

## Neuronal Ceroid-lipofuscinosis in a Japanese Domestic Shorthair Cat

Mitsuru KUWAMURA<sup>1)</sup>, Masanori NAKAGAWA<sup>2)</sup>, Mikoto NABE<sup>1)</sup>, Jyoji YAMATE<sup>1)</sup>, Michito INOUE<sup>2)</sup>, Hiroyuki SATOH<sup>3)</sup> and Osamu YAMATO<sup>4)</sup>

<sup>1)</sup>Laboratory of Veterinary Pathology, Osaka Prefecture University, Sakai, Osaka 599–8531, <sup>2)</sup>Matsubara Animal Hospital, Taijyo, Matsubara, Osaka 580–0044, <sup>3)</sup>Laboratory of Internal Medicine, Department of Veterinary Clinical Sciences, Graduate School of Veterinary Medicine, Hokkaido University, Sapporo 060–0818 and <sup>4)</sup>Laboratory of Clinical Pathology, Department of Veterinary Clinical Sciences, Faculty of Agriculture, Kagoshima University, Kagoshima 890–0065, Japan

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**ABSTRACT.** A female Japanese domestic shorthair cat showed gait abnormality at 5 months of age, and head shaking and tremor became apparent from 6 months of age. Serum biochemistry at 13 months of age revealed markedly elevated ALT and ALP. The cat died at 16 months of age. Histopathologic examination revealed prominent cytoplasmic swelling of neurons with accumulation of yellowish pigments. The storage pigments stained positively with periodic acid Schiff reaction, Schmorl method, and Oil red O stain. Ultrastructurally, the neuronal storage consisted of aggregates of dense materials, similar to the granular osmiophilic deposits in infantile ceroid-lipofuscinosis in humans. Hepatocytes were markedly swollen and contained faintly eosinophilic inclusion. To our knowledge this case is the sixth case of feline ceroid-lipofuscinosis, which is characterized by granular osmiophilic dense bodies in the neurons and prominent involvement of hepatocytes.

**KEY WORDS:** ceroid-lipofuscinosis, hepatocyte lesion, ultrastructure.

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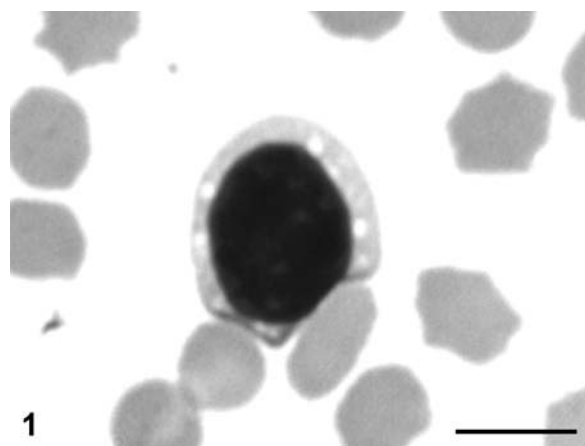
Neuronal ceroid-lipofuscinosis (NCL) is a heterogeneous metabolic disease characterized by an abnormal lipopigment accumulation in the neurons and glial cells [5, 6]. Accumulated materials show autofluorescence, and subunit c of mitochondrial adenosine triphosphate (ATP) synthase or sphingolipid activator proteins (SAPs) were detected in the lysosomes of neurons of NCL [8, 10]. Therefore, it is quite likely that the pathogenesis may involve mitochondrial abnormality rather than a primary defect of lysosomal catabolism. NCLs have been described in many animal species such as dogs [4, 5, 7] and cats [1, 3, 9, 13]; canine cases have most frequently been reported. In many animal cases, storage materials were exclusively found in the central nervous system (CNS) [6], however, storage materials also showed a systemic distribution with a heavy accumulation in the smooth muscle; the intestine and other organs have a brown discoloration [4]. This report describes the clinical and pathological findings of a feline NCL which involves the hepatocyte.

A female Japanese domestic shorthair cat was presented to the private veterinary hospital with gait abnormality at 5 months of age. Head shaking and tremor became apparent from 6 months of age and the cat was presented to another private veterinary hospital. Blood smear examination revealed cytoplasmic vacuoles in some lymphocytes (14%) (Fig. 1). The tremor gradually worsened and the cat exhibited recumbency, convulsion and constricted pupil from 13 months of age. Serum biochemistry at 13 months of age revealed markedly elevated ALT (180 U/l; normal range 0–

80), ALP (321 U/l; normal range 10–44), and CK (>2000 U/l; normal range 30–300). At 14 months of age, the cat showed abnormal nystagmus and difficulty in urination. The cat died at 16 months of age and necropsy was conducted.

At necropsy, hematoid contents were found throughout the alimentary tract, being especially prominent from the ileum to colon. Congestive pulmonary edema was noted and foamy fluid was occupied in the lumen of the bronchus and bronchiolus. Severe congestion was found in the liver and kidneys.

Light microscopic examination revealed prominent cytoplasmic swelling of neurons (Fig. 2) throughout the CNS and swollen neurons possessed yellowish pigments in the



1

Fig. 1. Blood smear image in the present feline case of ceroid-lipofuscinosis. Note cytoplasmic vacuoles in lymphocyte. Bar=10  $\mu$ m.

\* CORRESPONDENCE TO: KUWAMURA, M., Laboratory of Veterinary Pathology, Osaka Prefecture University, Sakai, Osaka 599–8531, Japan.  
e-mail: kuwamura@vet.osakafu-u.ac.jp

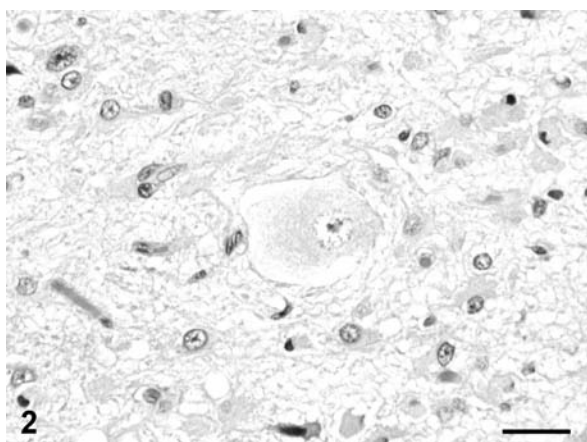


Fig. 2. Cerebral cortex. Swollen neurons with storage material in the cytoplasm. HE. Bar=25  $\mu$ m.

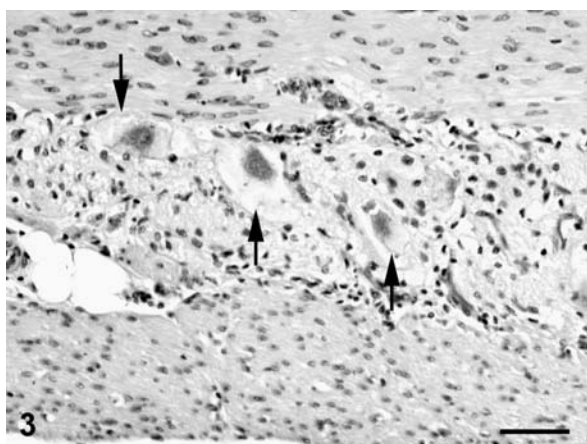


Fig. 3. Auerbach's plexus in the ileum. Swollen neurons with storage material in their cytoplasm (arrows). HE. Bar=50  $\mu$ m.

cytoplasm. The deposition of pigments was prominent in the neurons of the cerebral cortex, the pyramidal cells of the hippocampus, and neurons of the cerebellar nuclei and medulla oblongata. Neurons in the retina and in the submucosal plexus of the intestine also showed swelling with the pigment deposits (Fig. 3). A neuronal loss was evident throughout the CNS and especially severe in the cerebral cortex, and Purkinje and granule cells in the cerebellum. A few remaining Purkinje cells had many cytoplasmic pigments. Prominent astrogliosis consisting of gemistocytes was observed throughout the gray matter of the CNS and a laminar gliosis was often found in the cerebral cortex. Reactive Bergmann glial cells were present adjacent to the depleted Purkinje cell zone. Yellowish pigment-laden macrophage infiltration was observed in the neuropil of the CNS while considerable number of foamy macrophages was seen around the blood vessels in the cerebrum.

The storage pigments stained positively with periodic acid Schiff reaction (PAS) and Schmorl method for lipofus-

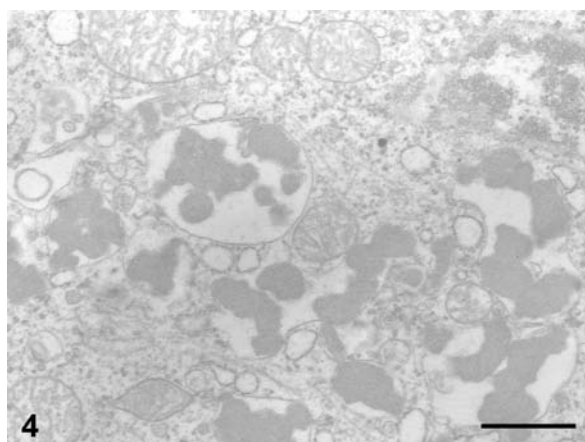


Fig. 4. Electron microscopy of the storage materials in the neuron. Aggregates of globule or irregularly shaped dense materials surrounded by a unit membrane. Bar=1  $\mu$ m.

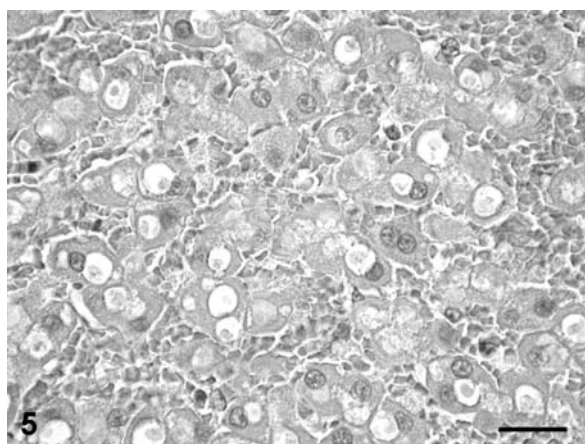


Fig. 5. Liver. Swollen hepatocytes containing eosinophilic inclusions with surrounding halo. HE. Bar=25  $\mu$ m.

cin. Oil red O stain for lipid demonstrated positive reactions in the neurons and macrophages in both frozen and paraffin-embedded sections. Fluorescence microscopic examination revealed autofluorescent granules in the swollen cytoplasm of the affected neurons and macrophages.

Ultrastructurally, the storage materials in the neurons consisted of aggregate of globule or irregularly shaped dense materials surrounded by a unit membrane (Fig. 4). These ultrastructural characteristics are similar to the granular osmiophilic deposits (GROD) found in the infantile onset form of ceroid-lipofuscinosis in human [8].

Hepatocytes were markedly swollen and contained faintly eosinophilic inclusions with halo in the HE stained section (Fig. 5). In the epon-embedded toluidin blue stained section, hepatocytes contained various sizes of clear vacuoles without halo, suggesting the halo found in the HE stain was an artifact during processing of tissue preparation. Ultrastructurally, these inclusions in the hepatocyte con-

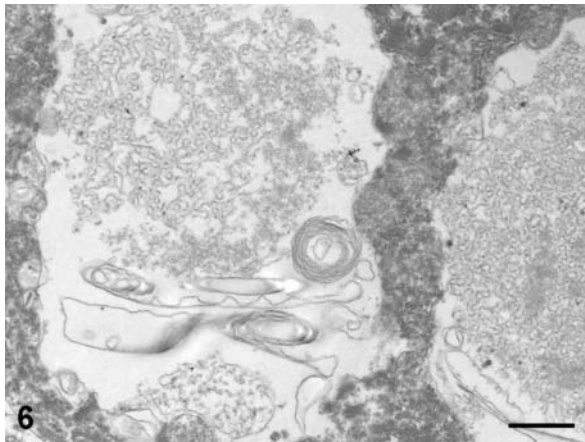


Fig. 6. Electron microscopy of the storage materials in the hepatocyte. Aggregations of curvilinear bodies and occasional cytoplasmic lamellar structures. Bar=1  $\mu$ m.

sisted of aggregations of curvilinear bodies and occasional cytoplasmic lamellar structures (Fig. 6).

Kupffer cells in the liver possessed yellowish pigments in the cytoplasm while foamy macrophages were observed frequently in the peripheral lymph nodes and lymphoid system in the alimentary tract.

Vacuoles were found in some lymphocytes on blood smears, however no obvious vacuoles were detected in the paraffin-embedded sections. This may reflect the differences of processing procedures. The presence of vacuoles in the smear samples would be valuable for clinical diagnosis of this storage disease.

The storage materials in the present case showed staining and fluorescent properties similar to those of ceroid and lipofuscin. The ceroid-lipofuscinoses are divided into two categories according to the presence or absence of subunit c storage and the subunit c forms of ceroid-lipofuscinosis are best classified separately and by descriptive clinical names [6]. In the infantile onset form in humans, the dominant storage material is SAPs A and D rather than subunit c [6, 12]. This form of ceroid-lipofuscinosis characterized by GRODs, the absence of subunit c, and the accumulation of SAPs has been reported in Miniature Schnauzer dogs with an adult onset [11]. Although we have not investigated the accumulation of SAPs and subunit c, the present cat had a granular electron-dense ultrastructure in the affected neurons, similar to GRODs.

Lipopigment storage in the mononuclear phagocyte system has been reported in a Japanese domestic cat [9]. In addition to the mononuclear phagocyte system, accumulated materials were also distributed in the hepatocytes in the present cat. Elevated serum ALT and ALP levels seem to be related to a hepatic dysfunction caused by abnormal proteolipid accumulation. A high activity of serum CK may be due to bedsores at the end of this disease. Electron microscopic findings of the storages have different structures

between the neurons and hepatocytes; the hepatocyte contained both curvilinear and membranous bodies, while the neurons mainly possessed dense bodies. Extensive storage in hepatocytes has been reported in some cases of adult ceroid-lipofuscinosis in humans [2]. Granular materials in the liver have been found in the miniature Schnauzer dog with accumulation of SAPs [11]. The presence of these different ultrastructures in this cat may reflect a heterogeneity of the accumulations.

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