MRI findings of neuronal ceroid lipofuscinosis in a cat

Crystal White¹, Jeremy Mortier¹, Ranieri Verin¹, Thomas Maddox¹, Rita Goncalves¹ and Daniel Sanchez-Masian¹

Abstract

Case summary A 2-year-old male domestic shorthair cat presented to the University of Liverpool Small Animal Teaching Hospital with a 2 week history of altered mentation, blindness and focal epileptic seizures. MRI examination revealed generalised cerebral and cerebellar atrophy, diffuse T2-weighted hyperintensity of the white matter and meningeal thickening. Neuronal ceroid lipofuscinosis was confirmed on post-mortem examination.

Relevance and novel information This is the first report of the MRI findings of neuronal ceroid lipofuscinosis in a cat.

Accepted: 12 January 2018

Case description

A 2-year-old, 3.4 kg male neutered domestic shorthair cat presented to the University of Liverpool Small Animal Teaching Hospital with a 2 week history of progressive weight loss, altered mentation, blindness and suspected focal epileptic seizures. Relevant history included a single visit to the referring veterinary surgeon 6 months prior to presentation for non-specific behavioural changes. On neurological examination, the cat’s mentation was obtunded and disoriented. The menace response was markedly reduced bilaterally with intact pupillary light reflexes. Jaw clattering and hypersalivation were demonstrated intermittently, consistent with focal epileptic seizure activity. Stimulus resulted in hyper-reactivity and hypertonicity of all limbs, thus hindering a comprehensive neurological examination, including ophthalmological and visual assessment. Gait analysis was not possible owing to the cat’s obtunded status and hyper-reactivity. The remaining general examination was unremarkable. Based on the clinical signs and limited neurological examination, a diffuse forebrain neurolocalisation was suspected. The main differential diagnoses for a 2-year-old cat with diffuse forebrain neurolocalisation included metabolic disease (lysosomal storage disease, thiamine deficiency, hepatic encephalopathy), infectious causes (feline infectious peritonitis, toxoplasmosis, bacterial meningitis), immune-mediated disease (meningoencephalitis of unknown origin), developmental disease (lissencephaly, microencephaly) and degenerative disease (neuroaxonal dystrophy and leukoencephalomyelopathy). Biochemistry, including fasting ammonia and preprandial bile acids, and haematology were within normal limits. Serological testing for feline leukaemia virus, feline immunodeficiency virus, feline coronavirus and toxoplasmosis were negative. Cerebrospinal fluid analysis, including total protein, total nucleated cell count and cytology, was unremarkable.

MRI of the brain was performed using a 1.5 T magnet (Philips Ingenia CX). All slices were 3 mm thick with a 0.3 mm slice gap. Sagittal, dorsal and transverse T2-weighted (T2W) images were acquired. Transverse images for fluid-attenuated inversion recovery (FLAIR), T2*W, T1-weighted before and after intravenous administration of contrast medium (gadobutrol 0.1 mmol/kg bodyweight [Gadovist;
Bayer) and proton-density weighted sequences were obtained.

On all sequences there was thinning of the cerebral and cerebellar cortices with widening of the corresponding sulci, demonstrating diffuse cortical atrophy. The corpus callosum was markedly thin and only partially visualised with absence of a visible rostrum, genu and body. Moderate, generalised symmetrical dilation of the ventricular system was present, with complete suppression of contents on FLAIR, indicating that the cerebrospinal fluid was not markedly abnormal. Mild, generalised, symmetrical and homogeneous increased T2W signal intensity of the cerebral white matter was visible, with consequent decrease in the distinction between the white and grey matter (Figure 1). The pachymeninges were mildly and diffusely thickened.

There were no regions of abnormal contrast enhancement. Generalised and marked thickening of the calvarium and osseous tentorium cerebelli was visible (calvarial hyperostosis) with reduction of the fat signal of the diploe (Figure 2). The remaining bones of the skull were normal. Given the MRI findings and the signalment of the cat the presumptive diagnosis was an inherited neurodegenerative disorder, most likely neuronal ceroid lipofuscinosis or other lysosomal storage disease.

Following the presumptive diagnosis of neurodegenerative lysosomal storage disease and worsening of clinical signs the cat was euthanased on humane grounds and underwent a full post-mortem examination. Grossly, the meninges were diffusely moderately thickened and the brain showed moderate diffuse and bilateral cortical atrophy with narrowing of gyri and widening of sulci.
Subjectively, a mild dilation of the ventricular system was observed. Sections of meninges and relevant areas of the brain and cerebellum, including motor, somatosensory, limbic, vestibular and visual system, were sampled for histopathology and transmission electron microscopy (TEM). Samples of the spinal cord at the level of the cervical and lumbosacral intumescences (C5–T1 and L3–L6, respectively) were also obtained for histopathology.

Meninges showed diffuse moderate thickening due to deposition of palely eosinophilic mature collagen interpreted as fibrosis and confirmed with Masson’s trichrome stain. All cerebral areas examined showed mild-to-moderate gliosis and moderate loss of neurons (more severe in cortical areas) with numerous neurons distended and enlarged by botryoid, palely acidophilic to glassy cytoplasmic inclusions (Figure 3b), with frequent marginalisation of nuclei. Intracytoplasmic vacuoles resulted markedly positive to Luxol fast blue stain (Figure 3c), moderately positive to periodic acid–Schiff (Figure 3d) and stained red with Masson’s trichrome (Figure 3e). When observed under a fluorescent microscope (excitation 465–495 nm) the cytoplasmic material showed green autofluorescence (Figure 3f). The cerebellum and the spinal cord were the least affected areas with well-represented neuronal cells showing few (very rare in the spinal cord) intracytoplasmic inclusions. Ultrastructural examination of the intracytoplasmic neuronal deposits in the occipital cortex showed electron-dense, membrane-bound material (Figure 3g) composed of small curvilinear lamellar stacks (Figure 3h) and electron-dense, variably sized granular material (Figure 3i), consistent morphologically with previously described intra-neuronal lipofuscins in cats.1–4 No further gross and histopathological changes were observed in the main thoracic and abdominal organs, including autonomic ganglia. Furthermore, both eyes were thoroughly examined to rule out the presence of retinal neuronal inclusions and to confirm the suspect of central blindness. Neither eye showed histopathological changes.

**Discussion**

To our knowledge this is the first report of MRI findings in a cat with confirmed neuronal ceroid lipofuscinosis. The neuronal ceroid lipofuscinoses represent a heterogeneous group of genetically determined neurodegenerative lysosomal storage diseases. The disease is characterised by abnormal accumulation of autofluorescent lipopigments within the neuronal and extraneural tissue.5 Neuronal ceroid lipofuscinosis has been described in several domestic species, including dogs, cats, cattle, sheep, goats, monkeys and mice.6–13 Clinical signs are similar in all species, including humans, and involve progressive cognitive decline, motor dysfunction, vision deficits and epileptic seizures, ultimately resulting in death or euthanasia. Symptomatic management is generally the mainstay of treatment.14 Encouraging results have, however, been seen using enzyme-replacement therapy and gene therapy.15 Carnitine is a breakdown product of subunit c protein, a major component of the storage material that accumulates in a number of neuronal ceroid lipofuscinoses. Delays in cognitive dysfunction have been reported in English Springer Spaniels supplemented long term with carnitine.15 However, despite these therapeutic advances, neuronal ceroid lipofuscinoses remain an incurable group of diseases.

The cat in this case demonstrated generalised and symmetrical brain cortical atrophy and secondary dilation of the ventricular system and intracranial subarachnoid space (hydrocephalus ex vacuo). These MRI findings have been consistently described in humans and dogs and are considered cardinal signs for neuronal ceroid lipofuscinosis.10,16–19 The cat in this report also showed moderate meningeal thickening. This finding, not described in the human form of the disease, was also reported in three Chihuahuas and a Dachshund with neuronal ceroid lipofuscinosis.9,20 An autoimmune cause of meningeal thickening has been alluded to; however, in the case of the Dachshund, a subdural haematoma was also identified and the findings of thickened meninges could represent reactive meningitis. In our case, the moderate meningeal thickening was due to the diffuse moderate hypertrophy and fibrosis, and could be interpreted as an adaptive change due to brain atrophy.
Diffuse T2W white matter hyperintensity with reduction in the white/grey matter distinction has not been previously described in the veterinary literature. A possible explanation for this could be that animals previously reported were scanned with lower field strength MRI magnets. Better contrast resolution of higher field magnets and newer MRI systems could account for this new finding. In humans, periventricular white matter T2W hyperintensity is a common finding in neuronal ceroid lipofuscinosis, confirmed as gliosis and demyelination on histology. In one study, the authors also described a diffuse increase in T2W signal intensity of the white matter. Our patient showed a thinned and partially visualised corpus callosum, which, in this case, likely resulted from the generalised brain atrophy. Atrophy of the corpus callosum has also been described in dogs and humans with neuronal ceroid lipofuscinosis. The cat in our report had generalised calvarial hyperostosis. No skeletal abnormality has been previously described in the human or animal form of the disease. We believe calvarial hyperostosis developed in response to the chronic ex vacuo negative pressure induced by the brain cortical atrophy. Gross and histopathological findings were consistent with previous cases of neuronal ceroid lipofuscinosis in cats, and confirmed the presence of lysosomal storage neuronal disease with autofluorescent material. TEM showed the presence of characteristic small curvilinear lamellar stacks and electron-dense granular material, consistent morphologically with previously described intra-neuronal lipofuscin in cats, making the diagnosis
ances in the mutations account for the variable pheno-
neuronal ceroid lipofuscinosis remain unidentified.23,25–28
orthologues to the human causative mutations.23,25–28
genes have been identified in dogs, eight of which are
central in origin.
therefore the blindness reported in this cat was likely
no lesions were observed bilaterally in the retina and
CLN9
different CLN9 gene, which remains elusive.23 The differ-
neuronal ceroid lipofuscinosis types in humans, with the exception
analysis has identified the genetic loci for the neuronal
cical course is slightly altered for each one. Molecular
lipofuscinosis (CLN1–CLN14). Traditionally and still
commonly encountered in the literature, nomenclature
was based on the age of clinical presentation, for example
infantile/juvenile/adult. The storage material, along
with its ultrastructure, is variable for each neuronal
ceroid lipofuscinosis type, and, although similar, the
clinical course is slightly altered for each one. Molecular
analysis has identified the genetic loci for the neuronal
ceroid lipofuscinosis types in humans, with the exception
of the CLN9 gene, which remains elusive.23 The differ-
ences in the mutations account for the variable pheno-
types between the types.24 Mutations in nine different
genes have been identified in dogs, eight of which are
orthologues to the human causative mutations.23,25–28
Sequencing of the exons of CLN1, CLN3, CLN5, CLN8
and CLN10 in a confirmed case of feline neuronal ceroid
lipofuscinosis failed to identify the molecular cause in
that patient.4 The genes involved in the development of feline
neuronal ceroid lipofuscinosis remain unidentified.
In humans and dogs, neuronal ceroid lipofuscinosis has been
shown to be a recessively inherited disease with the progeny of
two carrier parents having a one in four chance of developing the disease.14 A second cat,
from the same litter as the cat in this case, developed
similar clinical signs 4 weeks after its littermate and was
euthanased owing to severity of clinical signs. Histopathology and TEM confirmed neuronal ceroid
lipofuscinosis in this second cat with central nervous
system morphological changes similar to the cat
described in this case. MRI was not performed in this
second case, but pathological findings and the relation-
ship between the two littermates reinforce the hypothe-
sis of an inherited mutation in cats.3

Conclusions
Without knowledge of the causative genes involved in
feline neuronal ceroid lipofuscinosis, definitive diagnosis
is based on necropsy and histopathology results alone.
MRI findings, combined with clinical signs, are the main-
stay of the ante-mortem presumptive diagnosis. To sup-
port the use of this imaging modality in the ante-mortem
diagnosis of feline neuronal ceroid lipofuscinosis, recruit-
ment of more feline subjects with confirmed disease into
similar descriptive studies is essential in identifying dis-
tinct MRI findings within this population. Owing to the
similarities in the MRI findings in this cat and those
seen in humans and dogs it could be assumed that the imag-
ning features would be the same or similar in other cases
of feline neuronal ceroid lipofuscinosis. It would seem
prudent, therefore, to consider neuronal ceroid lipofusci-
nosis within the list of differentials when faced with a cat
showing similar neurological signs as our case, with evi-
dence of generalised cerebral and cerebellar atrophy and
diffuse T2W hyperintensity of the white matter on MRI.

Conflict of interest
The authors declared no potential conflicts of interest with respect to the research, authorship, and/or
publication of this article.

Funding
The authors received no financial support for the
research, authorship, and/or publication of this article.

References
1 Weissenbeck H and Rossel C. Neuronal ceroid-lipofuscinosis in a domestic cat: clinical, morphological and
immunohistochemical findings. J Comp Pathol 1997; 117: 17–24.
2 Kuwamura M, Nakagawa M, Nabe M, et al. Neuronal
ceroid-lipofuscinosis in a Japanese domestic shorthair
cat. J Vet Med Sci 2009; 71: 665–667.
3 Furusawa Y, Mizukami K, Yabuki A, et al. Mutational
analysis of the feline CLN3 gene and an ultrastructural
evaluation of lysosomal storage material in a cat with
neuronal ceroid lipofuscinosis: an investigation into the
molecular basis of the disease. Vet J 2012; 194: 425–428.
4 Chalkley MD, Armien AG, Gilliam DH, et al. Characteri-
zation of neuronal ceroid-lipofuscinosis in 3 cats. Vet Pathol
2014; 51: 796–804.
5 Kohlschutter A and Schulz A. Towards understanding the
neuronal ceroid lipofuscinoses. Brain Dev 2009; 31: 499–502.
6 Tammen I, Houweling PJ, Frugier T, et al. A missense
mutation (c. 184C>T) in ovine CLN6 causes neuronal
ceroid lipofuscinosis in Merino sheep whereas affected
South Hampshire sheep have reduced levels of CLN6
mRNA. Acta Biochim Biophys Pol 2006; 1762: 898–905.
7 Fiske RA and Storts RW. Neuronal ceroid-lipofuscinosis
in Nubian goats. Vet Pathol 1988; 25: 173–174.
8 Harper PA, Walker KH, Healy PJ, et al. Neurovisceral ceroid lipofuscinosis in blind Devon cattle. Acta Neuro-pathol 1988; 75: 632–636.

9 Nakamoto Y, Yamato O, Uchida K, et al. Neuronal ceroid-lipofuscinosis in longhaired Chihuahua: clinical, pathological, and MRI findings. J Am Anim Hosp Assoc 2011; 47: 4.

10 O’Brien DP and Katz ML. Neuronal ceroid lipofuscinosis in 3 Australian shepherd littermates. J Vet Intern Med 2008; 22: 472–475.

11 Bildfell R, Matwichuk C, Mitchell S, et al. Neuronal ceroid-lipofuscinosis in a cat. Vet Pathol 1995; 32: 485–488.

12 Bronson RT, Lake BD, Cook S, et al. Motor neuron degeneration of mice is a model of neuronal ceroid lipofuscinosis (Batten’s disease). Ann Neurol 1993; 33: 381–385.

13 Jasty V, Kowalski RL, Fonseca EH, et al. An unusual case of generalized ceroid-lipofuscinosis in a Cynomolgus monkey. Vet Pathol 1984; 21: 46–50.

14 Bennett M and Rakheja D. The neuronal ceroid-lipofusinoses. Dev Disabil Res Rev 2013; 17: 254–259.

15 Katz ML, Rustad E, Robinson GO, et al. Canine neuronal ceroid lipofuscinosis: a promising model for preclinical testing of therapeutic interventions. Neurobiol Dis 2017; 108: 277–287.

16 Jarvela I, Autti T, Lamminranta S, et al. Clinical and magnetic resonance imaging findings in Batten disease: analysis of the major mutation (1.02-kb deletion). Ann Neurol 1997; 42: 799–802.

17 D’Incerti L. MRI in neuronal ceroid lipofuscinosis. Neuroradiol Sci 2000; 21: S71–S73.

18 Autti T, Ranininko R, Vanhanen SL, et al. MRI of neuronal ceroid lipofuscinosis. Neuroradiol 1996; 38: 476–482.

19 Koie H, Shibuya H, Sato T, et al. Magnetic resonance of neuronal ceroid lipofuscinosis in a Border collie. J Vet Med Sci 2004; 66: 1453–1456.

20 Asakawa M, G, Mackillop E, Olby NJ, et al. Neuronal ceroid lipofuscinosis with a chronic subdural hematoma. Vet Radiol Ultrasound 2010; 51: 155–158.

21 Rieger D, Auerbach S, Robinson P, et al. Neuroimaging of lipid storage disorders. Dev Disabil Res Rev 2013; 17: 269–282.

22 Hasegawa D, Tamura S, Nakamoto Y, et al. Magnetic resonance findings of the corpus callosum in canine and feline lysosomal storage diseases. PLOS One 2013; 8: 12.

23 Nita DA, Mole SE and Minassian BA. Neuronal ceroid lipofuscinosis. Epileptic Disord 2016; 18 Suppl 2: S73–S88.

24 Mole SE, Williams RE and Goebel HH. Correlations between genotype, ultrastructural morphology and clinical phenotype in neuronal ceroid lipofuscinoses. Neurogenetics 2005; 6: 107–126.

25 Sanders DN, Farias FH, Johnson GS, et al. A mutation in canine PPT1 causes early onset neuronal ceroid lipofuscinosis in a Dachshund. Mol Genet Metab 2010; 100: 349–356.

26 Melville SA, Wilson CL, Chiang CS, et al. A mutation in canine CLN5 causes neuronal ceroid lipofuscinosis in Border collie dogs. Genomics 2005; 86: 287–294.

27 Katz ML, Khan S, Awano T, et al. A mutation in the CLN8 gene in English Setter dogs with neuronal ceroid lipofuscinosis. Biochem Biophys Res Commun 2005; 327: 541–547.

28 Wohlke A, Philipp U, Bock P, et al. A one base pair deletion in the canine ATP13A2 gene causes exon skipping and late-onset neuronal ceroid lipofuscinosis in the Tibetan terrier. PLoS One 2001; 7: 10.