TAR-DNA Binding Protein 43 in Pick Disease

Stefanie H. Freeman, MD, Tara Spires-Jones, DPhil, Bradley T. Hyman, MD, PhD, John H. Growdon, MD, and Matthew P. Frosch, MD, PhD

Abstract

Pick disease (PiD) is a frontotemporal dementia characterized by frontal and temporal atrophy, neuronal loss, gliosis, ballooned neurons that are positive for α-B crystallin and neurofilament, and the presence of tau- and ubiquitin-positive Pick bodies. TAR-DNA binding protein 43 (TDP-43) has been found to be a component of ubiquitinated inclusions in other neurodegenerative diseases, including frontotemporal lobar degeneration with ubiquitinated inclusions and amyotrophic lateral sclerosis. Fifteen cases of PiD were examined using immunohistochemical methods, and 5 cases with both Pick bodies and smaller intracytoplasmic inclusions that showed staining for ubiquitin, tau, and TDP-43 were observed. The presence of TDP-43 inclusions in PiD suggests that TDP-43 accumulation may be an important component of many neurodegenerative diseases, and that its presence in only some cases of PiD may indicate different pathways of disease development.

Key Words: Frontotemporal dementia, Immunohistochemistry, Neurodegeneration, Pick bodies, Pick disease, TDP-43.

INTRODUCTION

Ubiquitin-positive inclusions are found in a number of neurodegenerative diseases. Pick disease (PiD) is a frontotemporal dementia characterized by extensive atrophy most prominent in the frontal and temporal lobes—a form of frontotemporal lobar degeneration. Histologically, there is cortical gliosis, spongiosis, and the presence of Pick bodies and ballooned neurons. Pick bodies are stained with the immunohistochemical markers tau and ubiquitin and demonstrate positivity using the Bielschowsky silver preparation (1). The pattern of severe frontal and temporal atrophy is similar to that in frontotemporal lobar degeneration with ubiquitinated inclusions (FTLD-U). By contrast, neuronal cytoplasmic and intranuclear inclusions in FTLD-U are tau negative and ubiquitin positive.

TAR-DNA binding protein 43 (TDP-43) has recently been shown to be a major factor comprising the inclusions in FTLD-U (2). It has also been described in the white matter of FTLD-U cases (3); strong TDP-43 immunopositivity has also been found in FTLD-U cases with progranulin mutations (4). Contrary to early expectations that TDP-43 immunopositivity was specific to FTLD-U, it has subsequently been found in other neurodegenerative diseases. For example, TDP-43 is a component of the skin-like inclusions, neuronal intranuclear inclusions, and glial inclusions found in cases of amyotrophic lateral sclerosis (2, 5–8). TAR-DNA binding protein 43 colocalization with ubiquitin intranuclear inclusions and dystrophic neurites has been found in cases with VCP gene mutations with frontotemporal dementia, inclusion body myopathy, and Paget disease of the bone (9). Twenty-three percent of Alzheimer disease cases and 71% of hippocampal sclerosis cases have demonstrated immunoreactivity for TDP-43 (10). Recently, TDP-43 has also been found to label cytoplasmic and intranuclear inclusions in the brains of Guamanians with the parkinsonism-dementia complex (11). TAR-DNA binding protein 43 positivity has also been reported in cases with Lewy body pathology, and Arai et al (5) and Nakashima-Yasuda et al (12) reported staining of Pick bodies in PiD. Because of the close relationship between FTLD-U and PiD, we further evaluated the presence of TDP-43 in PiD and found a subset of cases in which intracytoplasmic inclusions label with antibodies to tau, ubiquitin, and TDP-43. These results further support the view that TDP-43 inclusions are not specific to FTLD-U and expands the list of neurodegenerative diseases associated with TDP-43 accumulations to include PiD.

MATERIALS AND METHODS

Tissue Collection

We examined tissue from 15 clinically and neuropathologically defined cases of PiD and 5 age-matched controls obtained from the Neuropathology Core of the Massachusetts Alzheimer Disease Research Center. At the time of autopsy, the brains were hemisected, and coronal slices from 1 hemisphere were frozen between dry ice-cooled aluminum plates, whereas the opposite hemisphere was fixed in 10% buffered formalin with subsequent processing and paraffin embedding. From the formalin-fixed

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TABLE. Clinical and Pathologic Features of PiD Cases

<table>
<thead>
<tr>
<th>Age of Death</th>
<th>Age of Onset (years)/Early Symptoms</th>
<th>Gender</th>
<th>Brain Weight (g)</th>
<th>Tau-Positive Pick Bodies</th>
<th>Ballooned Neurons (Pick Cells) LFB/H and E</th>
<th>Ubiquitin-Positive Pick Bodies</th>
<th>TDP-43-Positive Cytoplasmic Inclusions</th>
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<tbody>
<tr>
<td>84</td>
<td>69, memory impairment and tardive dyskinesia</td>
<td>F</td>
<td>840</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>−</td>
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<tr>
<td>76</td>
<td>64, memory impairment, “nervousness”</td>
<td>F</td>
<td>821</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
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<tr>
<td>71</td>
<td>65, depression, anxiety, and memory impairment</td>
<td>F</td>
<td>936</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>73</td>
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<td>M</td>
<td>1,110</td>
<td>+</td>
<td>+</td>
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<td>+</td>
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<td>85</td>
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<td>+</td>
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<td>−</td>
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<td>80</td>
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<td>M</td>
<td>986</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
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<tr>
<td>64</td>
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<td>F</td>
<td>1,310</td>
<td>+</td>
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<td>+</td>
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</table>

F, female; LFB/H and E, Luxol fast blue/hematoxylin and eosin; M, male; PiD, Pick disease; TDP-43, TAR-DNA binding protein 43.

hemisphere, blocking consisted of samples from frontal, temporal, parietal, and occipital lobes, hippocampus, amygdala, basal ganglia, thalamus, hypothalamus, and cingulate gyrus. Cerebellum with dentate nucleus and brainstem sections were also examined. All sections were stained with Luxol fast blue/hematoxylin and eosin. Bielschowsky silver preparation, β-amyloid, and α-synuclein immunostaining were performed on selected blocks. Ubiquitin, tau, and

FIGURE 1. Case of Pick disease demonstrating tau-positive Pick bodies in frontal cortical neurons (A). Tau inclusion in dentate gyrus (B). Numerous ubiquitin-positive inclusions in the dentate gyrus (C) and TAR-DNA binding protein 43-positive inclusions in dentate gyrus (D) (magnification: 60×).
TDP-43 immunostaining were performed on sections of frontal cortex, temporal cortex, hippocampus, and amygdala. Neuropathologic diagnoses were made according to established diagnostic criteria (1, 13–15).

**Immunostaining**

Paraffin sections (5 μm) were deparaffinized in xylene, rehydrated with graded alcohols, pretreated with 70% formic acid (5 minutes, room temperature) for Aβ immunostaining only, and blocked with 5% bovine serum albumin (Fisher Scientific, Pittsburgh, PA) in Tris-buffered saline (TBS; 1 hour). Sections were incubated overnight at 4°C with rabbit anti-tau (1:3000; Dako, Glostrup, Denmark), rabbit anti-ubiquitin (1:200; Dako), rabbit anti-TAR-DNA binding protein/anti-TDP-43 (1:500; ProteinTech Group, Inc., Chicago, IL), mouse anti–α-synuclein (1:1600; Zymed, San Francisco, CA), or mouse anti-Aβ clone 6F/3D ba4 (1:1200; Dako) in 1.5% normal goat serum in TBS. Sections were washed in TBS (3 × 5 minutes), incubated with biotinylated anti-rabbit or anti-mouse (α-synuclein, Aβ) immunoglobulin G (1:200; Invitrogen, Carlsbad, CA; 1 hour; room temperature), and treated with preformed avidin-biotin-conjugated enzyme complex (Vector Laboratories, Burlingame, CA). The staining was visualized with diaminobenzidine (Vector). The slides were then counterstained with hematoxylin. Sections were cleared in alcohols and xylene and coverslipped with Permount mounting media (Fisher Scientific). The cases that were initially negative for TDP-43 staining were restained after pretreatment with 70% formic acid for 5 minutes.

**FIGURE 2.** Elongated TAR-DNA binding protein 43-positive inclusions in temporal white matter (magnification: 60×).

**FIGURE 3.** (A–C) Separate ubiquitin (green) and TAR-DNA binding protein 43 (TDP-43) (red) intracytoplasmic inclusions in the dentate gyrus. (D–F) Colocalized (yellow), TDP-43 (red), and ubiquitin (green) intracytoplasmic inclusions in the temporal cortex. (G–I) Largely colocalized intracytoplasmic TDP-43 and ubiquitin inclusion in the dentate gyrus (all panels’ original magnification: 63×; scale bars = 30 μm).
TDP-43, Phospho-tau, and Ubiquitin Confocal Microscopy

Sections were deparaffinized as above and blocked in 5% normal goat serum in TBS. Sections were incubated at 4°C overnight in rabbit anti-TDP-43 (1:500; ProteinTech) and mouse plant homeodomain finger protein 1 (PHF-1; 1:200; generously provided by Dr. Peter Davies, Albert Einstein College of Medicine; antibody directed against tau phosphorylated at serines 396 and 404) or anti-TDP-43 and mouse anti-ubiquitin (1:100; Abcam 61013) in 1.0% normal goat serum in TBS. Sections were washed in TBS (3 × 5 minutes) and incubated with 1:200 anti-mouse Alexa Flour 488 (Invitrogen) and 1:200 anti-rabbit Cy3 (Jackson ImmunoResearch, West Grove, PA) in 1.0% normal goat serum. Sections were then washed in TBS and coverslipped with TBS containing 0.01 mg/mL of 4',6-diamidino-2-phenylindole (Sigma, St. Louis, MO) and observed with a Zeiss LSM-510-META confocal microscope mounted on a Zeiss inverted Axioscope 200 (Carl Zeiss, Inc., Oberkochen, Germany). To observe double labeling of TDP-43 (Cy3) and tau or ubiquitin (Alexa 488), excitation was provided by 488- (Argon) and 543-nm (Helium Neon) lasers. To ensure that double labeled structures were not caused by bleed through artefacts, each channel was excited and collected sequentially using the Zeiss multitrack feature. To excite 4',6-diamidino-2-phenylindole, a tuneable ti-sapphire 2-photon chameleon laser was used at 750-nm wavelength, and images were collected with a photomultiplier tube (390–430 nm). Images were merged and contrast enhanced in Image J (free software from National Institutes of Health).

RESULTS

All 15 cases had the neuropathologic diagnosis of PiD. Grossly, the cases demonstrated circumscribed frontal and temporal atrophy (asymmetric in many) and reduced brain weights (Table). Immunohistochemical staining revealed that all 15 of the cases had tau- and ubiquitin-positive Pick body inclusions (Fig. 1A). The Pick bodies were most numerous within the dentate gyrus. In all cases, the Pick bodies demonstrated positive staining with Bielschowsky silver impregnation.

![Image of Tau-only and Colocalized Inclusions](https://example.com/image.png)

**FIGURE 4.** (A–C) Tau-only (plant homeodomain finger protein [PHF-1], green) inclusion in a hippocampal neuron. (D–F) Colocalized tau and TAR-DNA binding protein 43 (TDP-43) inclusion in hippocampus and single TDP-43-only (red) inclusion. (G–I) Colocalized TDP-43 and tau inclusion in temporal cortex (all panels’ original magnification: 63×; scale bars = 30 μm).
Five control cases, ranging in age from 57 to 91 years, were examined. Anti-TDP-43 staining in all of these cases demonstrated strong nuclear staining of neurons, glia, and ependymal cells and no intracytoplasmic staining. In addition to tau- and ubiquitin-positive Pick bodies, 5 of the 15 PiD cases also had smaller intracytoplasmic inclusions that showed immunoreactivity for TDP-43 (Fig. 1). Many of the smaller inclusions (non-Pick bodies) also showed staining for tau and ubiquitin in PiD cases with and without TDP-43 positivity. The areas of highest concentration of Pick bodies were found in the dentate gyrus, with inclusions also present in the amygdala, temporal, and frontal cortex. The smaller intracytoplasmic inclusions were also seen within these regions. There was strong nuclear staining with TDP-43 in the cases that lack TDP-43-positive inclusions, indicating the normal distribution of this protein. However, in the 5 TDP-43-positive cases, the nuclear staining was variable with weaker staining in nuclei and strong staining observed in the cytoplasmic inclusions, as reported in other diseases with TDP-43-positive inclusions (2, 7, 16). Elongated, threadlike, and rare glial TDP-43 inclusions were also present in the white matter in 2 of the 5 cases (Fig. 2). The 10 cases that did not show TDP-43-positive intracytoplasmic inclusions were negative whether or not antigen retrieval approaches were taken.

From the available clinical histories, there were no clear differences in the mean age of onset, the duration of disease, or the presenting clinical manifestations between cases of PiD with and without TDP-43 inclusions (Table), although the sample size was small. The mean age of onset in those with TDP-43-positive inclusions was 76 ± 13 years, and those without TDP-43-positive inclusions was 61.5 ± 8.8 years. The mean duration of disease was 6.6 ± 3.6 years in those with TDP-43-positive inclusions and 7.9 ± 5.1 years for cases without TDP-43-positive inclusions. Two of the cases had neurofibrillary tangles in the entorhinal cortex and corresponded to Braak stage II/VI. Of the 5 cases, 1 had more abundant tangles (Braak stage IV/VI) along with moderate numbers of diffuse and neuritic plaques.

After observing cases of PiD with both TDP-43 and ubiquitin immunoreactivity, we investigated whether these markers of neurodegenerative pathology were associated with the same structures. Using confocal microscopy with antibodies to these 2 markers, we observed that most Pick bodies and many of the smaller intracytoplasmic inclusions, as defined by the presence of nuclear staining with 4',6-diamidino-2-phenylindole, were seen to colocalize for TDP-43 and PHF-1 (Fig. 3). There were also occasional inclusions that were positive for only 1 of the 2 markers. In contrast, double staining for TDP-43 and PHF-1 in these same anatomic regions revealed several different patterns. Most Pick bodies and many of the smaller intracytoplasmic inclusions, as defined by the presence of nuclear staining with 4',6-diamidino-2-phenylindole, were seen to colocalize for TDP-43 and PHF-1 (Fig. 4). A subset of these inclusions, however, showed only partial colocalization because different parts of the same inclusions stained for the different proteins (Fig. 5). Rare cells demonstrated faint nuclear staining with TDP-43 and cytoplasmic staining with tau. Similar to the staining with ubiquitin and TDP-43, confocal microscopy revealed only occasional intracytoplasmic inclusions positive for a single protein of only tau or TDP-43.

**DISCUSSION**

TAR-DNA binding protein 43 is a nuclear protein involved in regulation of transcription and splicing (5). It is found in cell nuclei and has been shown to be a component
of the ubiquitinated inclusions of FTLD-U and amyotrophic lateral sclerosis. TAR-DNA binding protein 43 immunoreactivity has been reported in Alzheimer disease, cases of hippocampal sclerosis, PiD, and corticobasal degeneration (5, 10). It has also been described in patients with Guam parkinsonism-dementia complex and in a family with FTLD-U and progranulin mutation, and along with tau and alpha-synuclein pathology (4, 11, 17). TAR-DNA binding protein 43 staining has even been observed in a case with ubiquitin-negative glial inclusions (16). We have found that TDP-43 may be a component of Pick bodies in a subset of patients with PiD, confirming and expanding the report by Arai et al (5). Five of our 15 cases demonstrated positivity with TDP-43. These cases also show staining with both tau and ubiquitin.

In a study examining Alzheimer disease cases, double labeling and confocal microscopy demonstrated phospho-tau, and TDP-43 showed some areas of colocalization. In other areas, there was labeling of intermingled yet separate structures. Some neurons also contained tau-positive neurofibrillary tangles with separate inclusions in the same neuron demonstrating staining for TDP-43 (10). Our double labeled staining with phospho-tau and TDP-43 also showed a similar variable pattern of staining for TDP-43 and tau. Most intracytoplasmic inclusions colocalized with confocal microscopy. Some of these inclusions demonstrated only partial colocalization with TDP-43 or tau. Occasional intracytoplasmic inclusions did not colocalize. The double staining with TDP-43 and ubiquitin showed most intracytoplasmic inclusions colocalized and only occasionally contained either TDP-43 or ubiquitin. Thus, the intracytoplasmic TDP-43 aggregates in these cases of PiD are associated primarily with ubiquitin and tau inclusions.

What separates a subset of PiD with immunoreactivity for TDP-43 from cases without TDP-43 is not clear from the clinical presentation or pathology. The presence of TDP-43 aggregates in only some cases with PiD pathology may suggest that there may be more than 1 disease pathway leading to the same clinical manifestations and end point. This has also been seen in cases of amyotrophic lateral sclerosis with superoxide dismutase 1 mutation that lack TDP-43 staining (18). It also suggests the possibility that some cases may express mixed pathology with combined FTLD-U pathology (10).

The finding of TDP-43 associated with some PiD indicates that this alone is not a reliable way to distinguish between FTLD-U and PiD. It does indicate, however, that TDP-43 accumulation in cytoplasmic inclusions may be an important component in the pathogenesis of many neurodegenerative disorders.

REFERENCES