

Neuropathology Education

A 2-year-old boy with hypoactivity of neonatal onset and profound developmental delay

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CLINICAL HISTORY

This male patient was born at 38 weeks after an uneventful pregnancy. Delivery was complicated by fetal bradycardia lasting for 10 min. Immediately after birth, he had respiratory distress, and underwent mechanical ventilation for 10 days. Facial expression and body movements were decreased. Although he followed objects with his eyes and smiled socially at 2 months of age, no further development was noted thereafter. At 5 months of age, he started to have epileptic seizures, and was admitted under the diagnosis of West syndrome. On admission, he was lethargic and somnolent with hypothermia and poor sucking. Series of tonic spasms were seen despite treatment with anti-epileptic drugs. The skin was whitish and eczematous, and the hair was short, sparse, thin, twisted and hypopigmented. The muscles were hypotonic with a frog-leg posture and an absence of head control. On biochemical examination of the blood, the serum levels of copper and ceruloplasmin were low. Multifocal spikes and spike-waves were seen on electroencephalography. Radiography revealed wormian bones in the skull, generalized osteoporosis, anterior flaring of the ribs, and metaphyseal spurring of the limb bones. Copper assay of cultured skin fibroblasts was abnormally high. DNA analysis of the *ATP7A* gene disclosed a C to T mutation at codon 3101 (Arg986X).

From 7 months of age, he was treated with s.c. injection of copper histidine. The serum copper and ceruloplasmin levels soon normalized, and his hair became straight and black. However, other systemic and neurologic symptoms showed no improvement, with persistence of epileptic sei-

zures and absence of developmental milestones. At 1 year and 3 months of age, he suffered from pneumonia, and transiently became critically ill because of ventricular fibrillation. Ultrasonography showed the presence of multiple diverticula in the urinary bladder. Subsequently he suffered from recurrent respiratory and urinary tract infections, and multiple bone fractures. At 2 years and 9 months of age, he died of massive hemorrhage from the urinary bladder.

NEURORADIOLOGIC FINDINGS

Cranial CT at 3 months of age showed a cystic lesion in the left putamen, and slight hypodensity of the bilateral thalami. At 5 months, the bilateral temporal lobes became swollen and markedly hypodense. On cranial magnetic resonance imaging (MRI), signal intensity of the temporal lobes was low on T1-weighted and fluid-attenuated inversion recovery images, and high on T2-weighted images (Fig. 1a). There was a subdural hematoma over the left frontal lobe. The cerebral arteries had excessive tortuosity, confirmed by magnetic resonance arteriography. At 7 months (prior to the onset of copper treatment), the temporal lobes showed spontaneous disappearance of the abnormal intensity, and became atrophic (Fig. 1b). During the subsequent period, there was progressive brain atrophy (Fig. 1c).¹

PATHOLOGICAL FINDINGS

The patient's brain was small, weighing 690 g (average, 1100 g). On gross examination, the cerebrum, brainstem and cerebellum were diffusely atrophic. Leptomeninges around the bilateral Sylvian fissures was thickened, and the blood vessels were tortuous. On sectioning, the cerebral and cerebellar white matter was reduced in volume. There was a cystic infarct of the left putamen, 10 × 4 mm in size. The thalamus and medial temporal lobe had brownish discoloration (Fig. 2).

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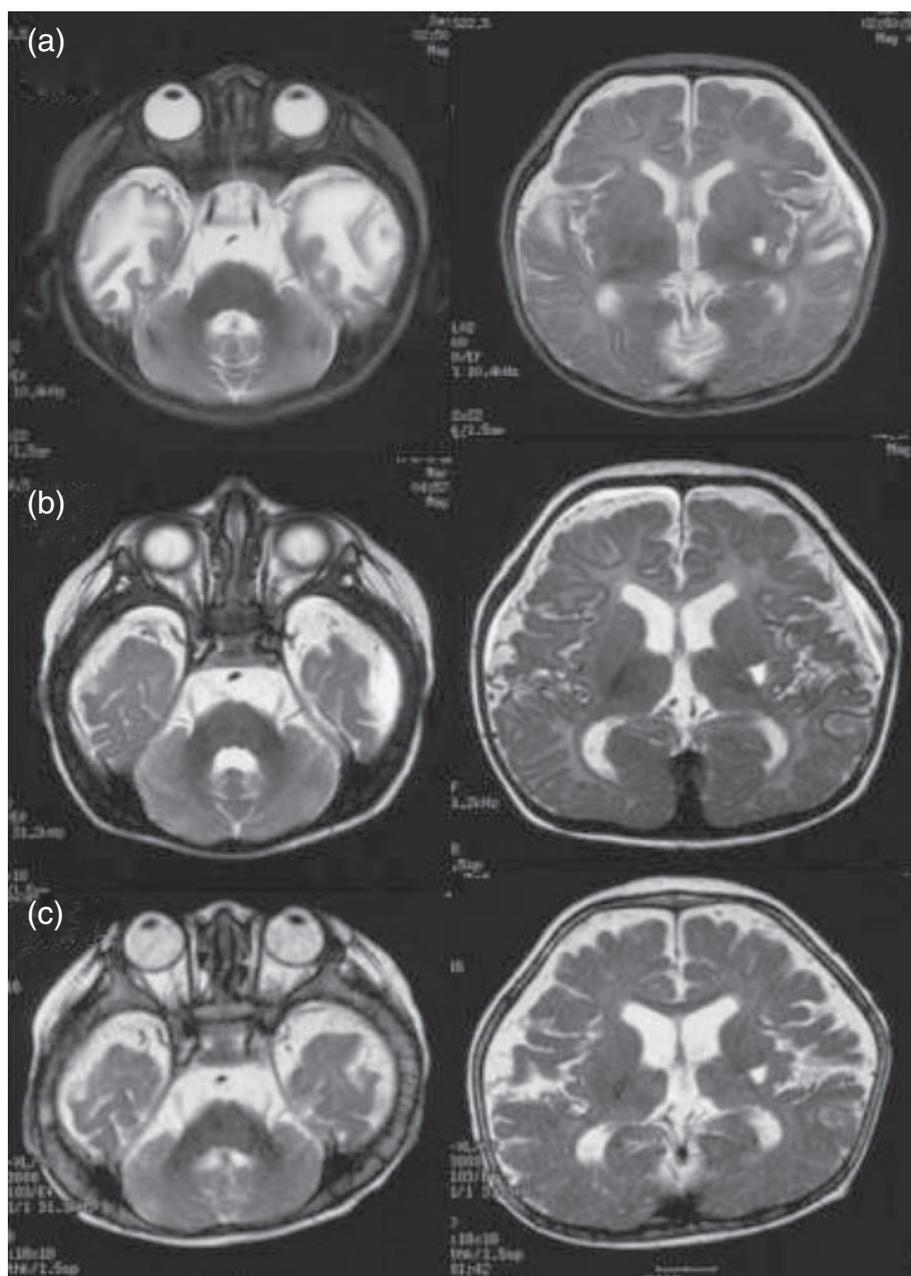


Fig. 1 Serial cranial MRI findings (T2-weighted images). (a) At 5 months of age there was a small cystic infarct in the left putamen. The bilateral temporal lobes had marked T2 prolongation of both the gray and white matter. (b) At 7 months of age the temporal lobes became mostly isointense and atrophic. (c) At 13 months, diffuse brain atrophy became more prominent. Myelination was severely delayed.

Histologically, the cerebral white matter showed spongy change with loss of myelin sheaths and axons, varying in degree from region to region. The temporal and frontal lobes were most severely affected, whereas the corpus callosum was spared. Fibrillary gliosis was only minimal. In the cortical gray matter overlying the severely affected white matter, there was mild atrophy and neuronal loss. The cerebral arteries were tortuous, ectatic, and thin-walled. In the left putamen, such an artery was surrounded by an old cystic infarct rimmed by gliotic tissue (Fig. 3a). Several tiny old infarcts were also noted in the right putamen, caudate head and periventricular white matter, some showing calcification. In the thalamus, there was neuronal

loss, astrogliosis and rarefaction of perivascular cerebral tissue (Fig. 3b), showing marked regional difference in their severity.

The cerebellum had cortical atrophy involving both the gray and white matter. In the cortical gray matter, there was marked rarefaction of the molecular layer, a moderate loss of Purkinje cells with abnormal arborization of apical dendrites (“weeping willow” appearance), growth of perisomatic dendrites (somal sprouts) and swelling of proximal axonal segments (torpedoes), and a severe loss of granule cells (Fig. 4). In the cerebral white matter, there was moderate to severe loss of myelin sheaths and axons, associated with fibrillary gliosis and/or rarefaction. The dentate

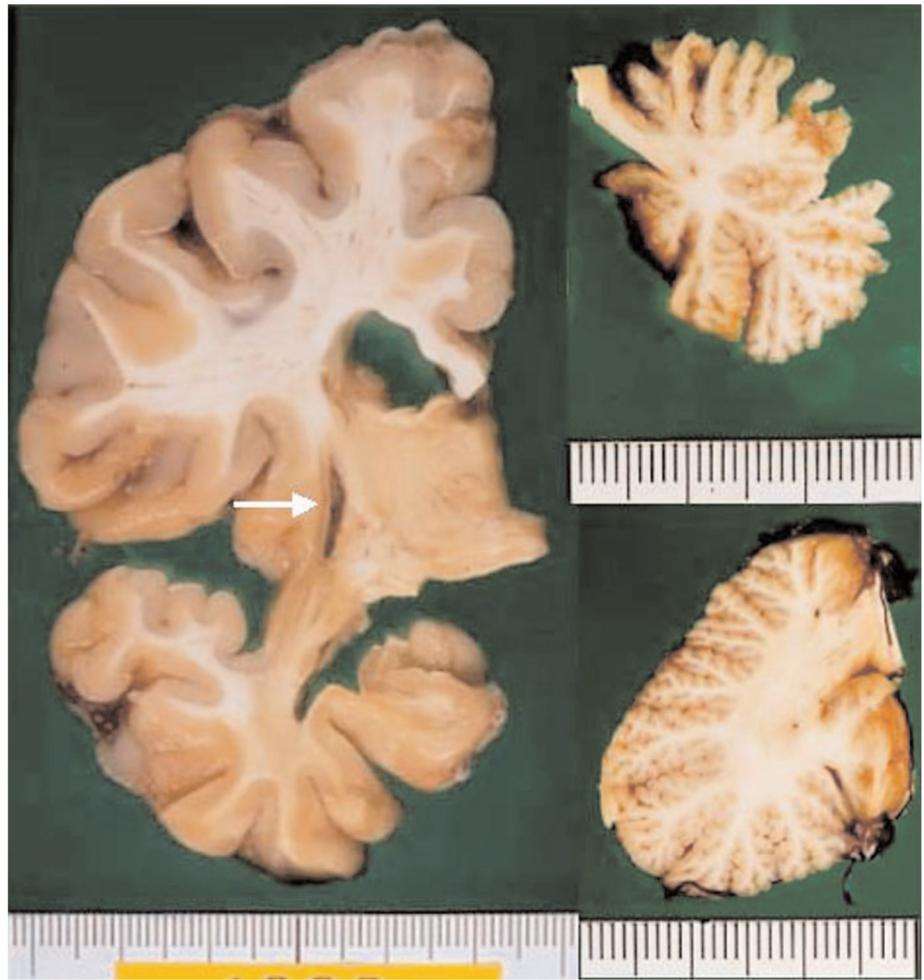


Fig. 2 Macroscopic findings of brain sections. The cerebral and cerebellar white matter was atrophic and sclerotic. The medial temporal lobe, thalamus and cerebellar dentate nucleus were brownish in color. A cystic infarct (arrow) was noted in the putamen.

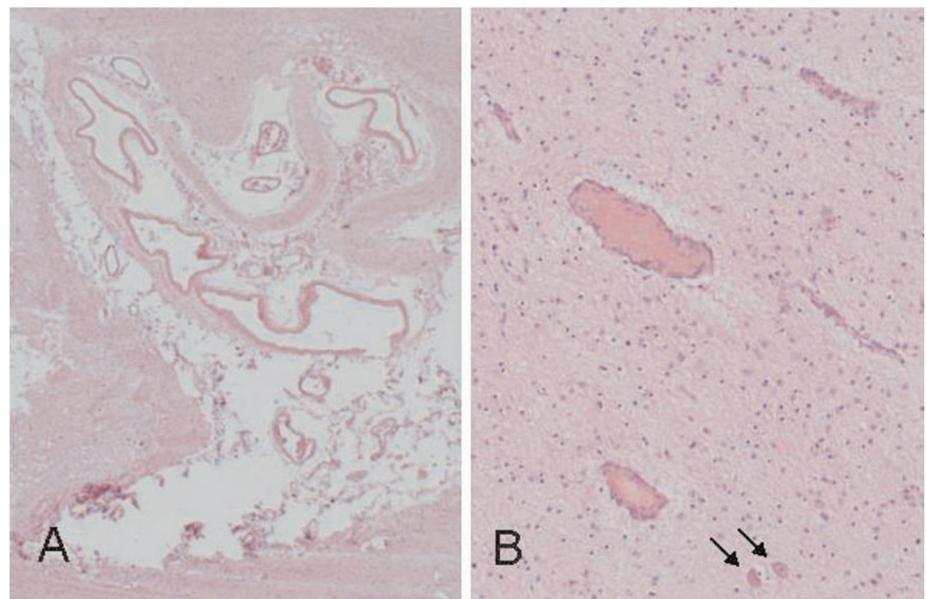


Fig. 3 Histologic findings of (a) the basal ganglia and (b) the thalamus (HE). (a) In the left putamen, there was a cystic infarct around an artery with an irregular lumen, a thin wall, and excessive kinking. The cerebral tissue surrounding the cyst was gliotic. (b) The ventrolateral nucleus of the left thalamus had a severe loss of neurons (arrows), astrogliosis, and loosening of the perivascular tissue.

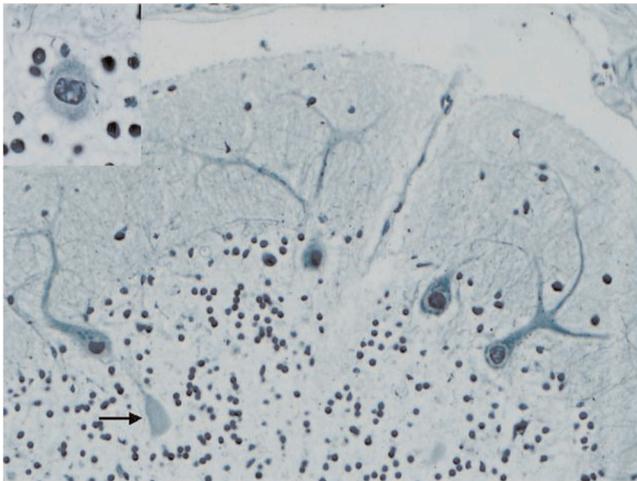


Fig. 4 Histologic findings of the cerebellar cortex (Bodian staining). The molecular layer showed rarefaction. Purkinje cells were moderately decreased in number, and the remaining cells had a “weeping willow” pattern, somal sprouts (left upper inset), and a torpedo (arrow). Granular cells were markedly reduced.

nucleus showed moderate neuronal loss and severe astrogliosis.

The midbrain had rarefaction of the periaqueductal tissue, neuronal loss of the red nucleus, and myelin loss of the superior cerebellar peduncle. In the pons, myelin pallor was noted in the central tegmental, pontocerebellar and pyramidal tracts, and in the medial and lateral lemniscus. In the medulla oblongata, neuronal loss and astrogliosis were observed in the inferior olivary nucleus.

The body was small, measuring 9.0 kg in weight (average, 13.3 kg) and 82 cm in height (average, 91 cm). The lungs (right, 35 g; left, 47 g) were unremarkable except for small lesions of focal pneumonia. The liver (296 g) was yellow in color, and had a loss of hepatocytes and marked fibrosis. The urinary bladder had two large diverticula surrounded by a huge hematoma, resulting from submucosal hemorrhage.

DIAGNOSIS

Menkes disease, followed by perinatal hypoxic–ischemic encephalopathy, and massive hemorrhage from the urinary bladder diverticulum.

DISCUSSION

Menkes disease is an X-linked recessive disorder caused by a loss-of-function mutation of the *ATP7A* gene, encoding a copper-transporting P-type ATPase expressed in virtually all organs except the liver. *ATP7A* is located in the trans-Golgi network, pumping copper from the cytosol into the Golgi apparatus. In the presence of excess copper, this protein relocates to the plasma membrane, pumping copper out of the cells.

In Menkes disease, the defect of *ATP7A* reduces copper uptake across the small intestine. The resultant systemic deficiency of copper is clinically recognized as low serum levels of copper and ceruloplasmin. In contrast, copper accumulates in the small intestine and kidney, as well as in cultured cells, owing to the copper efflux defect. In the brain, the failure of copper to cross the blood–brain barrier makes copper deficiency even worse. All non-hepatic tissues are affected by defective intracellular transport of copper. The lack of copper in the Golgi apparatus results in hypoactivity of various copper-dependent enzymes, such as tyrosinase (melanin synthesis), sulfhydryl oxidase (cross-linking of keratin), cytochrome *c* oxidase (mitochondrial oxidative phosphorylation), lysyl oxidase (cross-linking of collagen and elastin) and ascorbic acid oxidase (ossification), accounting for systemic manifestations of Menkes disease in the skin, hair, blood vessels, urinary bladder and bones. Neurologic symptoms are produced by deficiency of cytochrome *c* oxidase, superoxide dismutase (free radical detoxification), monoamine oxidase and dopamine- β -hydroxylase (neurotransmitter synthesis), and other enzymes. Menkes disease is treated by parenteral administration of copper, which quickly restores the serum copper and ceruloplasmin to normal levels. However, neither the brain tissue nor Golgi apparatus of systemic cells have significant recovery of copper deficiency; therefore this treatment often fails to ameliorate most of the clinical symptoms.²

The present patient had most of the clinical and biochemical findings typical of Menkes disease. Even after the introduction of copper replacement therapy, systemic and neurological abnormality relentlessly progressed, terminating in early death. Neuropathologic studies also indicated most of the characteristic findings of Menkes disease, except that “cactus-like” Purkinje dendrites were absent. Thus, both the clinical and neuropathologic features of the present patient were essentially the same as those of previously reported cases not undergoing copper supplementation.

In the present patient there were additional lesions of perinatal hypoxic–ischemic encephalopathy in the cerebral deep gray matter and periventricular white matter (Figs 2,3a), attributed to intrapartum fetal asphyxia and neonatal respiratory distress. From a clinical viewpoint, however, these events are not serious enough to produce these lesions by themselves. It is therefore plausible that a defect in lysyl oxidase had already caused cerebrovascular liability *in utero*, rendering the brain more vulnerable to minor perinatal hypoxic–ischemic insults.

Cranial CT and MRI in the present case depicted a dynamic change of the temporal lobe lesions: transient severe edema followed by progressive atrophy (Fig. 1); to date, descriptions of this finding have been scarce.^{1,3} On his-

tologic examination of this patient, the temporal lobes had unexpectedly mild findings: a spongy state with no evidence of tissue necrosis. Such discrepancy in the severity of imaging and pathologic findings is reminiscent of MELAS. In Menkes disease, there is a defect of cytochrome *c* oxidase, a copper-dependent enzyme critical for mitochondrial function. Previous ultrastructural studies have also found abnormal morphology of mitochondria in the brain and skeletal muscles: swelling, deformed or irregular cristae, and electron-dense deposits. In this context, Menkes disease should be regarded as a mitochondrial disorder. The temporal lesions of the present patient may provide additional evidence for the critical pathogenetic role of mitochondria in the progressive brain damage of Menkes disease.

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