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Case study

α-Synuclein coaggregation in familial amyotrophic lateral sclerosis with *SOD1* gene mutation

Yo-ichi Takei MD[a], Kenya Oguchi MD[a], Hiroshi Koshihara MD[a], Akiyo Hineno MD[b], Akinori Nakamura MD[b], Shinji Ohara MD[a,*]

[a]*Department of Neurology, Matsumoto Medical Center, Chushin-Matsumoto Hospital, Kotobuski 811, Matsumoto, 399–0021 Japan*
[b]*Department of Medicine (Neurology and Rheumatology), Shinshu University School of Medicine, Asahi 3-1-1, 390–8621, Japan*

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Summary Immunohistochemical studies were performed on postmortem brain and spinal cord from a patient with familial amyotrophic lateral sclerosis characterized by a C111Y mutation in the *Cu/Zn superoxide dismutase* gene. Clinically, the patient presented with classical amyotrophic lateral sclerosis and died of respiratory failure at age 53 years without ventilator dependence, 4 years after the onset. Pathologically, loss of motor neurons was more extensive than upper motor neurons. Lower motor neurons developed massive intracellular cytoplasmic neuronal inclusions, which were immunoreactive for Cu/Zn superoxide dismutase and phosphorylated α-synuclein, often colocalized. The inclusions were TAR DNA-binding protein 43 negative. The clinicopathologic significance of coaggregation of α-synuclein and Cu/Zn superoxide dismutase protein, a novel finding in neurodegenerative disorders, needs further investigation.

1. Introduction

Approximately 10% of amyotrophic lateral sclerosis (ALS) cases are familial, 20% of which are caused by a mutation in the gene encoding Cu/Zn superoxide dismutase (*SOD1*). Misfolding and aggregation of SOD1 are related to a gain of toxic function in SOD1-related ALS [1]. We report the autopsy findings of a case with familial motor neuron disease associated with a *SOD1* C111Y mutation. Unlike previous reports of patients with a *SOD1* C111Y mutation, there was no immunoreactivity for 43-kd TAR DNA-binding protein (TDP-43), which is commonly found in cytoplasmic aggregates in sporadic ALS and SOD1-unrelated familial ALS [2,3]. Moreover, we found that the cytoplasmic aggregates in the present case were often immunoreactive for α-synuclein, which was colocalized with SOD1 protein, despite the absence of clinical and pathologic evidence of concurrent Parkinson disease.

2. Case report

The detailed genetic and clinical features of the family, showing marked intrafamilial phenotypic variations, have been previously reported [4]. The patient noticed weakness of his right hand muscle at age 49 years and, a year later, experienced similar changes in his left hand. Neurologic findings included fasciculation of bilateral arm muscles,

* Corresponding author.
E-mail address: oharas@cmatumoto.hosp.go.jp (S. Ohara).

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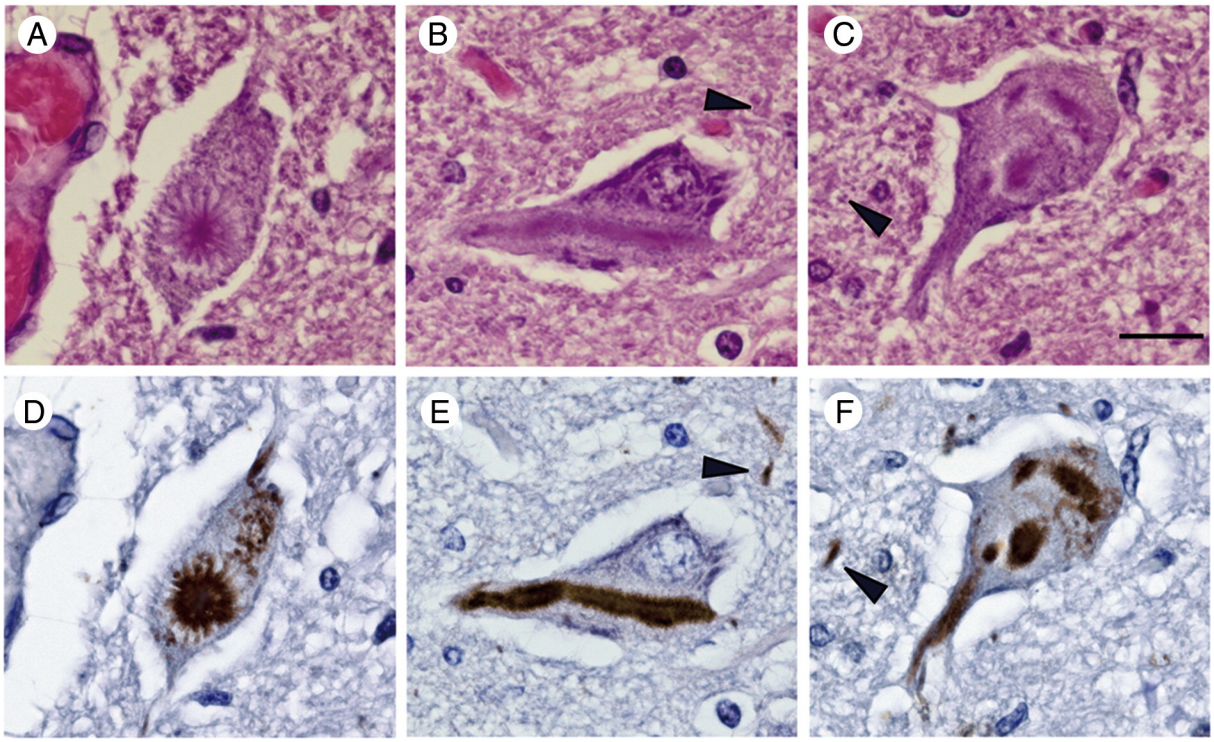

Fig. 1 H&E stain and SOD1 immunohistochemistry of neuronal cytoplasmic inclusions. These sections were firstly stained with H&E (A-C) and destained and processed for SOD1 immunohistochemistry (D-F). The fibrillar inclusions show asteroid-like (A and D) or brushed-bar (B and E) appearance and often found multiple within a cell (C and F). SOD1 immunohistochemistry could reveal intracellular and extracellular fibrils (arrowheads) not well identified with conventional H&E. Bar, 20 μm.

atrophy of dorsal interosseus muscles, increased deep tendon reflexes, and bilaterally positive Babinski signs. Electromyogram revealed neurogenic patterns involving his leg muscles. He gradually developed bulbar symptoms but continued to walk until 1 year before his death. He died of respiratory failure after 4 years of illness.

3. Materials and methods

The brain and spinal cord were fixed in 10% buffered formalin, and multiple tissue blocks were embedded in paraffin. Histologic examination was performed on 4-μm-thick sections using hematoxylin and eosin (H&E) and Kluver-Barrera staining. Selected sections were immunostained by the streptavidin-biotin method and double immunofluorescence with the following primary antibodies: mouse monoclonal antibodies against human SOD1 (0.5 μg/mL; MBL, Aich, Japan), phosphorylated α-synuclein (1:5000; WAKO, Osaka, Japan; 1:5000 LB509; Santa Cruz Biotech, Santa Cruz, CA), phosphorylated TDP-43 (1:5000 pS409/410; CosmoBio, Tokyo, Japan), a sheep polyclonal antibody against human SOD1 (1:100; Calbiochem, San Diego, CA), and a rabbit polyclonal antibody against TDP-43 (1:3000; Protein Tech Group, Chicago, IL). Reaction products were visualized with diaminobenzidine (DAB). For double immunofluorescence, deparaffinized sections were incubated with Sudan Black B to suppress autofluorescence, and fluorescein isothiocyanate (FITC)- or Cy3-labeled secondary antibodies were applied (Jackson Labs, Pittsburgh, PA) and were mounted in 50% glycerol in phosphate-buffered saline. Sections of midbrain from a case of classical Parkinson disease (65-year-old man, Hoehen-Yahr Scale, stage II) harboring numerous Lewy bodies were used as positive control.

To examine ultrastructural features of α-synuclein immunoreactive inclusions, areas of paraffin sections

Fig. 2 Immunostaining for SOD1 (A, C, E, G, and H) and phosphorylated α-synuclein (B, D, F, I, and J) examined on serial sections. A and B, Nucleus prehypoglossi. The intracytoplasmic inclusions (arrowhead) are immunoreactive for both SOD1 and α-synuclein. A neuron (arrow) undergoing neuronophagia contains 2 cytoplasmic inclusions, which are positive for SOD1 but negative for α-synuclein. Bar, 20 μm. C and D, Cervical anterior horn. Fibrillar aggregates in 2 neurons (indicated by arrow and arrowhead) are immunoreactive both for α-synuclein and SOD1. Bar, 50 μm. E and F, Lumbar anterior horn. SOD1 and α-synuclein are colocalized in one neuron (thin arrow), but not another (thick arrow). Bar, 100 μm. G-J, Enlarged photos of the area indicated by the arrows. The neuronal surface containing SOD1-positive inclusions (H) is covered by α-synuclein–positive cell processes (arrowheads in J). Bar, 20 μm.