Familial Amyotrophic Lateral Sclerosis With a Novel Leu126Ser Mutation in the Copper/Zinc Superoxide Dismutase Gene Showing Mild Clinical Features and Lewy Body–Like Hyaline Inclusions

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Background: Mutations in the SOD1 gene are responsible for approximately 25% of all familial amyotrophic lateral sclerosis (ALS) cases. However, the correlation between the clinical and pathological features and the various SOD1 gene mutations has not been well characterized.

Objectives: To screen the SOD1 gene in search of potential mutations and to obtain clinical and pathological data for 2 Japanese families with ALS.

Design: Clinical histories and neurological findings, gross and microscopic pathological features, and DNA analysis of the SOD1 gene.

Results: The 2 families with ALS showed a novel missense mutation in the SOD1 gene, which was heterozygous for point mutation TTG to TCG, causing substitution of leucine for serine at codon 126 (Leu126Ser) in exon 5. Clinically, patients showed slower disease progression and lack of upper motor neuron signs. Neuropathologically, the autopsied patient showed the form of familial ALS with posterior column involvement, and the pontocerebellar tract and the dentate nuclei of the cerebellum were also involved. Furthermore, abundant Lewy body–like hyaline inclusions were observed in the affected motor and nonmotor neurons.

Conclusions: Familial ALS with a novel Leu126Ser mutation in the SOD1 gene showed mild clinical features and lack of upper motor neuron signs. We believe that Leu126Ser might be associated with the clinical features and that the mutation site in the SOD1 gene and disease duration might be associated with the formation of Lewy body–like hyaline inclusions.

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IN APPROXIMATELY 5% to 10% of individuals, amyotrophic lateral sclerosis (ALS) is inherited as an autosomal dominant trait.1 Approximately 25% of familial ALS (FALS) cases are associated with mutations in the copper/zinc superoxide dismutase (SOD1) gene2,3 encoded on chromosome arm 21q22.1.4 More than 60 kinds of mutations in the SOD1 gene have been identified,5,6 mostly single base pair (bp) substitutions in an exon or at a splice junction, except for 3 cases with 2- or 4-bp deletions.7-9

The correlation between the clinical and pathological features and the various SOD1 gene mutations has not been well characterized. Patients with FALS and the Ala4Val mutation show defined clinical features and rapid progression, and they usually die within 1 year of disease onset.5,9 However, other mutations in the SOD1 gene produce a wide spectrum of clinical features, including age of onset, disease duration, and severity and distribution of clinical symptoms.3 We describe 4 patients with ALS in 2 families with a novel mutation at codon 126 of the SOD1 gene and show their characteristic clinical and pathological features.

MOLECULAR GENETICS

The probands had a heterozygous point mutation in exon 5 of the SOD1 gene (codon 126 TTG to TCG) that resulted in an amino acid substitution from leucine to serine (Leu126Ser) (Figure 1C). No mutations were detected in the other exons. The mutation was confirmed by sequencing the complementary strand. Because this mutation created an AvaI restriction site, it was analyzed using polymerase chain reaction–restriction fragment length polymorphism (Figure 1D). AvaI digestion in exon 5 of the SOD1 gene was found in all 3 patients examined but not in 108 controls (mean age, 68.7 years).
PATIENTS AND METHODS
Pedigrees of family A and B are shown in Figure 1A-B.

FAMILY A
Patient 1
A 52-year-old man first noticed weakness of the left lower limb that extended to the other limbs during the next 6 months. At age 54 years he developed dysphagia and dysarthria. Neurological examination revealed muscular weakness, atrophy and fasciculation of all limbs, and decreased deep tendon reflex. The Babinski sign was absent. Cognitive function, the cranial nerves, sensation, and the autonomic system were intact. Electromyography revealed active denervation discharge in muscles of all limbs. At age 58 years he was given respirator support because of dyspnea; he died 4 days later. The pyramid sign was not observed until his death. Disease duration was 6 years. An autopsy was performed.

Patient 2
A 28-year-old man (the nephew of patient 1) first noticed right lower limb weakness that progressed to the other limbs during the next year. Neurological examination revealed weakness, atrophy, and fasciculation of all limbs and decreased deep tendon reflex. The Babinski sign was absent. Electromyography showed a neurogenic pattern in all muscles examined. Muscle biopsy of the quadriceps femoralis pathologically revealed neurogenic change. Patient 2 died at age 36 years, with a disease duration of 8 years.

FAMILY B
Patient 3
A 74-year-old man who experienced right hemiparesis by cerebral infarction at age 71 years first noticed lower limb weakness that progressed to the upper limbs and dysarthria and dysphagia during the next year. Deep tendon reflex was decreased and the Babinski sign was absent. Patient 3 died of pneumonia at age 76 years, and disease duration was expected to be 20 months if we assume the age when he noticed lower limb weakness to be the onset of disease.

Patient 4
A 54-year-old woman (the sister of patient 3) first noticed lower limb weakness that progressed to the upper limbs during the next 2 years. On neurological examination, the muscles of all limbs showed marked weakness and atrophy with fasciculation and decreased deep tendon reflex. The Babinski sign was absent. Patient 4 died at age 68 years.

MOLECULAR GENETIC STUDIES
Genomic DNA was extracted from heparin-anticoagulated peripheral blood samples from patients 1 and 3 and from paraffin-embedded tissue from the quadriceps femoralis biopsy sample from patient 2. The region that encoded the 5 exons of the SOD1 gene was amplified by polymerase chain reaction, which was performed with pairs of primers described previously. Polymerase chain reaction products were sequenced by the protocol of the ABI PRISM Dye Terminator Cycle Sequencing Ready Reaction Kit (Perkin-Elmer Applied Biosystems, Santa Clara, Calif). The brain and spinal cord of patient 1 were fixed in 10% buffered formalin. Multiple tissue blocks were embedded in paraffin; sectioned at 7-µm thickness; and stained with hematoxylin-eosin, Klüver-Barrera, and Holzer stains. For immunohistochemical analysis, polyclonal primary antibodies were used against ubiquitin (Dako, Glostrup, Denmark), α-synuclein (Dako), neurofilament (Dako), and rabbit SOD1 (provided by M. Nishibori, MD, PhD, Department of Pharmacology, Okayama University Medical School, Okayama, Japan). Immunostaining was performed using the avidin-biotin peroxidase complex method with a Vectastain ABC Elite kit (Vector, Burlingame, Calif).

NEUROPATHOLOGICAL FINDINGS
Degeneration of the cerebral cortices, basal ganglia, thalamus, and midbrain was unremarkable. There were no abnormalities in the striatum, globus pallidus, substantia nigra, thalamus, hypothalamus, and red nuclei. There was mild loss of neurons with gliosis in the pontine, hypoglossal nerve, and vestibular nuclei. The tracts of the pontine transverse fibers showed severe degeneration accompanied by fibrillar gliosis. In the cerebellum, the dentate nucleus showed granule degeneration, and patchy loss of Purkinje cells was also observed. Histological examination showed diffuse cell loss in the anterior horn, middle root zone of the posterior column, spinocebellar tracts, and Clark column nuclei, with preservation of Onuf nuclei (Figure 2). Demyelination of the corticospinal tract was observed. Bunina bodies were not found. We found Lewy body–like hyaline inclusions (LBHIs) in neurites and neurons in the anterior horn, hypoglossal nerve nucleus, dentate nucleus of the cerebellum, and pontine transverse fibers. The LBHIs were labeled by the antibody against SOD1, ubiquitin, and neurofilament but not labeled by the antibody against α-synuclein (Figure 3).

COMMENT
We described 4 patients with FALS in 2 families with a novel Leu126Ser mutation in exon 5 of the SOD1 gene. We could not examine the SOD1 genes of other unaffected members of the family because they declined gene analysis. Therefore, it is likely that Leu126Ser can be a rare polymorphism. However, other mutations at codon 126 have been found to be associated with FALS, and 108 control subjects did not have such mutant alleles. This result strongly indicates that Leu126Ser must be responsible for the pathogenesis of FALS in these patients.

Clinically, the patients’ ages of onset were disparate; however, they showed similar mild progression of illness and long disease duration of 6, 8, and 14 years (excluding patient 3) compared with the duration in patients with FALS.
and the SOD1 mutation (mean duration, 3.9 years). Disease onset in patient 3 is late (age 74 years); however, he experienced cerebral infarction at age 72 years. We expect that he might have noticed disease onset lately because of aging and his complication of cerebral infarction; therefore, it is difficult to compare simply patient 3 with the other patients with Leu126Ser. Although the mother of patient 2 must be carrying the mutant gene, she is presently healthy at age 65 years and has declined gene analysis. Moreover, none of the patients had clinical features of upper motor neuron involvement in life, but the autopsy case showed corticospinal tract involvement. The lack of upper motor neuron features in life probably reflects the severe early lower motor neuron degeneration and late upper motor neuron changes.

Familial ALS is neuropathologically classified into 2 forms. The classical form is similar to sporadic ALS, in which degeneration is restricted to motor neurons only. The other form has posterior column involvement and is characterized by degeneration of middle root zones of the posterior column and spinocerebellar tracts, in addition to the lesion of the motor neuron system. Contrary to the mild clinical features, the neuropathological findings in patient 1 showed the form of FALS with posterior column involvement, and the degeneration extended to the pontocerebellar tract and the dentate nuclei of the cerebellum, which are usually spared in FALS. The reason for the discrepancy between the clinical and neuropathological findings in patient 1 is not clear, but it is possible that the mutation site of the SOD1 gene and disease duration might be significant. Different types of mutations at codon 126 have been described in 4 patients (Table 1). Three patients had 2-bp deletions and the other had Leu126stop. Although few clinical data are provided, at least 2 of these patients showed rapid progression compared with the patients with Leu126Ser in this study, and their duration was less than 2 years. The difference of progression might be because the 2-bp deletion in codon 126 generates a frameshift that introduces a premature stop codon. The patient with the 2-bp deletion in the report by Kato et al showed longer duration (11 years) than the other 2 patients; however, he was given respirator support within 1 year of disease onset and it is impossible to compare this patient with the other 2.
Approximately 20 autopsied FALS cases with SOD1 mutation, including patient 1 in this study, are currently available for analysis of neurological findings. Nine kinds of SOD1 mutations were found in autopsied cases: Ala4Thr, Ala4Val, His46Arg, His48Glu, Glu100Gly, Ileu113Thr, Val118Leu, a 2-bp deletion in codon 126, and Leu126Ser (patient 1 in this study) (Table 2). Among them, LBHIs are observed only in patients with Ala4Thr, Ala4Val, His46Arg, a 2-bp deletion in codon 126, and Leu126Ser. The major components of the LBHIs are 15- to 25-nm granule-coated fibril and granular materials, which are positive for SOD1 antibody by immunoelectron microscope; LBHIs include SOD1 as core protein. In patients with FALS and posterior column degeneration, LBHIs are characteristically found in the lower motor neuron and are rarely

Table 1. Comparison of Clinical Findings in Patients With FALS and a Mutation in Codon 126 of the SOD1 Gene

<table>
<thead>
<tr>
<th>Gene analysis</th>
<th>Leu126Ser</th>
<th>Leu126Ser</th>
<th>Leu126Ser</th>
<th>NE</th>
<th>2-bp deletion</th>
<th>2-bp deletion</th>
<th>2-bp deletion</th>
<th>Leu126stop</th>
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<tbody>
<tr>
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<td>M</td>
<td>M</td>
<td>F</td>
<td>F</td>
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<td>ND</td>
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<td>Age at onset, y</td>
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<td>28</td>
<td>74</td>
<td>54</td>
<td>42</td>
<td>46</td>
<td>54</td>
<td>ND</td>
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<tr>
<td>Disease duration, y</td>
<td>6</td>
<td>8</td>
<td>2</td>
<td>14</td>
<td>2</td>
<td>2</td>
<td>1.5</td>
<td>11†</td>
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<td>Neuropathological findings</td>
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<tr>
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<tr>
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<td>+</td>
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<td>+</td>
<td>+</td>
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<td>−</td>
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<td>−</td>
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<td>−</td>
<td>−</td>
<td>Urinary incontinence, eye movement disorder</td>
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<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>ND</td>
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</tbody>
</table>

*FALS indicates familial amyotrophic lateral sclerosis; ND, not described; bp, base pair; UMN, involvement was defined by the presence of spasticity on examination (spasticity criteria included the presence of brisk deep tendon reflexes, a jaw jerk, Hoffman reflex, finger jerks, increased muscle tone, and pseudobulbar affect); LMN, involvement was indicated by muscle atrophy and weakness on examination and evidence of active or chronic motor unit denervation on electromyography; minus sign, negative; and plus sign, positive.

†The patient received respirator support 1 year after disease onset.
seen beyond the motor neuron system. In our patient 1, LBHIs are observed in the affected nonmotor neurons. In patients with FALS, LBHIs are observed not only in long survivors with His46Arg, a 2-bp deletion in codon 126, and Leu126Ser (present study), but also in short survivors with Ala4Thr and Ala4Val. In contrast, astrocytic hyaline inclusions are observed only in long-surviving patients with FALS who have 2-bp deletions in codon 126 or Leu126Ser. Astrocytic hyaline inclusions are not found in short-surviving patients with FALS who have 2-bp deletions in codon 126. The essential common constituents between LBHIs and astrocytic hyaline inclusions are immunoelectron microscopically SOD1-positive granular-coated fibril. These common properties of the 2 types of inclusions might reflect a representation of the same disease process. Because the published literature concerning FALS autopsy cases with mutation of the SOD1 gene is remarkably limited in number, we cannot conclude but assume that the mutation site of the SOD1 gene and disease duration might be related to the formation of LBHIs. Further pathological and molecular analyses of a large number of patients with FALS are necessary to confirm this assumption.

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