TDP-43 Pathologic Lesions and Clinical Phenotype in Frontotemporal Lobar Degeneration With Ubiquitin-Positive Inclusions

Murray Grossman, MD; Elisabeth M. Wood, MS; Peachie Moore, BA; Manuela Neumann, MD, PhD; Linda Kwong, PhD; Mark S. Forman, MD, PhD; Christopher M. Clark, MD; Leo F. McCluskey, MD; Bruce L. Miller, MD; Virginia M.-Y. Lee, PhD, MBA; John Q. Trojanowski, MD, PhD

Background: TDP-43 is a major ubiquitinated disease protein in the pathologic condition of frontotemporal lobar degeneration with ubiquitin-positive inclusions (FTLD-U).

Objective: To investigate the demographic, clinical, and neuropsychological features associated with subtypes of FTLD-U with TDP-43 inclusions (FTLD-U/TDP-43).

Design: Retrospective clinical-pathologic study.

Setting: Academic medical center.

Patients: Twenty-three patients with histopathologically proven FTLD-U.

Main Outcome Measures: Demographic, symptom, neuropsychological, and autopsy characteristics.

Results: There are notably different clinical and neuropsychological patterns of impairment in FTLD-U subtypes. Patients with FTLD-U/TDP-43 characterized by numerous neuronal intracytoplasmic inclusions have shorter survival; patients with FTLD-U/TDP-43 featuring numerous neurites have difficulty with object naming; and patients with FTLD-U/TDP-43 in whom neuronal intranuclear inclusions are present have substantial executive deficits. There are also different anatomical distributions of ubiquitin pathologic features in FTLD-U subgroups, consistent with their cognitive deficits.

Conclusion: Distinct TDP-43 profiles may affect clinical phenotypes differentially in patients with FTLD-U.

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F RONTOTEMPORAL DEMENTIA (FTD) is a progressive neurodegenerative disease manifesting as language dysfunction or as a disorder of social comportment and executive functioning.1,2 This condition is as common as Alzheimer disease in individuals younger than 65 years.3-5 The single most common histopathological diagnosis associated with FTD is frontotemporal lobar degeneration with ubiquitin-positive inclusions (FTLD-U),6,8 characterized by ubiquitin-positive and tau-negative and α-synuclein-negative inclusions. Similar pathologic inclusions are seen in motor neuron disease (MND), a neurodegenerative disease affecting motor neurons in which a disorder of social, executive, or language functioning may co-occur in up to half of these patients.9,10

Until recently, the ubiquitinated protein in FTD-U was unidentified. TDP-43 is now known to be a major disease protein in FTLD-U and MND, and pathologic TDP-43 is ubiquitinated, phosphorylated, and fragmented in these disorders.11,12 TDP-43 is specific to the ubiquitinated inclusions of FTLD-U, and the diagnostic inclusions in Parkinson disease (Lewy bodies) or in Alzheimer disease (neurofibrillary tangles and senile plaques) are not detected with antibodies to TDP-43. Several FTLD-U subtypes have been described using staining with antibodies to ubiquitin13,14 and TDP-43.11,12 As summarized in Table 1, FTLD-U1 (type 1 in the scheme by Sampathu et al,14 equivalent to type 2 in the study by Mackenzie et al13) consists of numerous neurites, few neuronal cytoplasmic inclusions (NCIs), and no neuronal intranuclear inclusions (NIIs). FTLD-U2 (type 2 in the scheme by Sampathu et al,14 equivalent to type 3 in the scheme by Mackenzie et al13) consists of numerous NCIs, rare neurites, and no NIIs. FTLD-U3 (type 3 in the scheme by Sampathu et al,14 equivalent to type 3 in the study by Mackenzie et al13) contains numerous NCIs, rare neurites, and no NIIs. FTLD-U3 (type 3 in the scheme by Sampathu et al,14 equivalent to type 3 in the study by Mackenzie et al13) contains numerous NCIs, rare neurites, and no NIIs. FTLD-U3 (type 3 in the scheme by Sampathu et al,14 equivalent to type 3 in the study by Mackenzie et al13) contains numerous NCIs, rare neurites, and no NIIs. FTLD-U3 (type 3 in the scheme by Sampathu et al,14 equivalent to type 3 in the study by Mackenzie et al13) contains numerous NCIs, rare neurites, and no NIIs.
TDP-43 inclusions (FTLD-U/TDP-43), although descriptions such as semantic dementia, progressive nonfluent aphasia, and social-executive disorder can be unstable over time and difficult to distinguish later in the course of the disease. In this study, we report the clinical features of patients with subtypes of FTLD-U pathologic features as defined by immunostaining for TDP-43. We focus on quantitative neuropsychological performance, and we relate this to the neuroanatomical distribution of histopathological disease.

**METHODS**

**SUBJECTS**

Twenty-three patients with the pathological diagnosis of FTLD-U were investigated in this study. The brains were identified in the consecutive pathological series collected between 1995 and 2006 at the Center for Neurodegenerative Disease Research at the University of Pennsylvania School of Medicine and represent about 29% of a series of patients with the diagnosis of an FTD spectrum disorder. All patients were diagnosed by experienced neurologists in the departments of neurology at the University of Pennsylvania School of Medicine (M.G., C.M.C., and L.F.M.) or at the University of California, San Francisco (B.L.M.), according to published criteria. Subgroup diagnosis was assigned using a consensus mechanism based on a modification of published criteria. All cases were carefully screened for MND. Clinical diagnosis was based on informant interview, medical history, neurological examination, neuropsychological evaluation, laboratory screening, and brain imaging when available (including magnetic resonance imaging, single-photon emission computed tomography, or positron emission tomography). Because the patients came from multiple clinics by different investigators during a 10-year period, there was variability in the clinical data obtained and in the approach to clinical diagnosis. Clinical data were obtained from medical record review in patients in whom autopsy occurred before 2000 and were collected prospectively in patients with autopsy since 2000. Demographic characteristics are summarized in Table 1. Disease duration (survival) was computed from the time of symptom onset until death. Symptom onset was based on a family report of the earliest persistently abnormal clinical feature in the domains of language, memory, executive functioning, visual-spatial functioning, movement disorder or weakness, and social function or personality change.

Symptoms tabulated at presentation included focal weakness, language dysfunction, movement disorder, social or behavioral changes, and other cognitive complaints (eg, memory loss, inattention, planning disorder, or visual-spatial complaints). A limited battery of neuropsychological measures was obtained on a subset of patients as part of the routine evaluation at the University of Pennsylvania School of Medicine and at the University of California, San Francisco. This included the following 6 measures: (1) dementia (Mini-Mental State Examination [a 30-point scale surveying dementia severity]), (2) visual perceptual-spatial functioning (geometric design [copying geometric designs graded in difficulty]), (3) social functioning (social scale [a 6-point scale surveying disorders of social comportment and personality]), (4) language (confrontation naming [correct confrontation naming of black-and-white line drawings from an abbreviated version of the Boston Naming Test]), (5) memory (delayed recall [correct recall of 10 words after a brief delay following presentation during 3 learning trials] and recognition [correct recognition of the 10 words interspersed among 10 foils, probed following delayed recall]), and (6) executive functioning (digit span forward [the longest series of numbers repeated correctly in the presented order], digit span reverse [the longest series of numbers repeated correctly in an order reversing the order of presentation], and category naming fluency [the number of different animals named in 60 seconds]).

**NEUROPATHOLOGICAL EVALUATION**

The neuropathological evaluations were performed as described previously. All cases were reviewed by 3 board-certified neuropathologists (M.N., M.S.F., and J.Q.T.) in a manner blinded to the clinical diagnoses. Using established criteria, we identified brains with ubiquitin-positive and tau-negative and α-synuclein-negative inclusions (ie, FTLD-U). All cases without any inclusions were classified as dementia lacking distinctive histology and were excluded from this study. We analyzed 8 regions, including subcortical nuclei (striatum with nucleus basalis), limbic system (hippocampus and amygdala with entorhinal cortex), and cortex (midfrontal gyrus, inferoparietal lobule, superior and middle temporal gyr, anterior cingulate gyrus, and calcareous cortex). The spinal cord was not available in most of these cases, so we cannot exclude the possibility that clinically unapparent MND was underdiagnosed in this cohort. Semiquantitative methods were used to assess the density of immunostained ubiquitin lesions, and grading was assigned (0, no or rare pathologic findings; 1, low pathologic findings; 2, moderate pathologic findings; and 3, high pathologic findings) in each analyzed brain region. As described in detail elsewhere and as summarized in Table 1, subsets of FTLD-U were established on the basis of monoclonal antibodies. Frontotemporal lobar degeneration with ubiquitin-positive inclusions type 1 (FTLD-U1, equivalent to type 2 in the study by Mackenzie et al) was recognized by monoclonal antibody 182 and consisted of frequent neurites and some NCIs but no NIIs; FTLD-U type 2 (FTLD-U2, equivalent to type 3 in the study by Mackenzie et al) was recognized by monoclonal antibody 406 and was characterized by frequent NCIs and some neurites but no NIIs; and FTLD-U type 3 (FTLD-U3, equivalent to type 1 in the study by Mackenzie et al) was identified by ubiquitin inclusions staining for neither of these monoclonal antibodies and contained NCIs, neurites, and NIIs. These analyses were based on an analysis of 1 section per
region, and agreement between raters was good (κ statistic, 0.75; 95% confidence interval, 0.52-0.98). The definition of these FTLD-U subtypes, all of which are characterized by TDP-43 pathologic lesions, was also confirmed using polyclonal antibodies to TDP-43.12

**STATISTICAL ANALYSIS**

Because of the few patients constituting the FTLD-U/TDP-43 subgroups, nonparametric statistical tests were used to evaluate the demographic characteristics, frequencies of clinical features, neuropsychological performance, and severity of the histopathological abnormalities. Neuropsychological measures were converted to z scores in each individual relative to 25 age-matched and education-matched healthy control subjects so that relative performance could be compared across measures with different numbers of items and different inherent levels of difficulty and so that statistically significant deficits could be identified in individuals constituting these small subgroups.

**RESULTS**

Clinical and demographic characteristics of patients with FTLD-U/TDP-43 are summarized in Table 2. Age at onset did not differ among patients with the FTLD-U/TDP-43 subtypes. The mean±SD age at onset of patients with FTLD-U/TDP-43 was 61.5±8.9 years (range, 46-78 years). This is similar to tauopathies (mean age at onset, 61.0 years)10 but is somewhat older than the mean age at onset of 54.8 years among patients with dementia lacking distinctive histopathology and is younger than the mean age at onset of 71.4 years among patients with Alzheimer disease.11 The mean±SD survival in FTLD-U/TDP-43 is 87.3±32.5 months (range, 20-156 months). The difference in survival among FTLD-U/TDP-43 subtypes approaches significance (χ² = 4.81, P < .09). Although the small numbers of patients in these subgroups limit interpretation, survival among patients with FTLD-U2 is half as long as that among patients with FTLD-U1 and is substantially less than that among patients with FTLD-U3. This could not be entirely attributed to the presence of MND in 1 patient with FTLD-U2 because the disease duration associated with this TDP-43 subtype (20 months in the MND case and mean durations of 48 months and 84 months in the other 2 subtypes) was less than the disease duration of most patients with FTLD-U1 pathologic lesions (the disease duration in only 1 patient was <72 months). Disease duration in the MND case with FTLD-U3 was 24 months. PGRN mutations occurred only in association with FTLD-U3. A statistical comparison of patients with FTLD-U3 with PGRN mutations compared with those without PGRN mutations failed to reveal any differences. Likewise, comparisons of the clinical and pathological features associated with these FTLD-U3 subgroups (described herein) failed to reveal any differences.

**Table 3. Clinical Symptoms at Onset in Patients With Frontotemporal Lobar Degeneration With Ubiquitin-Positive Inclusions (FTLD-U) and TDP-43 Inclusions**

<table>
<thead>
<tr>
<th>Symptom</th>
<th>FTLD-U1 (n=9)</th>
<th>FTLD-U2 (n=3)</th>
<th>FTLD-U3 (n=11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Social</td>
<td>6 (66.7)</td>
<td>2 (66.7)</td>
<td>10 (90.9)</td>
</tr>
<tr>
<td>Language</td>
<td>6 (66.7)</td>
<td>1 (33.3)</td>
<td>6 (54.5)</td>
</tr>
<tr>
<td>Memory</td>
<td>3 (33.3)</td>
<td>0</td>
<td>6 (54.5)</td>
</tr>
<tr>
<td>Executive</td>
<td>2 (22.2)</td>
<td>1 (33.3)</td>
<td>3 (27.3)</td>
</tr>
<tr>
<td>Visual-perceptual</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Motor-pyramidal</td>
<td>0</td>
<td>0</td>
<td>1 (9.1)</td>
</tr>
<tr>
<td>Motor-extra pyramidal</td>
<td>0</td>
<td>1 (33.3)</td>
<td>1 (9.1)</td>
</tr>
</tbody>
</table>

αThe primary initial symptom in both cases of motor neuron disease was speech difficulty.

**Table 2. Clinical and Demographic Characteristics of Patients With Frontotemporal Lobar Degeneration With Ubiquitin-Positive Inclusions (FTLD-U) and TDP-43 Inclusions**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>FTLD-U1 (n=9)</th>
<th>FTLD-U2 (n=3)</th>
<th>FTLD-U3 (n=11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male sex</td>
<td>5</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Clinical diagnosis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Motor neuron disease</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Other</td>
<td>1</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>FTD</td>
<td>8</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>Clinical FTD phenotype</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Progressive nonfluent aphasia</td>
<td>1</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Semantic dementia</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Social-executive disorder</td>
<td>4</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>PGRN mutation present</td>
<td>0</td>
<td>0</td>
<td>4</td>
</tr>
</tbody>
</table>

| Age at onset, y | 61.6±9.0 | 57.6±13.9 | 60.5±8.9 |
| Age at testing, y | 66.2±9.0 | 60.7±13.6 | 63.9±9.3 |
| Education, y | 13.7±2.2 | 14.7±2.3 | 15.2±2.9 |
| Disease duration, mo | 100.3±39.0 | 50.7±13.6 | 74.8±29.5 |

Abbreviation: FTD, frontotemporal dementia.

αData are given as number of patients or as mean±SD.

b Three patients were given the clinical diagnosis of Alzheimer disease, and 2 patients were given the clinical diagnosis of Lewy body disease.

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The pathological assessment of these patients is summarized in Table 5. Ubiquitin and TDP-43 pathologic lesions differed in their anatomical distribution across FTLD-U/TDP-43 subgroups ($\chi^2 = 14.7, P < .04$), although the overall histopathological burden did not differ between subgroups ($\chi^2 = 3.33, P = .07$). Pathologic lesions were dense in lateral temporal cortex and entorhinal portions of the medial temporal lobe in patients with FTLD-U1, while the densest pathologic change in FTLD-U3 was in midfrontal cortex. Ubiquitin and TDP-43 pathologic findings differed statistically significantly across subgroups in the hippocampal region ($\chi^2 = 6.2, P < .05$) because of substantial disease in FTLD-U1 and FTLD-U2. Ubiquitin and TDP-43 pathologic lesions were also moderately dense in the amygdala region in all subgroups but were less dense in parietal, cingulate, and deep gray regions, including the striatum. These findings must be interpreted cautiously because of the small numbers of samples in each subgroup.

We examined the clinical phenotypes associated with subtypes of FTLD-U defined by immunostaining for pathologic TDP-43. Differences were found in the clinical characteristics of FTLD-U/TDP-43 subtypes, implicating pathologic TDP-43 in the phenotype associated with patients with FTLD-U. Patients with FTLD-U seem to have executive dysfunction, and their pathologic lesions are dense in midfrontal cortex. By comparison, patients with FTLD-U1 have statistically significant impairment with naming, and their pathologic lesions are dense in temporal cortex. Therefore, distinct phenotypes are evident in FTLD-U/TDP-43 subgroups, and these seem to be determined at least in part by the distribution of their pathologic lesions.

A possible explanation for this finding is that specific conformations of pathologic TDP-43 or the extent of its abnormal ubiquitination, phosphorylation, or cleavage has a predilection for a particular anatomical distribution or differentially affects neuronal function or viability in a specific brain region. For example, the dense lateral temporal pathologic lesions in patients with FTLD-U1 who have the greatest naming difficulty is consistent with this possibility. This may be related in part to the increased frequency of the semantic dementia phenotype in this FTLD-U/TDP-43 subgroup. Findings from an-
other study associated the form of pathologic lesion seen in this subgroup with semantic dementia. Likewise, prefrontal pathologic lesions were abundant in the patients with FTLD-U3 with executive limitations in the present study, and Mackenzie and coworkers observed a disorder of social comportment and executive functioning in patients with these pathologic findings. We also found that episodic memory is most impaired in patients with FTLD-U1 and FTLD-U2, who have more extensive hippocampal pathologic findings than patients with FTLD-U3. Our use of quantitative observations allowed us to establish a direct relationship between clinical impairment and the neuroanatomical distribution of disease burden that is less sensitive to the shifting syndromic diagnoses known to occur in FTD. This is consistent with an extensive literature implicating regional disease burden in the cognitive profiles of patients with neurodegeneration. Our observations must be tempered by the fact that small numbers of patients were investigated in this study and that clinical evaluation was separated from the time of death by many months. Nevertheless, groupwise statistical contrasts seem to reflect individual patient performance profiles. Cognitive differences in FTLD-U/TDP-43 subgroups are unlikely to be due to the age at onset, the age at the time of testing, overall dementia severity, or overall histopathological burden because the subgroups were matched on these factors. These clinical differences are unlikely to reflect differences in disease duration between FTLD-U/TDP-43 subgroups. There may be greater naming difficulty in FTLD-U1 because these patients have longer disease duration, allowing the disease to compromise temporal cortical functioning for a longer period. However, this would not explain the statistically significantly greater executive difficulty in FTLD-U3 (ie, cognitive difficulties in patients with briefer survival). PGRN mutations were observed only in FTLD-U3, but a direct statistical comparison of FTLD-U cases with PGRN mutations compared with FTLD-U cases without PGRN mutations did not reveal regional or overall differences in histopathological severity. Although findings from previous studies associate more severe language difficulty with PGRN mutations, a recent series of patients with FTLD-U with PGRN mutations did not show more severe language difficulty than patients with FTLD-U without PGRN mutations. Regardless of the basis for cognitive difficulties associated with FTLD-U subtypes, selective impairment in specific cognitive domains suggests that TDP-43 pathologic lesions have a substantial effect on the clinical phenotype in FTLD-U/TDP-43. This emphasizes the disease-causing importance of this protein in FTD and underlines TDP-43 as an important target for drug development.

There has been considerable controversy in the literature examining survival in FTD. Some work suggests that tau-negative pathologic conditions such as FTLD-U are associated with a briefer survival. This finding persists after excluding cases with clinical MND, a condition with tau-negative pathologic lesions known to have a poor prognosis. We and others found a statistically significant difference in survival among subgroups of patients with different FTLD-U/TDP-43 pathologic patterns. This observation should be interpreted cautiously because of the difficulty in establishing the onset of a neurodegenerative disease with any precision. Nevertheless, patients with FTLD-U1 seemed to have longer disease duration than patients with FTLD-U2 and FTLD-U3 in the present study. This is unlikely to be entirely because of the poor survival in MND because of the small number of participants with MND in our study. A previous study described briefer survival in the subgroup of patients with FTLD-U/TDP-43 with numerous NCIs, few neurites, and no NIIs, consistent with the pathologic profile seen in FTLD-U2, although this may have been confounded in part by the large number of patients with clinical features of MND in this subgroup. Additional work with larger numbers of patients is needed to assess the role of FTLD-U/TDP-43 pathologic lesions on survival. Investigations found no difference in the survival of individuals with tau-negative pathologic conditions compared with tau-positive pathologic conditions such as Pick disease, but other work demonstrated that tau-negative pathologic conditions are associated with longer survival than tau-positive pathologic conditions. Some of these discrepancies seem in part to be related to the different kinds of pathologic lesions contributing to the tau-positive subgroups in these studies. Therefore, studies with larger numbers of patients with corticobasal degeneration seem to be associated with briefer survival in tau-positive patient subgroups. Findings from the present study suggest that an additional source of variability in the survival of patients with FTD is the specific type of TDP-43 pathologic lesion seen in patients with FTLD-U with tau-negative pathologic findings. Indeed, this may represent a notable confound in previous studies investigating survival because FTLD-U/TDP-43 represents such a large proportion of the cases in tau-negative subgroups.

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Correspondence: Murray Grossman, MD, Department of Neurology, University of Pennsylvania School of Medicine, 2 Gibson, 3400 Spruce St, Philadelphia, PA 19104-4283 (mgrossma@mail.med.upenn.edu).


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REFERENCES


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