TAR DNA-Binding Protein 43 Immunohistochemistry Reveals Extensive Neuritic Pathology in FTLD-U: A Midwest-Southwest Consortium for FTLD Study

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Abstract

TAR DNA-binding protein 43 (TDP-43) is a major component of the inclusions in frontotemporal lobar degeneration with ubiquitinated inclusions (FTLD-U). We studied TDP-43 pathology in the hippocampus and frontal cortex of autopsy brains from patients with FTLD-U (n = 68), dementia lacking distinctive histopathology (n = 4), other neurodegenerative diseases (n = 23), and controls (n = 12) using a sensitive immunohistochemistry protocol. Marked enhancement of staining of TDP-43-positive dystrophic neurites (DNs) was obtained, and we observed 2 previously unrecognized pathologic patterns (i.e. frequent long DNs in the CA1 region and frequent dot-like DNs in the neocortical layer 2) in 39% and 15% of the FTLD-U cases, respectively. Frequent long DNs, but not dot-like DNs, were significantly associated with progranulin mutations. Based on this evaluation, 4 FTLD-U cases showed no TDP-43 pathology and were reclassified as “FTLD-U, non-TDP-43 proteinopathy,” and 3 cases of dementia lacking distinctive histopathology were reclassified as FTLD-U. Of the cases with other neurodegenerative diseases, 43% showed TDP-43 pathology in the hippocampus, but only 4% showed TDP-43 pathology in the frontal cortex. No TDP-43 pathology was seen in controls. These results indicate that the sensitivity of the TDP-43 immunohistochemistry method affects both the extent and type of abnormalities detected. Moreover, assessment of abnormalities in both the hippocampus and frontal cortex may be diagnostically important in FTLD-U.

Key Words: Dementia lacking distinctive histopathology, Dystrophic neurites, Frontotemporal lobar degeneration with motor neuron disease, Frontotemporal lobar degeneration with ubiquitinated inclusions, Immunohistochemistry, Progranulin, TAR DNA-binding protein 43.

INTRODUCTION

In 1996, Jackson et al described a series of patients with frontotemporal dementia and atrophy of the frontal and temporal lobes (1). None of the patients had clinical evidence of either an upper or lower motor neuron disorder. In each case, brain autopsy revealed neuronal inclusions identical to those described previously in dementia associated with motor neuron disease (amyotrophic lateral sclerosis with dementia). Originally named as motor neuron disease inclusion dementia and believed to be rare, this disease is now referred to as frontotemporal lobar degeneration with ubiquitinated inclusions (FTLD-U) (2) and is recognized as the most common cause of frontotemporal dementia (3). The pathologic hallmarks of FTLD-U are neuronal cytoplasmic inclusions, dystrophic neurites (DNs), and occasional neuronal intranuclear inclusions, all of which are immunoreactive with antibodies to the relatively nonspecific marker ubiquitin and generally negative for other proteins such as tau, α-synuclein, Aβ, α-intermixin, and polyglutamine (4, 5).

Recently, TAR DNA-binding protein 43 (TDP-43), a nuclear protein involved in exon skipping and transcription regulation, was identified as a major component of the inclusions and DNs in FTLD-U, FTLD-U with motor neuron disease, and amyotrophic lateral sclerosis (4). Another major recent advance was the discovery that autosomal dominant null mutations in the progranulin gene are the genetic basis for most cases of familial FTLD-U linked to chromosome 17 (6–9).

Dementia lacking distinctive histopathology (DLDH) and the closely related hippocampal sclerosis dementia are terms currently used for cases of dementia in which no
inclusions are found that permit further pathologic classification (10–13). Frontal or temporal lobe atrophy and neuron loss are usually present in DLDH, whereas pronounced hippocampal atrophy and neuron loss are seen in hippocampal sclerosis dementia. Frontotemporal dementia is the most common clinical presentation (10, 14). Dementia lacking distinctive histopathology was previously thought to be the most common type of pathology underlying frontotemporal dementia, but studies using ubiquitin immunohistochemistry have demonstrated that most cases of DLDH and hippocampal sclerosis dementia represent FTLD-U (3, 15, 16). The term hippocampal sclerosis refers simply to a severe loss of neurons in the hippocampal pyramidal cell layer; hippocampal sclerosis can be associated with many neurodegenerative diseases, including Alzheimer disease (AD), although it is particularly common in FTLD-U (17).

In this study, we used a highly sensitive immunohistochemistry method that permitted increased detection of the extent of TDP-43 abnormalities compared with a previously described routine TDP-43 immunohistochemistry protocol. We then combined all available autopsy brains with FTLD-U and DLDH from 3 centers and used the enhanced immunohistochemistry protocol to characterize TDP-43 pathology in the hippocampus and frontal cortex of these cases. Two control groups (i.e. cases with other neurodegenerative diseases and nondemented controls) were also evaluated. The enhanced immunohistochemistry method permitted determination of the prevalence and specificity of TDP-43 pathology in the FTLD-U cases. In addition, we wanted to know if the types and severity of TDP-43 pathology differed between FTLD-U cases with progranulin mutations and FTLD-U cases without progranulin mutations. We also investigated whether any of the DLDH cases could be reclassified as FTLD-U based on the presence of TDP-43-pathology.

MATERIALS AND METHODS

Case Material

Autopsy brains diagnosed as either FTLD-U (n = 68) or DLDH (n = 4) were selected from 3 centers based on availability (Table 1). All elderly control brains without history of dementia or neurodegenerative diseases (n = 12) were selected from 1 institution (UTSW). Other neurodegenerative disease cases (n = 23) were selected to include a broad range of diagnoses. They consisted of cases diagnosed as AD (n = 8), Lewy body variant of AD (n = 1), neurofibrillary tangle dementia (n = 3), vascular dementia (n = 1), sporadic multiple system tauopathy with dementia (2) (n = 1), basophilic inclusion body disease (n = 1), FTLD with Pick bodies (2) (n = 3), progressive supranuclear palsy (n = 4), and cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (n = 1). Diagnostic workup of these cases had been performed according to published criteria (2) and included examination of hematoxylin and eosin sections, thioflavin S preparations, and immunohistochemical stains for α-synuclein in all cases. Immunohistochemistry for phosphorylated tau, Aβ, and ubiquitin had been performed in all cases of FTLD-U. When indicated, immunohistochemistry had also been performed for α-intermem, polyglutamine, 3R tau, 4R tau, and Notch3. The diagnosis of AD was based on tangle and plaque densities meeting the Consortium to Establish a Registry for Alzheimer’s Disease “definite” (18) and National Institute on Aging–Reagan Institute “high-likelihood” (19) criteria. Two cases of FTLD-U with valosin-containing protein mutation, a very rare type of FTLD, were available. Because of the small number of FTLD-U cases with valosin-containing protein mutations and the previous evidence that they represent their own frontal pathology type (Type 4) (20), these cases were included in the analyses of the FTLD-U types but excluded from other analyses. A signed consent for brain autopsy had been obtained from the next of kin or legal representative in each case.

Sequencing of the Progranulin Gene

High molecular weight DNA was extracted from blood or frozen brain tissue of 58 cases according to standard procedures. The PGRN gene was sequenced as previously described (8, 9).

Antibodies for Immunohistochemistry

The primary antibodies were rabbit polyclonal antibodies for TDP-43 at a dilution of 1:1000 (ProteinTech, Chicago, IL) and ubiquitin at a dilution of 1:500 (Dako, Carpinteria, CA). A mouse monoclonal TDP-43 antibody (2E2-D3; Novus Biologicals, Littleton, CO) was used in some cases for verification purposes.

Routine Immunohistochemistry Protocol

Formalin-fixed, paraffin-embedded tissue blocks from the posterior hippocampus at the level of the lateral

<table>
<thead>
<tr>
<th>Case Diagnoses Before and After TDP-43 Immunohistochemistry</th>
<th>Mean Age at Death (±SEM)</th>
<th>PGRN Mutations in Tested Cases (+/−)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FTLD-U</td>
<td>68 → 67</td>
<td>66 ± 1.5</td>
</tr>
<tr>
<td>FTLD-U, non-TDP-43</td>
<td>0 → 4</td>
<td>57 ± 4.5</td>
</tr>
<tr>
<td>FTLD-U with VCP mutations</td>
<td>2 → 2</td>
<td>54 ± 6.5</td>
</tr>
<tr>
<td>DLDH</td>
<td>4 → 1</td>
<td>98</td>
</tr>
<tr>
<td>Other neurodegenerative diseases</td>
<td>23 → 23</td>
<td>74 ± 2.5</td>
</tr>
<tr>
<td>Controls</td>
<td>12 → 12</td>
<td>77 ± 2.7</td>
</tr>
</tbody>
</table>

+−/−: Mutations present/absent; DLDH, dementia lacking distinctive histopathology; FTLD-U, frontotemporal lobar degeneration with ubiquitinated inclusions; PGRN, progranulin; SEM, standard error of mean; TDP, TAR DNA-binding protein; VCP, valosin-containing protein.
geniculate nucleus and from the middle frontal gyrus were used. As previously described (4, 21), the slides underwent a pretreatment step with formic acid (5 minutes), the detection system was the avidin-biotin system, the chromogen was diaminobenzidine, and the procedure was performed manually. Ubiquitin immunohistochemistry, which served as the basis of the original FTLD-U diagnoses, was performed according to this routine method.

**Enhanced Immunohistochemistry Protocol**

In the enhanced immunohistochemistry protocol for TDP-43, the detection system was the alkaline phosphatase-based ultraView Red detection system (Ventana Medical Systems, Tucson, AZ), the chromogen was Fast Red/Naphthol, and the procedure was performed using the Benchmark XT automated stainer (Ventana). This automated stainer is commercially available, and more than 5,500 units are currently in use worldwide (Don Green, Ventana, personal communication, September 2007). No pretreatment was found to be necessary. Ubiquitin immunohistochemistry using the enhanced protocol was not systematically performed or studied in all cases; the results of ubiquitin immunohistochemistry reported here refer only to the routine method.

**Evaluation of the Immunohistochemical Staining**

The results were evaluated semiquantitatively using a scale of 0 to 3, where 0 corresponds to “none,” and 3 corresponds to “frequent” detection of specific features such as DNs. The evaluator was a neuropathologist (K.J.H.) blinded to clinical data and diagnoses.

**Double Labeling Immunohistochemistry**

Double labeling immunohistochemistry for phosphorylated tau and TDP-43 was performed in selected cases using the Benchmark XT automated stainer. After pretreatment with the Cell Conditioning Solution (Ventana), the sections were exposed to a mouse monoclonal paired helical filament antibody (AT8, Innogenetics, Ghent, Belgium) at a dilution of 1:200. The detection system was the ultraView Universal diaminobenzidine, and the chromogen was diaminobenzidine. The sections were then processed for TDP-43 immunohistochemistry as described above.

**FTLD-U Frontal Cortex Pathology Types**

Frontotemporal lobar degeneration with ubiquitinated inclusion frontal cortex pathology types were determined as previously described (20).

**Statistics**

Comparisons of frequency data were performed using the Fisher exact probability test (22). The Student t-test assuming unequal variances was used for comparisons of continuous variables. All tests were 2-tailed. The level of statistical significance was set at p = 0.05.

Statistical tests involving the DNs variable, originally collected as a 4-class ordered category variable (none, sparse, moderate, frequent), were performed by 2 alternative methods, which, as it turned out, produced very similar results. The first method consisted of dichotomization of the variable into 2 classes (none to moderate and frequent), followed by the Fisher exact probability test. This approach was chosen because of its simplicity and because one of the specific research aims of our study was to compare the frequent category to all other categories. In addition, the DN variable followed a nearly dichotomous distribution in the hippocampus, that is, cases with moderate numbers were rare. A second alternative statistical approach (i.e. the Mantel-Haenszel χ² test [23]) was also performed using the SAS software (SAS Institute Inc., Cary, NC). All of the original category data were used in this test. As shown in the Results section, the p values obtained with both approaches were similar: all p values that were significant with the first method remained significant with the second method, and all p values that were nonsignificant with the first method remained nonsignificant with the second method.

Agreement of frontal pathology subclassification based on routine and enhanced immunohistochemistry protocols was evaluated using Cohen unweighted κ (22).

**RESULTS**

**Increased Sensitivity of the Immunohistochemistry Method**

In some FTLD-U cases, use of the enhanced immunohistochemistry method led to dramatic increases in the staining intensity and frequency of visible TDP-43-positive DNs compared with the routine method. The difference was particularly striking in the hippocampal CA1 region in which the DNs were strongly positive and frequent in 39% of the FTLD-U cases by the enhanced method (Fig. 1). The area of frequent DNs was typically well demarcated and limited to the CA1 region (Fig. 2); in some cases, frequent DNs were also found in the prosubiculum. In contrast, routine immunohistochemistry revealed either no DNs or weakly positive, sparse DNs in CA1 (Fig. 1A). Similar results were obtained with the routine method performed at 2 centers. The enhanced method did not result in significant increases in the staining intensity or frequency of cytoplasmic inclusions or intranuclear inclusions. The nondemented control cases remained negative for TDP-43 pathology with the enhanced method. Unless specified otherwise, further analyses were performed using data only from the enhanced immunohistochemistry method.

**Prevalence of TDP-43 Pathology**

Three of the 4 DLDH cases showed TDP-43 pathology both in the hippocampus and frontal cortex and were reclassified as FTLD-U (Table 1). No TDP-43 pathology was found in the remaining DLDH case. Of the cases with other neurodegenerative diseases, 43% showed TDP-43 pathology in the hippocampus, but only 4% (a single case) showed TDP-43 pathology in the frontal cortex. In contrast, 97% of FTLD-U cases had TDP-43 pathology in both the hippocampus and the frontal cortex. No TDP-43 pathology was seen in the controls without neurodegenerative diseases or history of dementia. Four FTLD-U cases (6%) failed to...
A dichotomy was not observed in the frequency distribution of DNs. They were uncommon in the hippocampal CA1 region (6%). A similar distribution in that most cases showed either no to sparse neurites (55%) or frequent neurites (39%). Moderate numbers were seen in the CA1 region of 39% of the FTLD-U cases. The DNs are predominantly elongated, rather than dot-like in appearance. Note that in both (A) and (B), the nuclei are negative, which is consistent with displacement of TDP-43 from the nucleus to the neurites. In a normal control case, the enhanced immunohistochemistry protocol shows nuclear positivity but no neurites. Original magnification: 200×.

The frequency of TDP-43-positive DNs in the hippocampal CA1 region of FTLD-U cases showed a dichotomous distribution in that most cases showed either no to sparse neurites (55%) or frequent neurites (39%). Moderate numbers of DNs were uncommon in this region (6%). A similar dichotomy was not observed in the frequency distribution of the cytoplasmic inclusions in the hippocampal dentate gyrus or the frequency distribution of DNs in the frontal cortex in which 32% and 27% of cases, respectively, were in the moderate category.

Frequent TDP-43-positive DNs in the CA1 region were significantly associated with progranulin mutations (Table 2). Progranulin mutations were found in 9 of 15 (60%) cases with frequent DNs in CA1 and in 5 of 29 (17%) cases with zero to moderate numbers of DNs in CA1 (Fisher exact probability test, p = 0.007; Mantel-Haenszel $\chi^2$ test, p = 0.001).

For verification purposes, the hippocampi of 2 FTLD-U cases with frequent DNs in CA1 were also stained using the mouse monoclonal TDP-43 antibody. A similar pattern of frequent DNs was observed with the monoclonal TDP-43 antibody, although the staining intensity was weaker compared with the polyclonal antibody.

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**FIGURE 1.** Improvements in the immunohistochemistry (IHC) protocol lead to pronounced increases in visible TAR DNA-binding protein (TDP-43) pathology in some cases, particularly in the hippocampal CA1 region. (A) Routine immunohistochemistry for TDP-43 shows thin, weakly labeled dystrophic neurites (DNs) (brown chromogen) in the CA1 region of an frontotemporal lobar degeneration with ubiquitinated inclusions (FTLD-U) case. (B) In the same field of the same FTLD-U case, the enhanced TDP-43 immunohistochemistry method reveals frequent, strongly labeled neurites (red chromogen). Similarly frequent neurites were seen in the CA1 region of 39% of the FTLD-U cases. The DNs are predominantly elongated, rather than dot-like in appearance. (C) In a nondemented control case, the enhanced immunohistochemistry protocol shows nuclear positivity but no neurites. Original magnification: 200×.

**FIGURE 2.** Frequent TAR DNA-binding protein (TDP) 43-positive dystrophic neurites (DNs) in the CA1 region of a frontotemporal lobar degeneration with ubiquitinated inclusion (FTLD-U) case. (A) At low magnification, the DNs are evident as a dense band covering the CA1 region (400×). (B) The area of frequent DNs is well demarcated and, in most cases, limited to CA1 (400×).

**FIGURE 3.** An example of frontotemporal lobar degeneration with ubiquitinated inclusions (FTLD-U), non-TAR DNA-binding protein (TDP-43) pathology. (A) Ubiquitin-positive neuronal cytoplasmic inclusions (arrows) in the dentate gyrus (400×; ubiquitin immunohistochemistry). (B) No cytoplasmic inclusions are evident in the same area by TDP-43 immunohistochemistry (400×).

**TABLE 2.** Clinical and Genetic Associations of Frequent Versus Absent or Moderate TDP-43+ DNs in Hippocampal CA1

<table>
<thead>
<tr>
<th></th>
<th>Frequent DNs</th>
<th>Absent to Moderate DNs</th>
</tr>
</thead>
<tbody>
<tr>
<td>N (% of total examined)</td>
<td>25 (39)</td>
<td>39 (61)</td>
</tr>
<tr>
<td>Age (years ± SEM)</td>
<td>67 ± 1.7</td>
<td>66 ± 1.9</td>
</tr>
<tr>
<td>Male/female</td>
<td>13/12</td>
<td>21/18</td>
</tr>
<tr>
<td>Family history (+/−)</td>
<td>12/10</td>
<td>14/19</td>
</tr>
<tr>
<td>PGRN mutation (+/−)</td>
<td>9/6*</td>
<td>5/24</td>
</tr>
<tr>
<td>MND (+/−)</td>
<td>1/24</td>
<td>6/33</td>
</tr>
<tr>
<td>Solid CI predominant (+/−)</td>
<td>12/11</td>
<td>25/12</td>
</tr>
<tr>
<td>Language presentation (+/−)</td>
<td>4/17</td>
<td>7/25</td>
</tr>
</tbody>
</table>

*, p = 0.007; +/−, Feature present/absent; CI, cytoplasmic inclusions in the dentate gyrus; DN, dystrophic neurite; MND, motor neuron disease; PGRN, progranulin; SEM, standard error of mean.
the long neurites were generally more widely dispersed throughout the entire thickness of the cortex compared with the dot-like neurites. Frequent long DNs were present in the frontal cortex in 35% of FTLD-U cases and in either frontal cortex or CA1 in 50% of FTLD-U cases.

Frequent TDP-43-positive, long DNs in the frontal cortex were significantly associated with progranulin mutations (Table 3). Progranulin mutations were found in 71% of FTLD-U cases with frequent long neurites and in 4% of FTLD-U cases with zero to moderate long neurites in the frontal cortex (Fisher exact probability test, \( p = 0.00008; \) Mantel-Haenszel \( \chi^2 \) test, \( p < 0.0001 \)). Solid cytoplasmic inclusions (Fig. 5) were the predominant type of cytoplasmic inclusions in 100% of FTLD-U cases with frequent long neurites and in 56% of cases with none to moderate long neurites in the frontal cortex (Fisher exact probability test, \( p = 0.0003; \) Mantel-Haenszel \( \chi^2 \) test, \( p < 0.0001 \)).

Frequent TDP-43-positive, dot-like DNs were seen in an additional 15% of FTLD-U cases in the frontal cortex. Unlike frequent long neurites, the dot-like neurites were not significantly associated with progranulin mutations and were associated with granular, rather than solid, cytoplasmic inclusions in the frontal cortex (Table 4). Granular inclusions (Fig. 5) were the predominant cytoplasmic inclusion type in

![FIGURE 4](image)

**FIGURE 4.** Two types of TAR DNA-binding protein (TDP) 43-positive dystrophic neurites were evident in the frontal cortex of frontotemporal lobar degeneration with ubiquitinated inclusion (FTLD-U) cases by immunohistochemistry. (A) Long neurites were frequent in the frontal cortex of 35% of FTLD-U cases (400×). (B) Dot-like neurites were frequent in an additional 14% of FTLD-U cases (400×). (C) The dot-like neurites were concentrated in layer II (arrows; 40×).

67% of FTLD-U cases with frequent dot-like neurites and in 20% of FTLD-U cases without frequent dot-like inclusions (\( p = 0.01 \)).

**Localization of TDP-43 Pathology Relative to Tau Pathology**

Double labeling immunohistochemistry for phosphorylated tau and TDP-43 was performed on a case of neurofibrillary tangle dementia with coexistent TDP-43 pathology in the hippocampus (Fig. 6). Because some neurons contained inclusions composed of tau only or TDP-43 only, the tau-positive tangles and TDP-43-positive inclusions seemed to be independent.

**TABLE 4.** Clinical and Genetic Associations of Frequent Versus Absent or Moderate TDP-43+ DNs in Frontal Cortex

<table>
<thead>
<tr>
<th></th>
<th>Frequent Long DNs</th>
<th>Absent to Moderate Long DNs</th>
</tr>
</thead>
<tbody>
<tr>
<td>N (% of total examined)</td>
<td>22 (35)</td>
<td>41 (65)</td>
</tr>
<tr>
<td>Age (years ± SEM)</td>
<td>66 ± 1.8</td>
<td>66 ± 1.6</td>
</tr>
<tr>
<td>Male/female</td>
<td>12/10</td>
<td>22/19</td>
</tr>
<tr>
<td>Family history (+/−)</td>
<td>12/7*</td>
<td>11/25</td>
</tr>
<tr>
<td>PGRN mutation (+/−)</td>
<td>10/4†</td>
<td>3/27</td>
</tr>
<tr>
<td>MND (+/−)</td>
<td>0/22</td>
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<td>Solid CI predominant (+/−)</td>
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<tr>
<td>Language presentation (+/−)</td>
<td>5/15</td>
<td>7/26</td>
</tr>
</tbody>
</table>

\* \( p = 0.02; \) † \( p = 0.00008; \) ‡ \( p = 0.0003 \).

\( +/− \), Feature present/absent; CI, cytoplasmic inclusions in the frontal cortex; DN, dystrophic neurite; MND, motor neuron disease; PGRN, progranulin; SEM, standard error of mean.

![FIGURE 5](image)

**FIGURE 5.** Examples of TAR DNA-binding protein (TDP) 43 inclusion types (TDP-43 immunohistochemistry; 400×).
routine ubiquitin and enhanced immunohistochemistry methods showed that there was substantial agreement between the 2 methods among the cases classifiable by both methods (\(J = 0.61\)); however, 10 cases were unclassifiable by the enhanced method because of the presence of frequent dot-like DNs in the frontal cortex. (A) Cytoplasmic TDP-43-positive inclusions (arrows) are associated with loss of nuclear TDP-43 immunoreactivity due to displacement of TDP-43 from the nucleus to the cytoplasm. (B) In another microscopic field of the same tissue section, neurons with cytoplasmic tau-positive inclusions (arrows) maintain their nuclear TDP-43 immunoreactivity (100×).

**TDP-43 Pathology and Hippocampal Sclerosis**

Among the FTLD-U cases, the prevalence of hippocampal sclerosis, including severe neuronal loss limited to the CA1/subiculum border zone (24), was 25% in the posterior hippocampus at the level of the lateral geniculate nucleus and 57% in the anterior hippocampus at the level of the pes hippocampi (\(p = 0.003\); Table 6). Hippocampal sclerosis was not disproportionally more common in any particular FTLD-U frontal pathology type. The anterior hippocampus was available for examination from only 30 FTLD-U cases, and this may have limited the determination of a possible correlation between hippocampal sclerosis in the anterior hippocampus and the FTLD-U frontal cortex pathology types.

**FTLD-U, non-TDP-43 Proteinopathy**

A minority of FTLD-U cases (\(n = 4\)) showed inclusions and DNs by ubiquitin immunohistochemistry but not by routine or enhanced TDP-43 immunohistochemistry. These 4 cases were reclassified as FTLD-U, non-TDP-43 proteinopathy. Except for the lack of TDP-43-immunopositive inclusions and neurites, the clinical and pathologic features of the 4 cases overlapped with those seen in FTLD-U cases with TDP-43 proteinopathy (FTLD-U) (Table 7). However, the average age of onset was significantly younger in the 4 cases (42 years) compared with the other FTLD-U cases (59 years; \(p = 0.006\)). Of the 4 cases, 2 were tested for progranulin mutations, and both were found to be negative.

**DISCUSSION**

Our results show that a greater extent of TDP-43 neuritic pathology can be visualized by increasing the

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**TABLE 5. Numbers of FTLD-U Cases Classified into Frontal Cortex Pathology Type Based on Routine Ubiquitin and Enhanced TDP-43 IHC**

<table>
<thead>
<tr>
<th>Routine IHC</th>
<th>Unclassifiable</th>
<th>No frontal pathology</th>
<th>Type 1</th>
<th>Type 2</th>
<th>Type 3</th>
<th>Type 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unclassifiable</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>No frontal pathology</td>
<td>3</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
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<tr>
<td>Type 1</td>
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<td>1</td>
<td>8</td>
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<tr>
<td>Type 2</td>
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<td>0</td>
<td>3</td>
<td>9</td>
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<td>0</td>
</tr>
<tr>
<td>Type 3</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>21</td>
<td>0</td>
</tr>
<tr>
<td>Type 4</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
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</tbody>
</table>

*See Reference 20 for FTLD-U type classification criteria.

**TABLE 6. FTLD-U Cases with Hippocampal Sclerosis and Frequent CA1 DNs in Each Frontal Cortex Pathology Type**

<table>
<thead>
<tr>
<th>No. Cases With Hippocampal Sclerosis (/Total Examined)</th>
<th>Cases With Frequent DNs in CA1 (/Total Examined)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Posterior Hippocampus</td>
<td>Anterior Hippocampus</td>
</tr>
<tr>
<td>Unclassifiable</td>
<td>4/9</td>
</tr>
<tr>
<td>No frontal pathology</td>
<td>2/2</td>
</tr>
<tr>
<td>Type 1</td>
<td>3/13</td>
</tr>
<tr>
<td>Type 2</td>
<td>2/12</td>
</tr>
<tr>
<td>Type 3</td>
<td>5/27</td>
</tr>
<tr>
<td>Type 4</td>
<td>1/2</td>
</tr>
<tr>
<td>Total</td>
<td>17/69 (25%)</td>
</tr>
</tbody>
</table>

*See Reference 20 for FTLD-U type classification criteria.

†, \(p = 0.003\) (posterior vs anterior hippocampus).

DN, dystrophic neurite; FTLD-U, frontotemporal lobar degeneration with ubiquitinated inclusions.
sensitivity of the immunohistochemistry protocol (Fig. 1), and that FTLD-U is characterized by more extensive TDP-43-positive neuritic pathology than previously appreciated. We speculate that the TDP-43-positive neuritic pathology in FTLD-U, possibly due to its potential to interfere directly with synaptic function, may closely correlate with clinical dementia.

Using a commercially available automated stainer, a detection system that contains an amplification step, and a high-contrast red chromogen, we demonstrate increased sensitivity for detection of TDP-43 abnormalities over the routine avidin-biotin immunohistochemistry method. Several other highly sensitive immunohistochemistry methods are available (25, 26). These methods do not require an automated stainer and have been shown to be more than 10 times more sensitive than the avidin-biotin method. It is possible that other sensitive immunohistochemistry methods would have produced results similar to those presented here. Indeed, Mackenzie et al (16) performed ubiquitin immunohistochemistry on cases previously diagnosed as DLDH using a similar automated stainer but with a different detection system. They reported increased numbers of ubiquitin-positive cytoplasmic inclusions in the dentate gyrus and increased numbers of DNs in the frontal and temporal cortex. The variations in detection of types (neurites vs cytoplasmic inclusions) and localization in certain anatomic areas (CA1 vs neocortex) of TDP-43 pathology highlighted in the present study are consistent with biochemical heterogeneity of TDP-43 pathology. Posttranslational modifications such as phosphorylation and truncation, previously reported in TDP-43 pathology (4), may result in variations in the affinity of the antibody for TDP-43 that are differentially affected by different detection methods.

The localization of frequent TDP-43-positive DNs in the hippocampus (CA1 or CA1-prosubiculum) in FTLD-U we identified corresponds to the area affected by severe neuronal loss in hippocampal sclerosis. Hippocampal sclerosis can be seen in many neurodegenerative diseases but is particularly common in FTLD-U (15, 17); in the present study, 42% of FTLD-U cases had hippocampal sclerosis. In view of the similar anatomic localization of frequent TDP-43-positive DNs and hippocampal sclerosis in FTLD-U, we hypothesize that frequent DNs may be a precursor lesion to hippocampal sclerosis. An additional finding in support of this hypothesis is the dichotomous severity distribution of the DNs (i.e. in most FTLD-U cases [94%]), the DNs in the CA1 region were either absent/sparse or very frequent. Neuronal loss in the CA1 region occurs in a similar dichotomous severity distribution in FTLD-U, that is, being either unrecognizable/very mild or very severe (hippocampal sclerosis) in most cases (unpublished observation).

Frequent TDP-43-positive, long DNs in CA1 and frontal cortex were significantly associated with progranulin mutations, whereas no such association was found between frequent dot-like DNs in the frontal cortex and those mutations (Fig. 4; Tables 2–4). These results are consistent with those of Josephs and Dickson (17), who used ubiquitin to show a greater density of ubiquitin-positive DNs in the

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**TABLE 7. Clinical and Pathologic Features of Cases of FTLD-U, Non-TDP-43 Proteinopathy**

<table>
<thead>
<tr>
<th>Case</th>
<th>Age at Onset</th>
<th>Sex</th>
<th>Clinical Presentation</th>
<th>Clinical Data</th>
<th>Ubiquitin Immunohistochemistry</th>
<th>Hippocampal Sclerosis</th>
<th>Intraneuronal Inclusions</th>
<th>Cytoplasmic Inclusions</th>
<th>DNs</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>59</td>
<td>M</td>
<td>FTD</td>
<td>1</td>
<td>++</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>33</td>
<td>M</td>
<td>FTD</td>
<td>1</td>
<td>++</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>54</td>
<td>F</td>
<td>FTD</td>
<td>1</td>
<td>++</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>39</td>
<td>M</td>
<td>FTD</td>
<td>1</td>
<td>++</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>+</td>
</tr>
</tbody>
</table>

*DNs, dystrophic neurites; F, female; FTD, frontotemporal dementia; M, male.*

† present = 1; absent = 0.
neocortex of FTLD-U cases with progranulin mutations than in cases without these mutations. Cairns et al. (20), using the avidin-biotin immunohistochemistry protocol, reported a strong association between progranulin mutations and Type 3 pattern of neocortical TDP-43 pathology (i.e. with numerous neuronal cytoplasmic inclusions and DNAs), whereas no association was found between progranulin mutations and the Type 1 pattern (i.e. long DNAs with few or no neuronal cytoplasmic or intranuclear inclusions). Using the enhanced immunohistochemistry method, we found that all cases with frequent long DNAs in the frontal cortex also had at least moderate cytoplasmic inclusions. Thus, the cases with frequent long DNAs in the frontal cortex would probably correspond to Type 3 in the classification of Cairns et al. (20) and would be unclassifiable in an earlier version of the same classification in which the Type 3 pattern included the requirement of “short neurite profiles” (21). The cases with frequent dot-like neurites would be unclassifiable by both classification systems. When the criteria of Cairns et al. were used and the unclassifiable cases were excluded, there was substantial agreement between the routine and enhanced immunohistochemistry methods (Table 5).

Four FTLD-U cases (6%) did not show any TDP-43 pathology, although ubiquitin-positive inclusions were present (Fig. 3; Table 7). The ages at onset of these unrelated patients were 33, 39, 54, and 59 years, whereas the average age at onset among the other FTLD-U patients was 59 years (p = 0.006). A younger age of onset suggests a genetic component in the pathogenesis. Indeed, a mutation in the charged multivesicular body protein 2B gene in chromosome 3 was recently shown to be the cause of FTLD-U, non-TDP-43 proteinopathy in a large Danish pedigree (20, 27), but the charged multivesicular body protein 2B mutation status of our 4 cases is currently unknown. Progranulin mutation screening was performed in 2 of the cases, and both were negative for progranulin mutations.

Of the 4 cases originally classified as DLDH based on a diagnostic workup that included ubiquitin immunohistochemistry by the routine method, only 1 case remained as DLDH after TDP-43 immunohistochemistry by the enhanced method. The patient died aged 98 years after a 10-year clinical history of Alzheimer-like dementia. Further review of the pathologic findings in that remaining DLDH case raised the possibility of vascular dementia as an alternative diagnosis.

Among the cases with other neurodegenerative diseases, 35% showed mild to moderate TDP-43 pathology in the hippocampus but not in the frontal cortex. In contrast, 97% of FTLD-U cases had TDP-43 pathology in both the hippocampus and frontal cortex. Our results are consistent with a recent report of TDP-43 pathology in the hippocampus of 20% of AD cases (28).

The specificity of the TDP-43 staining observed with the enhanced method is supported by a number of findings, including the following: i) nondemented controls did not have any TDP-43 pathology, ii) TDP-43 pathology was anatomically restricted in cases with other neurodegenerative diseases, iii) results obtained with 2 different TDP-43 antibodies showed a similar pattern of staining, iv) nuclear staining was always present as an internal positive control, v) the presence of frequent long neurites in the CA1 and frontal cortex correlated with progranulin mutations (Tables 2–3), vi) substantial agreement was found between the routine and enhanced protocols in the frontal cortex when the cases with dot-like neurites were excluded (Table 5), and vii) the presence of frequent dot-like neurites in the frontal cortex correlated with granular cytoplasmic inclusions and younger age of the patients (Table 4).

Overall, our results demonstrate that the sensitivity of the immunohistochemistry protocol can have a pronounced effect on the numbers of visible TDP-43-positive neurites in FTLD-U. Extensive neuritic pathology is common in the hippocampal CA1 region and forms a well-demarcated, previously unrecognized lesion pattern that is readily identifiable only when a sensitive immunohistochemistry protocol is used. Interestingly, this pattern of pathology is usually either clearly present (i.e. visible even without a microscope) or absent; cases falling into the “moderate” category were rare. Frequent TDP-43-positive DNAs in the CA1 region and frontal cortex are associated with progranulin mutations. However, frequent dot-like neurites in the frontal cortex showed no such association. Although the presence of DNAs in FTLD-U was previously known, the patterns of pathology (frequent CA1 neurites and dot-like neurites) described here are novel, and their identification was made possible by the increased sensitivity of the detection method.

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REFERENCES

9. Mukherjee O, Pastor P, Cairns NJ, et al. HDDD2 is a familial...