Introduction

Degeneration of the substantia nigra is almost constantly found as a subsidiary finding in patients with amyotrophic lateral sclerosis-dementia (ALS-D)[1-6]. However, in patients with this disorder, clinical symptoms and signs of parkinsonism are usually not apparent[2,4,7]. They seem to be masked by clinical features of motor neuron disease[1,8]. Neuropathologically, degeneration of the substantia nigra found in this disorder does not exhibit α-synucleinopathy-related changes such as Lewy bodies, Lewy neurites, and pale bodies, and the pathogenetic mechanisms of nigral degeneration remain obscure.

In this report, we document an autopsy case of ALS-D, in which the clinical features simulated those of idiopathic Parkinson disease and they were moderately responsive to L-DOPA treatment. Motor neuron symptoms and dementia were very mild or almost absent, and the diagnosis of ALS-D could not be made antemortem. Autopsy revealed severe degeneration with neuronal mineralization of the substantia nigra. Pathological changes of the motor neuron system, hippocampus, and fronto-temporal lobes were typical of ALS-D but mild in degree, and they were considered to be in an early stage of the development.

Case Report

The patient is a 78-year-old man, a retired worker of a pharmaceutical company. His family history is noteworthy in that his mother died of a dementing illness after a clinical course of more than seven years and one of his sisters has been treated under

the diagnosis of Alzheimer disease. No further information is available concerning the medical history of these two persons. He was noticed to walk with short steps at the age of 76. One year later, he frequently dropped food during a meal and walked dragging the left foot. Resting tremors of both hands and swallowing difficulty also developed, and he was diagnosed idiopathic Parkinson disease by his family physician. Although magnetic resonance imaging of the brain at that time showed atrophy of the bilateral temporal lobes, impairment of memory or other intellectual abilities was not noted. He was a heavy smoker and was hospitalized for a short term because of pulmonary emphysema and pneumonia at the age of 77. During this hospitalization visual hallucinations appeared transiently. After discharge his activities of daily living deteriorated remarkably, and he could not walk nor take a meal without assistance.

He was admitted to our hospital 7 months before death. The intelligence was approximately at the normal level and no evidence of dementia was present. Neither change of the personality nor abnormal behavior was noted. Muscle atrophy, weakness, and fasciculation were not observed. Hyperreflexia and spasticity in the extremities were also absent, and Babinski reflex was not elicited. Cogwheel rigidity and resting tremors were noted in the upper extremities, and the Myerson sign and retropulsion were also observed. These parkinsonian symptoms and signs were moderately responsive to the administration of L-DOPA (100 mg/day), and his activities improved from Yahr's stage V to IV. One month prior to death, he could make a short trip of two days, but after that he complained of shortness of breath and asthma-like respiratory difficulty and died of respiratory failure after a short term hospitalization. The clinical

course from the onset of neurological symptoms to death was about two years, and the final neurological diagnosis was idiopathic Parkinson disease.

General autopsy revealed severe emphysematous changes of both lungs and bronchopneumonia of the right lung. Remarkable emaciation and atrophy of the heart and liver were noted. Atrophy of skeletal muscles was not pronounced, but the diaphragm showed mild neurogenic atrophy of muscle fibers.

Neuropathological Findings

The brain weighed 1,380 grams and showed mild atrophy of the tips of the bilateral temporal lobes. Otherwise the cerebrum retained its normal appearance, and atrophy of the motor cortex, hippocampus, and amygdala was not found. Remarkable depigmentation of the substantia nigra was the most prominent change (Fig. 1), whereas pigmentation of the locus ceruleus was preserved. The cerebellum and spinal cord appeared grossly normal.

On histopathological examination, the substantia nigra showed severe neuronal loss accompanied by astrocytosis and rarefaction of the neuropil (Fig. 2). These changes were accentuated in the ventro-medial portion. There were many free pigment granules in the neuropil, and small aggregates of pigment-laden macrophages were also observed. Deeply basophilic and finely granular or stellate-shaped mineralization in the zona compacta, probably coinciding with necrotic neurons, was a unique finding (Fig. 3). This mineralization was not stained with the von Kossa method. No Lewy bodies, Lewy neurites, and pale bodies were detected by use of

immunohistochemistry employing an anti-α-synuclein antibody (Affinity Research Products, Exeter, UK). Neurofibrillary tangles were not observed, but a few Marinesco bodies were seen. No ubiquitinated abnormal structures were identified in the substantia nigra with immunohistochemistry using an anti-ubiquitin antibody (DakoCytomation, Glostrup, Denmark). Neurons of the locus ceruleus were well preserved, but a few neurons contained tangles. The other structures in the midbrain and pons did not show any remarkable alterations. In the medulla oblongata, the hypoglossal nucleus showed mild neuronal loss, and a few neurons contained Bunina bodies. The pyramids did not show axonal loss or myelin pallor.

The spinal cord exhibited mild loss of anterior horn cells accompanied by astrocytosis at the level of the cervical cord (Fig. 4). Bunina bodies were detected in several anterior horn cells of the cervical and lumbar cords (Fig. 5), and many axonal spheroids and a few chromatolytic neurons were also noted. Neither globular nor skein-like, ubiquitin-positive inclusions were detected in anterior horn cells. The lateral and anterior cortico-spinal tracts did not show degenerative changes.

In the cerebrum, Betz cells in the motor cortex were well preserved. Circumscribed neuronal loss with astrocytosis was found in the transitional zone between the CA1 of the hippocampus and the subiculum (Fig. 6). A small number of ubiquitin-positive intracytoplasmic inclusions were found in neurons of the dentate fascia of the hippocampus and also of the temporal and frontal cortices (Fig. 7). These inclusions were of globular or semicircular shapes and showed finely fibrillary internal structures. Many ubiquitin-positive, dot-like structures were also found in the

neuropil of the affected cortices. Scattered neurofibrillary tangles and ghost tangles were found in the hippocampus, parahippocampal cortex, temporal cortex and amygdala. The superficial layers of the parahippocampal cortex and the anterior dorso-medial portion of the superior temporal gyrus showed mild microvacuolation of the neuropil and astrocytosis, but neuronal loss was not apparent. Similar changes were not observed in the frontal cortex. Many senile plaques, mainly of the diffuse type, were distributed throughout the cerebral cortex.

The striatum, globus pallidus, thalamus, subthalamic nucleus, and cerebellum did not show any significant pathological alterations.

Discussion

ALS-D or motor neuron disease with dementia is a disease entity which has well-defined clinico-pathological features, although its nosological position within the disease groups of fronto-temporal dementia or motor neuron disease is still in controversy[6,7,9,10,11]. Clinically the disease is characterized by dementia and predominantly lower motor neuron symptoms and signs[6,7]. Neuropathologically, in addition to degeneration of the motor neuron system, degeneration of the hippocampus and the anterior dorso-medial portion of the temporal cortex is a constant finding[10], and ubiquitinated intraneuronal inclusions are seen in the dentate fascia of the hippocampus and some other regions[12]. Emphasizing the pathological significance of these inclusions, the term "motor neuron disease-inclusion dementia" has been advocated recently[13].

Degeneration of the substantia nigra has been frequently described in patients with ALS-D[1,2,5,8,9], and in autopsy series studied by Yoshida[6] and by Tsuchiya et al.[3] the involvement of this nucleus was a constant finding. Rare cases of ALS-D combined with degeneration of the striato-nigral and pallido-luysian systems have also been reported[14,15]. In these cases, neither α-synucleinopathy-related alterations nor neurofibrillary tangles are seen in the nigral neurons, and the pathogenesis of nigral degeneration remains obscure, although ubiquitinated inclusions were demonstrated in nigral neurons in some cases[4,5,6,11]. The absence of degeneration of the locus ceruleus in these cases also suggests the involvement of a mechanism different from that operating in idiopathic Parkinson disease. There are presumably several different

mechanisms leading to neuronal death of the substantia nigra, and several case reports attest the presence of primary nigral degeneration not associated with α-synucleinopathy[16,17,18]. In the present case, finely granular or stellate-shaped mineralization of necrotic neurons (encrustation) in the substantia nigra was a unique finding which has not been described previously. It remains unknown whether the mineralization is essentially related to the pathogenesis of nigral degeneration or merely a coincidental finding secondary to neuronal death. Mineralization of necrotic neurons is not an uncommon finding in ischemic lesions of the cerebrum, but it is not usually seen in the substantia nigra in various degenerative conditions affecting this nucleus. Severe neuronal loss with mineralization in the substantia nigra suggests that nigral degeneration occurred in the early period of the disease progression, probably preceding degeneration of the motor neuron system and the temporal lobe. In the present case degeneration of the substantia nigra was not merely a subsidiary lesion but rather a principal lesion both clinically and neuropathologically. As suggested by Sudo et al.[14], ALS-D might be a multisystem degenerative disorder which constantly involves the dopaminergic system.

In spite of the almost constant nigral degeneration, patients with ALS-D are seldom presented with clinical features of Parkinson disease[1,2,4,7]. In the autopsy series studied by Yoshida[6], which comprises 28 cases, parkinsonism was a prominent symptom apparently in only one case. Parkinsonian clinical features seem to be usually masked by symptoms and signs of motor neuron disease in this disorder[1,8]. The present case is therefore very instructive clinically and indicates that in some

patients with ALS-D the neurological features are dominated by parkinsonism and it can be improved by L-DOPA treatment. Itoh et al.[19] reported a clinical case of ALS-D in which the concentration of homovanillic acid, a dopamine metabolite, in the cerebrospinal fluid was abnormally low and the administration of L-DOPA brought the improvement of dementia, and suggested the involvement of the dopaminergic system in this disorder.

In contrast to the symptoms and signs of parkinsonism, those of motor neuron disease were not apparent in the present case, although mild swallowing difficulty suggestive of bulbar palsy was noted. Corresponding with these clinical features, neuropathological changes of the motor neuron system were mild. Neuronal loss with astrocytosis was mild, and Bunina bodies and axonal spheroids were found in the anterior horn. These findings are in common with the early changes in the classical ALS[20] and suggest that the pathogenetic mechanisms of anterior horn cell degeneration are at least partly common in the two disorders.

The lack of clinically overt dementia is explicable by the mild degree of neuronal loss in the hippocampus and the temporal lobe, and the relative paucity of ubiquitinated inclusions. These suggest that the lesions were in the early stage of the development. The lack of overt dementia in spite of the presence of temporal lobe degeneration in patients with ALS-D has been reported by a few authors, and the possibility of the presence of the *form fruste* of ALS-D is suggested[21].

References

1. Horoupian DS, Thal L, Katzman R, Terry RD, Davies P, Hirano A, DeTeresa R, Fuld PA, Petito C, Blass J, Ellis JM. Dementia and motor neuron disease. Morphometric, biochemical, and Golgi studies. Ann Neurol 1984;16:305-313.
2. Kato S, Oda M, Tanabe H. Diminution of dopaminergic neurons in the substantia nigra of sporadic amyotrophic lateral sclerosis. Neuropathol Appl Neurobiol 1993;19:300-304.
3. Tsuchiya K, Ikeda K, Mimura M, Takahashi M, Miyazaki H, Anno M, Shiotsu H, Akabane H, Niizato K, Uchihara T, Tomonaga I, Nakano I. Constant involvement of the Betz cells and pyramidal tract in amyotrophic lateral sclerosis with dementia. A clinicopathological study of eight autopsy cases. Acta Neuropathol 2002;104:249-259.
4. Al-Sarraj S, Maekawa S, Kibble M, Everall I, Leigh N. Ubiquitin-only intraneuronal inclusion in the substantia nigra is a characteristic feature of motor neurone disease with dementia. Neuropathol Appl Neurobiol 2002;28:120-128.
5. Mackenzie IR, Feldman H. Extrapyramidal features in patients with motor neuron disease and dementia. A clinicopathological correlative study. Acta Neuropathol 2004;107:336-340.
6. Yoshida M. Amyotrophic lateral sclerosis with dementia. The clinicopathological spectrum. Neuropathology 2004;24:87-102.

7. Mitsuyama Y. Presenile dementia with motor neuron disease in Japan. Clinico-pathological review of 26 cases. J Neurol Neurosurg Psychiat 1984;47;953-959.

8. Hudson AJ. Amyotrophic lateral sclerosis and its association with dementia, parkinsonism and other neurological disorders. A review. Brain 1981;104:217-247.

9. Yuasa R. Amyotrophic lateral sclerosis with dementia (in Japanese with English abstract). Clin Neurol (Tokyo) 1970;10:569-577.

10. Nakano I, Iwatsubo T, Hashizume Y, Mizutani T, Mannen T. Amyotrophic lateral sclerosis with dementia. Lesions in the apical cortex and some deeper structures of the temporal lobes. Neuropathology 1992;12:69-77.

11. Mackenzie IRA, Feldman HH. Ubiquitin immunohistochemistry suggests classic motor neuron disease, motor neuron disease with dementia, and frontotemporal dementia of the motor neuron disease type represent a clinicopathologic spectrum. J Neuropathol Exp Neurol 2005;64:730-739.

12. Okamoto K, Hirai S, Yamazaki T, Sun X, Nakazato Y. New ubiquitin-positive intraneuronal inclusions in the extra-motor cortices in patients with amyotrophic lateral sclerosis. Neurosci Lett 1991;129:233-236.

13. Jackson M, Lennox G, Lowe J. Motor neuron disease-inclusion dementia. Neurodegeneration 1996;5:339-350.

14. Sudo S, Fukutani Y, Matsubara R, Sasaki K, Shiozawa M, Wada Y, Naiki H, Isaki K. Motor neuron disease with dementia combined with degeneration of striatonigral and pallidoluysian systems. Acta Neuropathol 2002;103:521-55.

15. Toyoshima Y, Tan CF, Kozakai T, Tanaka M, Takahashi H. Is motor neuron disease-inclusion dementia a *forme fruste* of amyotrophic lateral sclerosis with dementia? An autopsy case further supporting the disease concept. Neuropathol 2005;25:214-219.

16. Oyanagi K, Nakashima S, Ikuta F, Homma Y. An autopsy case of dementia and parkinsonism with severe degeneration exclusively in the substantia nigra. Acta Neuropathol 1986;70:190-192.

17. Rajput AH, Uitti RJ, Sudhakar S, Rozdilsky B. Parkinsonism and neurofibrillary tangle pathology in pigmented nuclei. Ann Neurol 1989;25:602-606.

18. Ishihara K, Nonaka A, Fukui T, Kawamura M, Shiota J, Nakano I. Lewy body-free nigral degeneration. A case report. J Neurol Sci 2002;198:97-100.

19. Itoh H, Nakamura S, Akiguchi I, Yamao S, Kameyama M. Levodopa therapy for a patient with ALS-dementia complex (in Japanese with English abstract). Clin Neurol (Tokyo) 1987;27:1145-1149.

20. Inoue K, Hirano A. Early pathological changes of amyotrophic lateral sclerosis. Autopsy findings of a case of 10 months' duration (in Japanese with English abstract). Clin Neurol (Tokyo) 1979;11:448-455.

21. Tsuchiya K, Takahashi M, Shiotsu H, Akiyama H, Haga C, Watabiki S, Taki K, Nakano I, Ikeda K. Sporadic amyotrophic lateral sclerosis with circumscribed temporal atrophy. A report of an autopsy case without dementia and with ubiquitinated intraneuronal inclusions. Neuropathol 2002;22:308-316.

Figure Legends

Fig. 1: Coronal sections of the midbrain show remarkable depigmentation of the substantia nigra.

Fig. 2: Severe neuronal loss accompanied by astrocytosis is seen in the substantia nigra. (Hematoxylin-Eosin stain)

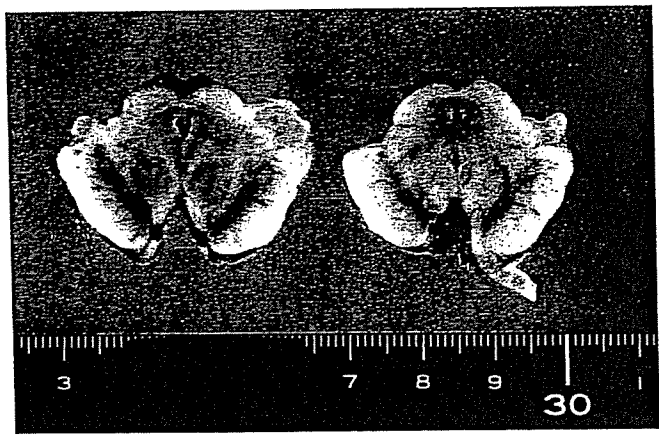
Fig. 3: Deeply basophilic, finely granular or stellate-shaped mineralization, probably coinciding with necrotic neurons, is seen in the substantia nigra. (Hematoxylin-Eosin stain)

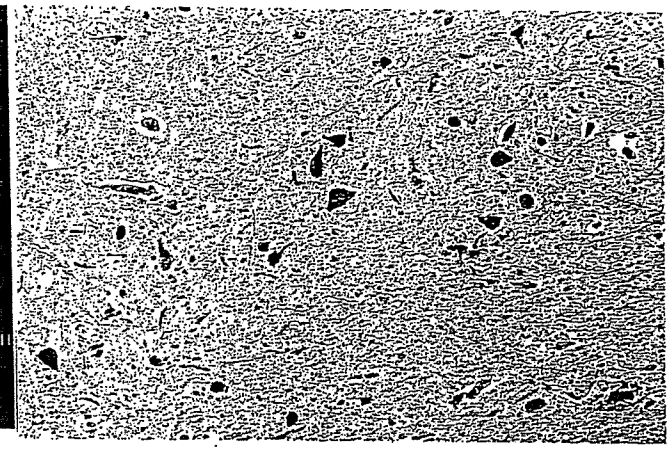
Fig. 4: The anterior horn of the cervical cord exhibits mild loss of large motor neurons and astrocytosis. (Nissl stain)

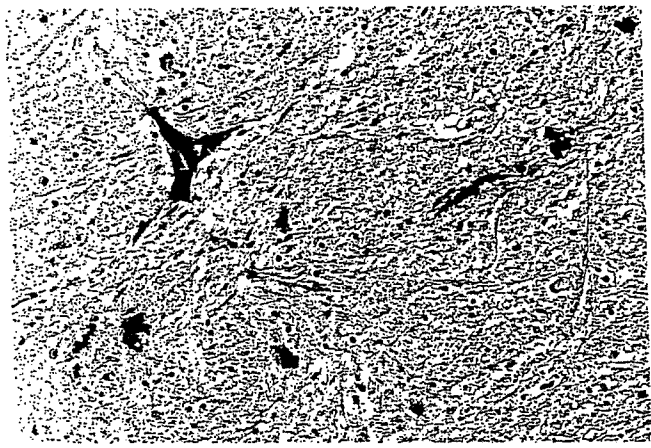
Fig. 5: Bunina bodies are found in several anterior horn cells. (Hematoxylin-Eosin stain)

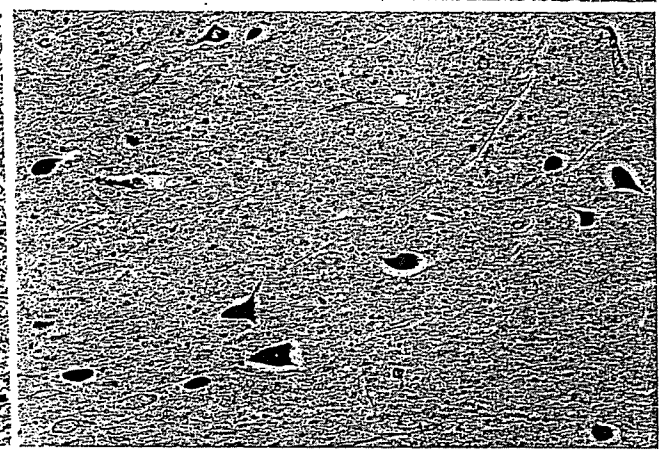
Fig. 6: Circumscribed neuronal loss with astrocytosis is found in the transitional region between CA1 of the hippocampus and the subiculum. (Hematoxylin-Eosin stain)

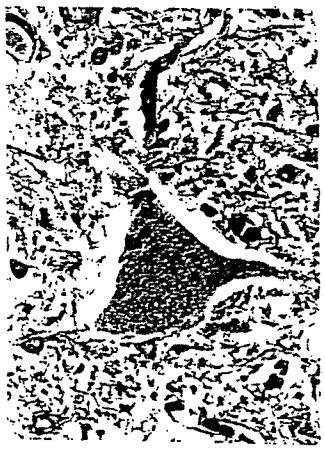
Fig. 7: A small number of ubiquitin-positive intraneuronal inclusions are found in the temporal cortex. (Immunostain for ubiquitin)

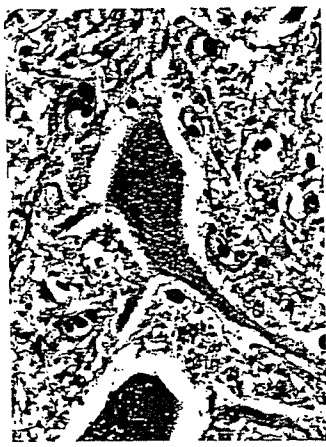


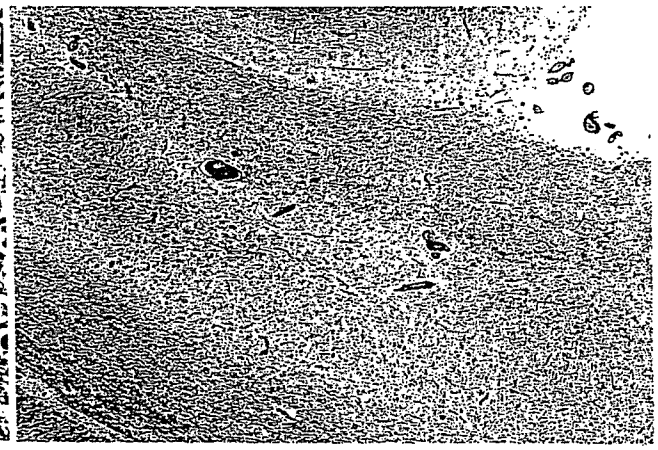












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Neuropathology, in press.

Forme fruste or incipient form of widespread-type ALS, or MND with PNLA? An autopsy case report

Running title: An ALS of widespread type or with PNLA?

Tomoyo Hashimoto,[1,2] Shiro Matsubara,[3] Yoko Mochizuki,[4,5] Sadatoshi Tsuji,[2] Toshio Mizutani,[4] Kiyomitsu Oyanagi,[1,4]

[1]Department of Neuropathology, Tokyo Metropolitan Institute for Neuroscience, Tokyo, Japan, [2]Department of Neurology, University of Occupational and Environmental Health, Fukuoka, Japan, [3]Department of Neurology, and [4]Department of Pathology, Tokyo Metropolitan Neurological Hospital, Tokyo, Japan, [5]Department of Internal Medicine, Tokyo Metropolitan Kita Medical and Rehabilitation Center, Tokyo, Japan

Correspondence to :

Kiyomitsu Oyanagi, MD, PhD,

Department of Neuropathology,

Tokyo Metropolitan Institute for Neuroscience,

2-6 Musashidai, Fuchu, Tokyo 183-8526, Japan.

E-mail: k123ysm@tmin.ac.jp,

Phone: +81-42-325-3881, Fax: +81-42-321-8678

Abstract

We describe a 52-year-old man with body weight loss and bulbar palsy, who exhibited muscle atrophy and weakness with fasciculation especially in the respiratory muscles 4 years prior death, necessitating respiratory support for 4 years, but who was able to walk until the end stage. He had no significant family history. Neuropathological examination revealed severe loss of motor neurons in the spinal cord and brainstem, and ubiquitin-positive skein-like inclusions and Bunina bodies in the remaining neurons. In addition, prominent degeneration of the anterolateral funiculus and severe loss of neurons in the intermediate zone of the spinal cord were evident, without marked alteration of the corticospinal tracts. Degeneration of the subthalamic nucleus, increased iron deposition in the substantia nigra, and axonal swelling, residual nodules and acidophilic granules in the spinal ganglia were found. The patient's condition was considered to have been a forme fruste or incipient form of widespread-type amyotrophic lateral sclerosis (ALS) or motor neuron disease (MND) with pallido-nigro-luysian atrophy (PNLA). The neuropathological features of the present case appear to be important for understanding the nature of widespread-type ALS and MND with PNLA.

Key words: amyotrophic lateral sclerosis, anterolateral funiculus, pallido-nigro-luysian atrophy, subthalamic nucleus, spinal ganglia.

Introduction

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disorder in the elderly, clinically manifested by weakness and wasting of the affected muscles with pyramidal signs. The pathological hallmarks of sporadic ALS are severe degeneration of the spinal anterior horn cells and corticospinal tracts (CST) of the spinal cord and characteristic Bunina bodies and ubiquitinated inclusions.[1,2]

Some patients with sporadic ALS who survive for a long time with respiratory support develop a totally locked-in state, or widespread-type ALS.[3-8] Such patients show extensive pathological involvement far beyond the motor neuron system, and usually show impairment of voluntary ocular movements.[3-5,7,8] In widespread-type ALS, the pallidoluysian system is one of the most vulnerable regions, and the spinal ganglia show frequent degeneration.[3] The pallidoluysian system has been reported to be involved also in motor neuron disease (MND) combined with pallido-nigro-luysian atrophy (PNLA).[9-14]

We report here the neuropathological findings in a 52-year-old male patient with lower-motor-neuron-predominant ALS, who showed unusual pathological features such as prominent degeneration of the anterolateral funiculus (ALF) in the spinal cord without marked alteration of the corticospinal tract (CST), degeneration of the subthalamic nucleus, increased iron deposition in the substantia nigra, and axonal swelling, residual nodules and acidophilic granules in the spinal ganglia. The significance of these findings is discussed.

Clinical summary

A Japanese man with no family history of neurological or psychiatric disease or relevant medical history suffered body weight loss of about 11 kg (from 67 to 56 kg) during a one-year period at the age of 48. One year later, mild to moderate muscle atrophy and weakness with fasciculation became evident in the tongue, sternocleidomastoid, upper limb, and intercostal muscles. The deep tendon reflexes of the bilateral lower limbs were mildly exaggerated, but no pathological reflexes were seen. Dysarthria and dysphagia were not observed, but arterial blood gas examination revealed moderate hypercapnia and hypoxia. His pulmonary vital capacity was only 59%. Electromyography (EMG) showed systemic neurogenic changes in the tongue, truncal muscles, arms and legs. He was diagnosed clinically as having ALS. Thirteen months after onset, respiratory support (non-invasive positive pressure ventilation) was initiated for night apnea. These neurological symptoms gradually progressed, and bulbar palsy and weakness of the facial muscles were evident at the age of 51. Arterial oxygen partial pressure decreased progressively, and the respiratory support time was extended. However, the patient remained capable of swallowing food and walking without support until just before his death. He was found dead in his home at the age of 52 years, 42 months after disease onset. Throughout the clinical course of the disease, the patient's mental status had remained unimpaired and extrapyramidal symptoms such as resting tremor, akinesia and rigidity were not observed.

The serum creatine kinase level was slightly elevated (455 IU/L), but HbA1c was normal. Results of other investigations, such as cerebrospinal fluid analysis and a nerve conduction study, were normal.

Pathological findings

A general autopsy was performed 5 h after the patient's death. Pulmonary emphysema was observed, but no other visceral organs exhibited significant pathological abnormality.

The brain with the dura mater weighed 1517g before fixation. The brain and spinal cord were fixed with 20% buffered formalin, and some parts of the cervical and lumbar enlargements were fixed in 2.5% glutaraldehyde-1% paraformaldehyde in 0.1 M cacodylate buffer solution (CB) at autopsy. Coronally cut surfaces of the brain showed that the subthalamic nucleus was small and brownish in color, and the pigmentation of the substantia nigra and locus ceruleus was mildly decreased. In terms of size, the pyramis of the medulla oblongata and the cerebral peduncle looked well preserved, but the volume of the spinal cord and the anterior roots at the cervical and lumbar enlargements appeared moderately decreased.

Histological examinations were performed using 10-μm-thick sections stained with hematoxylin and eosin (HE), Klüver-Barrera (KB), Bodian, Holzer, Gallyas-Braak, Berlin blue, periodic-acid Schiff (PAS), Luxol fast blue (LFB) and cresyl violet (CV).

Selected sections were immunostained using the labeled streptavidin-biotinylated antibody (LSAB) method (Dako, Kyoto, Japan) or avidin-biotin-peroxidase (ABC) method (Vector, Berlingame, CA, USA) with diaminobenzidine as the chromogen. The primary antibodies used were rabbit

polyclonal antibody against ubiquitin (Dako, Glostrup, Denmark; 1:600), goat polyclonal antibody against α-synuclein (N-19, SantaCruz, CA; 1:200), and mouse monoclonal antibodies against phosphorylation-dependent tau (AT8; Innogenetics, Ghent, Belgium; 1:500), neurofilament (Dako, Glostrup, Denmark; 1:100), glial fibrillary acidic protein (GFAP) (Novocastra, Newcastle-upon-Tyne, UK; 1:100) and expanded polyglutamine stretches (1C2; Chemicon, Temecula, CA; 1:8000, stained by Dr. M. Yamada, Department of Pathology, Brain Research Institute, Niigata University, Japan).

For the ultrastructural study, the cervical and lumbar segments of the spinal cord and dorsal root ganglia were post-fixed with 4% osmium tetroxide in 0.2 M CB, followed by dehydration through a graded ethanol series and embedding in Epon 812. Toluidine blue-stained semithin sections were observed by light microscopy, and ultrathin sections of the selected areas were stained with uranyl acetate and lead citrate, and examined using a transmission electron microscope (H-9000, Hitachi, Tokyo, Japan).

Histologically, severe volume loss and degeneration of the ALF was noted in the spinal cord, and atrophy of the anterior horn (AH) and intermediate zone (IMZ) was observed especially in the cervical enlargement. The ALF showed severe loss of myelinated fibers. The lateral corticospinal tract (LCS) appeared to be preserved in K-B-stained sections, but showed slight loss of myelinated fibers in Epon-embedded toluidine blue-stained sections. Severe loss of neurons and myelinated fibers was observed in the spinal AH and IMZ (Fig. 1A-C, 2A, B), and remaining neurons in the

IMZ were severely shrunken (Fig. 2C). The neurons of Clark's column and the intermediolateral nucleus were relatively well preserved. Bunina bodies (Fig.1D) and ubiquitin-immunopositive skein-like inclusions (Fig.1E) were detected in the lumbar and sacral anterior horn cells. Several spheroid bodies and globules were also observed in the anterior horn, especially in the lumbar segments (Fig.1F). Severe loss of large myelinated fibers and GFAP-immunopositive glial bundles were noted in the anterior spinal nerve roots. Mild degeneration was also observed in the fasciculus cuneatus (Fig. 1A).

In the brainstem, severe loss of neurons and gliosis were observed in the hypoglossal nucleus, and moderate loss in the trigeminal motor, facial and ambiguous nuclei, with relative sparing of the oculomotor and trochlear nuclei. There was moderate loss of myelinated fibers in the reticular formation, and mild loss in the pyramid of the medulla oblongata (Fig. 2D). In the motor cortex, Betz cells appeared atrophic, but their number was relatively well preserved.

In the muscles, grouped atrophy was confirmed, being severe in the sternocleidomastoideus and basophilic fibers, moderate in the 4th intercostal muscles, diaphragm and tongue, and mild in the illiopsoas.

The subthalamic nucleus showed marked gliosis with moderate neuronal loss (Fig. 3A, B). In the medial part of the substantia nigra, mild neuronal loss, several foamy spheroid bodies, and an increased number of Berlin blue-positive granules were observed (Fig.3C, D). Some iron granules were found in the astrocytes, neurons (Fig. 3D) and foamy spheroid bodies in the substantia nigra. A few iron granules were also