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Ubiquitin-positive intraneuronal inclusions in the extramotor cortices of presenile dementia patients with motor neuron disease

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Summary. Ubiquitin-positive intraneuronal inclusions were found in the extramotor cortices of ten presenile dementia patients with motor neuron disease. There were inclusions in the hippocampal granular cells and in the small neurons of the superficial layers of the temporal and frontal cortices. Bunina bodies were present in the anterior horn cells in all cases. These results suggest that ubiquitin-related cytoskeletal abnormalities are common in cerebral non-motor small neurons in these patients.

Key words: Motor neuron disease – Dementia – Ubiquitin – Amyotrophic lateral sclerosis

Introduction

Presenile or progressive dementia with motor neuron disease (MND) has been increasingly reported [3, 14, 15, 17]. The clinicopathological features of these patients can be summarized as follows [3, 14, 15, 17]. There is progressive dementia with insidious onset in the presenile period. There are also bulbar symptoms, neurogenic muscular wasting, and occasional pyramidal tract signs, which have been diagnosed as amyotrophic lateral sclerosis (ALS) or spinal progressive muscular atrophy (SPMA). Changes in personality and loss of capacity for social interaction usually antedate the neurogenic atrophy, but in some cases neurological symptoms appear before mental symptoms. CT usually shows frontotemporal atrophy. The pathological findings are nonspecific slight degenerative changes in the central nervous system (CNS), especially in the frontotemporal cortices. Neuronal loss and degeneration, and slight sponginess in the upper layers, are the main pathological features in the cortex. There are no pathognomonic inclusions such as Pick bodies, cortical Lewy bodies, Alzheimer neurofibrillary tangles or plaques in the cortex. Degeneration of the substantia nigra and basal ganglia are frequently reported. Spinal cord pathological findings are consistent with MND.

However, no specific biological or histological marker has been found, and the relationship between this disorder and classic ALS is unclear. There are many opinions on the nosological status of this illness. It has been viewed as a new disease entity [14, 15], a variant of ALS [21] and a form of Creutzfeld-Jakob disease [4], and these patients have been said to resemble those with "frontal lobe dementia", "progressive subcortical gliosis" or "dementia lacking distinctive histological changes" [5].

Recently, we reported that there were ubiquitin-positive intraneuronal inclusions in the extramotor cortex of 7 out of 27 sporadic ALS patients [20]. In one patient who suffered from dementia with ALS, similar inclusions were also observed in many small neurons in the second layer of the frontal cortex. We therefore studied ten similar dementia patients with MND and found that all ten had ubiquitin-positive intraneuronal inclusions in the temporal and frontal cortices.

Materials and methods

We examined the brains and spinal cords of ten clinically and pathologically confirmed presenile dementia patients with MND (44–64 years at onset; average age, 55.4 years). Table 1 shows clinical and pathological characteristics of these patients. Several formalin-fixed, large paraffin-embedded 5-μm-thick sections from temporal cortex including hippocampal formation and frontal cortex were stained by the haematoxylin and eosin , Klüver-Barrera, Nissl, phosphotungstic acid-haematoxylin, Mallory, periodic acid-Schiff, Congo red, Bodian and modified Bielschowsky methods. We examined many specimens from the CNS in cases 2 and 3. Immunohistochemical examinations were done with various anti-

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Table 1. Summary of ten dementia patients with motor neuron disease. B, Bulbar symptoms; D, dementia (mental changes); U/E, upper extremities; ubiq.(+) granul., percentage of ubiquitin-positive hippocampal granular cells in the remaining granular cells; ubiq.(+) front., degree of ubiquitin-positive small neurons of second layer of frontal lobe; Bunina bodies, presence or absence of Bunina bodies in anterior horn cells of the spinal cord; senile plaques and NFT, degree of senile plaques examined by immunostaining with anti-β protein antiserum and NFT (Alzheimer neurofibrillary tangles) examined by immunostaining with anti-tau antiserum in the temporal cortex

Case	Age (years)	Sex	Duration (months)	Onset	Brain (g)	Ubiq. (+)		Bunina bodies	Senile plaques	NFT
						Granul.	Front.			
1	44	M	31	D	1190	14%	++	+	–	–
2	61	M	29	U/E	1200	12	++	+	–	–
3	50	M	36	D	1200	10	++	+	–	–
4	46	M	18	D	–	10	++	+	–	–
5	64	F	20	B+D	1220	10	Rare	+	+	Rare
6	51	M	28	D	1470	8	++	+	–	–
7	59	F	31	U/E	1090	8	++	+	–	–
8	52	M	28	B+D	1160	6	Rare	+	–	–
9	64	F	12	B+D	1110	3	+	+	–	Rare
10	63	M	47	D	1230	1	Rare	+	+	+

bodies, including monoclonal (1:1000, Chemicon, Calif.) and polyclonal (1:400, Dakopatts, Denmark; 1:20, Sigma, St. Louis, Mo.) anti-ubiquitin antiserum, anti-neurofilament (monoclonal, 160 kDa, 1:80, 200 kDa, 1:80, Sigma; polyclonal, 200 kDa, 1:400, Dr. H. Yamaguchi), anti-tau (monoclonal, 1:1000, Sigma), anti-α tubulin (monoclonal, 1:1000, Sigma), anti-β tubulin (monoclonal, 1:200, Sigma), anti-synthethic β protein (1–28) (1:500, our laboratory), anti-cystastin C (1:200, Dakopatts), anti-microtubule associated proteins (1:400, Sigma), anti-actin (1:200, Advance, Tokyo), anti-glial fibrillary acidic protein (1:1000, Dr. H. Yamaguchi), anti-swine vimentin (monoclonal, 1:200, Dako) and anti-desmin (1:100, Dakopatts) antiserum. Sections were stained by the avidin-biotin-peroxidase method.

Results

Clinical features

The initial symtoms of five patients were character changes and abnormal behaviour, followed by dysarthria, dysphagia, and muscular weakness and atrophy in the extremities (Table 1). The upper extremities were more frequently affected than the lower extremities. Three patients began with dementia and bulbar palsy. In two patients the initial symptom was hand muscle weakness. No patient had symptoms of parkinsonism, or a family history of MND. Seven patients were examined by brain CT. Three had slight to moderate frontal lobe atrophy (cases 2, 6, 9); three had frontal and temporal lobe atrophy (cases 1, 5, 8); and one had marked temporal lobe atrophy (case 10).

Neuropathological findings

In the spinal cords of all ten patients the anterior horn cells had degenerated and there were Bunina bodies. Central chromatolysis-like neuronal changes, spheroids and gliosis were also found. Pyramidal tract degeneration was present in all patients except cases 1, 3 and 8. Clarke's nuclei and posterior columns were intact. All patients had slight to moderate neuronal loss in the substantia nigra. Lewy bodies were not seen in the substantia nigra or cerebral cortex. Cases 5 and 10 had a few senile plaques in the frontal and temporal cortices. There were a few Alzheimer neurofibrillary tangles in the hippocampal areas of cases 5, 9, 10, but we could not observe the tangles in the frontal cortices of these three cases. Slight to moderate sponginess was observed in the frontal and temporal cortices in all patients. Cortical neuronal loss was slight, and slight to moderate subcortical gliosis was also seen in those lobes.

Ubiquitin-positive intraneuronal inclusions

All ten patients had ubiquitin-positive intracytoplasmic inclusions in neurons of the hippocampal granular cell layers (Fig. 1a). All three anti-ubiquitin antisera reacted positively to the inclusions. The granular cells were well preserved. The inclusions formed a crescent or circular pattern around the nucleus, and were seen in 1–14% of the remaining granular cells (Table 1). They were not seen under the routine light microscope stainings, and were not anilinophilic, argentophilic or congophilic. Many of the antibodies other than the anti-ubiquitin antibodies failed to show the inclusions (Fig. 1b). Eosinophilic inclusions, Lewy body-like inclusions and Alzheimer neurofibrillary tangles were not seen in the remaining granular cells.

These ten patients also had similar inclusions in small neurons of the second layer of the temporal cortices including entorhinal, occipitotemporal, inferior temporal, middle temporal and superior temporal gyri. The inclusions were more frequent in the second and third layers of the lateral part of the entorhinal cortex (Fig. 2a). The ubiquitin-positive and tau-negative inclusions were not seen in the pyramidal neurons of the hippocampus. Their incidence was almost similar in the granular cell layer and in the entorhinal cortex.

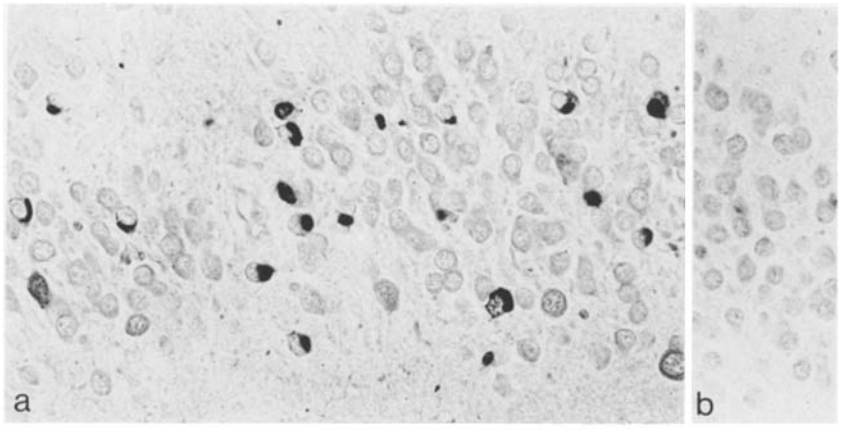

Fig. 1a, b. Adjacent sections of the granular cell layer of the hippocampus from case 1. **a** Immunostaining with ubiquitin (monoclonal), 1:1000. Many ubiquitin-positive intraneuronal inclusions formed a crescent or circular pattern around the nucleus (×200). **b** Tau immunostaining (1:1000) + haematoxylin. The inclusions were negative for tau. ×200

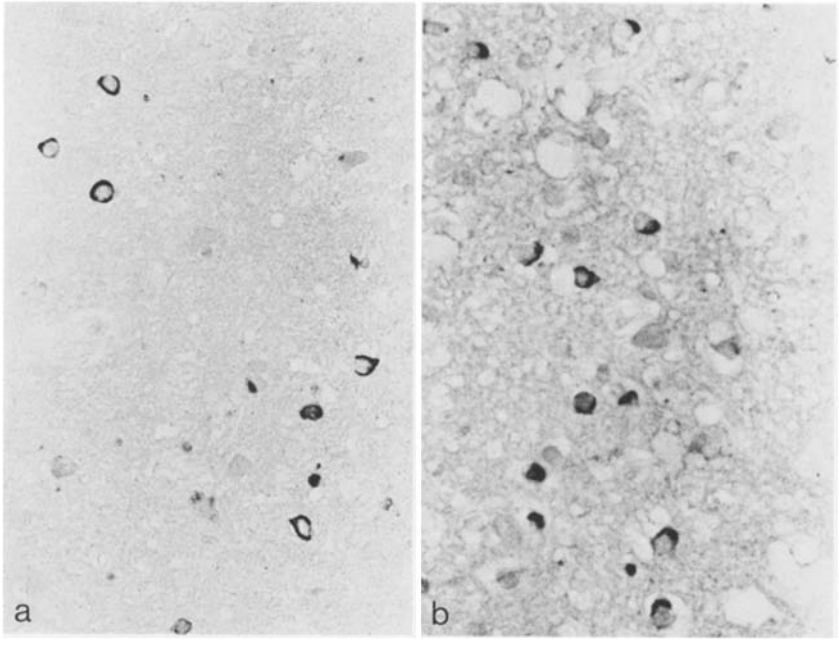

Fig. 2. a Ubiquitin-positive inclusions were seen in small neurons in the second and third layers of the lateral part of the entorhinal cortex of case 1. ×200. **b** Similar inclusions were also seen in small neurons in the second layer of the frontal cortex of case 6. Sponginess was observed in this layer. Ubiquitin (monoclonal) immunostaining. ×200

Similar inclusions were observed in small neurons in the second layer (external granular layer) of the frontal cortex (Fig. 2b), but their incidence was less than in the entorhinal cortex. The distribution of the inclusions was almost similar in each gyrus of the frontal cortex. The incidence of the inclusions seemed to be unrelated to the degree of sponginess of the second layer. In cases 5, 8 and 10 the inclusions in the frontal cortices were few.

We examined many specimens from the CNS in cases 2 and 3, but found no inclusions in the parietal and occipital cortices, basal ganglia, cerebellar granular layer, olfactory granular layer or brain stem.

Discussion

The major neuropathological finding in the CNS in ALS is the loss and degeneration of the large motor neurons. Bunina bodies, spheroids, central chromatolysis-like neuronal changes, Lewy body-like inclusions and skein-like inclusions are said to be associated with the early stages of ALS [10, 16, 19]. Of these, the presence of Bunina bodies is the only histological finding specific to ALS known at present. In all of the present cases, we observed Bunina bodies in the anterior horn cells, in addition to non-specific degeneration. Judging from the neuropathology of the motor neuron system we could not differentiate these patients from classic ALS or SPMA patients.

We have reported that there are ubiquitin-positive intraneuronal inclusions in the hippocampal granular cell layer and entorhinal cortex of about 25% of ALS patients [20]. Similar inclusions were not seen in the same areas in controls, including several with neurodegenerative disorders. Immunoelectron microscopy by the pre-embedding method with anti-ubiquitin antibody showed that those inclusions consist of loosely arranged linear filaments and granular materials [20]. We differentiated these inclusions from known intracytoplasmic bodies, including Alzheimer neurofibrillary tangles, Pick

bodies, cortical Lewy bodies and basophilic inclusions [20].

Ubiquitin, a protein implicated in non-lysosomal degradation of abnormal and short-lived cellular proteins, has recently been associated with abnormal cytoplasmic filaments characteristic of neurodegenerative diseases [7, 12]. Ubiquitin-associated filamentous inclusions in the anterior horn cells appear to be specific to ALS, and may be pathognomonic for this disease [6, 8, 9, 13]. Lowe et al. [10] reported that ubiquitin-positive intracytoplasmic bodies were also found in both large pyramidal cells and small neurons in the motor cortex in ALS. Recently, Nihei et al. [18] reported similar ubiquitin-positive intraneuronal perinuclear inclusions, in small neurons in the second layer, and to a lesser extent in the fifth layer, of the motor cortex of two familial ALS patients.

In our previous study, we found no clinical or pathological differences between the inclusion-positive and inclusion-negative cases [20].

In the present study, all ten dementia patients with MND had ubiquitin-positive inclusions in the temporal and frontal cortices. These results suggest that ubiquitin-related cytoskeletal abnormalities are common in cerebral non-motor small neurons in this group, and this is important in considering the pathogenesis and nosology of presenile dementia with MND. It raises the possibility that clinically dementia-free patients with ubiquitin-positive inclusions in the hippocampal areas (granular cell layer and entorhinal cortex) might have mental changes. Mental tests were usually not done in the advanced stages of this disease. Recent studies have shown that some classic ALS patients have impaired mental functions [2] and that cerebral blood flow and metabolism can be low, as measured by single photon emission computed tomography [11] and positron emission tomography [1]. The hippocampal changes may be related to the cognitive impairment sometimes seen in ALS. Another possibility is that there are various types of ALS, based on these ubiquitin-positive inclusions in the cerebrum: a ubiquitin-negative group which may be classic ALS, a group in which ubiquitin-positive inclusions are seen mainly in the granular cell layer and in the entorhinal cortex, and a group in which ubiquitin-positive inclusions are more widely distributed in the temporal and frontal cortices. From our results we believe that a majority of presenile dementia patients with MND belong to the last group.

To determine if ubiquitin-positive inclusions are specific to these patients, it will be necessary to look for ubiquitin-positive inclusions in the temporal and frontal cortices of the patients with similar pathological changes in the CNS, who have been described as having "frontal lobe dementia", "progressive subcortical gliosis" or "dementia lacking distinctive histological changes" [5].

Two of our ten cases had a few senile plaques in the frontal and temporal neocortices, and a few tangles were confined to the hippocampal areas in the three cases. Their incidence and densities were almost similar in our age-matched controls, so we thought that these senile findigns were only concomitant with aging.

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