NEUROPATHOLOGY

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Neuropathology Education

A case of a girl with poor school achievement, ataxia and neurological deterioration

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CLINICAL HISTORY

The patient[1] was born uneventfully at 40 weeks gestation and weighed 3000 g. She was able to walk alone at the age of 12 months and to speak several words at 10 months. Her developmental milestones were normal until the age of 6 years. After she entered elementary school, she had difficulty keeping up with school studies. At the age of 9, her school achievement became worse. In addition, she showed slurred speech and was prone to falls. Incontinence, drooling and memory disturbance were apparent at the age 13 years, and thus she was referred to our clinic.

On examination, she showed no abnormalities in the chest and abdomen, which included no hepatosplenomegaly. Neurological examination revealed alternating exotropia, scanning speech, bilateral pes excavatus, increased deep tendon reflexes in the lower extremities and no pathologic reflexes. She also had finger oscillation in the finger-to-finger test and the finger-to-nose test, truncal instability during one-leg standing and a positive Romberg test. We interpreted these findings as cerebellar symptoms and funiculus posterior symptoms of the spinal cord. MRI showed atrophy of the cerebellar vermis (Fig. 1). She was diagnosed as having spinocerebellar degeneration transiently and received a close follow-up in the out-patient clinic. Her electroencephalography turned abnormal at the age of 14, and she was administered an anticonvulsant although no seizure attacks occurred. At age 15, her cerebellar symptom aggravated, deep tendon reflexes increased markedly, and she suffered a dystonic posture. She showed athetotic movements in her neck and upper extremities and disturbance of vertical eye movements. She was admitted to our clinic again.

Laboratory examination revealed no abnormalities in complete blood count, chemistry, WBC, CSF, urine and lysosomal enzyme assay, including arylsulfatase A, beta-galactosidase, beta-hexosaminidase, alpha-mannosidase, alpha-galactosidase, beta-glucronidase, alpha-glucosidase and beta-glucosidase. We detected no CAG repeat in the DNA analysis. Motor nerve conduction velocity was 51.7 m/sec in the median nerve and 41.9 m/sec in the peroneal nerve. Fundoscopy showed no abnormalities. Her intelligence quotient was 40 by the Wechsler Intelligence Scale for Children – Revised.

After discharge from the hospital, her illness progressed relentlessly. She lost the ability to walk independently at the age of 16. Generalized tonic-clonic convulsions commenced. At age 17, she had affective incontinence and dysphasia and underwent gastrostomy. Her neurological symptoms, such as involuntary movements and pyramidal tract signs, aggravated further, and she became bedridden. At age 19, she received a tracheostomy owing to the sticking of sputum to her throat and frequent pneumonia. She expired at the age of 20 years.

PATHOLOGICAL FINDINGS

We detected sea-blue histiocytes in bone marrow aspirations at the age of 15 years (Fig. 2). This finding became the clue of the diagnosis. Acid sphingomyelinase activities were 22.01 ± 2.02 nmol/mg protein in this patient, compared with 0.53 ± 0.092 in a Niemann–Pick disease type A patient and 71.34 ± 9.74 in normal controls. On the other hand, filipin staining was positive in the patient's cultured skin fibroblasts (Fig. 3). This patient showed preserved sphingomyelinase activities and an accumulation of cholesterol in skin fibroblast cells. The diagnosis was made to her and her family at age 15 years.

At autopsy, each visceral organ such as liver, spleen, bone marrow, kidney and nerve cells of the intestine showed foam cells, which contained accumulated materials. Neuropathologically, the cerebrum weighed 760 g, the cerebellum weighed 114 g and the brain stem weighed 18 g. The total brain weighed less than the total expected weight

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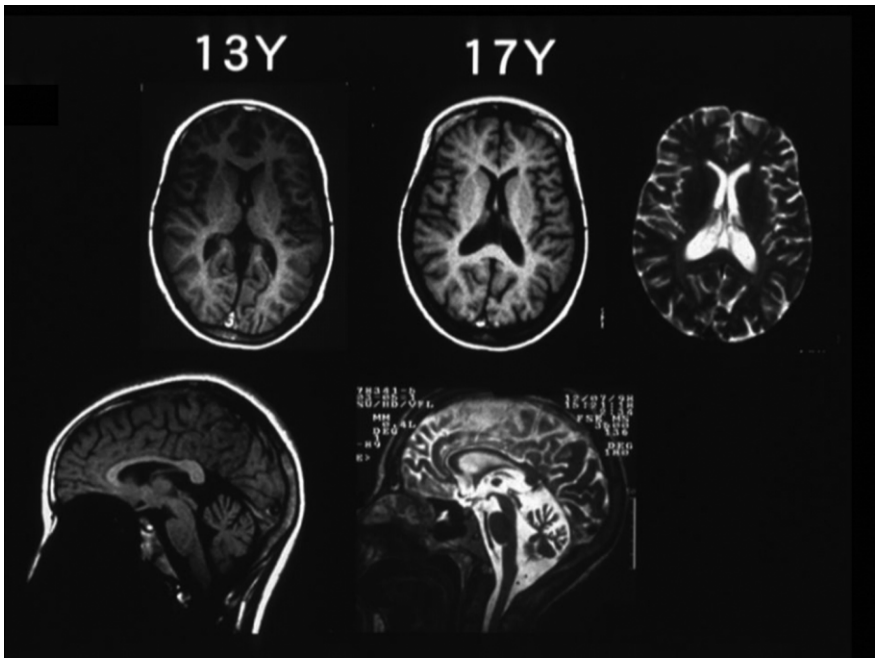

Fig. 1 MRI. Left top: T1-weghted axial image; left bottom: T1-weighted sagittal image at patient age 13 years. Both show cerebellar atrophy. Middle top: T1-weighted axial image; middle bottom: T2-weighted sagittal image and right: T2-weighted axial image. All at the patient age of 17 years, these images demonstrate progressive cerebellar atrophy and no abnormal intensity alterations despite the presence of mild atrophy in the cerebrum.

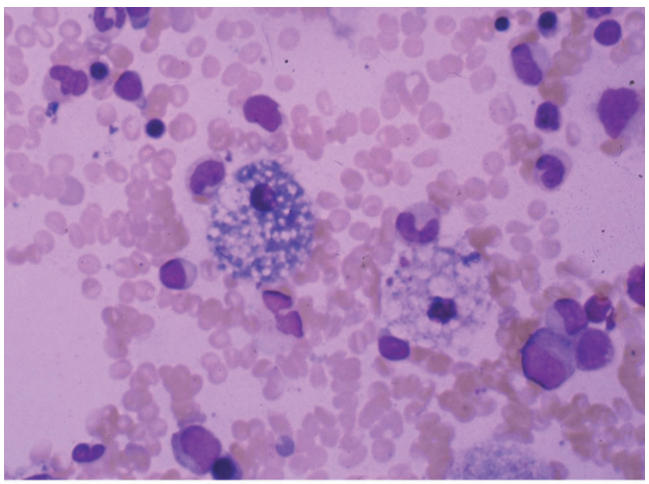

Fig. 2 Bone marrow film. Wright-Giemsa stain. Sea-blue histiocytes (Niemann–Pick cells) are observed in the bone marrow aspiration.

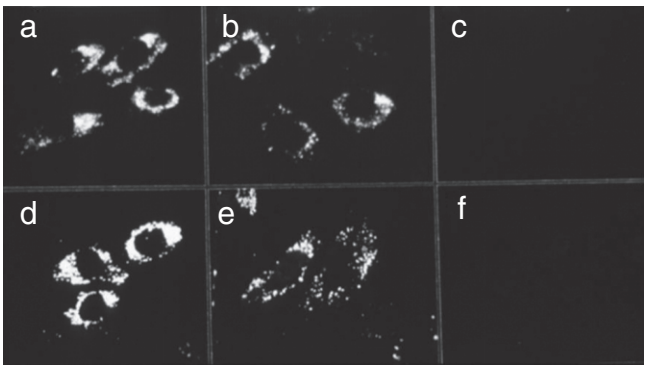

Fig. 3 Filipin staining of skin fibroblast cells. Left: positive control of Niemann–Pick disease type C; middle: this case; right: negative healthy child control. Top panels: filipin stain; bottom panels: GM2 stain. Fibroblast cells of this case are positive for both filipin and GM2 staining.

of 1400 g. There were neuronal loss and gliosis in the entire brain, which were remarkable in the dentate nucleus and Purkinje cells of the cerebellum, and in the dorsal nucleus more than medial nucleus of the thalamus. Neuronal ballooning was observed in all regions and was particularly prominent in the cerebral cortex, especially in layers 4, 5 and 6 where remarkable gliosis accompanied the hippocampus, the substantia nigra, the tegmental nuclei in the brain stem (Fig. 4) and the anterior horn cells of the spinal cord. Electron microscopic examination revealed many polymorphous membranous cytoplasmic bodies and dense osmiophilic inclusions in the ballooning neuronal cells (Figs 5, 6). Many spheroid bodies formed by axonal swelling were seen in the globus pallidus, thalamus, substantia nigra, tegmental nuclei of the brain stem and in the gray matter of the spinal cord. Although gliosis was minimal and the myelin structure was preserved in the cerebral white matter, remarkable gliosis was seen in the cerebellar white matter detected by GFAP staining.

Neurofibrillary tangles (NFTs) were observed in the neurons of the hippocampus, which were positive for tau antibodies (AT8 and PHF1) (Fig. 7) and α-synuclein (Fig. 8), whereas antibodies for amyloid β protein and ApoE4 by immunohistochemical staining were negative. Lewy bodies were not identified in the substantia nigra because of destructive cytoplasmic lesions caused by the remarkable ballooning.

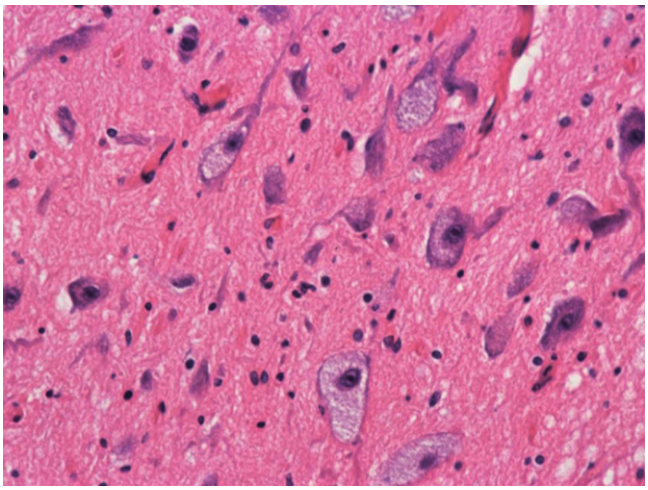

Fig. 4 Balloon neuron in the dorsal vagus nucleus. HE stain. Remarkable balloon neurons are seen.

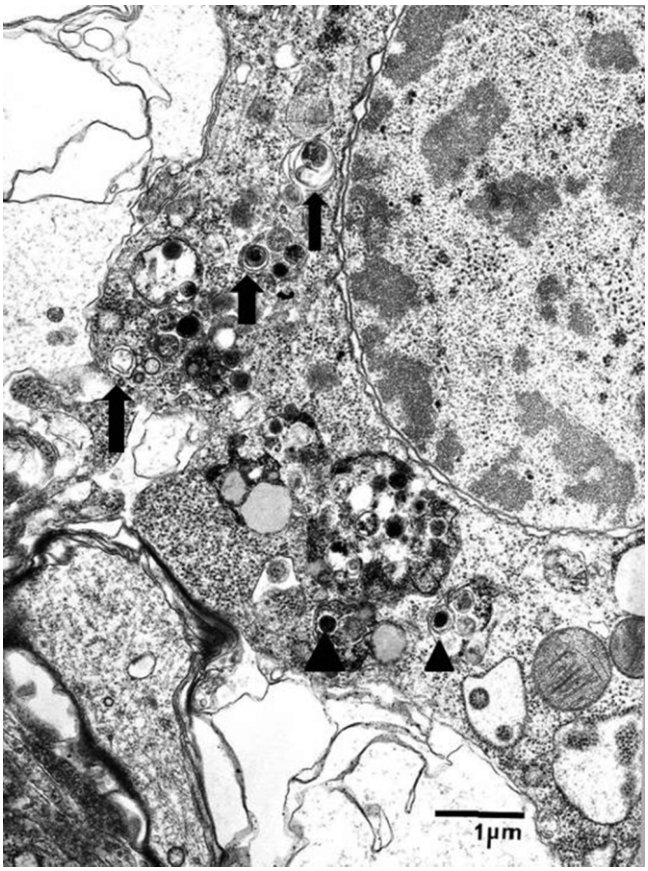

Fig. 5 Electron micrograph in the neuron of the cerebral cortex. Electron microscopic examination shows many polymorphous membranous cytoplasmic bodies (↑) and dense osmiophilic inclusions (▲) in the cytoplasm.

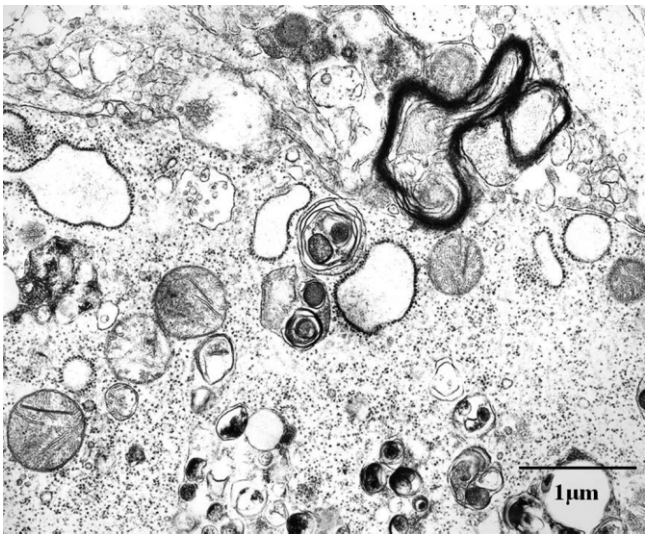

Fig. 6 Higher magnification of an electron micrograph in the neuron of the cerebral cortex. Typical polymorphous membranous cytoplasmic bodies (center) are detected.

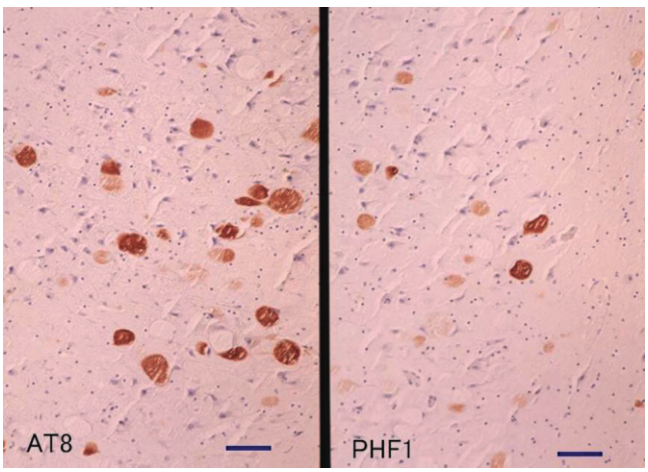

Fig. 7 Immunohistohemical staining using anti-tau antibodies in the pyramidal neurons of the hippocampus. Left: tau antibody AT8, Right: tau antibody PHF1. (bar = 100 μm)

DIAGNOSIS

We diagnosed the patient as Niemann–Pick disease type C (NPC), juvenile form.

DISCUSSION

Niemann–Pick disease includes a heterogenous group of lysosomal lipid storage diseases. Patients with type A have severe neural and visceral symptoms, and patients with type B have chronic visceral symptoms, due to deficient sphingomyelinase activity. NPC is a subacute progressive neurodegenerative disorder with cholesterol and sphingolipid accumulation in endosomes (E) and lysosomes (L)

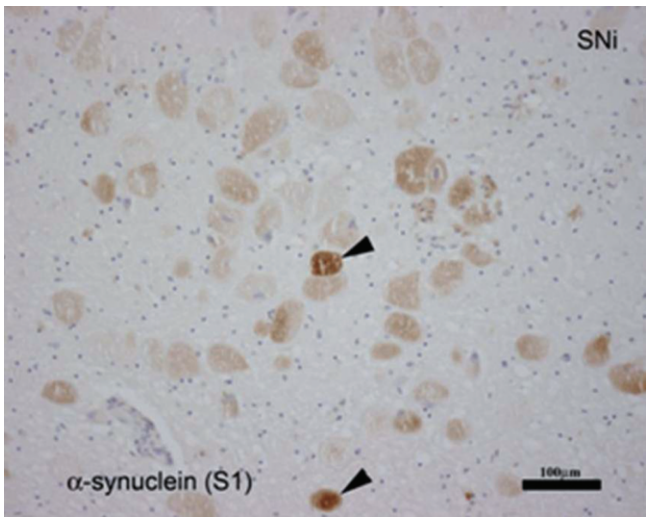

Fig. 8 Immunohistochemical staining using a polyclonal antibody against α-synuclein in the neurons of the substantia nigra. (bar = 100 μm)

of neurons due to erroneous cholesterol trafficking. Currently, the clinically identical forms of NPC disease are caused by defects in either of two different proteins. Ninety-five percent of NPC cases arise from mutation of the protein NPC1 (gene locus 18q11-q12), and the remainder by mutation of the protein NPC2 (gene locus 14q24.3).[2] The former is a large transmembrane protein localized to the endosomal membrane, the latter is an intra-lysosomal soluble protein which binds cholesterol with high affinity.[3]

Neurological symptoms in younger children begin with cerebellar ataxia and older children present with learning difficulties. Dystonia and other basal ganglia symptoms appear commonly, and myoclonic movements, dystonia and tonic-clonic seizures also are observed. A supranuclear paralysis of vertical gaze is observed in later life and is the characteristic phenomenon of the disorder. Neuropathologically, these symptoms are derived from neuronal involvements of disease progression. Remarkable gliosis was seen in the cerebellum and neuronal storage material was found in the basal ganglia in our case, which may explain the correlation with neurological symptoms.[2] Fortunately, we were able to make a diagnosis during the patient's lifetime through the observation of sea-blue histiocytes in the bone marrow aspirate, which were called "Niemann–Pick cells" and contain accumulated materials.

We detected a gene abnormality: a 2-bp deletion in exon 1 of the NPC1 gene located at 18q11-q12. This deletion leads to a frame shift, resulting in deficient NPC1 protein.[4,5] Zervas *et al*. proposed "a trafficking defect theory".[6] In normal neurons, gangliosides and cholesterol are synthesized in the Golgi apparatus and endoplasmic reticulum (ER), respectively, and are transported to the plasma membrane. They are endocytosed to early endosomes, and trafficked to late endosomes (LE) and lysosomes (L). Some of the unesterized cholesterol is hydrolyzed in L and is recycled to the ER. In NPC, aberrant NPC1 vesicles are unable to clear gangliosides or unesterized cholesterol from LE and L. Consequently, these lipids accumulate. Co-localized storage of gangliosides and cholesterol in an expanded LR and L system can disturb neuronal function and contribute to the clinical phenotype.

In the present case, we observed abnormal structures such as membranous cytoplasmic bodies (MCB) and dense bodies in the lysosomes by electron microscopic examination. MCB were mimic those of GM2 gangliosidosis, but ultrastructurally accumulated materials seemed more heterogeneous and more polymorphic in this disorder than gangliosidosis. Consequently, it is appropriate for these ultrastructural findings of this disorder to be termed "polymorphous membranous cytoplasmic bodies".

In addition, the findings such as NFTs and tauopathy were obtained in the hippocampal neurons, which suggests an interesting correlation between cholesterol metabolism and Alzheimer's disease (AD), although amyloid β protein deposits were not detected in this case.[7] NFTs consist of paired helical filaments (PHFs). The PHF-tau in NPC brains is reported to be indistinguishable from PHF-tau in AD brains. Thereafter similar mechanisms may induce PHF-tau in NPC and AD.[8] Saito *et al*. described α-synucleopathy as being detected in association with tauopathy in 9/12 NPC1 patients; further, the intensity of immunoreactivity for α-synuclein increased in subjects with the apoE ε4 allele. The defect in intracellular cholesterol trafficking in NPC1 may provoke aberrant phosphorylation of α-synuclein and tau, and this phosphorylation is enhanced by the ApoE ε4 allele.[9] Intracellular high-level cholesterol is suggested to be a risk factor in CNS pathophysiology associated with the development of neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease.[3] It is unknown why cholesterol may bind Aβ leading to blocking the action of α-secretase, and also the machanisms of apoE ε4 still remain to be investigated.

According to recent studies by Lloyd–Evans *et al*., a number of NPC1-deficient cell lines point to a major role for sphingosine. Sphingosine storage is an initiating factor in NPC1 disease, when it inhibits calcium uptake into the LE and L. Calcium entry occurs through ATP-dependent processes. Reduction of calcium in LE can lead to defective endocytosis and subsequent storage of lipids such as cholesterol, sphingomyelin and glycosphingolipids. The authors also suggest curcumin as a therapeutic tool. Curcumin is a weak endoplasmic reticulum ATPase antagonist and compensates for lack of calcium release from the LE by elevating calcium levels in the cytosol. Curcumin is

expected to normalize trafficking in NPC1 patients. Consequently, curcumin-treated NPC1 mice survived 3.5 weeks longer than controls.[10] The mechanisms by which calcium regulates cholesterol transport from the lysosomes require further investigation.[3]

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