

Neurobiology

# TDP-43 in Familial and Sporadic Frontotemporal Lobar Degeneration with Ubiquitin Inclusions

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**TAR DNA-binding protein 43 (TDP-43) is a major pathological protein of sporadic and familial frontotemporal lobar degeneration with ubiquitin-positive, tau-negative inclusions (FTLD-U) with or without motor neuron disease (MND). Thus, TDP-43 defines a novel class of neurodegenerative diseases called TDP-43 proteinopathies. We performed ubiquitin and TDP-43 immunohistochemistry on 193 cases of familial and sporadic FTLD with or without MND. On selected cases, immunoelectron microscopy and biochemistry were performed.**

**Clinically defined frontotemporal dementias (FTDs) included four groups: 1) familial FTD with mutations in progranulin ( $n = 36$ ), valosin-containing protein ( $n = 5$ ), charged multivesicular body protein 2B ( $n = 4$ ), and linked to chromosome 9p ( $n = 7$ ); 2) familial cases of FTD with unknown gene association ( $n = 29$ ); 3) sporadic FTD ( $n = 72$ ); and 4) familial and sporadic FTD with MND ( $n = 40$ ). Our studies confirm that the spectrum of TDP-43 proteinopathies includes most cases of sporadic and familial FTLD-U with and without MND and expand this disease spectrum to include reported families with FTD linked to chromosome 9p but not FTD with charged multivesicular body protein 2B mutations. Thus, despite significant clinical, genetic, and neuropathological heterogeneity of FTLD-U, TDP-43 is a common pathological substrate underlying a large subset of these disorders, thereby implicating TDP-43 in novel and unifying mechanisms of FTLD pathogenesis. (Am J Pathol 2007, 171:227-240; DOI: 10.2353/ajpath.2007.070182)**

The frontotemporal dementias (FTDs) are a clinically, genetically, and neuropathologically heterogeneous group of diseases accounting for up to 20% of presenile dementia cases. FTD is characterized by behavioral and/or language dysfunction and may co-occur with motor neuron disease (MND).<sup>1,2</sup> Frontotemporal lobar degeneration (FTLD) with ubiquitin-positive, tau-negative inclusions (FTLD-U) is the most common underlying pathology in FTD with and without MND.<sup>3</sup> TAR DNA-

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binding protein 43 (TDP-43), a nuclear protein implicated in exon skipping and transcription regulation,<sup>4–6</sup> was recently identified as a major protein component of the ubiquitin-immunoreactive inclusions characteristic of sporadic and familial FTL-D-U, with and without MND, as well as in sporadic amyotrophic lateral sclerosis (ALS)<sup>7,8</sup> and has been rapidly confirmed by others.<sup>9–11</sup> TDP-43 in these disorders is abnormally phosphorylated, ubiquitinated, and cleaved to generate C-terminal fragments and is recovered only from areas with ubiquitin-immunoreactive inclusions, including hippocampus, neocortex, and spinal cord.<sup>8</sup> Therefore, the presence of abnormal aggregates of phosphorylated and ubiquitinated TDP-43 defines a novel class of neurodegenerative diseases that we propose to call “TDP-43 proteinopathies” that includes FTL-D-U, FTL-D-MND, and ALS. The neuropathology of these conditions is characterized by ubiquitin- and TDP-43-positive neuronal cytoplasmic inclusions (NCIs), neuronal intranuclear inclusions (NIIs), dystrophic neurites (DNs), and glial cytoplasmic inclusions<sup>8,10–12</sup> that are negative for tau,  $\alpha$ -synuclein,  $\beta$ -amyloid, neuronal intermediate filaments, and expanded polyglutamines. The variability in the morphological types of neuronal inclusions, their distribution, density, and immunohistochemical profile has led to the development of the classification of FTL-D-U into four pathological subtypes.<sup>8,10,13,14</sup> Recently, the molecular genetic basis of non-tau familial FTD linked to chromosome 17 was discovered as being mutations in the progranulin gene (*PGRN*).<sup>15–17</sup> The neuropathology in these cases is FTL-D-U with ubiquitin-positive neurites, NCI and, most characteristically, NII.<sup>17–19</sup> As demonstrated by immunohistochemical and biochemical investigation, the ubiquitinated pathological protein in these cases is not progranulin but TDP-43.<sup>8,9</sup> Pathological TDP-43 is detected biochemically in both affected gray and white matter, suggesting that both glial and neuronal pathology may contribute to the pathogenesis of FTL-D-U caused by *PGRN* mutations.<sup>8,11</sup>

Inclusion body myopathy associated with Paget's disease of bone and frontotemporal dementia is a rare autosomal dominant disorder caused by mutations in the valosin-containing protein gene (*VCP*).<sup>10,13,20</sup> *VCP*, a member of the AAA-ATPase gene super family (ATPase associated with diverse cellular activities), has multiple cellular functions, including acting as a molecular chaperone in endoplasmic reticulum-associated protein degradation, stress response, programmed cell death, and interactions with the ubiquitin-proteasome system.<sup>21</sup> The neuropathology in inclusion body myopathy associated with Paget's disease of bone and frontotemporal dementia is a unique subtype of FTL-D-U characterized by numerous NIIs and relatively few NCIs and DNs.<sup>10,13</sup> Once again, the ubiquitinated pathology is not primarily composed of the mutated protein (*VCP*) but rather TDP-43.<sup>10</sup> Phosphorylated TDP-43 is detected only in the insoluble brain extracts from affected regions, indicating that the *VCP* gene mutations cause a dominant-negative loss of function or alteration of *VCP* function, leading to impaired metabolism of TDP-43.<sup>10</sup>

Mutations in the charged multivesicular body protein 2B gene (*CHMP2B*) were recently identified as the cause of FTD linked to chromosome 3 in a large Danish pedigree.<sup>22</sup> Human *CHMP2B* is a protein of 213 amino acids with a predicted coil-coil domain and is a component of the endosomal secretory complex III required for transport. Neuropathology was originally described as “dementia lacking distinctive histopathology,” but more recent studies have revealed ubiquitin-positive granular NCIs in frontal neocortex and hippocampus.<sup>23</sup> TDP-43 immunohistochemistry, electron microscopy, and biochemistry have not previously been undertaken in these cases.

Recently, a new genetic locus on chromosome 9p for familial FTD-MND has been described.<sup>24,25</sup> In one family, candidate gene sequencing revealed the presence of a putative disease segregating stop codon mutation (Q342X) in the intraflagellar transport protein 74 gene (*IFT74*).<sup>26</sup> *IFT74* is a 600-amino acid protein with a coiled-coil domain-containing protein that localizes to the intracellular vesicle compartment and is a component of the intraflagellar transport system responsible for vesicular transport of material synthesized within the cell body into and along dendrites and axons. Neuropathology in a single case with the *IFT74* gene mutation was reported as showing all of the signs of FTL-D-U (ubiquitinated NCI, DN, and NII).<sup>26</sup> TDP-43 immunohistochemistry and biochemistry have not previously been reported in this or other chromosome 9-linked FTD families.

Therefore, previous studies indicate that TDP-43-immunoreactive inclusions constitute a common pathological finding linking many cases of sporadic FTL-D-U, familial FTL-D-U with *PGRN* and *VCP* mutations, and FTL-D-MND. However, each of the aforementioned studies included relatively small numbers of cases in each disease category. The aims of the present study were as follows: 1) to define the frequency of TDP-43 proteinopathy in a much larger collection of familial and sporadic cases of FTL-D-U and FTL-D-MND, collected at multiple sites in North America and Europe; 2) to determine whether FTL-D-U in reported families linked to chromosome 9p and FTL-D-U linked to chromosome 3 are TDP-43 proteinopathies; and 3) to examine the presence of pathological TDP-43 in a wider range of FTL-Ds and other neurodegenerative conditions.

## Materials and Methods

### Tissue Collection and Processing

Brain tissues from clinically and neuropathologically characterized cases of sporadic and familial FTL-D-U, with or without MND, other FTL-Ds, and other neurodegenerative diseases were obtained from Canada, Denmark, Germany, The Netherlands, and the United States (Table 1; Supplemental Table 1, see <http://ajp.amjpathol.org>). Cases of FTL-D-U showed characteristic pathology<sup>3,17–19,27</sup> and had a clinical diagnosis of one of the FTD subtypes (including frontal variant FTD, primary progressive aphasia, or corticobasal syndrome), MND

**Table 1.** Demographic, Clinical, Genetic, and Neuropathologic Data of Cases

Familial and sporadic FTDs	Demographic data			Diagnosis		
	Onset (years) [mean (range)]	Duration (years) [mean (range)]	Gender	Clinical diagnosis (n, %)	Pathological diagnosis (n, %)	Pathological diagnosis-other (n, %)
Familial FTD with <i>PGRN</i> mutation (n = 36)	59.8 (50 to 74)	7.06 (3 to 15)	20 F/16 M	FTD (27, 75) PPA (2, 6) FTD + CBD (2, 6) DAT/dementia (5, 14)	FTLD-U (36, 100)	AD (8, 22) AD, HS (2, 6) AD, MS (1, 3) CVD (4, 11) HS (1, 3) DLB, PD (1, 3)
Familial FTD with <i>VCP</i> mutation (n = 5)	51.4 (38 to 62)	7.2 (5 to 9)	4 F/1 M	FTD (5, 100)	FTLD-U (5, 100)	
Familial FTD with <i>CHMP2B</i> mutation (n = 4)	53.3 (50 to 48)	12.8 (9 to 21)	3 F/1 M	FTD (4, 100)	FTLD-U (4, 100)	
Familial FTD with/without MND linked to chromosome 9 (n = 7)	53.8 (39 to 59)	3.7 (2 to 11)	4 F/3 M	FTD (1, 14) FTD + MND (4, 57) MND (2, 28)	FTLD-U (2, 29) FTLD-MND (5, 71)	
Other familial FTD cases (n = 29)	57.1 (33 to 69)	7.4 (2 to 19)	15 F/14 M	FTD (21, 72) PPA (2, 7) DAT/dementia (6, 21)	FTLD-U (27, 93) FTLD-MND (2, 7) AD, DLB (1, 3)	AD (2, 7) AGD (3, 10) HS (1, 3)
Sporadic FTD cases (n = 72)	60.5 (33 to 89)	7.5 (2 to 18)	28 F/44 M	FTD (49, 68) PPA (6, 8) CBD (3, 4) PPA + CBD (1, 1) DAT/dementia (13, 18)	FTLD-U (61, 85) FTLD-MND (11, 15)	AD (9, 13) HS (3, 4) AGD (2, 3) CBD (1, 1)
Familial FTD and MND (n = 17)	51.0 (44 to 63)	6.6 (1 to 6)	5 F/12 M	PPA + MND (1, 6) FTD + MND (16, 94)	FTLD-MND (17, 100)	AD (2, 12) CVD (1, 6)
Sporadic FTD and MND (n = 23)	54.7 (35 to 72)	4.0 (1 to 11)	6 F/19 M	FTD + MND (21, 92) PPA + MND (2, 8)	FTLD-U (1, 4) FTLD-MND (22, 96)	
Other familial and sporadic FTD cases (non-FTLD-U) FTLN (n = 2)	75.5 (62 to 89)	6 (3 to 9)	2 F/0 M	FTD (1, 50) DAT (1, 50)	FTLD (2, 100)	Infarcts (1, 50)
	65 (NA)	8 (NA)	5 F/6 M	FTD (11, 84) DAT (1, 8) CJD (1, 8)	PICK (13, 100)	AD (3, 23)
Corticobasal degeneration (n = 19)	70.0 (57 to 78)	7.6 (5 to 12)	8 F/6 M	FTD (10, 53) CBD (9, 47)	CBD (19, 100)	AD, HS (1, 5)
Progressive supranuclear palsy (n = 4)	73 (NA)	3 (NA)	NA	FTD + PD (4, 100)	PSP (4, 100)	AD (1, 25)
FTD with <i>MAPT</i> mutation (n = 5)	60 (57 to 63)	14 (10 to 18)	0 F/2 M	FTD (4, 80) Dementia (1, 20)	FTD- <i>MAPT</i> (5, 100)	
Neuronal intermediate filament inclusion disease (n = 6)	37 (25 to 48)	3.7 (3 to 4)	2 F/2 M	FTD (5, 83) PLS (1, 17)	NIFID (6, 100)	
Basophilic inclusion body disease (n = 2)	29 (NA)	10 (NA)	0 F/2 M	FTD (1, 50) CBD (1, 50)	BIBD (2, 100)	

(table continues)

**Table 1.** Continued

Familial and sporadic FTDs	Demographic data			Diagnosis		
	Onset (years) [mean (range)]	Duration (years) [mean (range)]	Gender	Clinical diagnosis (n, %)	Pathological diagnosis (n, %)	Pathological diagnosis-other (n, %)
Hereditary diffuse leukoencephalopathy with spheroids (n = 2)	37.5 (36 to 39)	8.5 (7 to 10)	2 F/0 M	FTD (2, 100)	HDLS (2, 100)	
Other diseases						
Alzheimer's disease (n = 19)	73 (59 to 99)	8 (4 to 13)	7 F/9 M	DAT (18, 95) FTD (1, 5)	AD (19, 100) HS (1, 5)	Lewy bodies (3, 16) Infarct (1, 5)
Argyrophilic grain disease (n = 2)	70.5 (54 to 87)	8.5 (7 to 10)	2 F/0 M	FTD (1, 50) Dementia (1, 50)	AGD (2, 100)	
Tangle-only dementia (n = 2)	74 (NA)	4 (NA)	1 F/1 M	DAT/dementia (2, 100)	TOD (2, 100)	
Parkinson's disease (n = 3)	51 (35 to 67)	16 (10 to 20)	1 F/2 M	PD (3, 100)	PD (3, 100) HS (1, 33)	DLB (2, 67)
Dementia with Lewy bodies (n = 8)	65.5 (62 to 69)	13 (5 to 13)	4 F/4 M	DLB (3, 38) DAT + PD (2, 25) DAT/dementia (3, 38)	DLB (8, 100)	AD (7, 87) PD (1, 13)
Multiple system atrophy (n = 3)	NA	NA	NA	MSA (3, 100)	MSA (3, 100)	
Trinucleotide repeat disease (n = 3)	38 (NA)	6 (NA)	1 F/2 M	HD (1, 33) HD + FTD (1, 33) FTD (1, 33)	HD (1, 33) SCA (2, 67)	Tauopathy
Hippocampal sclerosis (n = 2)	43 (NA)	6	2 F/0 M	DAT (1, 50) PD + DAT (1, 50)	HS (2, 100)	AD (1, 50)
Normal adult brain (n = 19)	83.8** (73 to 98)	NA	8 F/11 M	NL (15, 79) Schizophrenia (4, 21)	NL (19, 100)	Gliosis (1, 5)

AGD, argyrophilic grain disease; BIBD, basophilic inclusion body disease; CVD, cerebrovascular disease; PGRN, progranulin; VCP, valosin-containing protein; CHMP2B, charged multivesicular body protein 2B; CJD, Creutzfeldt-Jakob disease; DAT, dementia of the Alzheimer's type; DLB, dementia with Lewy bodies; CBD, corticobasal degeneration; FTD, frontotemporal dementia; FTD-MAPT, FTD with tau mutation; FTLN, frontotemporal lobar degeneration; FTLN-MND, FTLN with ubiquitin-positive, tau-negative inclusions and MND; FTLN-U, FTLN with ubiquitin-positive, tau-negative inclusions but without MND; HD, Huntington's disease; HDLS, hereditary diffuse leukoencephalopathy with neuroaxonal spheroids; HS, hippocampal sclerosis; MS, multiple sclerosis; MND, motor neuron disease; MSA, multiple system atrophy; NIFID, neuronal intermediate filament inclusion disease; PD, Parkinson's disease; PSP, progressive supranuclear palsy; SCA, spinocerebellar ataxia; PICK, Pick disease; PPA, primary progressive aphasia; TOD, tangle-only dementia; NA, not available; NL, normal adult brain with no neurologic or psychiatric disease; F, female; M, male.

\*\* Age at death.

with dementia, or simply "dementia."<sup>1,2,27,28</sup> Those with the primary clinical diagnosis of either ALS or MND,<sup>29</sup> in the absence of clinical dementia, were excluded from this study. The FTLN-U group included 1) familial cases with *PGRN*,<sup>17-19,30</sup> *VCP*,<sup>11,13</sup> and *CHMP2B*<sup>22</sup> mutations and cases linked to chromosome 9p,<sup>24,25</sup> including one case with *IFT74* gene mutation<sup>26</sup>; 2) other familial cases of FTLN-U in which the genetic defect was not known; 3) cases with sporadic FTLN-U; and 4) familial and sporadic cases of MND with dementia.<sup>1,2,27</sup> FTLNs with tauopathy included Pick disease,<sup>27</sup> corticobasal degeneration,<sup>27,28</sup> progressive supranuclear palsy,<sup>27</sup> FTLN with *MAPT* mutations, also called frontotemporal dementia with parkinsonism linked to chromosome 17,<sup>27</sup> tangle-only dementia,<sup>27</sup> and argyrophilic grain disease.<sup>31</sup> Other cases fulfilling clinical and/or neuropathological diagnostic cri-

teria for FTLN included neuronal intermediate filament inclusion disease,<sup>32</sup> hereditary diffuse leukoencephalopathy with spheroids,<sup>33</sup> and basophilic inclusion body disease.<sup>34</sup> Synucleinopathies included dementia with Lewy bodies,<sup>35</sup> Parkinson's disease,<sup>35-37</sup> and multiple system atrophy.<sup>38,39</sup> Other neurological controls included Alzheimer's disease (AD),<sup>40</sup> polyglutamine expansion diseases (Huntington's disease and spinocerebellar ataxia),<sup>41,42</sup> dementia lacking distinctive histopathology,<sup>43</sup> and hippocampal sclerosis (HS).<sup>44</sup> In addition, normal aged controls were studied. Clinical, genetic, and neuropathological data and tissue samples were obtained from the following collaborating centers: Alzheimer's Disease Research Center, Washington University School of Medicine (St. Louis, MO); Northwestern University Cognitive Neurology and Alzheimer Disease Center (Chicago, IL); Cen-



ter for Neurodegenerative Disease Research, Department of Pathology and Laboratory Medicine, University of Pennsylvania School of Medicine (Philadelphia, PA); Center for Neuropathology and Prion Research, Ludwig-Maximilians University (Munich, Germany); Department of Pathology and Laboratory Medicine, Vancouver General Hospital (Vancouver, BC, Canada); Department of Pathology, Aalborg Hospital (Aalborg, Denmark); Department of Neuropathology, Academic Medical Centre (Amsterdam, The Netherlands); University of Texas Southwestern School of Medicine (Dallas, TX); and Rush Alzheimer's Disease Center, Rush University Medical School (Chicago, IL). After death, the consent of the next-of-kin was obtained for brain removal, following approved Local Ethics Committee procedures in accordance with state law. Brain tissue was preserved in either buffered 10% formal saline for up to 3 weeks or 4% paraformaldehyde for 30 to 72 hours followed by cryopreservation in sucrose or glycerol. When available, tissue was frozen at  $-70^{\circ}\text{C}$  for biochemistry.

### *Histology and Immunohistochemistry*

Paraffin sections from all cases of FTL-D-U included the frontal lobe (middle frontal gyrus and Brodmann areas 8, 9, and 46), temporal lobe (middle/superior temporal gyrus and Brodmann areas 21 and 22), parahippocampal gyrus, and hippocampus and, when available, striatum, precentral gyrus, medulla oblongata, and spinal cord. TDP-43 immunohistochemistry (IHC) was also performed on sections with disease-representative pathology from cases with other neurodegenerative conditions and normal aged control cases.

Antigen retrieval was performed by microwaving tissue sections in a solution of 0.1 mol/L citrate buffer, pH 6.0, at  $100^{\circ}\text{C}$  for 10 minutes. IHC was undertaken on 4- to 10- $\mu\text{m}$ -thick sections prepared from formalin-fixed, paraffin-embedded tissue blocks using the avidin-biotin complex detection system (Vector Laboratories, Burlingame, CA) and the chromogen 3,3'-diaminobenzidine, and sections were counterstained with hematoxylin as previously described,<sup>8,14</sup> or sections were immunostained with an automated staining procedure.<sup>45</sup> Antibodies used included those that recognized epitopes of ubiquitin (rabbit polyclonal, 1:1000; Dako, Glostrup, Denmark) (mouse monoclonal, 1510; Chemicon, Temecula, CA) and TDP-43 (rabbit polyclonal, 1:1000; ProteinTech Inc., Chicago, IL). In addition, in selected cases of FTL-D-U, novel monoclonal antibodies 137 and 182 were used, as previously described.<sup>8,10,14</sup>

The pattern of FTL-D-U pathology was subclassified, based on the system proposed by Sampathu et al.<sup>14</sup> Type 1 cases were characterized by an abundance of long DNs predominantly in superficial cortical laminae, with few or no NCIs or NIIs. Type 2 was characterized by numerous NCIs in both superficial and deep cortical laminae as well as infrequent DNs and sparse or no NIIs. Type 3 was characterized by pathology predominantly in the superficial cortical layers with numerous NCIs, DNs, and variable numbers of NIIs. An additional

class was added (type 4) that was distinguished by numerous NIIs and infrequent NCIs and DNs in neocortical areas with relative sparing of the hippocampus, consistent with the pathology previously described in cases with VCP mutations.<sup>10,13</sup> Each center performed its own severity rating of pathology and classification of cases using ubiquitin and TDP-43 IHC using established FTL-D-U subtype criteria. The severity of ubiquitin- or TDP-43-positive inclusions was rated semiquantitatively where 0 = no inclusions; 1 = rare to mild; 2 = moderate; and 3 = severe. To determine intercenter reliability of FTL-D-U subclassification, three or more cases (where available) classified in each subtype group, from Washington University School of Medicine and from University of British Columbia, were blindly reviewed by Dr. Neumann (Ludwig-Maximilians University, Munich, Germany). Agreement between the raters was good with  $\kappa = 0.75$  (95% CI, 0.52 to 0.98).

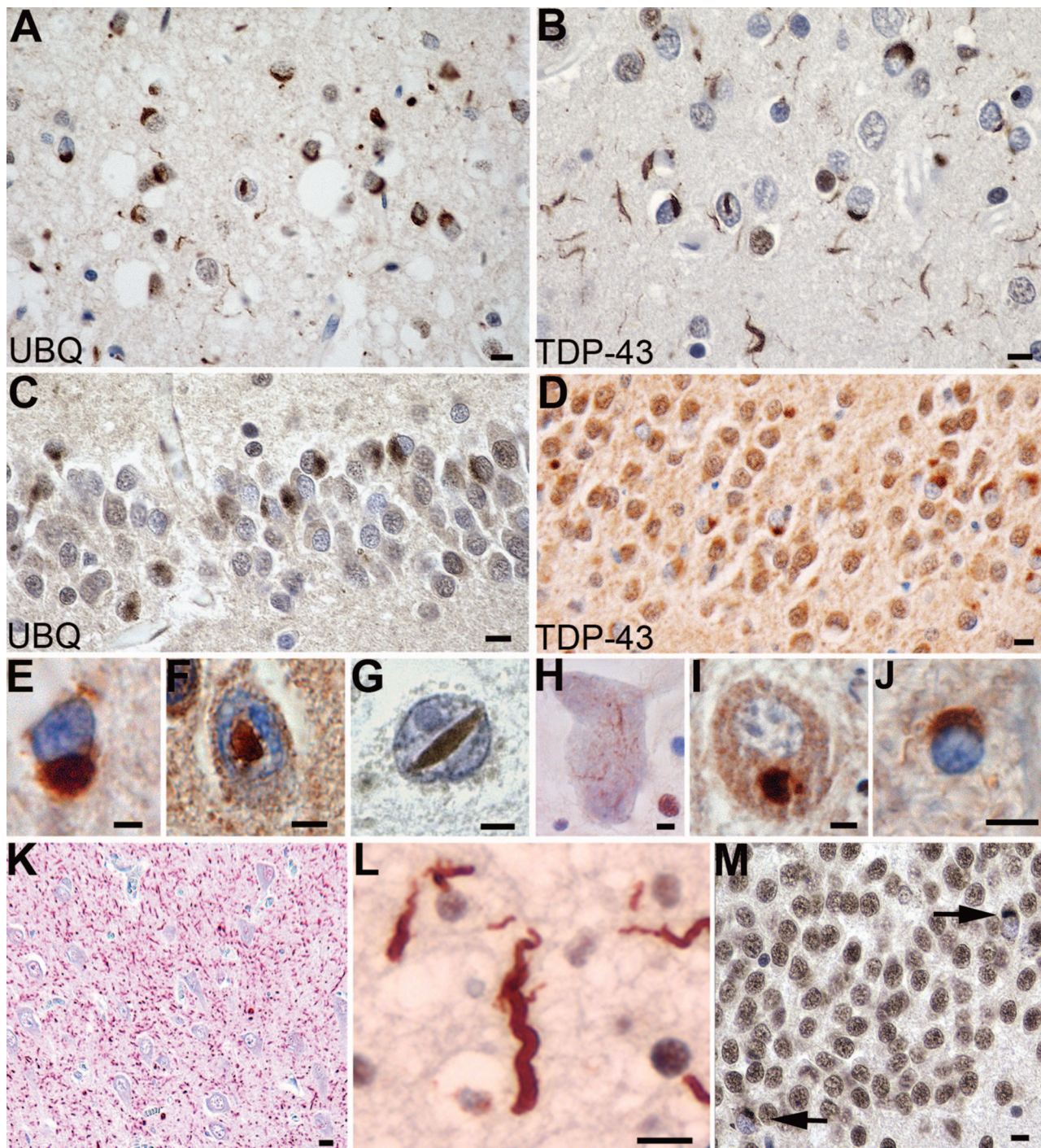
### *Electron Microscopy*

Samples of hippocampus and temporal cortex collected at autopsy were fixed overnight in 0.1% glutaraldehyde and 4% paraformaldehyde in 0.1 mol/L phosphate-buffered saline, sliced on a vibratome, and stored in cryoprotection solution at  $-20^{\circ}\text{C}$ . Tissue for routine electron microscopy was washed and fixed for an additional 20 hours at  $4^{\circ}\text{C}$  in 2.5% glutaraldehyde in 0.1 mol/L cacodylate buffer, pH 7.4, postfixed for 1 hour on ice in 1% osmium tetroxide and 1.5% potassium ferrocyanide in cacodylate buffer, dehydrated in ethanol, and embedded in Epon. Vibratome sections of hippocampus were fixed for an additional 15 minutes in 2.5% glutaraldehyde cacodylate buffer before immunostaining. Electron microscopy immunostaining was performed according to the method of Llewellyn-Smith and Minson.<sup>46</sup> The primary antibodies used were a rabbit polyclonal antibody to TDP-43 and normal rabbit IgG. The 3,3'-diaminobenzidine reaction was completed by incubating for 15 minutes in 0.05% 3,3'-diaminobenzidine with 10 mmol/L imidazole in 0.1 mol/L Tris buffer, pH 7.4, without  $\text{H}_2\text{O}_2$  and for 5 to 10 minutes with 0.1%  $\text{H}_2\text{O}_2$  added. Sections were postfixed for 2 hours in 1% osmium tetroxide and 1.5% potassium ferrocyanide in 0.1 mol/L cacodylate buffer, dehydrated in ethanol, and embedded in Araldite. Selected semithin sections were re-embedded for thin sectioning.

### *Biochemistry*

Postmortem brain tissue was dissected, weighed, and sequentially extracted with buffers of increasing strength as previously described.<sup>8,10,11</sup> In brief, gray and white matter was extracted at 5 ml/g (v/w) with low-salt buffer (10 mmol/L Tris, pH 7.5, 5 mmol/L ethylenediamine tetraacetic acid, 1 mmol/L dithiothreitol, 10% sucrose, and a cocktail of protease inhibitors), high-salt Triton X buffer (low-salt buffer, 1% Triton X-100, and 0.5 mol/L NaCl), myelin floatation buffer (Triton X buffer containing 30% sucrose), and sarkosyl buffer (low-salt buffer, 1% *N*-lauroyl-sarcosine, and 0.5



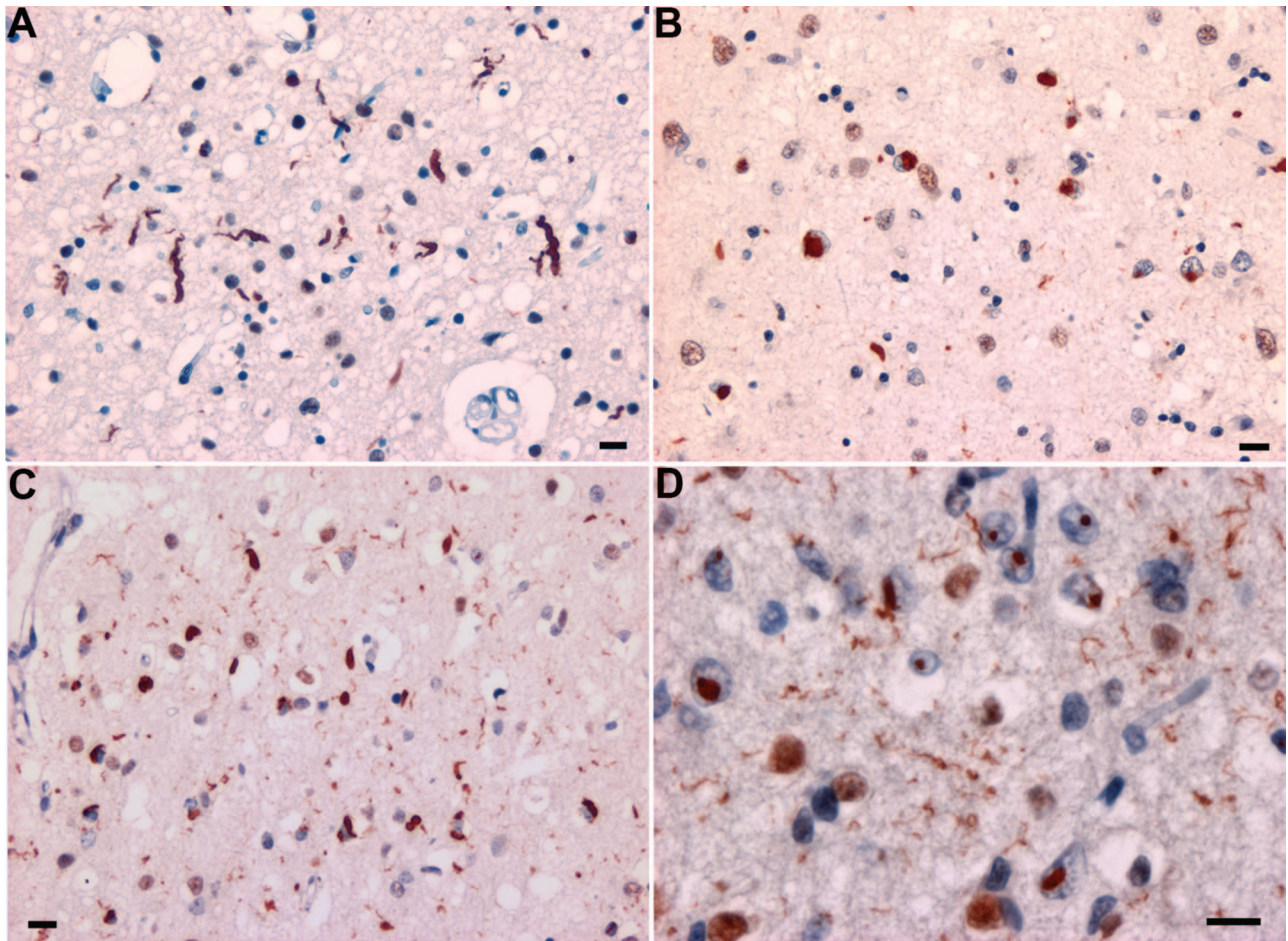


**Figure 1.** Spectrum of TDP-43 pathology in FTLD-U. Adjacent sections of superficial frontal neocortex showing NCIs, DNs, and isolated NIIs, stained for both ubiquitin (A) and TDP-43 (B). NCIs in the dentate granule cells stained for ubiquitin (C) and TDP-43 (D). Neuronal and glial inclusions include NCIs (E), round and lentiform NIIs (F and G), skein-like (H) and compact round (I) NCIs in lower motor neurons; and a glial cytoplasmic inclusion (J). Low-power micrograph showing numerous DNs in the hippocampus CA1 subfield (K). High-power micrograph showing a tortuous DN in a case of FTLD-U, subtype 1 (L). NCIs in the dentate fascia of a case of hippocampal sclerosis (M). A and C: Ubiquitin immunohistochemistry. B, D, E-M: TDP-43 immunohistochemistry. Bars = 10  $\mu$ m (A-D and K-M); 5  $\mu$ m (E-J).

mol/L NaCl). The detergent-insoluble materials were extracted in 0.25 ml/g urea buffer (7 mol/L urea, 2 mol/L thiourea, 4% 3-[(3-cholamidopropyl) dimethylammonio]-1-propanesulfonate, and 30 mmol/L Tris, pH 8.5). For Western blot analysis, protein extracts were resolved in Tris-glycine 5 to 20% gradient sodium dodecyl sulfate-polyacrylamide gel electrophoresis,

transferred to polyvinylidene difluoride membranes (Millipore, Billerica, MA), and probed with antibodies to TDP-43. Primary antibodies were detected with alkaline phosphatase-conjugated anti-mouse or anti-rabbit IgG (Dako) and visualized by incubation with nitro blue tetrazolium/5-bromo-4-chloro-3-indolyl phosphate (Roche Molecular Biochemicals, Mannheim,





**Figure 2.** FTLD-U subtypes 1 to 4. **A:** Type 1 is characterized by long and tortuous DNIs in laminae II/III with relatively few NCIs and no NIIs. **B:** Type 2 has numerous NCIs, relatively few DNIs, and no NIIs. **C:** Type 3 has numerous NCIs and DNIs and an occasional NII in lamina II. **D:** Type-4 pathology is characterized by numerous NIIs and DNIs but few NCIs. TDP-43 immunohistochemistry. Bar = 10  $\mu$ m.

Germany). Where indicated, TDP-43 was dephosphorylated by dialysis (50 mmol/L Tris and 0.2 mmol/L ethylenediamine tetraacetic acid, pH 8.0) and treated with *Escherichia coli* alkaline phosphatase (Sigma-Aldrich, St. Louis, MO) for 2 hours at 56°C.

## Results

### *TDP-43 Immunohistochemistry Indicates Neuropathological Heterogeneity in FTLD-U*

Immunohistochemistry for ubiquitin and TDP-43 revealed similar findings, which included a spectrum of neuronal (NCIs, DNIs, and NIIs) and glial inclusions (Figure 1). Most NCIs, DNIs, and NIIs that were ubiquitin immunoreactive were also TDP-43-positive. In contrast, glial cytoplasmic inclusions, which were readily seen by TDP-43 IHC, were only variably labeled with ubiquitin, possibly indicating an early stage in pathogenesis. When present, DNIs were most numerous in neocortical areas of the frontal and temporal lobes; rarely, numerous DNIs were seen in the CA1 subfield of the hippocampus (Figure 1K). In agreement

with our previous studies, four distinct patterns of FTLD-U pathology were identified, and a number of correlations with clinical and genetic subgroups were apparent (Table 1; Figure 2; Supplemental Table 1 at <http://ajp.amjpathol.org>).

We found that the majority (97.4%) of cases of FTLD-U with or without MND (Table 2) could be classified according to the previously published schemes.<sup>10,13,14</sup> Most cases in this large, multicenter series showed type-3 pathology (49.2%), whereas type-2 and -1 pathologies represented 28.5 and 17.1%, respectively (Table 2). The pathological subtypes distinguished familial cases with different known gene defects. Cases with *PGRN* mutations were exclusively type 3 (100%), those with *VCP* mutations all had type-4 pathology, and cases linked to chromosome 9 were all type 2 (see below). Familial FTLD-U and FTLD-MND in which the genetic association was unknown were mostly type 3 (55.2%), whereas type-2 cases represented 34.5%, and 10.3% were type 1 (Table 2). Sporadic FTD with FTLD-U and/or FTLD-MND were almost evenly split between type-1 (36.1%), type-2 (30.6%), and type-3 (33.1%) pathology. Most cases of sporadic MND with dementia had either type-2 (39.1%)

**Table 2.** TDP-43 and FTLD-U Subtypes

Familial and sporadic FTDs	Neuropathologic diagnosis	FTLD-U subtype	
		Ubiquitin type (n, %)	TDP-43 type (n, %)
Familial FTD with <i>PGRN</i> mutation (n = 36)	FTLD-U	3 (36, 100)	3 (36, 100)
Familial FTD with <i>VCP</i> mutation (n = 5)	FTLD-U	4 (5, 100)	4 (5, 100)
Familial FTD with <i>CHMP2B</i> mutation (n = 4)	FTLD-U	a (4, 100)	0 (4, 100)
Familial FTD with/without MND linked to chromosome 9 (n = 7)	FTLD-U	2 (2, 29)	2 (2, 29)
	FTLD-MND	2 (5, 71)	2 (5, 71)
Other familial FTD cases (n = 29)	FTLD-U	1 (3, 10)	1 (3, 10)
	FTLD-U	2 (8, 28)	2 (8, 28)
	FTLD-U	3 (16, 55)	3 (16, 55)
	FTLD-MND	2 (2, 7)	2 (2, 7)
Sporadic FTD cases with ubiquitin- or TDP-43-positive inclusions (n = 72)	FTLD-U	1 (26, 36)	1 (26, 36)
	FTLD-U	2 (15, 21)	2 (15, 21)
	FTLD-U	2 (1, 1)	0 (1, 1)
	FTLD-U	3 (18, 25)	3 (18, 25)
	FTLD-U	3 (1, 1)	0 (1, 1)
	FTLD-MND	2 (6, 8)	2 (6, 8)
	FTLD-MND	3 (5, 7)	3 (5, 7)
Familial FTD and MND (n = 17)	FTLD-MND	1 (2, 12)	1 (2, 12)
	FTLD-MND	2 (7, 41)	2 (7, 41)
	FTLD-MND	3 (7, 41)	3 (7, 41)
	FTLD-MND	b (1, 6)	b (1, 6)
Sporadic FTD and MND (n = 23)	FTLD-U	3 (1, 4)	3 (1, 4)
	FTLD-MND	1 (2, 9)	1 (2, 9)
	FTLD-MND	2 (9, 39)	2 (9, 39)
	FTLD-MND	3 (11, 48)	3 (11, 48)
Total of all familial and sporadic FTD cases with/without MND (n = 193)		1 to 4 (188, 97.4)	1 to 4 (186, 96.4)
Other familial and sporadic FTD cases (non-FTLD-U)			
FTLD (n = 2)	FTLD	0 (2, 100)	0 (2, 100)
Pick disease (n = 13)	PICK	0 (12, 100)	0 (12, 100)
Corticobasal degeneration (n = 20)	CBD	0 (20, 100)	0 (20, 100)
Progressive supranuclear palsy (n = 4)	PSP	0 (4, 100)	0 (4, 100)
FTD with <i>MAPT</i> mutation (n = 5)	FTD- <i>MAPT</i>	0 (5, 100)	0 (5, 100)
Neuronal intermediate filament inclusion disease (n = 6)	NIFID	c (6, 100)	0 (6, 100)
Basophilic inclusion body disease (n = 2)	BIBD	d (2, 100)	0 (2, 100)
Hereditary diffuse leukoencephalopathy with spheroids (n = 2)	HDLS	0 (2, 100)	0 (2, 100)
Other diseases			
Alzheimer's disease (n = 19)	AD	0 (19, 100)	0 (19, 100)
Argyrophilic grain disease (n = 2)	AGD	0 (2, 100)	0 (2, 100)
Tangle-only dementia (n = 2)	TOD	0 (2, 100)	0 (2, 100)
Parkinson's disease (n = 3)	PD	0 (3, 100)	0 (3, 100)
Dementia with Lewy bodies (n = 8)	DLB	0 (8, 100)	0 (8, 100)
Multiple system atrophy (n = 3)	MSA	0 (3, 100)	0 (3, 100)
Trinucleotide repeat disease (n = 3)	HD	0 (1, 33)	0 (1, 33)
	SCA	0 (2, 67)	0 (2, 67)
Hippocampal sclerosis (n = 2)	HS	e (2, 100)	e (2, 100)
Normal adult brain (n = 18)	NL	0 (18, 100)	0 (18, 100)

Cases that are inconsistent with FTLD-U subtypes 1 to 4: a, FTLD with *CHMP2B* mutation cases with ubiquitin-positive and TDP-43-negative inclusions; b, FTLD-MND case with ubiquitin- and TDP-positive inclusions but insufficient cortical pathology for FTLD-U subtyping; c, NIFID cases with NII or NCI that are ubiquitin-positive and TDP-43-negative; d, BIBD cases with NCI that are ubiquitin-positive and TDP-43-negative; and e, HS cases with NCI in dentate gyrus that are ubiquitin- and TDP-43-positive.

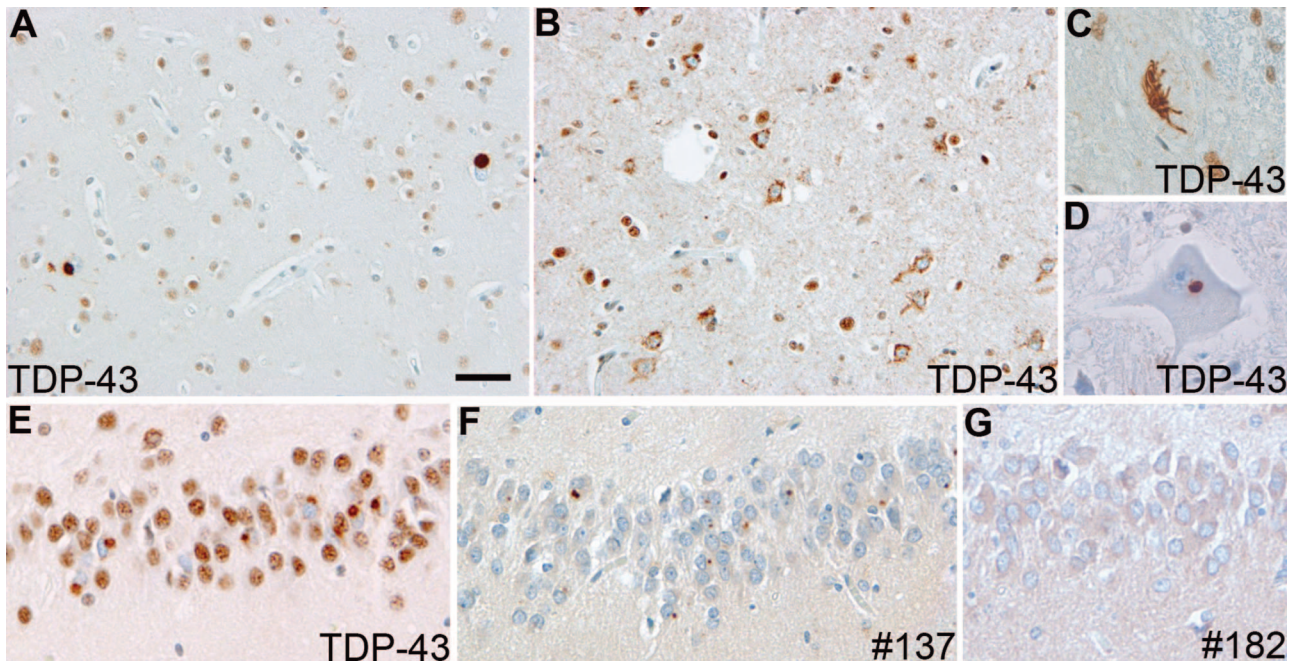
AD, Alzheimer's disease; AGD, argyrophilic grain disease; BIBD, basophilic inclusion body disease; DLB, dementia with Lewy bodies; CBD, corticobasal degeneration; FTD-*MAPT*, FTD with  $\tau$  mutation; HD, Huntington disease; HDLS, hereditary diffuse leukoencephalopathy with neuroaxonal spheroids; HS, hippocampal sclerosis; MSA, multiple system atrophy; NIFID, neuronal intermediate filament inclusion disease; PD, Parkinson's disease; PSP, progressive supranuclear palsy; SCA, spinocerebellar ataxia; PICK, Pick disease; TOD, tangle-only dementia.

or type-3 (52.2%) pathology, and a comparable distribution was found in cases of familial MND-dementia (41.2% were type 2, and 41.2% were type 3). One case of familial MND-dementia had too little cortical pathology to allow classification in this system.

### Chromosome 9p-Linked FTD with or without MND Is a TDP-43 Proteinopathy

Cases of familial FTD with genetic linkage to chromosome 9p showed moderate numbers of TDP-43-positive





**Figure 3.** Neuropathology of FTL-D-U linked to chromosome 9p. TDP-43 immunohistochemistry showing compact TDP-43-positive cytoplasmic inclusions in neurons in the lower cortical layers (A); numerous neurons with diffuse granular cytoplasmic TDP-43 immunoreactivity in superficial frontal cortex (B); skein-like (C) and round (D) inclusion in motor neurons; and cytoplasmic inclusions in dentate granule cells (E). Note the absence of physiological, nuclear TDP-43 immunoreactivity in neurons with NCI. Inclusions in the dentate gyrus stained with monoclonal antibody 137 (F) but were negative with MAB 182 (G). Bars = 50  $\mu$ m (A and B); 25  $\mu$ m (C–G).

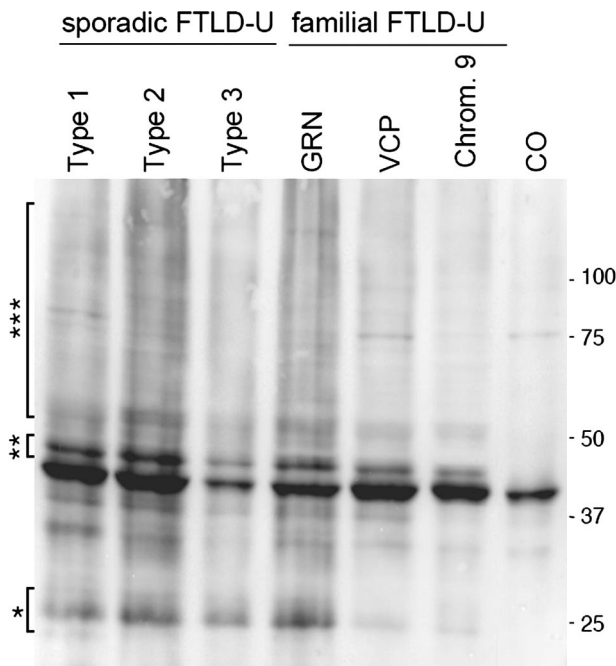
NCIs, relatively few DN and, in one case, rare NII (Figure 3). In addition to well-formed NCIs in the frontal and temporal neocortex and hippocampus, TDP-43 IHC revealed additional granular “pre-inclusions” (Figure 3B) that were not stained with ubiquitin. Both the NCIs and DN were most numerous in the upper cortical laminae but were also present in neurons throughout the entire cortical thickness. Consistent with findings in other subtypes of TDP-43 proteinopathy, neurons with inclusions showed loss of the normal physiological TDP-43 staining of nuclei (Figure 3E). The majority of chromosome 9p-linked cases also had evidence of ubiquitin- and TDP-positive inclusions in upper and lower motor neurons, identical to those encountered in sporadic ALS/MND (Figure 3, C and D) but were not seen in the one case with FTD only. Furthermore, the inclusions were immunostained with monoclonal antibody to FTL-D-U type 2 (#137) but not to type 1 (#182) (Figure 3, F and G). These data indicate that the pathology of FTD linked to chromosome 9p is a specific subtype of FTL-D-U (type 2) and that TDP-43 is the disease-associated protein.

To characterize TDP-43 biochemically, Western blot analysis was performed on samples of cortical gray matter from patients with sporadic FTL-D-U and FTL-D-U linked to chromosome 9p that were sequentially extracted with buffers of increasing strength. Full-length TDP-43 protein of ~43 kd was detected in all soluble and insoluble fractions of affected and unaffected brain regions from FTL-D-U linked to chromosome 9p cases, similar to sporadic FTL-D-U and control brains (data not shown). Additional protein bands of ~25 and 45 kd, as

well as a high molecular smear, were detected in detergent-insoluble, urea fractions from affected regions of cases of FTL-D-U linked to chromosome 9p and FTL-D-U but not controls (Figure 4). The quantity of these modified TDP-43 species was variable but correlated with the amount of pathology detected by immunohistochemistry. Furthermore, the 45-kd species was collapsed into the 43-kd band on dephosphorylation with alkaline phosphatase, indicating that TDP-43 is abnormally phosphorylated (data not shown). Thus, these data suggest that, despite the distinct genetic alterations and pattern of ubiquitin pathology in FTL-D-U linked to chromosome 9p, the molecular signature of the TDP-43 disease protein is similar to that seen in both sporadic and familial FTL-D-U, including those individuals with *PGRN* and *VCP* gene mutations.

### *Chromosome 3-Linked FTD Is Not a TDP-43 Proteinopathy*

Ubiquitin IHC revealed NCIs in the dentate fascia and sparse inclusions in the frontal neocortex of cases with familial FTD with *CHMP2B* mutation. However, the absence of DN and the presence of granular, ubiquitin-positive structures within the neocortex distinguished this FTL-D-U subtype from types 1 to 4 described above. In addition, TDP-43 IHC failed to label any of the ubiquitinated inclusions. Thus, familial FTD with *CHMP2B* mutation is a unique pathological subtype of FTL-D-U and is not a TDP-43 proteinopathy on the basis of IHC.



**Figure 4.** Biochemical analysis of TDP-43. Immunoblot analysis probed with anti-TDP-43 antibody demonstrated a distinct pathological profile of TDP-43 with variable presence of pathological ~25-kd bands (\*), 45-kd bands (\*\*), and high molecular smear (\*\*\*) in all sporadic and familial FTLD-U brains (FTLD-U subtypes 1 to 3), including those with progranulin (GRN) and VCP mutations and those linked to chromosome 9p (Chrom. 9). Pathological TDP-43 bands were not detected in control brains (CO).

### *TDP-43 Is a Component of the Ubiquitinated Inclusions of Hippocampal Sclerosis*

HS was found to be a coexisting pathology in seven cases of FTLD-U with or without MND. In an additional two cases classified as pathologically pure HS, small numbers of ubiquitin-positive and TDP-43-positive inclusions were found exclusively in the dentate granule cells (Figure 1M). In one case of corticobasal degeneration with HS, the NCIs were ubiquitin-positive but TDP-43-negative. The dense network of fine TDP-43-positive neurites seen occasionally in CA1 in FTLD-U (Figure 1K) was not observed in any case of HS with or without FTLD-U. These data indicate that some cases of HS are TDP-43 proteinopathies, but further studies on a larger sample of "pure HS" and biochemical studies are required to determine the nosological status of HS.

### *TDP-43 Is Not a Component of Inclusions of Other Neurological Diseases*

To determine the specificity of TDP-43 proteinopathy among the spectrum of FTLDs and other neurological diseases, we performed ubiquitin and TDP-43 IHC on sections with disease-representative pathology. In six cases of neuronal intermediate filament inclusion disease (Table 1), the ubiquitin-immunoreactive NCI and NII were not labeled with antibodies to TDP-43. In none of the FTLDs with tauopathy was TDP-43 a component of the inclusions as demonstrated by IHC. TDP-43 was also

absent from other FTLDs, including basophilic inclusion body disease ( $n = 2$ ) and hereditary diffuse leukoencephalopathy with spheroids ( $n = 2$ ), other neurological diseases including AD ( $n = 19$ ), argyrophilic grain disease ( $n = 2$ ), tangle-only dementia ( $n = 2$ ), synucleinopathies [dementia with Lewy bodies ( $n = 8$ ), Parkinson's disease ( $n = 3$ ), and multiple system atrophy ( $n = 3$ )], trinucleotide repeat diseases ( $n = 3$ ), and normal aged brain ( $n = 18$ ) (Table 1; Supplemental Table 1 at <http://ajp.amjpathol.org>).

### *Fine Structure of Inclusions of FTLD-U*

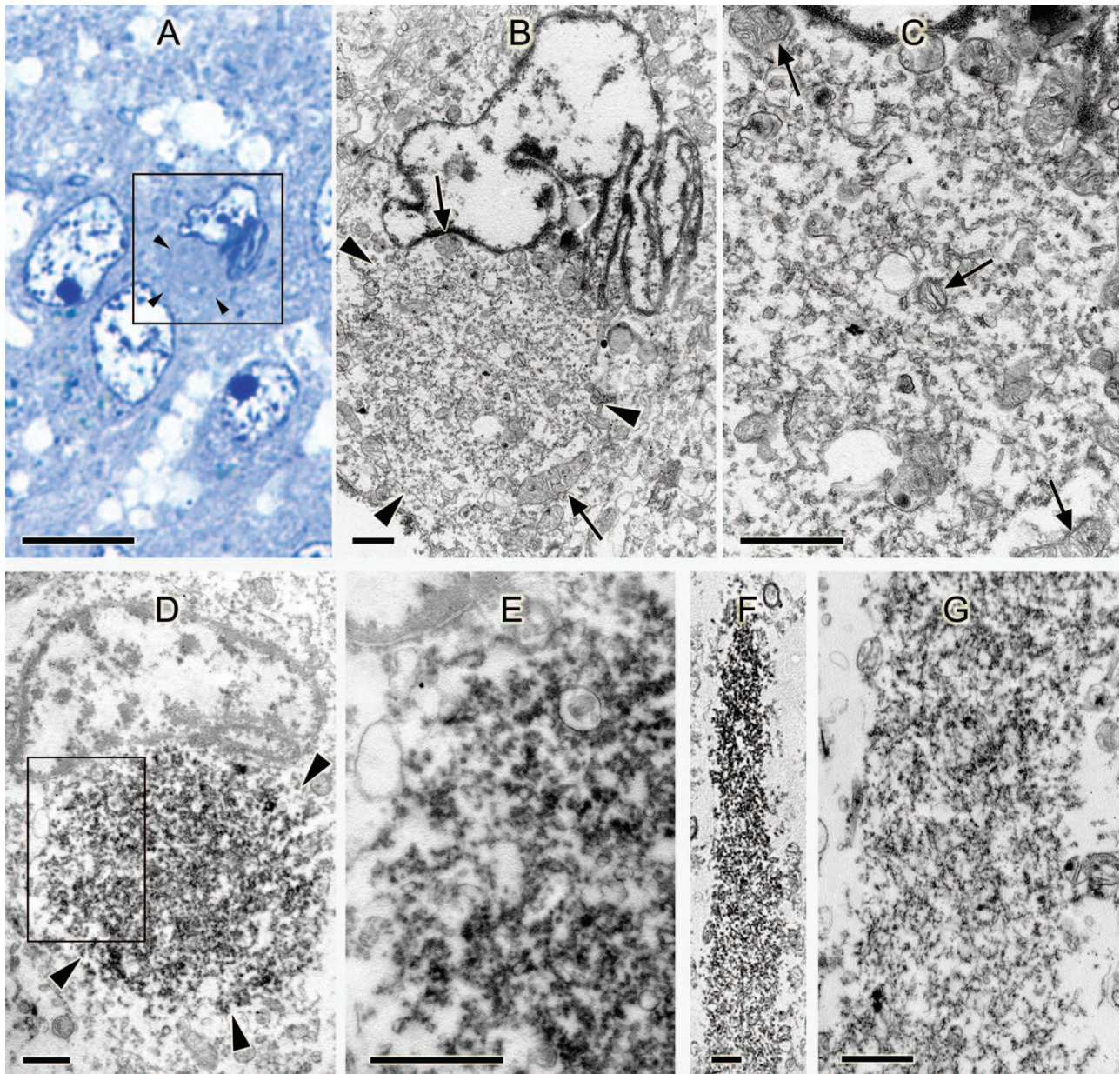
Light microscopic examination of semithin sections from the hippocampus of a case of FTLD-U revealed round cytoplasmic bodies identical to the NCIs immunolabeled with ubiquitin and TDP-43 antibodies in paraffin sections. Cells containing the NCIs often had a variably indented nucleus, sometimes clefted or corrugated (Figure 5A, inset). Electron microscopy of the same cells showed an accumulation of granular and membranous material with a few mitochondria. Mitochondria and lipofuscin surrounded the NCI (Figure 5, B and C). An occasional filament or microtubule was seen in the inclusions, but these were a minor component. Immunostained sections showed similar NCIs that were TDP-43-positive (Figure 5, D and E). Sections of the temporal lobe showed TDP-43-positive DNs that contained somewhat more filamentous material, but no dense or compact accumulations of filaments were seen (Figure 5, F and G).

### *Discussion*

TDP-43 is a recently identified pathological protein of the signature lesions of a spectrum of diseases, including FTLD-U, FTLD-MND, and sporadic MND.<sup>7,8,10,11</sup> The purpose of this study was to determine the frequency of TDP-43 proteinopathy in a broad spectrum of clinically and neuropathologically characterized cases of FTLDs obtained from several dementia research centers in Europe and North America. This study demonstrates that TDP proteinopathy is the most frequently found pathology in both familial and sporadic cases of FTLD-U with or without MND.

In our previous studies, we identified three subtypes of FTLD-U, which were distinguished by the morphology, immunohistochemical profile, and cellular localization of the inclusions (NCI, NII, or DN).<sup>8,14</sup> Recently, we have used ubiquitin and TDP-43 immunohistochemistry to characterize the inclusions of FTLD-U with VCP mutations.<sup>10,13</sup> Unlike FTLD-U subtypes 1 to 3, which have predominantly NCIs and/or DNs, FTLD-U subtype 4 is distinguished by predominantly NIIs.<sup>10,13</sup> Taken together, these studies show that four pathological subtypes of FTLD-U may be identified based on the morphology and distribution of ubiquitin-positive pathology.<sup>8,10,14</sup> In this study, the order of frequency was: type 3 > type 2 > type 1 > type 4. This differs from our previous study, in which we found that type 1 was the most frequent FTLD-U subtype.<sup>14</sup> However, in the present multicenter study, the





**Figure 5.** Fine structure of inclusions of FTLD-U. **A:** Light microscopy semithin section from the dentate gyrus of the hippocampus. Toluidine blue stain. **Inset:** Neuron with a round body (**arrowheads**) is adjacent to the nucleus. The nucleus is corrugated and indented compared with the other three normal neuronal nuclei. **B** and **C:** Re-embedded ultrathin section of the boxed cell in **A** and counterstained with uranyl acetate and bismuth subnitrate. **B:** Low-magnification electron micrograph showing a round body (**arrowheads**) of an NCI surrounded by mitochondria. **C:** Higher magnification of **B** shows an accumulation of granular and membranous material with a few mitochondria (**arrows**, mitochondria). **D:** Ultrathin section of a neuron from the dentate gyrus of the hippocampus, immunostained for TDP-43. An NCI (outlined by **arrowheads**) indenting the nucleus is labeled (no counterstain). **E:** Higher magnification of boxed area of **D**. **F:** Dystrophic neurite from the temporal lobe immunostained for TDP-43 (no counterstain). **G:** Same neurite as in **F** in an adjacent section counterstained with uranyl acetate and bismuth subnitrate. More filamentous material is seen than in the cell body of **C**, but no dense accumulations of filaments are present. Bars = 10  $\mu\text{m}$  (**A**); 1  $\mu\text{m}$  (**B-G**).

relatively low proportion of type 1 cases and high proportion of type 3 cases likely represents an acquisition bias and reflects the research interests in familial dementias at centers in this study. For instance, the present series included 37 cases (36 familial cases and one sporadic case) with *PGRN* mutations, which is characterized by type-3 pathology. Additional studies from other centers and population-based studies are required to determine the true prevalence of these subtypes.

A family history of dementia is present in up to 40% of FTD patients, implicating a strong genetic influence. To date, mutations in three genes have been linked to familial FTD with FTLD-U pathology: *VCP*, *CHMP2B*, and *PGRN*. We have previously shown that pathological TDP-43 is a component of the inclusions of FTLD-U with *PGRN* and *VCP* mutations.<sup>8,10,11</sup> Here, for the first time, we show that familial FTLD-U and FTLD-MND linked to chromosome 9p are also TDP-43 proteinopathies. In one



case, a putative mutation in the *IFT74* gene has been linked to this clinicopathological entity,<sup>26</sup> but this requires confirmation.

The seven cases with genetic linkage to chromosome 9p had a pattern of pathology consistent with FTL-D-U type 2 but with some additional, unique features. First, many neurons in neocortical and archicortical regions had granular TDP-43 immunoreactivity in the neuronal cytoplasm that was not seen with antibodies to ubiquitin. We speculate that this may represent an early stage in aggregate formation, analogous to pre-tangles commonly encountered in AD. Second, the majority of chromosome 9-linked cases also had evidence of ubiquitin- and TDP-positive inclusions in upper and lower motor neurons, identical to those encountered in sporadic ALS/MND.

A disease-specific biochemical signature of pathologically altered TDP-43 has previously been reported in sporadic and familial FTL-D-U.<sup>8,10,11</sup> In affected regions of cases of FTL-D-U linked to chromosome 9p, additional protein bands of ~25 and 45 kd and a high molecular smear identical to the pattern found in familial FTL-D-U with *PGRN* and *VCP* mutations were detected in sporadic FTL-D-U and MND but not in controls. Thus, these data suggest that despite the distinct genetic alterations and pattern of ubiquitin pathology in FTL-D-U linked to chromosome 9p, the molecular signature of the pathological TDP-43 protein is similar to that found in sporadic and other genetic causes of familial FTL-D-U.

In familial FTL-D-U, the morphology and distribution of ubiquitinated and TDP-43-positive inclusions correlated with genotype. Cases with *PGRN* mutations were exclusively FTL-D-U type 3, cases with *VCP* mutations were FTL-D-U type 4, FTL-D-U cases linked to chromosome 9 were FTL-D-U type 2, and cases with mutations in *CHMPB2* had a unique pattern of ubiquitin pathology that was negative for TDP-43. In contrast, sporadic cases with a similar clinical phenotype were much more heterogeneous according to FTL-D-U subtype, possibly reflecting variability in genotype and environmental factors.

TDP-43 was not a component of the inclusions of other FTLDs (neuronal intermediate filament inclusion disease, hereditary diffuse leukoencephalopathy with spheroids, and basophilic inclusion body disease), AD, the tauopathies (Pick disease, corticobasal degeneration, progressive supranuclear palsy, argyrophilic grain disease, tangle-only dementia, and FTLD with *MAPT* mutation), synucleinopathies (Parkinson's disease, dementia with Lewy bodies, and multiple system atrophy), and trinucleotide repeat diseases (Huntington's disease and spinocerebellar ataxia). The one exception was HS in which we found TDP-43-positive NCIs in the dentate gyrus. Although only one case of HS without coexisting neurodegenerative disease was available in this study, the presence of TDP-43 epitopes within the NCI indicates that a proportion of pure HS cases may be considered part of the spectrum of FTL-D-U.<sup>44</sup> The biochemistry of this disorder remains to be evaluated.

The mechanisms leading to inclusion formation in FTL-D-U remain unknown. However, we have shown for the first time by electron microscopy that the majority of inclu-

sions are composed of granular material that contains epitopes of TDP-43. The fine structure of the inclusions of FTL-D-U may be contrasted with the inclusions of other protein-folding diseases, including tauopathies and synucleinopathies, in which monomeric species aggregate to form fibrils that make up the inclusion. These fibrillary proteins are typically amyloidogenic, in that they stain with amyloid-binding dyes, including Congo red and thioflavin S. In contrast, the inclusions of FTL-D-U are not labeled by amyloid dyes, and this corresponds with the sparsity of fibrillary structure as visualized by electron microscopy. This biophysical property distinguishes the TDP-43 proteinopathies from other neurodegenerative diseases.

How defects in different genes cause TDP-43 protein aggregation remains to be elucidated. Furthermore, the common mechanisms leading to TDP-43 phosphorylation, ubiquitination, and aggregation in the nucleus, cytoplasm and processes or neurons are unknown. The ubiquitin pathology of FTL-D-U and FTL-D-MND may result from a primary defect of the ubiquitin-proteasome system.<sup>47</sup> Alternatively, there may be an abnormal metabolism of TDP-43, resulting in pathological species that then become ubiquitinated, a mechanism similar to that proposed for other neurodegenerative diseases, including the tauopathies, synucleinopathies, and trinucleotide repeat diseases.

TDP-43 is a ubiquitously expressed and highly conserved protein found mainly in the nucleus.<sup>48</sup> It may act as an activator of exon skipping,<sup>5,6</sup> a transcription repressor, and/or as a scaffold for nuclear bodies through interactions with survival motor neuron protein.<sup>49</sup> In unaffected neurons, TDP-43 IHC showed the normal diffuse nuclear staining pattern but did not reveal significant levels in the neuronal cytoplasm, axons, or dendrites. However, in cells with inclusions, there was a marked reduction of TDP-43 immunoreactivity within the nucleus.<sup>8</sup> The relocation of the nuclear protein TDP-43 resembles the relocation of peptidyl-prolyl cis-trans isomerase (Pin1) from the nucleus to the cytoplasm of inclusion-containing neurons in AD, FTLDs with tauopathy and neuronal intermediate filament inclusion disease.<sup>50</sup> This relocation of a nuclear protein may lead to loss of TDP-43 nuclear function or cause neurodegeneration by apoptotic mechanisms as with Pin1 translocation. The loss of nuclear TDP-43 immunoreactivity may result from reduced protein expression, relocation from the nucleus to the cytoplasm, and sequestration within ubiquitinated inclusions. However, because some TDP-43-positive neuronal and glial inclusions were detected that were not labeled by ubiquitin antibodies, abnormal TDP-43 phosphorylation and/or aggregation may precede ubiquitination in the pathogenesis of inclusions of FTL-D-U. The exact mechanism and pathological significance of the relocation of TDP-43 from the nucleus requires further study.

In summary, this study shows that TDP-43 is a major component of the pathological inclusions in four clinical groups characterized by FTL-D-U pathology: 1) familial FTD with *PGRN* and *VCP* mutations and cases linked to chromosome 9p; 2) familial FTD with unknown genetic association; 3) sporadic FTD; and 4) MND with dementia. Familial FTD with *CHMP2B* mutation is an exception, being a familial FTL-D-U without pathological TDP-43. Although pathological TDP-43 was not found to be a

component of the inclusions of any other FTD, other neurodegenerative disease, or normal aging, it was observed in most cases of HS, indicating that some of these cases have both clinical and pathological similarities with other TDP-43 proteinopathies. There was a remarkable correlation between genotype and FTLD-U subtype for familial cases with known gene defects. In contrast, there was much more heterogeneity in the pathological subtype of sporadic cases of FTD. The identification of granular TDP-43-positive pre-inclusions, particularly in FTD linked to chromosome 9p cases, may indicate that TDP-43 proteinopathy is an early event in pathogenesis and precedes ubiquitination.<sup>11</sup> In conclusion, the identification of TDP-43 proteinopathy in a diverse group of FTLDs illuminates a new target for investigation and therapeutic intervention.

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