Herein, we review advances in understanding a group of disorders collectively known as TAR-DNA binding protein 43 (TDP-43) proteinopathies since the report that TDP-43 is the major disease protein that mechanistically links frontotemporal lobar degeneration with ubiquitin-positive inclusions (FTLD-U) with and without motor neuron disease to amyotrophic lateral sclerosis. Because TDP-43 proteinopathy underlies sporadic and familial forms of FTLD-U and amyotrophic lateral sclerosis, they may share similar mechanisms linked to the abnormal hyperphosphorylation, ubiquitination, and cleavage of pathologic TDP-43 to generate C-terminal fragments in brain and spinal cord affected with FTLD-U and amyotrophic lateral sclerosis. TDP-43 proteinopathies are distinct from most other neurodegenerative disorders in which protein misfolding leads to brain amyloidosis, as pathologic TDP-43 forms neuronal and glial inclusions lacking the features of brain amyloid deposits. We discuss the implications of these distinct aspects of TDP-43 proteinopathies for developing better diagnostics and therapeutics for FTLD-U and amyotrophic lateral sclerosis.

TDP-43 Proteinopathy in Frontotemporal Lobar Degeneration and Amyotrophic Lateral Sclerosis

Protein Misfolding Diseases Without Amyloidosis

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H erein, we review advances in understanding a group of disorders collectively known as TAR-DNA binding protein 43 (TDP-43) proteinopathies since the report that TDP-43 is the major disease protein that mechanistically links frontotemporal lobar degeneration with ubiquitin-positive inclusions (FTLD-U) with and without motor neuron disease to amyotrophic lateral sclerosis. Because TDP-43 proteinopathy underlies sporadic and familial forms of FTLD-U and amyotrophic lateral sclerosis, they may share similar mechanisms linked to the abnormal hyperphosphorylation, ubiquitination, and cleavage of pathologic TDP-43 to generate C-terminal fragments in brain and spinal cord affected with FTLD-U and amyotrophic lateral sclerosis. TDP-43 proteinopathies are distinct from most other neurodegenerative disorders in which protein misfolding leads to brain amyloidosis, as pathologic TDP-43 forms neuronal and glial inclusions lacking the features of brain amyloid deposits. We discuss the implications of these distinct aspects of TDP-43 proteinopathies for developing better diagnostics and therapeutics for FTLD-U and amyotrophic lateral sclerosis.
protein misfolding in the absence of tive proteinopathy characterized by might be a unique neurodegenerative lack of affinity of amyloid-clearly shows that the inability to detect them readily and suggests that FTLD-U might be a unique neurodegenerative proteinopathy characterized by protein misfolding in the absence of brain amyloidosis, which is a signature of almost all other neurodegenerative diseases. Therefore, to consider the possibility of multiple disease proteins among the different FTLD-U subtypes, protein extracts enriched for insoluble proteins were prepared from brains with FTLD-U subtypes 1, 2, and 3, and high-molecular-mass material (≥250 kDa) was used to immunize mice to generate antibodies recognizing proteins in UBIs.11,12 More than 50 000 hybridomas were generated, which were all screened by immunohistochemistry (IHC) to select those that labeled UBIs in the same cases used for the immunizations for further analysis. Eventually, novel monoclonal antibodies (mABs) were successfully generated from subtype 1 cases (eg, mAB 182) and from subtype 2 cases (eg, mAB 137). These mABs immunolabeled all of the UBIs in the respective subtype of FTLD-U cases but not the UBIs in other subtypes. Most important, some of these Mabs also labeled disease-specific bands in insoluble protein extracts prepared from FTLD-U subtypes compared with control brains in immunoblot analyses. Therefore, these mABs allowed the performance of extensive protein analysis of extracts from the FTLD-U brains, including 2-dimensional sodium dodecyl sulfate–polyacrylamide gel electrophoresis and liquid chromatography coupled to tandem mass spectrometry analysis, which subsequently led to the identification of TDP-43 as the protein recognized by these novel mABs and the major component of UBIs in all forms of FTLD-U and ALS.11

TDP-43 is a 414–amino acid protein first cloned as a human protein capable of binding to the transactive response DNA of human immunodeficiency virus type 1 and later identified as part of a complex involved in splicing of the cystic fibrosis transmembrane conductance regulator gene.10 It is a highly conserved and ubiquitously expressed nuclear protein with 2 RNA recognition motifs and a glycine-rich C-terminal region,17 which may function as a transcription repressor and an initiator of exon skipping.18,19 Finally, TDP-43 may act as a scaffold for nuclear bodies through interaction with survival motor neuron protein.20

Despite the pathologic heterogeneity among FTLD-U subtypes and the specificity of novel mAbs for UBIs in specific FTLD-U subtypes, IHC using commercially available antibodies against TDP-43 allowed the demonstration that TDP-43 is the component of UBIs in all FTLD-U subtypes, while characteristic disease-specific inclusions of other neurodegenerative disorders such as the tau inclusions in different tauopathies and the α-synuclein inclusions in synucleinopathies were uniformly negative for TDP-43.11,14,21

Furthermore, biochemical analysis demonstrates that TDP-43 becomes abnormally modified in FTLD-U, suggesting that TDP-43 is directly involved in the pathogenesis of FTLD-U and does not simply represent a nonspecifically entrapped protein in UBIs. In addition to the normal 43-kDa protein, a disease-specific biochemical profile of TDP-43 was evident in detergent-insoluble, urea-soluble protein extracts from FTLD-U brains. Specifically, pathologic TDP-43 was found to be hyperphosphorylated, ubiquitinated, and N-terminally truncated, thereby generating abnormal species of TDP-43 migrating with a higher molecular mass at approximately 45 kDa, as well as a smear of high-molecular-mass proteins and C-terminal fragments of approximately 25 kDa, in immunoblots of FTLD-U extracts.11,12 The presence and extent of this pathology signature in affected cortical gray and white matter, as well as the spinal cord, roughly correspond with the density of TDP-43–positive inclusions detected by IHC.11,23

TDP-43 PATHOLOGY IN SPORADIC AND FAMILIAL FTLD-U
As demonstrated in the initial report and rapidly confirmed by several follow-up studies from different laboratories, TDP-43 is the most specific and sensitive marker to detect the characteristic ubiquitin-immunoreactive lesions in FTLD-U, including neuronal cytoplasmic inclusions (NCIs), dystrophic neurites, and neuronal intranuclear inclusions (NIIs).11,16,21,22 Moreover, TDP-43 IHC allows the detection of
previously unrecognized widespread and abundant white matter pathologic features with numerous oligodendroglial cytoplasmic inclusions in a subset of FTLD-U cases.\textsuperscript{23} This oligodendroglial neuropathological finding was not detected previously because most of the glial inclusions are not immunostained by antiubiquitin antibodies. Therefore, white matter pathologic features might contribute to the clinical symptoms in FTLD-U. Although physiologic TDP-43 is detectable in the nuclei of unaffected neurons and some glial cells, cells harboring NCIs show a dramatic loss of normal nuclear TDP-43 staining, raising the suspicion that some essential normal function of TDP-43 may be lost in FTLD-U.\textsuperscript{11,14,21,22}

It has been previously shown that FTLD-U pathologic features are heterogeneous with respect to morphologic structure, laminar distribution of ubiquitin, and TDP-43–positive inclusions and relative proportion of dystrophic neurites vs NCIs, leading to the description of 4 distinct subtypes (subtypes 1–4).\textsuperscript{11,14,24} The specificity of the novel mAbs (eg, mAb 182 and mAb 137) to immunolabel subtype 1– and subtype 2–related pathologic features, respectively, is a valuable tool in classifying FTLD-U pathologic conditions and provides further evidence for pathologic heterogeneity in FTLD-U.\textsuperscript{11,12,14} The relevance and the reasons for the distinct histologic distribution with respect to pathogenesis and clinical aspects remain unclear. However, a correlation of distinct histologic subtypes was observed among familial forms of FTLD-U with TDP-43 pathologic features and may underlie genetic defects. For example, FTLD-U subtype 3 is associated with mutations in the progranulin gene (PGRN),\textsuperscript{25,26} whereas subtype 4 is associated with mutations in the valosin-containing protein gene (VCP)\textsuperscript{27} and subtype 2 with an unidentified gene on chromosome 9.\textsuperscript{28,29} These and other recent findings further support the significance of these FTLD-U subtypes. Furthermore, the identification of 3 different mutant genes provides important clues to elucidate potential pathogenic pathways that lead to the accumulation of pathologic TDP-43. Representative images from TDP-43 staining patterns in distinct FTLD-U subtypes are shown in Figure 1A. Biochemically, extracted TDP-43 from all familial and sporadic subtypes of TDP-43 proteinopathies shows the characteristic disease-specific signature (Figure 1B), although there were subtle differences in these abnormal TDP-43 variants among the different subtypes, which may be the result of similar but not identical pathogenic mechanisms.\textsuperscript{11,14}

**SUBTYPE 1**

Histologic findings of subtype 1\textsuperscript{12} (similar to type 2 in the study by...
Mackenzie et al\textsuperscript{13}) are characterized by an abundance of long neuritic profiles predominantly in superficial cortical laminae with few or no NCIs or NIIs. Inclusions can be labeled with Mab 182 but not with mAb 137. Glial pathologic features are rare.\textsuperscript{23} Cases can occur sporadically or with familial inheritance, but no specific genetic defect has been identified in the familial cases, while PGRN mutations have been excluded. This subtype is the most common in patients with semantic dementia.\textsuperscript{13}

**SUBTYPE 2**

In subtype \textsuperscript{212} (similar to type 3 in the study by Mackenzie et al\textsuperscript{13}) cases, the predominant inclusions are NCIs in superficial and deep cortical layers with the presence of few neurites and few or no NIIs. mAB 137 but not mAB 182 specifically labels these inclusions. Affection of motor neurons in the hypoglossal nuclei and ventral horn of the spinal cord with inclusions is a common finding, correlating with the fact that patients with subtype 2 histologic findings often manifest additional clinical signs of motor neuron disease.\textsuperscript{13} Moreover, subtype 2 is often associated with abundant glial pathologic features in affected cortical, brainstem, and spinal cord regions.\textsuperscript{23} All examined familial FTLD cases with a confirmed linkage to a locus on chromosome 9 demonstrated subtype 2 histologic findings, while none of the examined familial cases with subtype 2 pathologic features had a PGRN mutation.\textsuperscript{14}

**SUBTYPE 3**

The abundance of small neuritic profiles and NCIs (often ring shaped) predominantly in the superficial cortical layers characterizes subtype \textsuperscript{312} (similar to type 1 in the study by Mackenzie et al\textsuperscript{13}) histologic findings. Especially in cases with positive family history, moderate numbers of lentiform NIIs can be found in affected cortical regions. Glial pathologic features are often present in affected cortical regions.\textsuperscript{23} All cases with PGRN mutations described so far have demonstrated subtype 3 histologic findings.\textsuperscript{13,13,19,30}

**SUBTYPE 4**

In subtype 4 (Forman et al\textsuperscript{31} and Neumann et al\textsuperscript{30}) mutations in VCP, which encodes for an AAA-type adenosine triphosphatase likely involved in endoplasmic reticulum-associated protein degradation, have been shown to cause FTLD with inclusion body myopathy and Paget disease of bone.\textsuperscript{27} The characteristic neuropathological feature is the abundance of ubiquitin and TDP-43 positive NIIs and dystrophic neurites with few NCIs in affected cortical regions and the absence of inclusions in the hippocampal dentate granule cells.\textsuperscript{29,31} So far, this histologic subtype has not been described in sporadic or other familial FTLD-U cases (to our knowledge). Although mutations in VCP are rare, the pathogenic mechanisms leading to TDP-43 accumulation in FTLD with inclusion body myopathy and Paget disease of bone may have broader significance to idiopathic FTLD-U.

Furthermore, the specificity of TDP-43 as a marker for FTLD-U lesions now permits the investigation of FTLD-U pathologic features in the setting of concurrent ubiquitin-positive pathologic findings such as neurofibrillary tangles and Lewy bodies in other neurodegenerative diseases. Surprisingly, additional TDP-43 pathologic features similar to those found in FTLD-U have been reported in up to 20% of patients with AD\textsuperscript{32} and in the brains of patients with Guam parkinsonism-dementia complex.\textsuperscript{33} However, additional studies among large cohorts are needed to further address the overlap of TDP-43 pathologic features in AD and other neurodegenerative disorders, as well as the clinical significance of concomitant TDP-43 pathologic features in these disorders.

**TDP-43 PATHOLOGY IN SPORADIC AND FAMILIAL ALS**

Amyotrophic lateral sclerosis is the most common adult-onset motor neuron disease, characterized by the destruction of upper and lower motor neurons that results in progressive weakness, muscular wasting, and spasticity leading to death within a few years after onset.\textsuperscript{34} Familial forms of ALS (fALS), which account for approximately 10% of ALS cases, have been associated with several genetic loci and mutations in specific genes.\textsuperscript{35,36} However, mutations in the copper-zinc superoxide dismutase gene (SOD1) are the most common, accounting for approximately 20% of fALS cases.\textsuperscript{35,36}

Although cognitive impairment was previously considered to be a rare event in ALS, several studies during the past 2 decades have provided a growing body of evidence for affection of extramotor cerebral regions in ALS. Detailed cognitive testing revealed a spectrum of frontal lobe dysfunction in approximately 50% of patients with ALS, with up to 20% showing abnormalities meeting Neary criteria for FTLD.\textsuperscript{34,37,39} Neuropathologically, ALS cases manifest protein inclusions in the cytoplasm of degenerating motor neurons, most often appearing as compact round Lewy body–like or skeinlike inclusions. Until recently, little was known about the specific biochemical composition of these inclusions except that the accumulating protein was ubiquitinated. This clinical and neuropathological overlap between FTLD, especially FTLD-U, and ALS prompted investigation of the role of TDP-43 in sporadic ALS (sALS) and fALS. TDP-43 IHC demonstrated that immunolabeling of round and skeinlike neuronal inclusions (Figure 1A) and additional glial inclusions in affected brain regions was a consistent finding in large series of sALS cases.\textsuperscript{11,40,41} Although pathologic TDP-43 is a consistent feature in non-SOD1 fALS, no TDP-43 immunoreactivity was present in the UBIs of any SOD1 fALS cases, including 15 cases with 7 different SOD1 mutations.\textsuperscript{41} Similar observations have been reported in 2 Japanese SOD1 fALS cases.\textsuperscript{30} Consistent with these findings is the reported absence of TDP-43 immunoreactivity in inclusions in mutant SOD1 (G93A, G37R, and G85R) transgenic mice.\textsuperscript{42}

Together with the fact that similar abnormal TDP-43 species as seen in FTLD-U can be extracted from affected brain and spinal cord from sALS and non-SOD1 fALS cases,\textsuperscript{20,41}
the results summarized herein provide histologic and biochemical evidence that ALS and FTLD-U represent a clinical spectrum of neurodegenerative disorders characterized by TDP-43 accumulation (Figure 2). However, the absence of TDP-43 pathologic features in SOD1 fALS implies that motor neuron degeneration in these cases of fALS may result from a different mechanism than that underlying sALS or fALS due to mutations in genes other than SOD1, thereby suggesting that fALS caused by SOD1 mutations may not represent the familial counterpart of sALS.

RELEVANCE OF TDP-43 PROTEINOPATHIES TO THE DIAGNOSIS AND THERAPY OF FTLD AND ALS

Based on initial discoveries and numerous ongoing studies confirming and extending the initial findings, it is evident that a new class of neurodegenerative disorders, TDP-43 proteinopathies, has emerged that includes familial and sporadic forms of FTLD-U with and without motor neuron disease, as well as sALS and non-SOD1 fALS (Figure 2). This will have notable implications for the diagnosis and treatment of FTLD and ALS. Preliminary data among small numbers of patients suggest that different FTLD-U subtypes might be correlated with different clinical features and survival. However, further studies among larger cohorts are necessary to confirm and validate these preliminary data. A critical issue for future drug trials regarding FTLD is to enroll subjects who have the underlying pathologic features for which the therapy has been identified or developed (e.g., tauopathies or TDP-43 proteinopathies). Development of assays to measure TDP-43 in plasma or cerebrospinal fluid might help establish biomarkers to distinguish FTLD with TDP-43 pathologic features from FTLD with tau pathologic features and other clinically similar neurodegenerative disorders. Furthermore, the development of imaging ligands that allow the detection of TDP-43 pathologic features in living patients will provide a powerful tool not only for diagnosing but also for monitoring disease progression and response to disease-modifying therapies. Finally, TDP-43 will be an important target for drug development, which should result in more effective therapies for FTLD and ALS. However, because TDP-43 proteinopathies may be distinct from all other neurodegenerative protein misfolding disorders as TDP-43 does not seem to form amyloid fibrils, the toxicity of TDP-43 aggregates may be due to toxic gains of function independent of those implicated in brain amyloidosis. Accordingly, strategies designed to reverse amyloidosis in AD, related tauopathies, and synucleinopathies may not be applicable to TDP-43 proteinopathies, although efforts to abrogate TDP-43 aggregates could inform efforts to target tau or Aβ oligomers in AD.

RELEVANCE TO THE STUDY OF NEUROSCIENCE

Although the physiologic function of TDP-43 in the brain and its specific role in neurodegeneration are unknown and speculative, the specificity of TDP-43 immunoreactivity for UBIs in FTLD-U and ALS as well as the demonstration of ubiquitinated, hyperphosphorylated, and N-terminally truncated TDP-43 species implicates TDP-43 in the pathogenesis of these conditions. Moreover, the redistribution of TDP-43 from the nucleus into the cytoplasm may represent loss of nuclear function for TDP-43. Hence, the loss of physiologic nuclear TDP-43 may disrupt key nuclear functions, thereby resulting in transcriptional deregulation, aberrant messenger RNA splicing, or disintegration of nuclear bodies. In addition, pathologic TDP-43 species may have aberrant biological activities, resulting in cell death due to gain of toxic functions. Future studies will need to address these and other mechanistic aspects of the aggregation of pathologic TDP-43 in cytoplasmic, neuritic, and nuclear inclusions, while additional studies will be required to understand the mechanisms linking TDP-43 accumulation with VCP and PGRN dysfunction.

The absence of TDP-43 in fALS with SOD1 mutations implies that pathomechanisms underlying motor neuron degeneration in sALS differ from those associated with SOD1 mutations. This will have a dramatic effect on future research.
The identification of TDP-43 as the major component of UBIs specific to familial and sporadic FTLD-U as well as sALS and non-SOD1 FALS resolves a long-standing enigma concerning the nature of the ubiquitinated disease protein in these disorders. The accumulation of ubiquitinated, phosphorylated, and N-terminally truncated TDP-43 defines a new class of neurodegenerative disorders (TDP-43 proteinopathies) and implicates TDP-43 in a novel and unifying mechanism of neurodegeneration in FTLD-U and ALS. Overlap of TDP-43 pathologic features provides neuropathologic and biochemical evidence that FTLD and ALS represent a spectrum of disorders that share similar pathologic mechanisms, culminating in the progressive degeneration of different selectively vulnerable neurons.

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**Announcement**

**Calendar of Events: A New Web Feature**

On the new Calendar of Events site, available at http://pubs.ama-assn.org/cgi/calendarcontent and linked off the home page of the Archives of Neurology, individuals can now submit meetings to be listed. Just go to http://pubs.ama-assn.org/cgi/cal-submit/ (also linked off the Calendar of Events home page). The meetings are reviewed internally for suitability prior to posting. This feature also includes a search function that allows searching by journal as well as by date and/or location. Meetings that have already taken place are removed automatically.