detected in the globus pallidus, putamen and dentate nucleus. No neurofibrillary tangles, as shown by Gallyas and Bodian staining, were present in the putamen, globus pallidus, substantia nigra, or subthalamic and dentate nuclei. Purkinje cells were well preserved and the dentate and inferior olivary nuclei appeared intact.

In the spinal ganglia, several residual nodules were found in the cervical segment (Fig. 4A). Axonal swelling was evident around the neurons (Fig. 4A, B), but onion bulbs were not detected. Many acidophilic granules, varying from 1 to 6 μm in diameter, appeared either singly or in groups in the neurons of the spinal ganglia (Fig. 4C). Histochemically, positive staining with PAS, LFB and CV suggested that these granules contained glycoprotein and phospholipids (Fig. 4D, E). Electron microscopy revealed that the acidophilic granules were electron-dense, homogeneous and amorphous round bodies, which showed fusion with each other. All of the fusing round bodies were surrounded by a double-layered limiting membrane, suggesting that they were mitochondria (Fig.4F). The surface of the round bodies was thorny (Fig. 4G).

There was no evidence of degeneration in the CA1 to subiculum transitional areas or motor neuron disease inclusions. There were no 1C2-immunopositive cytoplasmic and intranuclear inclusions or glial cytoplasmic inclusions in the brain and spinal cord.

Discussion

Severe involvement of the lower motor neurons in the brainstem and spinal cord with relative sparing of the oculomotor, trochlear and abducens nuclei and presence of

ubiquitin-immunopositive skein-like inclusions and Bunina bodies indicated that the present patient had sporadic ALS. The most noteworthy feature was prominent degeneration of the ALF, whereas only slight alteration of the LCS was evident. It has been reported that degeneration of the ALF is roughly correlated with the severity of degeneration of the LCS in patients with sporadic ALS.[15,16] However, it seems that the LCS and ALF of the spinal cord degenerate independently in some cases of sporadic ALS,[15,16] and only one case showing severe degeneration of the ALF despite mild degeneration of the CST has been reported previously.[17] In the experience of the authors during laboratory service, the proportion of patients showing prominent ALF degeneration with faint alteration of the LCS is approximately 2% among patients with sporadic ALS. In previous studies, the propriospinal neurons of the IMZ and neurons in the medullary reticular formation have been proposed to be the origin of the degenerated fibers in the ALF of ALS patients.[15,16,18,19] The present authors consider that the marked degeneration of the ALF in the present patient was due mainly to severe neuronal loss in the IMZ.

Some patients with ALS who survive for a long period with respirator support show widespread involvement beyond the motor neurons system. In these patients, Betz cells, the globus pallidus, subthalamic nucleus, red nucleus, substantia nigra, dentate nucleus, locus ceruleus, oculomotor, trochlear and abducens nuclei, reticular formation, medial longitudinal fasciculus (MLF), Clark's column, the intermediolateral nucleus, Onufrowicz nucleus, spinocerebellar tract and middle root zone of the posterior column[3-7] have been reported to be severely affected. In the present patient,

however, degeneration of the Betz cells was mild. Accordingly, the present case appears to have been a forme fruste or incipient form of the widespread type of sporadic ALS (Table 1).

MND patients with degeneration of the striatonigral-pallidoluysian systems have been reported previously.[9-13] Even in patients with a relatively short disease duration,[8-11. 14] all parts of the striatonigral-pallidoluysian systems were evidently involved, and the initial site of the degeneration has not yet been reported. Furthermore, iron deposition was evidently increased in the substantia nigra, putamen, globus pallidus and subthalamic nucleus in the affected patients. It has been claimed that these pathological findings are compatible with PNLA.[20] As the present patient showed iron deposition in the putamen, globus pallidus and substantia nigra, a forme fruste or an incipient form of MND may also have been combined with PNLA (Table 1). Iron deposition may play a role in generating free radicals, thus inducing neuronal degeneration in the PNLA lesions.[21]

There have been previous reports of onion bulbs being detected in the spinal ganglia in ALS,[22] and neuronal loss and residual nodules being evident in the spinal ganglia in widespread-type ALS.[3] However, axonal swelling in the spinal ganglia has not been reported in ALS. A recent study demonstrated that a neuronal intermediate filament protein "peripherin" was associated with axonal spheroids in ALS, and that its overexpression caused the death of not only motor neurons, but also spinal ganglia neurons in vitro.[23]

Many intracytoplasmic acidophilic granules were observed in the spinal ganglia

of the present patient. Similar granules have been reported in the locus ceruleus, substantia nigra and spinal ganglia in humans, and appear to be mitochondrial in origin.[24,25] The relationship between degeneration of the spinal ganglia and these acidophilic granules is still unclear.[26]

In conclusion, the present patient showing unusual degeneration of the spinal white matter with degeneration of the subthalamic nucleus and iron deposition in the putamen, globus pallidus and substantia nigra was considered to have had a forme fruste or incipient form of widespread-type ALS or MND with PNLA. The neuropathological findings in this case seem to be important for understanding the nature of widespread-type ALS and MND with PNLA.

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Legends

Fig. 1

A: Spinal cord at the 6th cervical (C6), 3rd thoracic (T3) and 5th lumbar (L5) segments; moderate atrophy of the spinal cord and anterior horns, and severe volume loss of the anterolateral region. Relatively well preserved anterior and lateral corticospinal tracts (CST), but severely degenerated anterolateral funiculus (ALF). Degeneration of fasciculus cuneatus in the cervical segment. (Klüver-Barrera (KB)). Bar; 1mm.

B: Severely decreased number of large myelinated fibers, in addition to moderate decrease of medium-sized and small myelinated fibers in the ALF (Toluidine blue preparation). Bar; 50 μm.

C: Decreased number of large myelinated fibers with relatively increased number of small myelinated fibers in the lateral corticospinal tract (LCS) (Toluidine blue preparation). Bar; 50 μm.

D: Bunina bodies in a lumbar anterior horn cell (Hematoxylin and eosin (HE)). Bar; 30 μm.

E: Ubiquitin-positive filamentous structures (skein-like inclusion) in a lumbar anterior horn cell (Immunohistochemistry for ubiquitin). Bar; 30 μm.

F: Spheroid in the lumbar anterior horn (HE). Bar; 30 μm.

Fig. 2

A, B: Moderate loss of neurons in the intermediate zone (IMZ) of the spinal cord at the 6th cervical (C6) (A) and 5th lumbar (L5) (B) segments ; (KB). Bar; 400 μm, (dotted line; boundaries between Rexed's lamina VI and VII, VIII and IX)

C: High-power micrograph of the IMZ of the spinal cord at the C6 segment; shrinkage of remaining neurons (KB). Bar; 50 μm.

D: Myelin pallor of longitudinal fibers in the reticular formation (asterisk) and that of the pyramis at the medulla oblongata (KB). Bar; 5 mm.

Fig. 3

A: Fibrillary gliosis is the subthalamic nucleus (arrowhead) (Holzer). Bar; 1mm.

B: Moderate neuronal loss with marked gliosis in the subthalamic nucleus (HE). Bar; 250 μm.

C: Mild neuronal loss in the medial part of the substantia nigra (double asterisk). Cerebral peduncle (asterisk) (KB). Bar; 400 μm.

D: Iron deposition in the neurons and astrocytes of the substantia nigra (Berlin blue). Bar; 30 μm.

Fig. 4

A: Degeneration of the cervical spinal ganglion. Scattered Nageott's residual nodules with loss of neurons. Arrow indicates swollen axon (HE). Bar; 100 μm.

B: A neuron (asterisk) in a dorsal root ganglion at the 7th cervical segment with axonal swelling (double asterisk) (immunohistochemistry for neurofilament). Bar; 50 μm.

C: Acidophilic granules in a neuron of the dorsal root ganglion (arrows) (HE). Bar; 50 μm.

D, E: Acidophilic granules in the spinal ganglion positive for periodic-acid Schiff (D), and luxol fast blue (E) staining. Bar; 30 μm.

F: Ultrastructure of acidophilic granules in the lumbar spinal ganglia. Amorphous and globular bodies of homogeneous material, and surrounded by a double-layered limiting membrane. Mitochondrial cristae (arrows) are evident. Bar; 1μm.

G: Surface of the bodies is thorny (F-G, Uranyl acetate and lead citrate). Bar; 1μm.

Table1 Neuropathological comparison between MND with iron deposited PNLA ,[14] ALS with widespread degeneration,[4] and present patient.

Report	Present patient	Sudo et al[14]	Oda et al[4]	
Age at death, gender	52 M	60 M	69 M	69 M
Duration	3y8m	5y	4y9m	9y9m
Respirator	2y7m	-	3y9m	8y5m
Brain weight (g)	1,517	1,040	1,190	1,050
Cerebral cortex	+/-	++/+	+++/+++	+++/+++
Putamen	+/+(Fe+)	++/++(Fe++)	-/-	-/-
GPi	+/+(Fe+)	+++/+++(Fe++)	++/++	++/++
GPe	+/+	++/+(Fe++)	+++/+++	+++/+++
Subthalamic n.	++/+++	+++/++(Fe++)	+/++	+/+++
Substantia nigra	+/+(Fe++)	+++/+++(Fe++)	++/++	++/++
Inferior olivary n.	-/-	-/-	-/-	-/-
Dentate n.	-/-	-/-	++/++	+++/+++
Hypoglossal n.	+++/+++	+/-	+++/+++	+++/+++
Pyramidal tract	+/+	++/+++	++/n.a.	++/n.a.
Spinal AHC	+++/+++	+/-	+++/+++	+++/+++
Bunina body	+	+	-	-
Skein-like inclusion	+	-	-	-

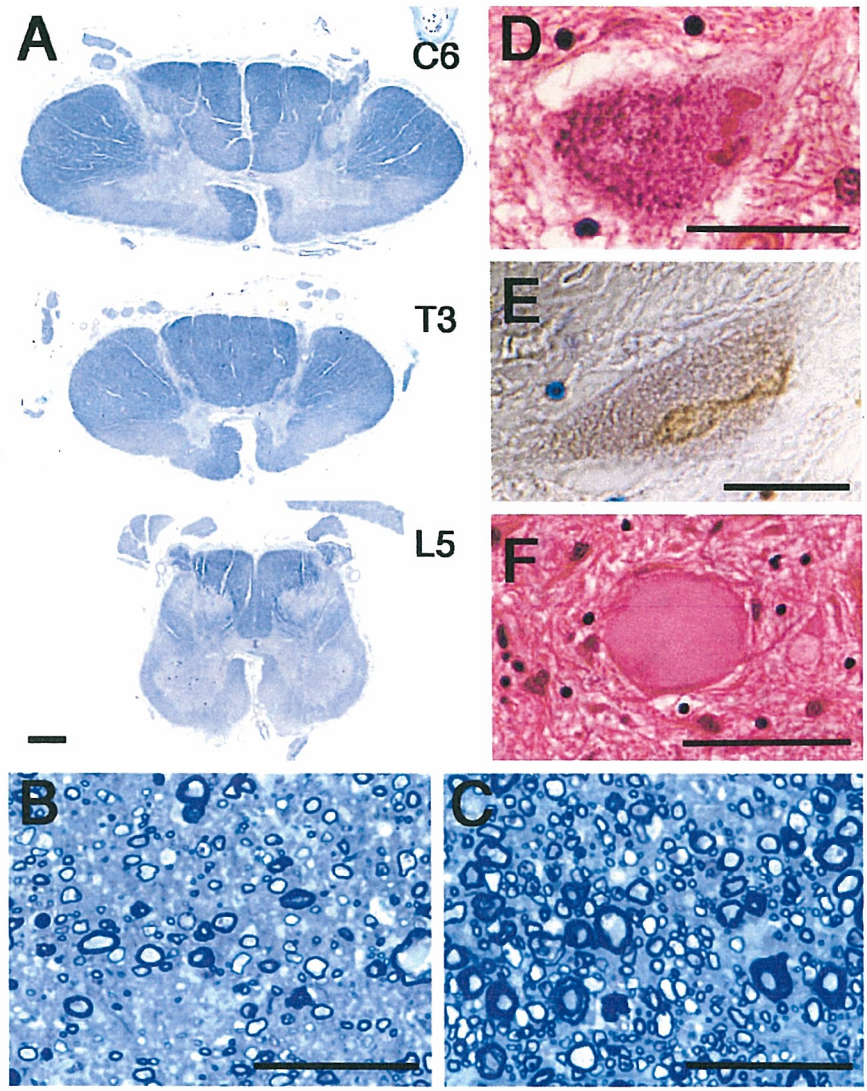
Neuronal or myelin loss /gliosis; - absent, + mild, ++ moderate, +++ severe,

Fe; iron deposition; – absent, + several, ++ many, n.a. not available

(MND: motor neuron disease, PNLA: pallido-nigro-luysian atrophy, M: male, y: year, m: month,

GPi: internal segment of globus pallidus, GPe: external segment of globus pallidus,

n.a.: not available, n.: nucleus, AHC: anterior horn cell)



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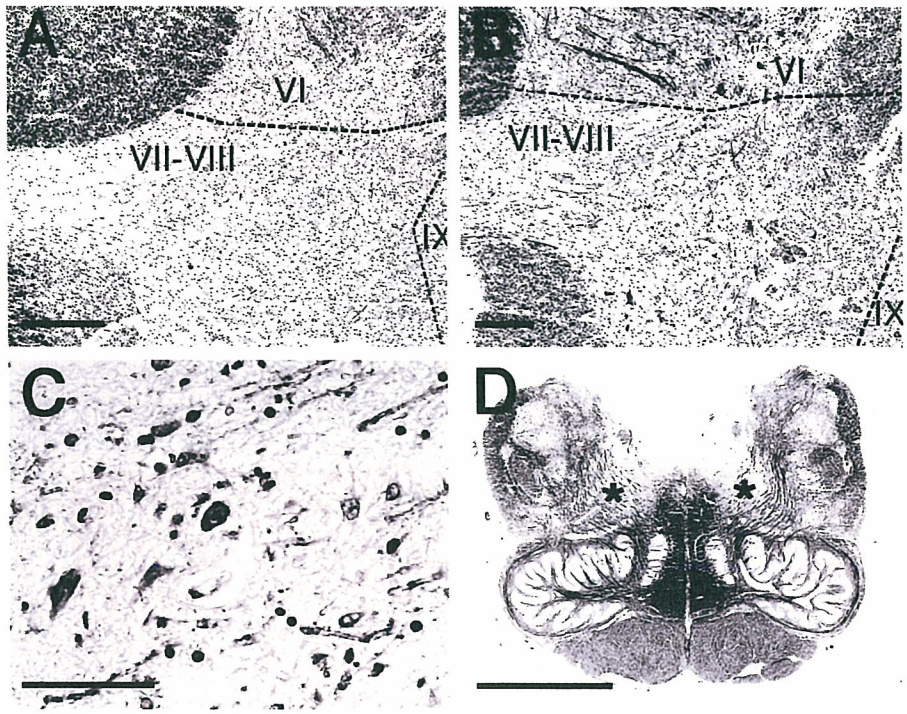

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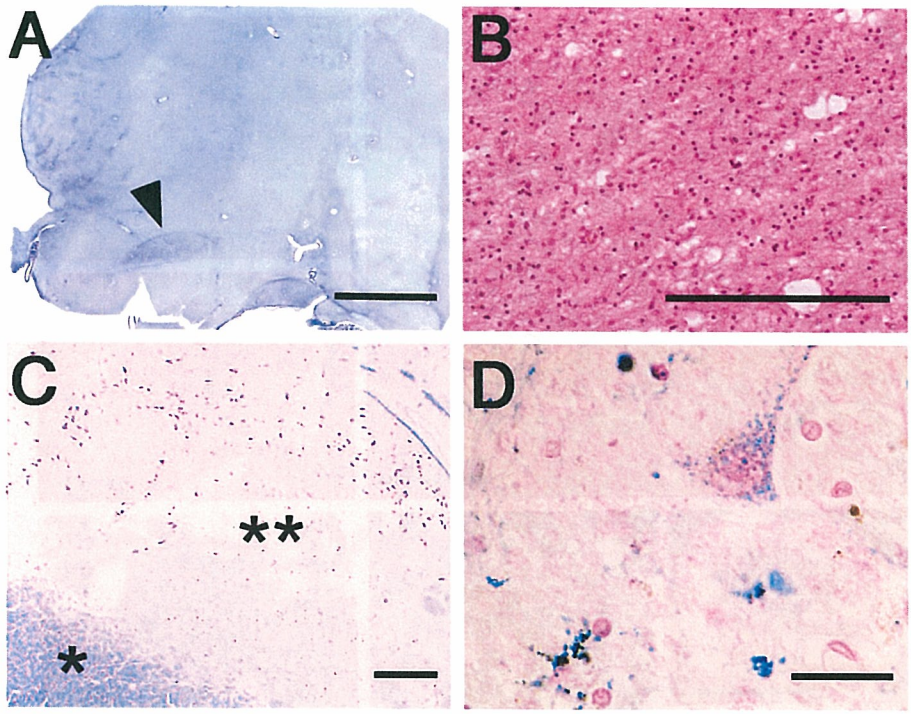

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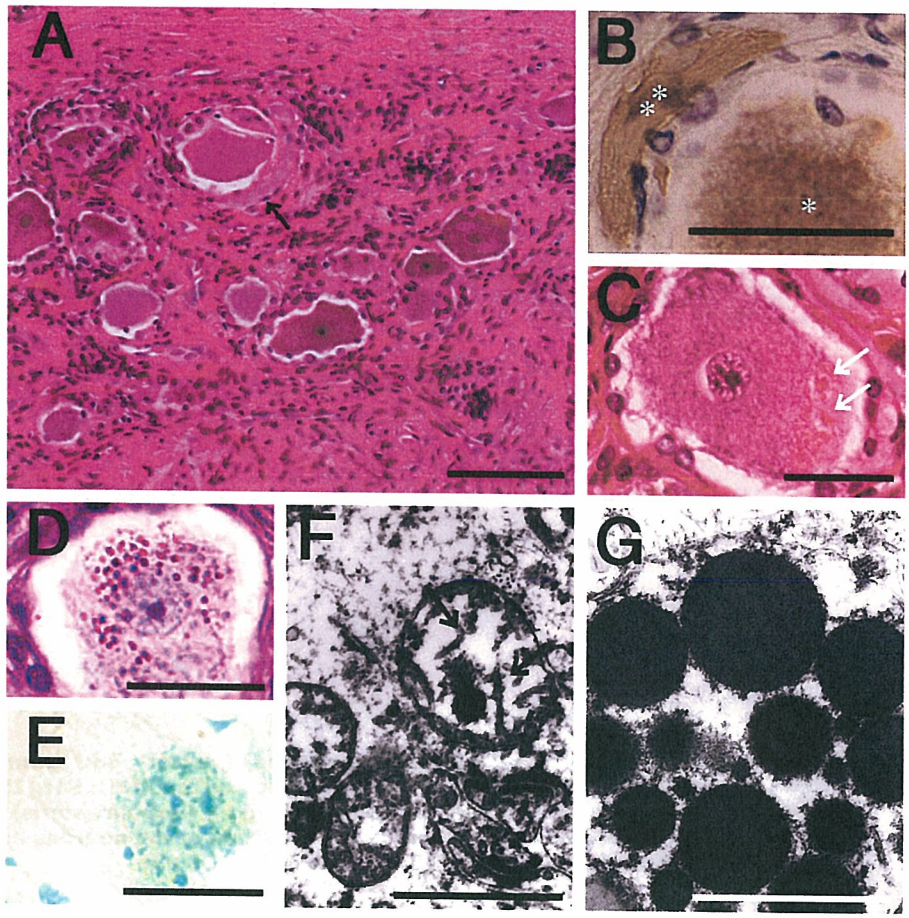

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1. Author Information Page:

Fate of disseminated dead neurons in the cortical ischemic penumbra: Ultrastructure indicating novel scavenger mechanisms by microglia and astrocytes

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3. Title Page

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Photographs	5
Line drawings	1

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ABSTRACT:

Background and purpose: Because the mechanism for scavenging acidophilic electron-dense dead neurons disseminated among the neuritic networks of surviving neurons in the ischemic penumbra of the cerebral cortex is still obscure, we investigated the fate of them up to 24 weeks after the ischemic insult. **Methods:** Stroke-positive animals were selected according to their stroke index score during the first 10-min left carotid occlusion done twice with a 5-hour interval. The animals were euthanized at various times after the second ischemic insult. Ultra-thin sections including the 2nd~4th cortical layers were obtained from the neocortex coronally sectioned at the infundibular level, in which the penumbra appeared, and observed by electron-microscopy. We determined the percentages of resting, proliferating/activated and phagocytic microglia and astrocytes in the specimens obtained at various time post-ischemia. **Results:** The electron-dense neurons had been fragmented into granular pieces by invading astrocytic processes from the periphery of the dead neurons and only the central portion remained. These granular pieces were dispersed along the extra-cellular spaces in the neuropils. By 8~24 weeks, the central core portion became a tiny vesicular particle (3.5~5.5μm in diameter) with a central dot. Microglia and astrocytes phagocytized these dispersed granular pieces. **Conclusions:** We found a novel scavenger mechanism in the ischemic penumbra, one by which dead neurons were fragmented by invading small astrocytic processes and only a thinned-out core portion remained, which finally became a tiny vesicular particle. The dispersed fragmented pieces were phagocytized by the microglia and astrocytes late, at 8 to 24 weeks post-ischemia.