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Basophilic cytoplasmic inclusions in a case of sporadic juvenile amyotrophic lateral sclerosis

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Abstract

A 24-year-old woman presented with progressive muscle atrophy and weakness of the right upper extremity. Subsequently her weakness rapidly extended to the left upper extremity, neck and lower extremities. Neurological examination disclosed involvement of the lower motor neuron system. She died 7 months after the onset. There were neuronal loss and reactive gliosis in the anterior horns of the spinal cord and much less frequently in the motor cortex. Basophilic cytoplasmic inclusions were observed in the thalamus and brain stem as well as the upper and lower motor neurons. Ultrastructurally, the inclusions lacked a limiting membrane and consisted of a meshwork of filamentous structures associated with granules. The inclusions failed to react with antibodies against phosphorylated neurofilament or cystatin C. Most of the inclusions show no reaction with anti-ubiquitin antibody, however, a few inclusions show granular reaction product deposits with this antibody. The inclusions were not immunostained with antibodies against TGN46 and MG-160, markers of the trans-Golgi network and the medial cisternae of the Golgi apparatus respectively, suggesting that they were not derived from the Golgi apparatus which was fragmented.

1. Introduction

Amyotrophic lateral sclerosis (ALS), which is characterized by progressive degeneration of motor neurons, is usually a disease of middle and late life, and juvenile sporadic ALS cases have rarely been reported. Characteristic basophilic cytoplasmic inclusions have been documented in several of these cases [1, 2, 3, 4, 5]. The basophilic cytoplasmic inclusions were also observed in an elder ALS case as well [6]. In this communication, we report another such a case and present immunohistochemical observation of basophilic cytoplasmic inclusions.

2. Patient and methods

2.1. Case report

A 24-year-old female noticed progressive muscle weakness of the right upper extremity. She rapidly developed weakness of the left upper extremity and neck and gait disturbance as well. A careful family history revealed no evidence of neurological disorders. Six months after the onset, neurological examination disclosed flaccid tetraparesis with muscle atrophy, neck weakness and hyporeflexia of the upper extremities. She had no eye movement disorders, facial weakness, dysarthria, dysphagea, tongue atrophy or Babinski signs. Laboratory examinations were unremarkable and showed no evidence of gammopathy. CSF protein content was elevated (82mg/dl).

Motor nerve conduction of the limbs was normal, but the amplitude of motor action potentials was decreased. Sensory nerve conduction was normal. Needle electromyography of the limbs revealed a decreased number of motor units and existence of occasional polyphasic long duration potentials and fibrillation potentials, suggesting active denervation. Brain and spinal cord MRI showed no abnormalities. She died 7 months after the onset.

2.2. Neuropathological methods

The tissues were fixed in buffered formalin and embedded in paraffin. Seven-μm sections were stained with hematoxylin and eosin, some sections were counterstained with luxol fast blue and some were used for immunohistochemical analyses. In addition, fresh segment of the lumbar spinal cord was fixed with 4% paraformaldehyde in 0.1M phosphate buffered saline (PBS, pH 7.4) for 2 hours, immersed in a sucrose series and quickly frozen on dry ice. Seven-μm frozen sections were stored at –80^{o}C until use.

For immunohistochemical studies, the sections mounted on slides were deparaffinized in xylene and ethanol, hydrated, and immersed in preheated Target Retrieval Solution (Dako) for 20 min. The sections were then washed with PBS and incubated with the primary antibody for 1 hour at room temperature. Polyclonal anti-ubiquitin (Dako; at a dilution of 1:200), monoclonal anti-phosphorylated neurofilament (SMI 31, Sternberger-Meyer, Inc; at a dilution of 1:10,000) and polyclonal anti-cystatin C (Dako; at a

dilution of 1:100) antibodies were used.

In addition, antibody against ubiquitin (Dako; at a dilution of 1:200) was applied to 7 μm frozen sections as well. Bound antibodies were detected by using an avidin-biotin-peroxidase complex (ABC) Kit (Vector Laboratories). Diaminobenzidine tetrahydrochloride was used as chromogen, and the sections were lightly counterstained with hematoxylin. Anti-trans-Golgi network (TGN46) antibody was raised against a synthetic polypeptide VPLLATESVKQEEAGVRPC (residues 18-35 of human TGN46 + a cystein residue [7, 8]). The polyclonal anti-TGN46 (at a dilution of 1:4000) and MG-160 (donated by Dr Gonatas to Dr Okamoto; at a dilution of 1:500) antibodies were also used for paraffin-embedded sections as reported previously [9, 10].

Several pieces of formalin-fixed lumbar anterior horn were processed for electron microscopy. The specimens were postfixed in 1% osmium tetroxide and embedded in epoxy resin. Ultrathin sections were cut with LKB-microtome and stained with 2% uranyl acetate and 0.2% lead citrate.

3. Results

At autopsy, the brain weighed 1,116g. Gross examination revealed no abnormalities of the brain or spinal cord except for brown discoloration of the thin cervical spinal anterior roots. There was marked neuronal loss of the spinal anterior horn with reactive astrocytosis. These changes were more prominent in the cervical segment than in the other segments of the spinal

cord. There were mild loss and degeneration of upper and brainstem motor neurons. In the lateral column of the spinal cord, degeneration or fiber loss was not apparent, although occasional reactive astrocytes were observed. Round, oval, lobulated, irregular-shaped or fragmented basophilic cytoplasmic inclusions were frequently observed (Fig. 1A-F). The inclusions were widely distributed in the motor cortex, spinal motor neurons, facial nucleus, hypoglossal nucleus, and less frequently in the thalamus, substantia nigra and midbrain tegmentum. In the motor cortex, the inclusions were observed in the small neurons as well as in the large Betz cells. The nuclei of neurons containing the inclusion were occasionally deformed and located on the periphery.

Most of the inclusions show no reaction with anti-ubiquitin antibody, however, a few inclusions show granular reaction product deposits with this antibody (data not shown). The inclusions showed no apparent immunoreactivity to antibodies against phosphorylated neurofilament, cystatin C, TGN46 or MG-160 (Fig. 1G). Sections stained with HE or anti-cystatin C antibody revealed no Bunina bodies in the motor neurons in the anterior horn of the spinal cord, although cystatin C-positive granules were scattered in the normal anterior horn cells [11]. Sections immunostained with anti-ubiquitin antibody disclosed no skein-like inclusions, Lewy body-like hyaline inclusions or other inclusions in the motor neurons in the anterior horn of the spinal cord. The neuron bearing the inclusion showed fragmentation of the Golgi apparatus (Fig. 1G). No neurofibrillary tangles were observed in the motor cortex or hippocampus.

Neurons bearing basophilic cytoplasmic inclusions were found in toluidine blue-stained sections. The inclusions, which had no limiting membrane, consisted of a meshwork of thick filaments (10 to 20 nm in diameter) associated with granules (15 to 30 nm in diameter) (Fig. 1H). No apparent neurofilamentous accumulation was observed among the inclusions.

4. Discussion

Neurological examination disclosed only the involvement of the lower motor neuron system in the present juvenile ALS patient, which seems to be consistent with the mild involvement of the upper motor neuron system at autopsy. However, basophilic cytoplasmic inclusions were frequently observed not only in the upper and lower motor neurons but also in other regions of the central nervous system, such as the thalamus, substantia nigra and midbrain tegmentum.

Basophilic cytoplasmic inclusions have been reported in 5 cases of pathologically confirmed sporadic juvenile ALS patients [1, 2, 3, 4, 5]. Electron microscopic examination in our case revealed that the inclusions were composed of a meshwork of thick filaments associated with granules, which appears to be identical to those described in previous reports [4, 5]. It has also been reported that the basophilic cytoplasmic inclusions contained RNA [3], endoplasmic reticulum and free ribosomes [4], microtubules and glycogen granules [12].

Clinically these sporadic juvenile ALS patients usually showed rapid

deterioration. In addition, they showed lower motor neuron involvement more frequently than upper motor neuron involvement (Table 1). Autonomic dysfunction was reported in two cases, and dementia in one case. Four out of 7 cases, including ours have shown basophilic cytoplasmic inclusions distributed widely in the central nervous system in addition to motor neurons [3, 4, 5]. It has been suggested that sporadic juvenile ALS is a distinct entity because of the clinical and neuropathological similarity of these cases [3].

Basophilic cytoplasmic inclusions are usually absent in sporadic adult-onset ALS [13, 14]. However, basophilic cytoplasmic inclusions indistinguishable from those seen in juvenile ALS have been reported in one case of adult-onset ALS [6]. This case presented a different clinical course (8.5 years) from other cases of juvenile ALS or sporadic adult-onset ALS.

Antibody against MG-160, a protein of the medial cisternae of the Golgi apparatus, is known to react reliably with the human Golgi apparatus [15]. Fragmentation of the Golgi apparatus has been observed in motor neurons in ALS using anti-TGN46 and anti-MG-160 antibody [9, 10, 16]. In the present study, the neurons with the basophilic cytoplasmic inclusion showed fragmentation of Golgi apparatus, however, the inclusions themselves were not immunostained by anti-TGN46 or anti-MG160 antibody, suggesting that they are not derived from Golgi apparatus.

The granulofilamentous structures of the basophilic cytoplasmic inclusions were similar to the Lewy body-like hyaline inclusion [17, 18] and the ubiquitin-positive inclusion in ALS with dementia [19]. However, both inclusions are apparently immunostained with anti-ubiquitin antibody [18, 19,

20, 21, 22], while the basophilic cytoplasmic inclusions show no or granular reaction product deposits with an anti-ubiquitin antibody [5, 6] as in our case. These observations indicate that ubiquitination seems to be less important for the formation of basophilic inclusions than the other two inclusions, although ubiquitin may be incorporated in the basophilic inclusions.

In conclusion, the present case suggests the basophilic cytoplasmic inclusions seen in rare sporadic juvenile ALS are unlikely to be ubiquitinated and not derived from Golgi apparatus. Further study is needed to elucidate the components of the inclusion.

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Table 1

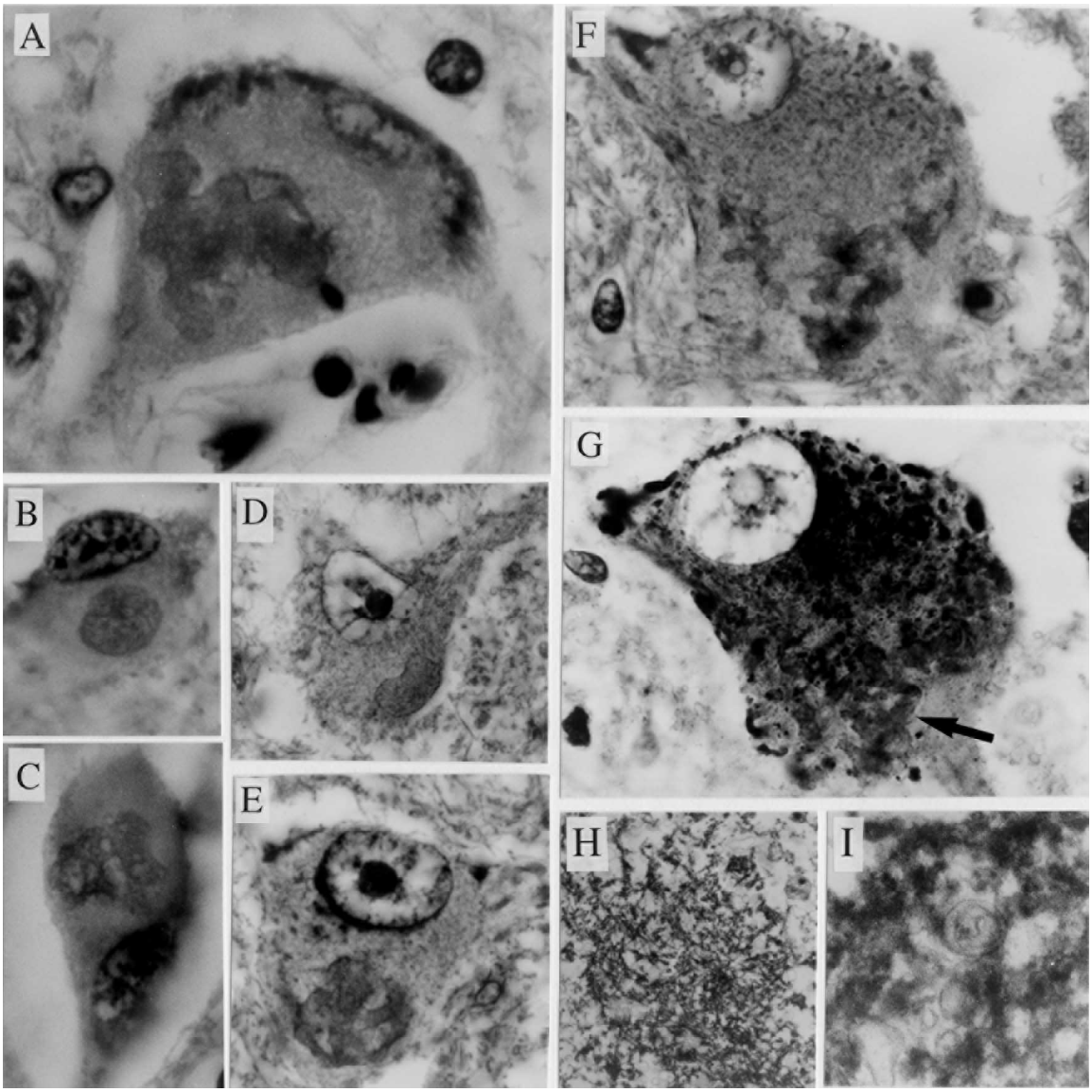
Cases of sporadic juvenile amyotrophic lateral sclerosis with cytoplasmic basophilic inclusion.

Case [citation]	Age at onset	Sex	Duration (months)	Clinical features UMN	LMN	B	others	Neuronal loss	BI
1 [1]	14	M	15	-	+	-		MN	lumber MN
2 [2]	15	M	18	(characteristic ALS)				MN	large MN
3 [3]	12	F	12	+	+	+		MN, dentate nucl	multisystem
4 [4]	16	F	12	+	+	+	autonomic dysfunction	MN	multisystem
5 [5]	22	F	32	?	+	?	dementia eye movement dysfunction autonomic symptoms	MN	multisystem
6 [5]	23	F	6	?	+	?	delayed development	MN	MN
7 (our case)	24	F	7	-	+	-		MN	multisystem

B, bulbar palsy; BI, basophilic inclusion; F, female; M, male; MN, motor neuron; LMN, lower motor neuron; UMN, upper motor neuron

Figure legend

Fig. 1. A, The degenerated neuron in the motor cortex have irregular-shaped basophilic cytoplasmic inclusion and deformed nucleus in the periphery (HE-LFB, original magnification x 330). B and C, The small cortical neurons also have oval or irregular-shaped basophilic cytoplasmic inclusions (HE-LFB, original magnification x 330). D and E, The anterior horn neurons in the lumbar segment of the spinal cord have bean-shaped or irregular-shaped basophilic cytoplasmic inclusions (HE, original magnification x 330). F, The anterior horn neuron in the lumbar segment of the spinal cord has irregular-shaped basophilic cytoplasmic inclusion (HE, original magnification x 330). G, The basophilic cytoplasmic inclusion (arrow) of the same neuron in the adjacent section is not stained with the anti-MG160 antibody, while the Golgi apparatus is fragmented. (Immunostain, original magnification x 330). H and I, Electron micrograph shows a meshwork of thick filaments associated with granules (original magnification x 5,000 and x 30,000).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