Neuronal Ceroid Lipofuscinosis Type 2 in Dachshunds

JR Coates[1], KL Narfström[1], JJ Cooper[1], DP O'Brien[1], GC Johnson[2], GS Johnson[2], ML Katz[2,3]

[1]Department of Veterinary Medicine and Surgery and [2]Veterinary Pathobiology College of Veterinary Medicine, and
[3]Mason Eye Institute School of Medicine, University of Missouri, Columbia, Missouri 65211, USA

Abstract

We recently identified a new form of autosomal recessive canine neuronal ceroid lipofuscinosis (NCL) in a juvenile Dachshund and determined that this disease resulted from a mutation in *TPP1*, the canine ortholog of human *CLN2* **(Figs. 1 and 2)**. Utilizing this DNA test, we genotyped a litter of 4 dogs from carrier parents. Genotyping indicated that one puppy was homozygous for the mutant allele, two were heterozygous and one was normal homozygous. The litter was followed with physical and neurologic examinations until 10.5 months of age. Funduscopy and electroretinography (ERG) were performed at 3, 7, 8, and 10 months. Brain magnetic resonance imaging was obtained on the affected dog at 10.5 months of age.

Physical examination remained normal for all 4 dogs. Neurologic examination **(Fig. 3)** in the affected dog revealed an abrupt onset of dull mentation and cerebellar ataxia beginning at 6.5 months. Intention tremors developed by 8 months. Menace response was absent after 7 months. Pupillary light reflex was incomplete at 7 months and absent by 8.5 months. The dog was blind by 8 months. Ocular motor abnormalities included positional downbeat nystagmus by 9 months. Whole body, myoclonic jerks developed by 10 months. Scotopic ERG a- and mainly b-wave responses were reduced at 7 months. Starting at 8 months of age, the funduscopic changes **(Fig. 4)** included patches of color changes and generalized vascular attenuation. By 10 months, the scotopic ERGs were non-recordable with only low amplitude photopic responses **(Fig. 5)**. MRI **(Fig. 6)** revealed diffuse cerebral and cerebellar atrophy. Histopathology **(Figs. 7 to 9)** on the affected dog at 10.5 months revealed autofluorescent storage material in the retina, cerebellum and cerebral cortex.

Genotyping enabled us to follow the course of this disease from prior to the onset of any clinical signs. The onset of signs was surprisingly abrupt for a progressive neurodegenerative disease suggesting a threshold effect. Cerebellar and visual deficits predominated early in the disease with progressive loss of retinal function both clinically and on ERG. Declining mental status and myoclonus necessitated euthanasia at 10.5 months. Dachshunds with similar signs should be tested for the *TPP1* mutation. Since other forms of NCLs occur in a wide variety of breeds **(Fig. 10)**, NCL should be considered as a diagnosis for dogs with signs of progressive visual loss, ataxia, mentation changes, and seizures. DNA tests are available for several breeds but otherwise diagnosis can only be made at post-mortem. Cortical atrophy may be apparent on brain imaging, but this is a relatively non-specific finding.

Pedigree

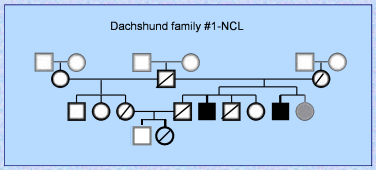

Fig. 1. Pedigree of a Dachshund family segregating NCL: open squares and circles represent clinically normal individuals; cross hatch indicates carriers; solid squares and circles represent individuals that exhibited clinical signs of NCL. DNA was not available for Dachshunds represented in gray.

Mutational Characterization

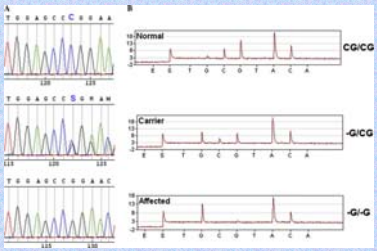

Fig. 2. Aligned sequence chromatogram from a segment of TPP1 exon 4 from a dog that is homozygous for the wild type allele (top), from the heterozygous sire of the propositus (middle), from the propositus homozygous for the c.325delC allele (bottom) **(A)**. Pyrosequegrams produced with DNA from the same three dogs **(B)**. *(Awano T et al., 2006a)*

Acknowledgments

We gratefully acknowledge Drs. T. Awano, S. Khan, I. Sohar, P. Lobel, U. Giger, L. Britt, D. Volkmann, E. Giuliano, and Ms. L. Castaner and L. Hansen. This research was supported by the University of Missouri Research Board, the College of Veterinary Medicine, and The Batten Disease Support and Research Association.

Neurologic Examination

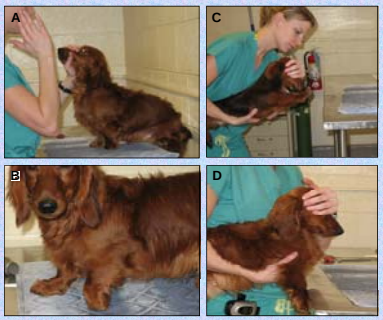

Fig. 3. Example of the neurologic examination: lack of menace response **(A)** and conscious proprioception **(B)** in affected dog; placing response in clinically normal dog **(C)** and lack of placing response in affected dog **(D)**. Other abnormal neurologic findings included cerebellar ataxia and tremor, myoclonic jerks, and cognitive dysfunction.

Ophthalmic Examination

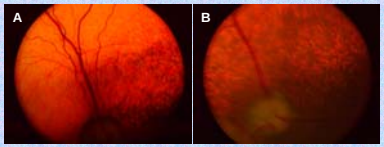

Fig. 4. External funduscopic examination of a normal **(A)** and affected dog **(B)** at 8 mos of age. CLN2 affected dog showing patches of minor color changes in the central and midperipheral fundus and generalized slight vascular attenuation.

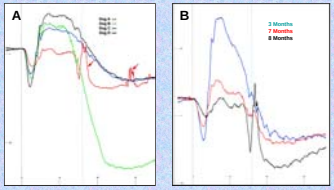

Fig. 5. ERG recordings from dogs (A-D) in litter for scotopic high intensity white light stimuli at age 8 months **(A)**; arrows indicate abnormal spikes in the affected dog (Dog A) ERG. Results from affected dog at 3, 7, and 8 mos for scotopic high intensity white light stimuli **(B)**.

Brain Imaging

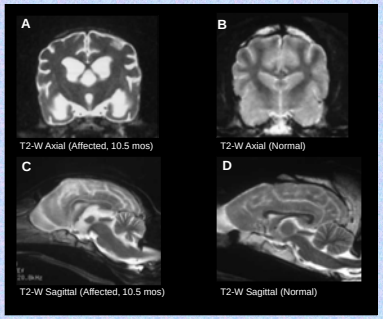

Fig. 6. Transverse MR image from a CLN2 affected dog **(A)** and normal dog **(B)** showing ventricular enlargement and sulci widening of the cerebral cortex in affected dog. Sagittal MR image from affected **(C)** and a normal dog **(D)** showing atrophy of cerebellar folia and enlargement of the third and fourth ventricle in affected dog.

Histopathology

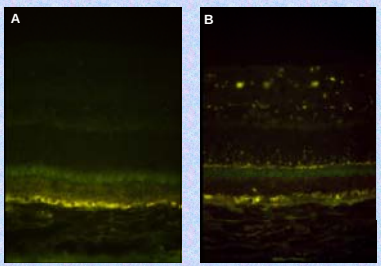

Fig. 7. Fluorescence micrographs of cryostat sections of retina from a normal **(A)** and affected **(B)** Dachshund at 10 months. Autofluorescent granules were present in retinal ganglion cells of the affected dog.

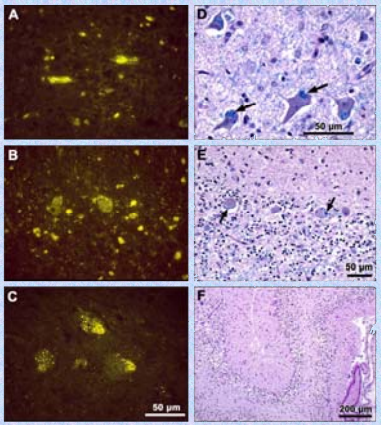

Fig. 8. Fluorescence micrographs of cryostat sections of cerebral cortex **(A)**, cerebellum **(B)**, and spinal cord **(C)** and sections of paraffin-embedded cerebral cortex **(D)** and cerebellum **(E and F)** from an affected dog. Paraffin sections were stained with Luxol fast blue and counter stained with hematoxyln and eosin. Cellular inclusions that were stained with Luxol fast blue were prominent in the cerebral cortical neurons **(D)** and cerebellar Purkinje cells. Cell loss was apparent in the cerebellar cortex **(F)**. *(Awano T et al., 2006a)*

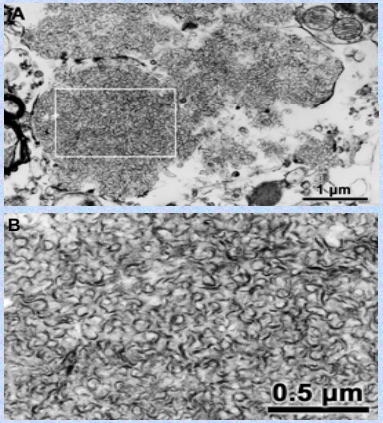

Fig. 9. Electron micrograph of storage bodies from the cerebral cortex **(A)**. Detailed electron micrograph of storage body contents of exclusively curvilinear form **(B)**. *(Awano T et al., 2006a)*

Summary of CLN2 Phenotype

- Juvenile onset, acute, progressive
- Multifocal intracranial disease
  - Asymmetric deficits at onset
  - Cerebellar ataxia
  - Blindness
  - Myoclonic activity
  - Cognitive decline
- Cerebral and cerebellar atrophy on MRI
- Diminished ERG precedes clinical blindness
- Curvilinear profiles in lysosomes

NCL Mutations Identified

Gene	Human Subtype(s)	Canine Ortholog
CLN1	INCL, LINCL, JNCL, ANCL	---
CLN2	LINCL, JNCL	Juvenile Dachshund
CLN3	JNCL	---
CLN4	ANCL	---
CLN5	LINCL Finnish variant, JNCL	Adult Border Collie
CLN6	LINCL, JNCL, other variants	---
CLN7	LINCL, Turkish variant	---
CLN8	LINCL, Northern epilepsy, Turkish variant	Adult English Setter
CSTD	Congenital NCL	Adult American Bulldog

Fig. 10. The genes responsible for NCL have now been identified in 4 breeds *(Awano T et al., 2006a; Awano T et al., 2006b; Melville SA et al., 2005; Katz ML et al., 2005)*. NCL has been reported in many other breeds including Australian Shepherds, Chihuahuas, Cocker Spaniels, Dalmatians, Miniature Schnauzers, Polish Lowland Sheepdogs, Salukis, Tibetan Terriers, Welsh Corgis, and others, but the mutations not yet identified.

Future Directions

Clinical signs and histopathology of NCLs in dogs are diverse and vary based on distribution of inclusions. The most common clinical signs of NCL are related to retinal degeneration and dysfunctions of the cerebral cortex that include altered mentation and cognitive function, and seizures. *(Mole SE et al. 2005)* Loss of coordination, head tremor and dysmetria are described. Classification is also based on age of onset and speed of progression: early onset – slow progression, young adult onset – rapid progression, and adult onset – slow progression. *(Jolly RD et al., 1994)* The later the onset of clinical signs, the more difficult it is to recognize that a disorder has a hereditary basis.

We continue to identify specific NCLs and the mutations responsible these disorders. Our approach consists of the following steps:

(1) Identify dogs with clinical signs suggestive of NCL
(2) Thoroughly characterize the disease phenotype
(3) Work with breeders to determine how widespread is the disease
(4) Determine the mode of inheritance through pedigree analyses
(5) Collect DNA and phenotype information from appropriate dogs
(6) Identify the disease mutation through a combination of candidate gene analyses and gene mapping
(7) Provide DNA testing to identify carriers and decrease the incidence

Please contact www.caninegeneticdiseases.net if you suspect NCL.

References

Awano T, et al. Mol Genet Metabol. 2006a;89:254-260.
Awano T, et al. Mol Genet Metabol. 2006b;87:341-348.
Katz ML, et al. Biochem Biophys Res Comm. 2005;327:541-547.
Melville SA, et al. Genomics. 2005;86:287-294.
Jolly RD, et al. J Sm Anim Prac.1994;35:299-306.
Mole SE, et al. Neurogenet. 2005;6:107-126.