Nuclear TAR DNA Binding Protein 43 Expression in Spinal Cord Neurons Correlates With the Clinical Course in Amyotrophic Lateral Sclerosis

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Abstract

TAR DNA binding protein 43 (TDP-43) has been considered a signature protein in frontotemporal dementia and amyotrophic lateral sclerosis (ALS), but not in ALS associated with the superoxide dismutase 1 (SOD1) gene mutations (ALS1). To clarify how TDP may be involved in ALS pathogenesis, clinical and pathological features in cases of sporadic ALS (SALS) were analyzed. In SALS patients with rapid clinical courses, TDP mislocalization (i.e., cytoplasmic staining and TDP-positive cytoplasmic inclusions) in anterior horn cells was frequent. In SALS patients with slow clinical courses, TDP-43 mislocalization was rare. In an ALS1 patient with the SOD1 gene mutation C111Y, there were numerous TDP-positive inclusions and colocalization of SOD1 and TDP. In mutant SOD1 transgenic (G93A) mice at the end stage (median, 256 days), TDP-positive inclusions and TDP colocalization with SOD1 were also observed; nuclear TDP-43 immunoreactivity was highly correlated with life span in these mice. In both humans and mice, nuclei that stained strongly for TDP were large and circular; weakly stained nuclei were atrophic or deformed. In conclusion, low levels of TDP expression in the nucleus correlate with a rapid clinical course in SALS and in ALS1 model mice, whereas high levels of TDP expression in the nucleus correlate with a slow clinical course in SALS.

Key Words: Amyotrophic lateral sclerosis, ALS, Anterior horn cell, ALS1, G93A transgenic mice, Lewy body–like hyaline inclusion, SOD1, TDP-43.

INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is a fatal motor neuron disease that causes progressive motor paralysis. The underlying pathogenetic mechanisms are largely unknown in 90% of ALS patients, that is, those with sporadic ALS (SALS). Of the 10% of ALS cases with familial ALS (FALS), approximately one fifth are associated with a mutation in the superoxide dismutase 1 (SOD1) gene; these patients are classified as ALS1 (1, 2). The pathogenesis of ALS1 is thought to involve aggregation of mutant SOD1 and subsequent oxidative stress (3). Another rare cause of juvenile autosomal recessive FALS is the gene that encodes ALS2, also known as alsin (4, 5). In most cases of FALS, however, the causative gene has not been identified because of low penetrance.

TAR DNA binding protein 43 (TDP-43), a nuclear protein, contains 2 fully functional RNA recognition motif domains and a C-terminal region that is capable of binding directly to several proteins of the heterogeneous nuclear ribonucleoprotein family (6–8); these ribonucleoproteins have a variety of functions including the modification, stabilization, and transport of RNA. The TDP modifies the splicing of exon 9 of the cystic fibrosis transmembrane conductance regulator gene (9) and of exon 3 of the apolipoprotein A-II gene (10). Recently, it also has been reported that loss of TDP in vitro results in nuclear dysmorphism, misregulation of the cell cycle, and apoptosis (11).

Neuronal inclusions, such as Lewy body–like hyaline inclusions (LBHIs), or the aggregation of mutant SOD1 in ALS1 (3, 12) are known to be important pathological features in the pathogenesis of neurodegenerative diseases. The TDP is a component of the ubiquitin-positive inclusions and neurites observed in frontotemporal dementia and ALS (13, 14). The TDP-positive round or filamentous inclusions in the cytoplasm and mislocalization of TDP from the nucleus to the cytoplasm have been observed in all cases of SALS (15) and FALS, but not in ALS1 (16, 17). A novel missense mutation in TDP was recently identified as causative in familial motor neuron disease and SALS (18, 19). Although the concept of TDP proteinopathy has been suggested (14, 20), the presence of TDP-positive inclusions in other diseases, including hippocampal sclerosis, Alzheimer disease (21), Parkinson disease (22), Pick disease (23), and neoplastic lesions (24), complicates this issue. Furthermore, Sanelli et al (25) have reported that TDP is not a major ubiquitinated target within the pathological inclusions of ALS.
TABLE. Clinical and Pathological Data*

<table>
<thead>
<tr>
<th>Patients</th>
<th>Age, years</th>
<th>Disease Duration, years</th>
<th>Initial Weakness Manifestation</th>
<th>Use of Respirator (Estimated Duration)</th>
<th>Cause of Death</th>
<th>Family History</th>
<th>No. Large Neurons†</th>
<th>No. Large Neurons With TDP Mislocalization†</th>
<th>Median (range)</th>
<th>Median (range)</th>
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<tr>
<td>SALS r-1</td>
<td>69</td>
<td>0.7</td>
<td>L</td>
<td>No</td>
<td>Pneumonia</td>
<td>No</td>
<td>13 (I)</td>
<td>6 (I)</td>
<td>40 (20–79)</td>
<td>2.0 (0.7–7.3)</td>
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<tr>
<td>SALS r-2</td>
<td>77</td>
<td>0.8</td>
<td>U</td>
<td>No</td>
<td>Resp. f.</td>
<td>No</td>
<td>23 (I)</td>
<td>8 (I–D)</td>
<td>34 (4–55)</td>
<td>4 (4–5)</td>
</tr>
<tr>
<td>SALS r-3</td>
<td>49</td>
<td>0.8</td>
<td>L, B</td>
<td>No</td>
<td>Resp. f.</td>
<td>No</td>
<td>9 (I)</td>
<td>4 (I)</td>
<td>35 (4–55)</td>
<td>6 (4–5)</td>
</tr>
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<td>60</td>
<td>1.5</td>
<td>B, U</td>
<td>No</td>
<td>Pneumonia</td>
<td>No</td>
<td>36 (D)</td>
<td>12 (D–I)</td>
<td>25 (4–55)</td>
<td>38 (4–55)</td>
</tr>
<tr>
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<td>59</td>
<td>1.5</td>
<td>U</td>
<td>No</td>
<td>Resp. f.</td>
<td>No</td>
<td>16 (D)</td>
<td>9 (D–I)</td>
<td>12 (4–55)</td>
<td>22 (4–55)</td>
</tr>
<tr>
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<td>2</td>
<td>B, U</td>
<td>No</td>
<td>Resp. f.</td>
<td>No</td>
<td>22 (D)</td>
<td>12 (D–I)</td>
<td>25 (4–55)</td>
<td>38 (4–55)</td>
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<tr>
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<td>B</td>
<td>No</td>
<td>Resp. f.</td>
<td>No</td>
<td>22 (D)</td>
<td>11 (D–I)</td>
<td>21 (4–55)</td>
<td>11 (4–55)</td>
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<td>71</td>
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<td>U</td>
<td>No</td>
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<td>No</td>
<td>21 (I)</td>
<td>4 (I–D)</td>
<td>69 (8–55)</td>
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<td>61</td>
<td>2</td>
<td>U</td>
<td>No</td>
<td>Resp. f.</td>
<td>No</td>
<td>8 (I)</td>
<td>3 (I–D)</td>
<td>12 (9–55)</td>
<td>9 (9–55)</td>
</tr>
<tr>
<td>SALS r-10</td>
<td>64</td>
<td>2</td>
<td>L</td>
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<td>7 (I)</td>
<td>0</td>
<td>9 (123)</td>
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<td>25 (D)</td>
<td>4 (I–D)</td>
<td>14 (7–55)</td>
<td>7 (7–55)</td>
</tr>
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<td>B, U</td>
<td>No</td>
<td>Resp. f.</td>
<td>No</td>
<td>12 (D)</td>
<td>7 (D–I)</td>
<td>18 (19–55)</td>
<td>19 (19–55)</td>
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<tr>
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<td>Resp. f.</td>
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<td>4 (I)</td>
<td>0</td>
<td>26 (26–55)</td>
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</tr>
<tr>
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<td>79</td>
<td>5</td>
<td>U</td>
<td>No</td>
<td>Resp. f.</td>
<td>No</td>
<td>2 (I)</td>
<td>0</td>
<td>0 (0–0)</td>
<td>0 (0–0)</td>
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<tr>
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<td>U</td>
<td>No</td>
<td>Resp. f.</td>
<td>No</td>
<td>9 (I)</td>
<td>0</td>
<td>4 (2–2)</td>
<td>2 (2–2)</td>
</tr>
<tr>
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<td>5.5</td>
<td>U, L</td>
<td>No</td>
<td>Resp. f.</td>
<td>No</td>
<td>4 (I)</td>
<td>0</td>
<td>2 (2–2)</td>
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<tr>
<td>SALS s-4</td>
<td>48</td>
<td>6.7</td>
<td>?</td>
<td>No</td>
<td>Resp. f.</td>
<td>No</td>
<td>15 (I)</td>
<td>0</td>
<td>0 (0–2)</td>
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<tr>
<td>SALS s-5</td>
<td>45</td>
<td>7.3</td>
<td>?</td>
<td>No</td>
<td>Resp. f.</td>
<td>No</td>
<td>2 (I)</td>
<td>0</td>
<td>0 (0–3)</td>
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*ALS1, amyotrophic lateral sclerosis (SOD-1 gene mutation); B, bulbar; D, diffuse staining pattern; D > I, predominantly diffuse staining pattern; I, inclusion pattern; I > D, predominantly inclusion pattern; L, lower limb; Ren. f., renal failure; Resp. f., respiratory failure; SALS r, sporadic ALS with a rapid clinical course (i.e. ≤2.5 years); SALS s, SALS with a slow clinical course; U, upper limb.

†Large neurons with cytoplasm exceeding 37 μm in diameter and clear nucleoli were counted. TAR DNA binding protein (TDP) mislocalization includes a diffuse staining pattern in the cytoplasm (D) and TDP-positive inclusions in the cytoplasm (I). Cell types containing TDP-positive inclusions were estimated as neuronal or glial on the basis of the morphology of their nuclei and cytoplasm.
To clarify how TDP may be involved in pathogenetic mechanisms in ALS, we examined SALS and ALS1 patients clinically and pathologically by immunohistochemistry using an anti-TDP antibody. We also examined the lumbar spinal cords of mutant SOD1 transgenic mice (G93A mice) that have lower copy numbers of the mutant SOD1 gene than the G93A mice previously examined by Robertson et al (26); G93A mice with low copy numbers of the mutant SOD1 gene show pathological changes that are similar to those in patients with ALS (27).

**MATERIALS AND METHODS**

**ALS Cases and Pathological Assessment**

Fixed paraffin-embedded 4-µm-thick sections through the lumbar spinal cord at the L5 level were obtained from Osaka University Graduate School of Medicine (Suita) for clinicopathologic analysis. These patients had SALS (n = 18; age at death 62 [median, 45–79] years; disease duration, 2 [0.7–7.3] years) or ALS1 (n = 6; age at death, 58 [42–71] years; disease duration, 9 [2–11] years; Table). All neuropathologic analyses were performed by trained neuropathologists. Sporadic ALS patients who did and did not have a history of respirator use and whose deaths were caused by respiratory failure or pneumonia were examined. Clinical data including the localization of initial symptoms, history of respirator use, cause of death, and family history are shown in the Table.

Deparaffinized sections were incubated for 30 minutes with 0.3% hydrogen peroxide to quench endogenous peroxidase activity and then washed with PBS. The primary antibodies used were rabbit polyclonal antibodies against TDP-43 (1:3000, Protein Tech Group, Chicago, IL) and ubiquitin (1:2000, Dako, Glostrup, Denmark), mouse monoclonal antibodies against human SOD1 (0.5 µg/mL, clone 1G2, MBL, Aichi, Japan) and phosphorylated neurofilament (1:10,000, SMI31, Covance, Berkeley, CA), and a sheep polyclonal antibody against human SOD1 (1:20,000, Calbiochem, San Diego, CA); these were applied to serial sections as primary antibodies. Goat anti-rabbit and anti-mouse immunoglobulins conjugated to peroxidase-labeled dextran polymer (ready to use, Dako Envision+, Dako Corp, Carpinteria, CA) and rabbit anti-sheep immunoglobulin (1:1000, Abcam PLC, Cambridge, United Kingdom) were used as secondary antibodies. Reaction products were visualized with 3,3'-diaminobenzidine tetrahydrochloride (ImmPACT DAB, Vector Laboratories, Burlingame, CA), and hematoxylin was used to counterstain cell nuclei.

To estimate the numbers of TDP-positive cytoplasmic inclusions, large neurons that had clear nucleoli and cell bodies with a diameter greater than 37 µm (28) (presumed to be α motoneurons) and the numbers of neurons with TDP mislocalization from the nucleus to the cytoplasm in the gray matter, from video images of each section obtained with a digital camera (Keyence VB-7010, Keyence, Osaka) attached to a light microscope (EclipseE800, Nikon, Tokyo), were counted. The diameters of the neurons were measured with the aid of image analysis software (VH-H1A5, Keyence).

Mislocalization of TDP was defined as the presence of a TDP-negative nucleus and TDP-positive cytoplasm. In mislocalizations of TDP, there can also be diffuse staining patterns in the cytoplasm and TDP-positive filamentous or round inclusions in the cytoplasm. Cells containing TDP-positive inclusions were classified as neurons or glia on the basis of the shape of their nuclei and cytoplasm.

**Animals**

Transgenic mice expressing the mutated human SOD1 (G93A) gene at a low level (B6SJ-L-TgN[SOD1]-G93A)1Gur1 (G1L) were obtained from Jackson Laboratory (Bar Harbor, ME). These mice carry 18 transgene copies because of a reduction in the copy number compared with (B6SJ-L-TgN[SOD1]-G93A)1Gur (G1H) mice, which express 25 copies (3). The G1L mice were bred and maintained as hemizygotes by mating with wild-type B6.SJL mice. Nontransgenic littermates were used as controls. All animals were genotyped and handled as previously described (29). We examined control (n = 6,292 [median, 240–296] days old) and G1L (n = 9,256 [224–281] days old, end stage) mice. One G1H mouse (120 days, end stage), also obtained from Jackson Laboratory, was examined to confirm the lack of TDP-43 abnormalities reported previously (26). End stage was defined as occurring when the mouse was so severely paralyzed that it could hardly move or drink water. The mice were killed with an overdose of sodium pentobarbital and perfused with PBS followed by 4% paraformaldehyde. The lumbar enlargement of the spinal cord was removed, immersed in 4% paraformaldehyde overnight at 4°C, and then dehydrated and embedded in paraffin blocks. Paraffin sections, 4-µm-thick, were prepared and stained with hematoxylin and eosin. Every fifth section (cut at 20-µm intervals) was obtained, and 4 sections from each mouse were used to count the total number of LBHIs in the sections. For immunohistochemistry, the primary antibodies used on the serial sections were against TDP-43 (1:600, Protein Tech Group) and human SOD1 (0.5 µg/mL, clone 1G2, MBL, Nagoya, Japan).

**Semiquantitative Analysis of Immunoreactivity for TDP**

Because variation in the TDP immunoreactivity (TDP-IR) was evident among G1L mice, the patterns of TDP immunostaining were divided into normal (0) and abnormal (1 to 4), the latter showing TDP-positive neurites and inclusions and varying degrees of nuclear positivity. The stages of normal or abnormal patterns were classified by TDP-IR of the neuron nuclei as follows: normal pattern (Stage 0): same as in normal littermates, with immunoreactivity apparent only in nuclei; abnormal pattern (Stages 1–4): Stage 1, weak immunoreactivity in nuclei; Stage 2, weak to moderate immunoreactivity in nuclei; Stage 3, moderate to strong immunoreactivity in nuclei; Stage 4, strong immunoreactivity in nuclei.

**Quantitative Analysis of LBHIs**

The numbers of LBHIs with a core and halo in neurons of the lumbar spinal cord were counted in hematoxylin and
eosin-stained sections (100× objective) from each G1L mouse as previously described (29).

**Statistical Analysis**

Differences in the numbers of TDP-positive inclusions in neurons and glia and in large neurons (>37 μm) between SALS patients with a rapid course and those with a slow course were analyzed by Wilcoxon rank sum test. The relationships among the numbers of large neurons and TDP-positive inclusions in neurons and glia were also analyzed using Spearman correlation coefficient with a 95% confidence interval (CI). In G1L mice, the relationships among age at the end stage, number of LBHIs, and TDP-IR were analyzed using Spearman correlation coefficient with 95% CI. Exploratory subgroup analyses for age at the end stage were done in the TDP-IR weak groups (Stages 1 and 2) and the TDP-IR strong groups (Stages 3 and 4) by Kolmogorov-Smirnov test. All statistical analyses were performed with SAS version 9.1 (SAS Institute Inc, Cary, NC).

**RESULTS**

**ALS Cases**

In the lumbar cords of SALS patients with disease durations less than or equal to 2.5 years (SALS r-1 to -13), mislocalization of TDP was frequently observed (Figs. 1B, C; Table); this was not evident in controls (Fig. 1A). There was a diffuse cytoplasmic staining pattern, especially in large neurons (>37 μm, Fig. 1B) and TDP-positive filamentous or round inclusions were evident in atrophic neurons and in glia (Figs. 1C, D); the nuclei in the neurons and glia were negative for TDP. In SALS patients with mild to moderate neuronal loss, there was often a diffuse TDP staining pattern in the cytoplasm of large neurons (Table). No extracellular TDP-positive inclusions were apparent. By contrast, in SALS patients with disease durations longer than 5 years (SALS s-1 to -5), neurons with diffuse cytoplasmic staining patterns were not evident, and TDP-positive inclusions were only rarely detected (Table). The TDP-IR of the nuclei of residual neurons appeared to be preserved.

In ALS1 patients, diffuse cytoplasmic TDP staining of large neurons was found only in 1 patient, i.e. ALS1-2 (126 2bp del), who had a clinical course of 2 years (Table). Some small neurons in Patients ALS1-2 and ALS1-3 (126 2bp del) also showed diffuse cytoplasmic TDP staining (Figs. 3A, C). The TDP-positive inclusions were prominent in the glia and in small neurons or their neurites in Patient ALS1-1 (C111Y), who had no remaining large neurons. The nuclei of cells with TDP-positive inclusions were TDP negative (Figs. 2A, E). Colocalization of TDP and SOD1 was frequently evident as

![Figure 1](http://jnen.oxfordjournals.org)
FIGURE 2. Colocalization of TAR DNA binding protein (TDP) and superoxide dismutase 1 (SOD1) in neurons of an amyotrophic lateral sclerosis 1 (C111Y) patient. (A, B) Heterogeneous cytoplasmic staining patterns for TDP (A) and for SOD1 (B). The nucleus is TDP negative. (C-F) Neuronal inclusions are positive for TDP (C, E) and SOD1 (D, F). (G, H) The halo portion of a Lewy body–like hyaline inclusion (LBHI) is stained strongly for TDP (G) and ubiquitin (H). The upper panels are high-magnification views of each LBHI. Immunohistochemistry: (A, C, E, G) = TDP; (B, D, F) = SOD1; (H) = ubiquitin. (B, D, F, and H) are adjacent serial sections of (A, C, E, and G), respectively. Scale bars = (A-F) 10 μm; (G, H) 50 μm; insets in (G, H) = 16 μm.
an aggregation pattern (Figs. 2A–F). The TDP and ubiquitin were also colocalized in inclusions (Figs. 2G, H). Patients ALS1-2 (30) and ALS1-3 (31) possessed the same SOD1 mutation; TDP-IR of the nuclei was weaker in Patient ALS1-3 who had more LBHIs than Patient ALS1-2. The TDP-IR of some of the LBHIs observed in these patients was weak, but stronger than that in most of the nuclei (Fig. 3A). Colocalization of TDP and SOD1 was demonstrated in LBHIs in serial sections (Figs. 3A, B). The LBHIs were scarce in other ALS1 patients. Although TDP-IR was retained in the nuclei of most of the residual neurons in Patients ALS1-4 (G37R) (32) and ALS1-5 (L126S) (33), there were a few neurons with TDP-negative nuclei and cytoplasm. The TDP-negative nuclei were atrophic or deformed (Fig. 3D), whereas most of the TDP-positive nuclei were circular (Fig. 3F). In ALS1 patients, there was no apparent relationship between nuclear TDP-IR and disease duration. The SMI31-positive conglomerate inclusions (Fig. 3E) in Patients ALS1-5 and ALS1-6 were mostly TDP negative (Fig. 3F) or had only very faint staining (Fig. 3D).

G93A Mice

In normal littermates, TDP-IR was found in the neurons and some glia in the gray matter (Fig. 4A); the white matter was negative for TDP. In G1L mice, the nuclei of neurons and reactive astrocytes were stained for TDP. The

FIGURE 3. TAR DNA binding protein (TDP) staining patterns in the other amyotrophic lateral sclerosis 1 patients. (A, B) Superoxide dismutase 1 (SOD1)-positive Lewy body–like hyaline inclusions (B) are weakly stained (left, arrow) and very faintly stained (right, arrow) for TDP (A). There is mislocalization in a small neuron (arrowhead) in (A). (C) The neuron cytoplasm is diffusely stained for TDP (arrows). (D, E) A conglomerate inclusion is positive for SMI31 (E arrowhead) and is negative for TDP (D arrowhead). The nucleus (D arrow) is atrophic and deformed. (F) The TDP-positive nucleus (arrow) in a neuron containing a TDP-negative conglomerate inclusion (arrowhead) appears round and intact. Immunohistochemistry: (A, C, D, F) = TDP; (B) = SOD1; (E) = phosphorylated neurofilament (SMI31). (B) and (E) are serial sections of (A) and (D), respectively. Scale bar = 20 μm.
cytoplasm of a few anterior horn cells showed a punctate TDP staining pattern, but most of the neuron nuclei were TDP positive (Figs. 4E, 5C). Neurons with TDP-negative nuclei and TDP-positive cytoplasm were rare (Fig. 4B). The TDP-positive inclusions and neurites were numerous (Figs. 4C, 5A–D), and the nuclear TDP-IR of these cells was weak (Fig. 5A). Some vacuoles were also stained for TDP (Fig. 4C). Colocalization of TDP and SOD1 was also detected in LBHIs in serial sections (Figs. 4C, D). Nuclear TDP-IR varied widely from mouse to mouse (Stages 1–4, Figs. 5A–D). In G1L mice showing a rapid clinical course and prominent LBHI-formation, nuclear TDP-IR was weak (Stage 1, Figs. 5A, E), whereas G1L mice showing a slow clinical course and less LBHI formation had strong nuclear TDP-IR (Stage 4, Figs. 5D, F). In Stage 4 mice, most of the nuclei were strongly positive and circular, but some weakly stained nuclei were atrophic or deformed (Fig. 4E). In G1H mice with a rapid disease course, TDP-IR of the neurons was weak, and nearly all LBHIs in the lumbar spinal cord were negative for TDP (Fig. 4F).

**Statistical Analysis**

The numbers of TDP-positive inclusions in neurons and glia were significantly lower in SALS patients with a

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**FIGURE 4.** TAR DNA binding protein (TDP) pathology in G93A mice. (A) Nuclei in large neurons in a normal littermate (arrows) are TDP positive; their nucleoli are not stained. (B–E) G1L mice. (B) A degenerated neuron containing small vacuoles (arrowhead) in the cytoplasm has a TDP-negative nucleus and TDP-positive cytoplasm. (C, D) There are numerous Lewy body-like hyaline inclusions (LBHIs) that are TDP positive (C) and superoxide dismutase 1 (SOD1) (D) (arrows). Some vacuoles that are SOD1 positive (D) are partly stained for TDP (C, arrowheads). (E) Most of the nuclei in the neurons are strongly positive for TDP. A few nuclei that are stained only weakly (arrows) are atrophic and deformed. (F) The LBHI in the lumbar cord of the G1H mouse stains extremely faintly for TDP (arrow). (A) Normal littermate (Stage 0), (B–E) G1L mice, (B–D) Stage 3, (E) Stage 4, (F) G1H mouse. Immunohistochemistry for TDP (A–C, E, F) and SOD1 (D). (D) is a serial section of (C). Scale bars = (A, C–E) 50 μm; (B, F) 20 μm.
slow course than in those with a rapid course (Wilcoxon rank sum test: neuronal inclusions, p = 0.0002; glial inclusions, p = 0.0002). The numbers of large neurons (>37 μm) were also lower in SALS patients with a slow course than in those with a rapid course, although the level of significance (p = 0.0264) was lower than that for TDP-positive inclusions. The relationships among the numbers of large neurons, and TDP-positive inclusions in neurons and glia were not significant in SALS patients as a whole (Spearman correlation coefficient with 95% CI).

In G1L mice, the TDP-IR stage was positively correlated with life span (Spearman correlation coefficient with 95% CI, r = 0.77; 95% CI, 0.22–0.95) and negatively correlated with the formation of LBHIs (r = −0.87; 95% CI, −0.97 to −0.47). The correlation between life span and the number of LBHIs was also high (r = −0.64; 95% CI, −0.92 to 0.04). A cumulative probability plot of age at the end stage (Fig. 6) showed a higher value for the group with strong TDP-IR (Stages 3 and 4) than for those with weak TDP-IR (Stages 1 and 2); age at the end stage in the strong TDP-IR group was significantly greater than that in the weak TDP-IR group (Kolmogorov-Smirnov test, p = 0.015).

**DISCUSSION**

The TDP mislocalization from the nucleus to the cytoplasm was previously considered to be a disease-specific
change not present in ALS1. Here, we analyzed TDP pathology in SALS and ALS1 patients and in ALS1 model mice. Our data suggest that the level of expression of TDP in the nucleus is associated with the clinical course and neurodegenerative changes in SALS patients and in ALS1 model mice.

Our observation that diffuse staining pattern was frequently observed in the cytoplasm of large neurons in SALS patients with rapid clinical courses showing mild neuronal loss suggests that TDP mislocalization starts gradually in the early phase of neurodegeneration. Most of the TDP-positive inclusions were found in atrophic neurons and glia, suggesting that the inclusions appeared later. Because no extracellular TDP-positive inclusions were apparent, neuronal TDP-positive inclusions likely disappear along with the death of the neurons.

In contrast, in SALS patients with slow clinical courses, no neurons with a diffuse TDP staining pattern in the cytoplasm were found, and TDP-positive inclusions in both neurons and glia were significantly less frequently found. Because relationships among the numbers of large neurons, those of TDP-positive inclusions in neurons, and those of TDP-positive inclusions in glia were not significant, the rarity of TDP pathology in SALS patients with a slow clinical course might not necessarily have resulted from severe neurodegeneration. The TDP pathology might be associated with a rapid clinical course in SALS. The influence of TDP-43 on the disease would then be less marked in SALS patients with a slow clinical course than in those with a rapid clinical course.

Previous studies have shown that LBHIs are not stained for TDP in ALS1 patients (16, 17, 26) and G1H mice (26).

**FIGURE 6.** Kolmogorov-Smirnov test of the weak TAR DNA binding protein immunoreactivity (TDP-IR) groups (Stages 1 and 2) and strong TDP-IR groups (Stages 3 and 4) of G1L mice. Age at end stage in the TDP-IR strong group was significantly greater than that in the TDP-IR weak group ($p = 0.015$).

**FIGURE 7.** Hypothetical course of neuronal degeneration associated with changes in nuclear TAR DNA binding protein (TDP) expression in sporadic amyotrophic lateral sclerosis (SALS). (A) A morphologically normal neuron is subjected to an insult associated with a disturbance of TDP nuclear trafficking. The upper neuron diagrammed, from a patient with SALS, showing a rapid clinical course has marked disturbance of TDP nuclear trafficking, whereas the lower diagrammed neuron from a patient with SALS showing a slow clinical course, is only mildly affected. (B-D) Images show degenerating neurons at the time of rapid disease progression. (B) Early occurrence of TDP redistribution, i.e. low expression in nuclei and high expression in cytoplasm. (C) Later occurrence of cytoplasmic TDP aggregate in an atrophic neuron. (D) Similar aggregate of cytoplasmic TDP in a more degenerative neuron than that in (C). (E, F) Images represent degenerative neurons at the time of slow disease progression. (E) Preservation of a high level of TDP expression in the nucleus of an atrophic neuron. (F) Successive maintenance of a high level of TDP expression in the nucleus of a more degenerative neuron. The lower 6 photographs are from SALS patients showing a rapid clinical course (A-D) and a slow course (E, F), which correspond to the diagrammatic illustrations for each letter.

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On the other hand, mislocalization of TDP to the cytoplasm in ALS1 cases (A4T, I113T) has been reported by Robertson et al (26). In the present study, TDP-positive LBHIs were clearly demonstrated in 1 ALS1 patient showing a slow disease progression, and in G1L mice, which also show slower disease progression than G1H mice. The ALS1 patients with TDP-negative LBHIs reported by Tan et al (17) showed very rapid progression within less than 1 year, and another ALS1 patient with TDP-negative LBHIs reported by Robertson et al (26) also showed rapid progression within 2 years. The difference in TDP immunoreactivity of LBHIs among ALS1 cases or between the 2 kinds of G93A mice might be a result of the difference in the clinical course or speed of SOD1 aggregation (34). The difference in morphology between TDP-positive inclusions in ALS1-1 and G1L mice and those in SALS patients would be caused by trapping of TDP-43 by SOD1 aggregation or LBHIs. The colocalization of TDP and SOD1 in LBHIs also suggests a biological relationship between SOD1 and TDP, although the specifics of that relationship are unclear.

Ayala et al (11) reported that loss of TDP in vitro results in nuclear dysmorphism, misregulation of the cell cycle, and apoptosis. Because the TDP-IR stage was positively correlated with life span in G1L mice, nuclei with low TDP-IR were atrophic and deformed in G1L mice and ALS1 patients, and an absence of TDP in the nucleus (such as that occurring through mislocalization) was frequently observed in ALS patients with a rapid clinical course, a high level of expression of nuclear TDP may play a protective role in neurons exposed to various insults. Because TDP-IR in the nucleus was inversely correlated with LBHI formation in G1L mice, TDP might have a suppressive effect on LBHI formation or toxic aggregation of SOD1, possibly through changes in the transcription and splicing of unknown genes (7, 8).

We hypothesized that rapid disease progression resulting from some insult to neurons might lead to disturbance of TDP nuclear trafficking (Fig. 7A) (35). Redistribution of TDP, with a low level of expression in the nucleus and a high level in the cytoplasm (Fig. 7B), occurs first, and cytoplasmic TDP later forms aggregates in the atrophic neurons (Figs. 7C, D). In contrast, neurons that succeed in maintaining a high level of expression of nuclear TDP (36) because of a slow shift of TDP (Fig. 7A, lower) show rather slower degeneration, and the disease progresses more slowly (Figs. 7E, F). It will be important to investigate the mechanism responsible for regulating the nuclear expression level of TDP, as this might yield a new strategy for treating not only ALS, but also other neurodegenerative disorders, including frontotemporal lobar degeneration.

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REFERENCES


46 © 2008 American Association of Neuropathologists, Inc.

27. Dal Canto MC, Gurney ME. A low expressor line of transgenic mice carrying a mutant SOD1 gene develops pathological changes that most closely resemble those in human amyotrophic lateral sclerosis. Acta Neuropathol 1997;93:537–50


34. Sato T, Nakanishi T, Yamanoto Y, et al. Rapid disease progression is correlated with instability of mutant SOD1 in familial ALS. Neurology 2006;65:1954–57
