Accumulation of Neurofilaments and SOD1-Immunoreactive Products in a Patient With Familial Amyotrophic Lateral Sclerosis With I113T SOD1 Mutation

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Objective: To report neuropathologic features of argyrophilic inclusions in the anterior horn cells, motor cortex Betz cells, and neurons of the medullary reticular formation, spinal posterior horn, and Clarke column in a Japanese case of familial amyotrophic lateral sclerosis with I113T substitution in exon 4 of the copper-zinc superoxide dismutase (SOD1) gene.

Methods and Results: These inclusions were stained pale pink on the hematoxylin-eosin stain and dark on the Bielschowsky stain. They were positive for antibodies to phosphorylated neurofilaments, ubiquitin, and SOD1. On electron microscopy, they consisted of abundant intermediate filaments of 10 to 20 nm in diameter with disordered array indicating neurofilaments.

Conclusion: These findings suggest that the I113T mutation induces accumulation of neurofilaments and SOD1 in the central nervous system neurons.

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MYOTROPHIC lateral sclerosis (ALS) is a fatal disease characterized by progressive degeneration of the upper and lower motor neurons. Approximately 10% of cases are inherited as an autosomal dominant trait, and 15% to 20% of cases of familial ALS (FALS) have mutations of copper-zinc superoxide dismutase (SOD1) gene on chromosome 21.1 We report neuropathologic findings of a Japanese case of FALS with the I113T mutation of the SOD1 gene. The gene analysis of this case was previously reported by Kikugawa et al.2

RESULTS

Mutation analysis of the SOD1 gene of the propositus disclosed I113T substitution in exon 4 as a heterozygous site.3 We failed to extract DNA of the wife from the paraffin-embedded tissues because of severe ischemic changes.

The brain of the propositus weighed 1305 g. Histological examination showed marked loss of the anterior horn cells with large axonal spheroids, more severely in the cervical cord than in the lumbar cord. There were many swollen anterior horn cells and a moderate number of Betz cells with heavily argyrophilic inclusions of redundant ribbonlike structures in the perikaryon. Similar inclusions were occasionally found in the reticular formation of the medulla oblongata and in the posterior horn and Clarke column of the spinal cord. The results of immunohistochemical examination of the argyrophilic inclusions of both the spinal motor neurons and the Betz cells are shown in Figure 1. There was a moderate to strong reaction with Bielschowsky stain, equivocal with Bodian stain, negative with Gallyas stain, strong with anti–phosphorylated neurofilament monoclonal antibody, moderate with anti–SOD1 polyclonal antibody, weak with anti–ubiquitin polyclonal antibody, and negative with anti–amyloid B protein antibody. On electron microscopy, the cytoplasmic inclusions in the anterior horn cells and the Betz cells consisted of accumulation of abundant intermediate filaments of disordered array and a variety of diameters between 10 and 20 nm, suggesting neurofilaments (NFs) (Figure 2). There were no neurofibrillary tangles, Bunina bodies, Lewy body–like inclusions, hyalin inclusions, skeinlike inclusions, or senile plaques.

The brain of the propositus’ wife weighed 990 g, and neurons of the brain and spinal cord were nearly gone because of the severe anoxic and ischemic incident. Argyrophilic nerve cells in the spinal cord, pons, and the pontine and...
PATIENTS AND METHODS

The propositus and his wife, who were first cousins, were both affected by ALS. The propositus developed muscle weakness and atrophy in the right hand in February 1994, when he was 68 years old. Neurologic examination in September 1994 showed marked atrophy with fasciculation and weakness in the upper and lower extremities, but no bulbar or pyramidal signs. He died of respiratory failure at age 69 years in July 1995, about 17 months after the onset of the disease.

The wife of the propositus developed muscle weakness of the right hand in June 1976, when she was 48 years old, and was diagnosed as having ALS. She was found in a deep coma and in cardiac arrest caused by respiratory failure on August 31, 1984, and required mechanical respiratory assistance for 10 months, until her death at age 57 years in June 1985.

Sections of the brains and spinal cords, fixed with formalin and embedded in paraffin, of the propositus and his wife were prepared for hematoxylin-eosin, Klüver-Barrera, Bodian, modified Bielschowsky, and Gallyas staining. The immunohistochemical investigations were done with the avidin-biotin-peroxidase complex technique by means of the following primary antibodies: anti-phosphorylated neurofilament monoclonal antibody (Sternberger Monoclonals Inc, Baltimore, Md; dilution, 1:500), anti-SOD1 polyclonal antibody (raised by Kohtaro Asayama, MD, Department of Pediatrics, Yamanashi Medical College, Yamanashi, Japan; 1:5000), anti-ubiquitin polyclonal antibody (Sigma-Aldrich Corp, St Louis, Mo; 1:100), anti-α antibody (raised by Yasuo Ihara, MD, Department of Neuropathology, University of Tokyo, Tokyo, Japan; 1:2000); and anti-amyloid β protein antibody (raised by Dr Yasuo Ihara; 1:1000). The anterior horn of the lumbar cord and the cerebral motor cortex of the propositus were examined by electron microscopy.

medullary reticular formation rarely existed, and they were weakly stained with anti-phosphorylated neurofilament monoclonal antibody and anti-SOD1.

The propositus is, to our knowledge, the first Japanese patient with FALS with I113T SOD1 gene mutation to undergo autopsy, in which argyrophilic cytoplasmic inclusions were verified neuropathologically not only in the anterior horn cells and the motor cortex Betz cells but also in the neurons of the medullary reticular formations, spinal posterior horns, and Clarke column. The argyrophilic inclusions reacted intensely with antibody to the phosphorylated epitope of NFs and consisted of abundant intermediate filaments of 10 to 20 nm in diameter with a disordered array suggesting NFs.

Four autopsy cases of FALS with I113T mutation were reported previously, 1 from Canada and 3 from the United Kingdom. The most characteristic feature common to them was accumulation of filamentous materials in nerve cells, either NFs or neurofibrillary tangles. Rouleau et al from Canada reported a case...
that coexisted with massive accumulations of NFs in the anterior horn cells. Ince et al from the United Kingdom recently reported 2 cases in which there were hyaline conglomerate inclusions in the motor and nonmotor neurons. These inclusions reacted strongly with antibodies to phosphorylated and nonphosphorylated epitopes of NFs, but not with anti-SOD1 antibody. Orrell et al from the United Kingdom described 1 patient who had survived for more than 20 years and showed neurofibrillary tangles in the neurons of the globus pallidus, substantia nigra, locus ceruleus, and inferior olivary nuclei.

The coexistent immunoreactivity with NFs and SOD1 may be unique to the present case or it may result from the different properties of the antibodies used. The existence of FALS that has the same I113T SOD1 mutation with similar neuropathologic findings among different ethnic groups and races suggests that the I113T mutation is firmly related to massive accumulation of NFs in the central nervous system that leads to degeneration of nerve cells.

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