-lipofuscinosis. *Biochemical and Biophysical Research Communications* **327**, 541–7.

Katz M.L., Farias F.H., Sanders D.N., Zeng R., Khan S., Johnson G.S. & O'Brien D.P. (2011) A missense mutation in canine *CLN6* in an Australian shepherd with neuronal ceroid lipofuscinosis. *Journal of Biomedicine and Biotechnology* **2011**, 198042.

Kolicheski A., Barnes Heller H.L., Arnold S. *et al.* (2017) Homozygous *PPT1* splice donor mutation in a cane corso dog with neuronal ceroid lipofuscinosis. *Journal of Veterinary Internal Medicine* **31**, 149–57.

Koppang N. (1973) Canine ceroid-lipofuscinosis—a model for human neuronal ceroid-lipofuscinosis and aging. *Mechanisms of Ageing and Development* **2**, 421–45.

Koppang N. (1992) English Setter model and juvenile ceroid-lipofuscinosis in man. *American Journal of Medical Genetics* **42**, 599–604.

Kousi M., Lehesjoki A.E. & Mole S.E. (2012) Update of the mutation spectrum and clinical correlations of over 360 mutations in eight genes that underlie the neuronal ceroid lipofuscinoses. *Human Mutation* **33**, 42–63.

Li H. & Durbin R. (2009) Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics* **25**, 1754–60.

Lingaas F., Aarskaug T., Sletten M. *et al.* (1998) Genetic markers linked to neuronal ceroid lipofuscinosis in English Setter dogs. *Animal Genetics* **29**, 371–6.

Lonka L., Kyttala A., Ranta S., Jalanko A. & Lehesjoki A.E. (2000) The neuronal ceroid lipofuscinosis CLN8 membrane protein is a resident of the endoplasmic reticulum. *Human Molecular Genetics* **9**, 1691–7.

Mahajnah M. & Zelnik N. (2012) Phenotypic heterogeneity in consanguineous patients with a common *CLN8* mutation. *Pediatric Neurology* **47**, 303–5.

McKenna A., Hanna M., Banks E. *et al.* (2010) The GENOME ANALYSIS TOOLKIT: a MapReduce framework for analyzing next-generation DNA sequencing data. *Genome Research* **20**, 1297–303.

Melville S.A., Wilson C.L., Chiang C.S., Studdert V.P., Lingaas F. & Wilton A.N. (2005) A mutation in canine *CLN5* causes neuronal ceroid lipofuscinosis in Border collie dogs. *Genomics* **86**, 287–94.

Mole S.E. & Cotman S.L. (2015) Genetics of the neuronal ceroid lipofuscinoses (Batten disease). *Biochimica et Biophysica Acta* **1852**, 2237–41.

Nita D.A., Mole S.E. & Minassian B.A. (2016) Neuronal ceroid lipofuscinoses. *Epileptic Disorders* **18**, 73–88.

Nolte A., Bello A., Drogemuller M., Leeb T., Brockhaus E., Baumgartner W. & Wohlsein P. (2016) Neuronal ceroid lipofuscinosis in an adult American Staffordshire Terrier. *Tierarztl Prax Ausg K Kleintiere Heimtiere* **44**, 431–7.

O'Brien D.P. & Katz M.L. (2008) Neuronal ceroid lipofuscinosis in 3 Australian shepherd littermates. *Journal of Veterinary Internal Medicine* **22**, 472–5.

Poet M., Kornak U., Schweizer M. *et al.* (2006) Lysosomal storage disease upon disruption of the neuronal chloride transport protein ClC-6. *Proceedings of the National Academy of Sciences of the United States of America* **103**, 13854–9.

Ranta S., Zhang Y., Ross B. *et al.* (1999) The neuronal ceroid lipofuscinoses in human EPMR and mnd mutant mice are associated with mutations in *CLN8*. *Nature Genetics* **23**, 233–6.

Sanders D.N., Farias F.H., Johnson G.S., Chiang V., Cook J.R., O' D.P., Hofmann S.L., Lu J.Y. & Katz M.L. (2010) A mutation in canine *PPT1* causes early onset neuronal ceroid lipofuscinosis in a Dachshund. *Molecular Genetics and Metabolism* **100**, 349–56.

Sleat D.E., Ding L., Wang S., Zhao C., Wang Y., Xin W., Zheng H., Moore D.F., Sims K.B. & Lobel P. (2009) Mass spectrometry-based protein profiling to determine the cause of lysosomal storage diseases of unknown etiology. *Molecular & Cellular Proteomics* **8**, 1708–18.

Thorvaldsdottir H., Robinson J.T. & Mesirov J.P. (2013) Integrative Genomics Viewer (IGV): high-performance genomics data visualization and exploration. *Briefings in Bioinformatics* **14**, 178–92.

Vantaggiato C., Redaelli F., Falcone S. *et al.* (2009) A novel *CLN8* mutation in late-infantile-onset neuronal ceroid lipofuscinosis (LINCL) reveals aspects of CLN8 neurobiological function. *Human Mutation* **30**, 1104–16.

Winter E. & Ponting C.P. (2002) TRAM, LAG1 and CLN8: members of a novel family of lipid-sensing domains? *Trends in Biochemical Sciences* **27**, 381–3.

Wohlke A., Philipp U., Bock P., Beineke A., Lichtner P., Meitinger T. & Distl O. (2011) A one base pair deletion in the canine *ATP13A2* gene causes exon skipping and late-onset neuronal ceroid lipofuscinosis in the Tibetan terrier. *PLoS Genetics* **7**, e1002304.

Supporting information

Additional supporting information may be found online in the supporting information tab for this article:

Figure S1 Histological detection of deposits, indicative of ceroid-lipofuscin.

Figure S2 Retina with ceroid-lipofuscin deposits in ganglion cells (arrows) shown with PAS and autofluorescence.

Figure S3 Pedigree showing the relationship between the two affected litters in Norway and Australia and potential inheritance of the mutant allele.

Figure S4 Short sequence alignments of the critical region in the parents and the affected dog.

Table S1 Human neuronal ceroid lipofuscinoses (NCL) loci and other loci and canine chromosomal positions checked for associated mutations.