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

Protein Misfolding Diseases Without Amyloidosis

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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.

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Frontotemporal lobar degeneration (FTLD) refers to a clinically, genetically, and neuropathologically heterogeneous group of neurodegenerative disorders and is the third most common form of dementia after Alzheimer disease (AD) and dementia with Lewy bodies. Current research criteria divide FTLD into the following 3 clinical syndromes: frontotemporal dementia, primary progressive nonfluent aphasia, and semantic dementia. Frontotemporal dementia, the most common clinical form, primarily manifests as personality and behavioral changes, while primary progressive nonfluent aphasia and semantic dementia manifest predominantly as language dysfunctions. In addition, patients may develop movement abnormalities such as parkinsonism and motor neuron disease.

The term frontotemporal lobar degeneration reflects the prominent frontal and temporal lobe atrophy seen in these patients by neuropathological examination. A characteristic feature in most FTLD brains is the formation of abnormal protein inclusions in neurons and glial cells. Immunohistochemically, FTLD can be broadly subdivided into disorders with tau-positive inclusions (eg, Pick disease, corticobasal degeneration, and progressive supranuclear palsy) and disorders with ubiquitin-positive, tau-negative, and α-synuclein-negative inclusions, termed “FTLD-U”, which is the most common neuropathological form underlying FTLD.

Tau has been known as the protein building block of the inclusions in tauopathies for many years, and its role in the pathogenesis of neurodegenerative disorders is established especially after identification of mutations in the microtubule-associated protein tau gene in familial tauopathies. However, the ubiquitinated protein forming the pathologic inclusions in FTLD-U remained unknown until recent antibody-based and biochemical...
brain amyloidosis, which is a signa-
tive proteinopathy characterized by
tions for the inability to detect them
tropic lateral sclerosis (ALS). Impli-
cations of these novel findings for the clini-
cial diagnosis and treatment of FTLD and the relevance of TDP-43
to the study of neuroscience will be
discussed.

IDENTIFICATION OF UBQUITINATED,
PHOSPHORYLATED, AND TRUNCATED TDP-43 AS THE
DISEASE PROTEIN IN FTLD-U

Although multiple biochemical tech-
niques, including fractionation of
disease brains to enrich for ubiqui-
tin-positive inclusions (UBIs) and
dimensional gel electrophoresis proteomic approaches, had been
used in attempts to identify the dis-
ease proteins in FTLD-U, they did
prove to be informative. For
these reasons, an alternative immu-
nologic approach was pursued that
was based on the assumptions that
the proteins in UBIs might behave
similarly with respect to changes in
solubility and ubiquitination as de-
scribed for other disease proteins
identified in inclusions in many neu-
rodegenerative diseases, including
tau in AD and related tauopathies and α-synuclein in synucleiopa-
thies. However, this approach was
complicated by the observa-
tions that inclusions in FTLD-U are
not fibrillar (eg, thioflavin S and sil-
ver negative) and that the morpho-
logic structure and distribution of
UBIs in FTLD-U brains were hetero-
genous, leading to the description of
different subtypes of FTLD-U
pathologic conditions (subtypes 1-4, described herein). Indeed, the
curious lack of affinity of amyloid-
undying dyes for UBIs may account
for the inability to detect them
readily and suggests that FTLD-U
might be a unique neurodegenera-
tive proteinopathy characterized by
protein misfolding in the absence of
brain amyloidosis, which is a signa-
ture of almost all other neurodegen-
erative diseases. Therefore, to con-
sider the possibility of multiple
inclusions in synucleinopathies were uni-
erve subtype of FTLD-U cases but not
the UBIs in other subtypes. Most im-
portant, some of these Mabs also la-
abeled disease-specific bands in in-
soluble protein extracts prepared
from FTLD-U subtypes compared with
control brains in immunoblot
analyses. Therefore, these Mabs al-
lowed the performance of exten-
sive protein analysis of extracts from
the FTLD-U brains, including 2-di-
menSional sodium dodecyl sulfate–
polyacrylamide gel electrophoresis and
liquid chromatography coupled to
tandem mass spectrometry analy-
sis, which subsequently led to the
identification of TDP-43 as the pro-
tein 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
restricted to the nucleus as a human protein
first cloned from a high molecular mass
DNA of human immuno-
posivity virus type 1 and later
identified as part of a complex in-
volved in splicing of the cystic fibro-
sis transmembrane conductance regu-
lator gene. It is a highly conserved
and ubiquitously expressed nuclear
protein with 2 RNA recognition mo-
tifs and a glycine-rich C-terminal re-
gion, which may function as a tran-
scriptional repressor and an inhibitor of
exon skipping. Finally, TDP-43
may act as a scaffold for nuclear bod-
ies through interaction with survival
motor neuron protein.20

Despite the pathologic heteroge-
nity among FTLD-U subtypes and
ubiquitinated, and N-terminally trun-
cated, thereby generating abnor-
mals of TDP-43 migrating
with a higher molecular mass at
approximately 45 kDa, as well as a
smear of high-molecular-mass pro-
teins and C-terminal fragments of
approximately 25 kDa, in immuno-
blots of FTLD-U extracts. The
presence and extent of this patho-
logic signature in affected cortical
gray and white matter, as well as the
spinal cord, roughly correspond with
the density of TDP-43–positive in-
cclusions detected by IHC, which

TDP-43 PATHOLOGY
IN SPORADIC AND FAMILIAL FTLD-U

As demonstrated in the initial re-
port and rapidly confirmed by sev-
eral follow-up studies from differ-
ent laboratories, TDP-43 is the most
specific and sensitive marker to de-
tect the characteristic ubiquitin-
inmunoreactive inclusions in FTLD-U,
including neuronal cytoplasmic in-
cclusions (NCIs), dystrophic neu-
rites, and neuronal intranuclear in-
cclusions (NIIs). The presence and extent of this patho-
logic signature in affected cortical
gray and white matter, as well as the
spinal cord, roughly correspond with
the density of TDP-43–positive in-
cclusions detected by IHC.
previously unrecognized widespread and abundant white matter pathologic features with numerous oligodendroglial cytoplasmic inclusions in a subset of FTLD-U cases. 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.

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). 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. 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), whereas subtype 4 is associated with mutations in the valosin-containing protein gene (VCP) and subtype 2 with an unidentified gene on chromosome. 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.

**SUBTYPE 1**

Histologic findings of subtype 1 (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 NLIIs. 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 \textit{PGRN} mutations have been excluded. This subtype is the most common in patients with semantic dementia.\textsuperscript{13}

\section*{SUBTYPE 2}

In subtype\textsuperscript{2} (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 NLIIs. 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 \textit{PGRN} mutation.\textsuperscript{14}

\section*{SUBTYPE 3}

The abundance of small neuritic profiles and NCIs (often ring shaped) predominantly in the superficial cortical layers characterizes subtype\textsuperscript{3} (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 NLIIs can be found in affected cortical regions. Glial pathologic features are often present in affected cortical regions.\textsuperscript{23} All cases with \textit{PGRN} mutations described so far have demonstrated subtype 3 histologic findings.\textsuperscript{11,13,19,30}

\section*{SUBTYPE 4}

In subtype 4 (Forman et al\textsuperscript{11} and Neumann et al\textsuperscript{12}), mutations in \textit{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 NLIIs 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 \textit{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.

\section*{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 1 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 (eg, 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.
SUMMARY

The identification of TDP-43 as the major component of UBIs specific to sporadic and familial FTLD-U as well as sALS and non-SOD1 ALS 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 neuropathological 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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REFERENCES


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.