Title

Detection of TDP-43 oligomers in frontotemporal lobar degeneration–TDP

Journal

Annals of Neurology, 78(2)

ISSN

0364-5134

Authors

Kao, Patricia F

Chen, Yun-Ru

Liu, Xiao-Bo

et al.

Publication Date

2015-08-01

DOI

10.1002/ana.24431

Peer reviewed

HHS Public Access

Author manuscript

Ann Neurol. Author manuscript; available in PMC 2016 April 06.

Published in final edited form as:

Ann Neurol. 2015 August ; 78(2): 211–221. doi:10.1002/ana.24431.

Detection of TDP-43 Oligomers in Frontotemporal Lobar Degeneration–TDP

Patricia F. Kao, PhD[1,2], **Yun-Ru Chen, PhD**[3], **Xiao-Bo Liu, PhD**[1], **Charles DeCarli, MD**[2,4], **William W. Seeley, MD**[5], and **Lee-Way Jin, MD, PhD**[1,2]

[1]Department of Pathology and Laboratory Medicine, University of California, Davis, School of Medicine, Sacramento, CA

[2]Alzheimer's Disease Center, University of California, Davis, School of Medicine, Sacramento, CA

[3]Genomics Research Center, Academia Sinica, Taipei, Taiwan

[4]Department of Neurology, University of California, Davis, School of Medicine, Sacramento, CA

[5]Departments of Neurology and Pathology, University of California, San Francisco, San Francisco, CA

Abstract

Objective—The proteinaceous inclusions in TDP-43 proteinopathies such as frontotemporal lobar degeneration (FTLD)-TDP are made of high–molecular-weight aggregates of TDP-43. These aggregates have not been classified as amyloids, as prior amyloid staining results were not conclusive. Here we used a specific TDP-43 amyloid oligomer antibody called TDP-O to determine the presence and abundance of TDP-43 oligomers among different subtypes of FTLD-TDP as well as in hippocampal sclerosis (HS), which represents a non-FTLD pathology with TDP-43 inclusions.

Methods—Postmortem tissue from the hippocampus and anterior orbital gyrus from 54 prospectively assessed and diagnosed subjects was used for immunostaining with TDP-O. Electron microscopy was used to assess the subcellular locations of TDP-O–decorated structures.

Results—TDP-43 inclusions staining with TDP-O were present in FTLD-TDP and were most conspicuous for FTLD-TDP type C, the subtype seen in most patients with semantic variant primary progressive aphasia. TDP-O immunoreactivity was absent in the hippocampus of HS patients despite abundant TDP-43 inclusions. Ultrastructurally, TDP-43 oligomers resided in granular or tubular structures, frequently in close proximity to, but not within, neuronal lysosomes.

Address correspondence to Dr Jin, Department of Pathology and Laboratory Medicine, 2805 50th Street, Sacramento, CA 95817. lee-way.jin@ucdmc.ucdavis.edu.

Authorship

P.F.K., Y.-R.C., C.D., W.W.S., and L.-W.J. conceived and designed the experiments. P.F.K. and X.-B.L. performed the experiments. P.F.K., X.-B.L., W.W.S., and L.-W.J. analyzed the data. P.F.K. and L.-W.J. wrote the paper.

Potential Conflicts of Interest

W.W.S.: consultancy, Summer Street Research Partners, Biogen Idec; grants/grants pending, National Institute on Aging, John D. French Alzheimer's Disease Foundation, Consortium for Frontotemporal Dementia Research, James S. McDonnell Foundation, Alzheimer's Drug Discovery Foundation, Association for Frontotemporal Dementia, Tau Consortium.

Interpretation—TDP-43 forms amyloid oligomers in the human brain, which may cause neurotoxicity in a manner similar to other amyloid oligomers. Oligomer formation may contribute to the conformational heterogeneity of TDP-43 aggregates and mark the different properties of TDP-43 inclusions between FTLD-TDP and HS.

The neuropathological diagnosis of major neurodegenerative diseases, including frontotemporal lobar degeneration (FTLD), is heavily based on the presence of proteinaceous inclusions.[1–3] FTLD is a heterogeneous group of disorders that manifest clinically as frontotemporal dementia (FTD), one of the most common forms of dementia in persons younger than 65 years. FTLD can be subdivided according to whether the protein inclusions found in neurons and glia contain tau (FTLD-tau), TDP-43 (TAR DNA-binding protein-43kDa; FTLD-TDP), or fused in sarcoma. TDP-43 inclusions are also found in nearly all patients with sporadic amyotrophic lateral sclerosis (ALS).[4]

TDP-43 is a DNA- and RNA-binding protein that serves multiple functions in gene transcription and translation.[5,6] In normal neurons, the majority of TDP-43 resides within the nucleus.[7,8] However, pathological TDP-43 inclusions commonly present as neuronal cytoplasmic inclusions (NCIs) and dystrophic neurites (DNs). Less commonly seen are neuronal intranuclear inclusions (NIIs). How abnormal TDP-43 causes neuronal dysfunction and forms NCI/DN pathologies is currently under investigation. There is evidence linking loss of TDP-43 function to neurotoxicity, and several studies using models overexpressing full-length or truncated TDP-43 have demonstrated neurotoxicity as well as formation of FTLD-like cytoplasmic inclusions, indicating a gain of toxic function from TDP-43 aggregation.[9–12] The 2 RNA recognition motifs RRM1[13,14] and RRM2[15] and the glycine-rich domain[16,17] in human TDP-43 have been implicated in aggregation of TDP-43 into pathological inclusions.

One prevailing hypothesis with regard to pathogenic proteins implicated in neurodegenerative disorders (for example, amyloid-β, tau, prion, α-synuclein, and huntingtin) is that they form amyloids, losing their normal function and causing neurotoxicity. In this regard, the high–molecular-weight aggregates of TDP-43 present in FTLD NCIs and DNs resemble amyloid inclusions found in other neurodegenerative disorders. The first line of supporting evidence came from our recent demonstration that human TDP-43 forms structurally stable, spherical oligomers that possess properties of amyloid.[12] Such TDP-43 oligomers exhibit reduced DNA-binding capability, are conformational and functionally distinct from native TDP-43, and are neurotoxic. Interestingly, TDP-43 oligomers were recognized by an amyloid oligomer-specific antibody A11,[18] indicating that they share structural and functional homology with many amyloid oligomers. Because a common neurotoxic mechanism associated with amyloid oligomers has been suggested for various neurodegenerative disorders,[19] we hypothesize that such a mechanism could play a role in FTLD-TDP. To obtain an initial confirmation of the pathological significance of TDP-43 oligomers in the human brain, we used a polyclonal TDP-43 oligomer antibody, TDP-O, the specificity of which was validated in our prior study,[12] to determine the presence and abundance of TDP-43 oligomers among different sub-types of FTLD-TDP. We found that TDP-O highlighted a subpopulation of conventional TDP-43 lesions such as NCIs and DNs in FTLD-TDP cases, with a particular abundance of

FTLD-TDP type C.[20] Remarkably, no TDP-O immunoreactivity was found in cases of isolated hippocampal sclerosis (HS), despite an abundant presence of TDP-43 NCIs in the hippocampus. Our data support the notion that TDP-43 aggregates into amyloid oligomers in FTLD-TDP, particularly FTLD-TDP type C, and suggest that TDP-O immunoreactivity profiles could aid in further refinement of the subclassification of TDP-43 proteinopathies.

Methods and Materials

Tissue Samples

Fixed human brain tissue and paraffin-embedded tissue used in this study were obtained from the Neurodegenerative Disease Brain Bank at the University of California, San Francisco, Memory and Aging Center, San Francisco, California and the Alzheimer's Disease Center at University of California, Davis, Sacramento, California. All human specimens were obtained through consented autopsies with institutional review board approval. The diagnosis of HS was based on clinicopathological correlation, following the National Institute on Aging–Alzheimer's Association guidelines,[21] and our previous criteria for HS.[22] FTLD-TDP subtypes were designated using consensus nomenclature[20] and diagnosed following the original descriptions.[23,24] Patients with FTLD-TDP spanned types A to D, and sporadic and genetic forms, including patients with *GRN*, *C9ORF72*, and *VCP* mutations (Table 1). Although such gene mutations were not routinely determined for HS and control cases, we stained the cerebellum of all HS and control cases with ubiquitin and p62 antibodies and did not find any inclusions, making it highly unlikely for these cases to have *C9ORF72* mutations.[25,26] The clinical diagnosis of ALS was made following the criteria described by de Carvalho et al.[27] All listed cases of ALS had pathological confirmation of motor neuron disease (MND) with essential manifestations of loss of anterior horn motor neurons and TDP-43–immunoreactive inclusions in neurons and/or glia in the spinal cord.

TDP-43 Oligomer Immunohistochemistry

Five-micrometer-thick sections of formalin-fixed, paraffin-embedded tissue from medial temporal lobe (including hippocampus) and anterior orbital gyrus were immunostained with anti–TDP-43 oligomer polyclonal antibody (TDP-O, 1:500) overnight at 4°C. The specificity of this antibody was validated in a prior study.[12] Slides were washed with ×1 phosphate-buffered saline (PBS; pH 7.4; Life Technologies, Carlsbad, CA) 5 times, then incubated in goat antirabbit horseradish peroxidase (1:500) for 1 hour at room temperature. Slides were washed with ×1 PBS 5 times, then incubated with Vectastain ABC kit (Vector Laboratories, Burlingame, CA). After washing with ×1 PBS, slides were placed in DAB substrate (Vector Laboratories) for 10 minutes and rinsed with water. Slides were counterstained in hematoxylin (Sigma-Aldrich, St Louis, MO) for 5 minutes, dipped in 0.05% lithium carbonate, rinsed, and coverslipped following dehydration in increasing concentrations of ethanol and clearing with xylene. A similar protocol was followed to stain TDP-43 cytoplasmic inclusions (Novus Biologicals, Littleton, CO)[1] at a dilution of 1:3,000 for 30 minutes at room temperature. Slides were scanned and images were taken using an Aperio camera (Leica Microsystems, Buffalo Grove, IL) and analyzed using ImageJ.

Electron Microscopy

Immunostained sections for electron microscopy (EM) were prepared as previously described.[28,29] Briefly, formalin-fixed brain tissue from the medial temporal lobe (including hippocampus) was cut on a vibratome. Fifty-micron-thick sections were collected in cold 0.1M phosphate buffer (PB) and used for immunocytochemistry (TDP-O antibody 1:50). Immunolabeled sections were osmicated and rinsed with PB, dehydrated, embedded in epoxy resin, and polymerized for 24 hours at 70°C in an oven. Ultrathin sections (70–80nm) were cut on a Leica Ultramicrotome (EM UC7) and were collected on Formvar-coated single-slot copper grids (EMS, Hatfield, PA). Sections were lightly stained with uranyl acetate and lead citrate and examined in with a Philips (Best, the Netherlands) CM120 electron microscope at 80kV. Electron microscopic images were acquired by a 2k × 2k high-resolution charge-coupled device camera attached to the microscope (Gatan, Pleasanton, CA). Images were processed using an EM software program (DigitalMicrograph; Gatan). Neuronal profiles were considered immunopositive when electron-dense immunoperoxidase reaction product could be detected in at least 3 serial thin sections.

Image Processing

Photoshop CS5 (Adobe Systems, San Jose, CA) was used for image processing in the current study. Image processing included adjustments in brightness and cropping of images to create layered images showing high magnification of stained cells.

Statistics

One-way analysis of variance (ANOVA) was performed in multiple group comparisons followed by Tukey post hoc analysis (Prism 5; GraphPad, La Jolla, CA). Data from the FTLD-TDP type D case was included in Figure 1, but was excluded from statistical analysis because there was only 1 patient in this group.

Results

Oligomeric TDP-43 Is Present in FTLD-TDP Brains

We examined TDP-O immunoreactivity in sections from 25 FTLD-TDP cases and 15 age-matched cognitively normal controls. Sections of medial temporal region and anterior orbital frontal cortex were chosen for this purpose, as TDP-43–immunoreactive NCIs and DNs are readily identifiable in these regions.[30] Figure 2 shows side-by-side comparisons of TDP-O and TDP-43 immunoreactivity. As expected, TDP-O did not stain the native monomeric TDP-43 normally present in the nucleus, but readily highlighted NCIs and DNs (Figs 2–4, arrows). No TDP-O–immunoreactive NIIs were identified. Morphologically, TDP-O–immunoreactive NCIs (TDP-O-NCIs) and DNs (TDP-O-DNs) were indistinguishable from those detected by anti–TDP-43, but they were much fewer in quantity in any given section, indicating that TDP-O epitopes are present only among sub-populations of NCIs and DNs. Our prior validation also ruled out the possibility that TDP-O cross-reacted with other types of amyloid oligomers such as amyloid-β.[12]

TDP-O Is Most Conspicuous in FTLD-TDP Type C

The widely adopted “harmonized” classification system for FTLD-TDP pathological subtypes is based on the profiles of TDP-43–immunoreactive NCIs and DNs.[20] Briefly, type A is characterized by abundant compact round or crescentic NCIs and short, thin neuropil threads, predominantly in superficial cortical layers. Type B has frequent diffuse, granular, or stippled NCIs throughout all cortical layers and few DNs. Type C has many long, swollen DNs and few NCIs in cortex. Type D has many short DNs, lentiform NIIs, and few NCIs found throughout all cortical layers.[20] This classification enables a moderate degree of clinicopathological correlation, suggesting relatively distinct pathological mechanisms underlying the 4 subtypes. Interestingly, in our initial 6 cases studied, we noticed that TDP-O–immunoreactive inclusions were more numerous in FTLD-TDP type C cases. Pursuing this initial impression, we expanded our study to include an additional 6 cases. Whereas all 12 cases (100%) of FTLD-TDP type C contained numerous TDP-O–immunoreactive NCIs and DNs in the dentate gyrus, these findings were less frequent in FTLD-TDP type A (50%) and FTLD-TDP type B (67%; Table 2). TDP-O-NCIs were quantified in 15 random microscopic fields, each covering a $0.01mm^2$ area of the dentate gyrus, amounting to a total area of $0.15mm^2$ examined for each case. Quantification by the experimenter was blind to the pathological diagnosis for each case. One-way ANOVA followed by Tukey post hoc analysis showed a significant increase in the number of TDP-O-NCIs found in type C cases as compared to control and types A and B (see Fig 1A). Figure 1B shows the number of TDP-O-NCIs in cases of type A, B, and C where inclusions were found. Figure 1C shows the number of TDP-O-NCIs in relationship to the frequency of TDP-43-NCIs in the dentate granules. The number of TDP-43-NCIs did not correlate with the number of TDP-O-NCIs. The finding of numerous TDP-O-NCIs in type C suggests that abnormal TDP-43 in type C pathology is prone to oligomerization. In the single case of type D,[31] a rare genetic subtype associated with *VCP* mutations, we did not identify TDP-O-NCIs or DNs (see Fig 2D).

Interestingly, 2 of the 15 control cases had minimal TDP-43 cytoplasmic inclusions, consistent with previous reports on the frequency of abnormal TDP-43 in normal controls. One study showed that 1 case out of 33 controls had TDP-43 inclusions limited to hippocampus and entorhinal cortex,[32] and a more recent study showed a 36.4% prevalence of pathologic TDP-43, mostly in the form of neurites, with inclusions being rare in cognitively normal elderly cases.[33] Between our 2 cases with TDP-43 inclusions, 1 showed a minimal number of TDP-ONCIs, and the other had none.

We similarly quantified TDP-O-NCIs and TDP-O-DNs in the anterior orbital gyrus, focusing on layer 2, where all FTLD-TDP subtypes show TDP-43 NCIs and DNs (see Fig 3). A total area of $0.45mm^2$ was examined for each FTLD-TDP case. The number of TDP-O-NCIs found in cortical layer 2 was relatively low and showed no significant difference between the type A, B, and C groups. However, considering that type A is defined by the presence of many TDP-43-NCIs and type C by few TDP-43-NCIs in layer 2 of the anterior orbital gyrus, the increased TDP-O staining in FTLD-TDP type C is clearly apparent. TDP-O stained significantly more DNs in FTLD-TDP type C and were significantly longer than type A and B DNs. We observed that TDP-O-DNs were shorter than TDP-43-DNs, suggesting that oligomers may constitute segments of TDP-43-DNs. These findings suggest

that TDP-43 oligomerization is present across the FTLD-TDP spectrum, although it may vary in degree and distribution by subtype.

Variable TDP-O Pathology in Other TDP-43 Proteinopathies

TDP-43 pathology is also found in other common neurodegenerative conditions,[1,34–36] especially in the mesial temporal region. Most patients with HS, for example, show abundant TDP-43-NCIs.[34,37] To determine whether TDP-43 oligomerization also occurs in non-FTLD pathologies, we examined the hippocampus of 9 cases of HS without FTLD pathology. Interestingly, whereas a large majority (8 of 9) of HS cases had abundant TDP-43-NCIs, none had TDP-O-NCIs (see Table 2 and Fig 4A, B). This observation, together with our findings among FTLD-TDP subtypes, indicates that the abundance of TDP-43-NCIs does not necessarily correspond to a similar abundance of TDP-O-NCIs (see Fig 1C).

ALS is a disease lying on a clinicopathologic spectrum with FTLD. In addition to the 5 cases of FTLD-TDP type B with ALS listed in Table 1, we also examined 5e cases of ALS with no clinical history of dementia. We found that 2 of these 5 cases had TDP-43 inclusions, and both cases had TDP-O-NCIs (see Fig 4C, D). In summary, among our series of 11 cases with ALS (see Table 1), 8 had TDP-43 proteinopathy (ALS-FTLD-TDP), and 5 of these 8 cases (62.5%) had TDP-O inclusions.

TDP-O Show Lysosome-Associated Granular and Tubular Ultrastructural Profiles in NCIs

We next examined TDP-43 oligomers in formalin-fixed, immunoperoxidase-labeled, Araldite-embedded tissue from FTLD-TDP type C cases using immunoelectron microscopy. TDP-43 oligomer immunoperoxidase labeling was first identified in the light microscope prior to electron microscopic study. At the EM level, light to moderate electron dense immunoperoxidase reaction products were found to be associated with neuronal lysosomes, consistent with the detection of NCIs at the light microscopic level (Fig 5). Serial EM section analysis from the same labeled neuron confirmed a cytoplasmic immunolabeling pattern. In some cases, nuclei of immunolabeled neurons appeared to be pushed in and oblong in shape as opposed to the normal rounded shape found in unlabeled (without NCIs) neurons. At higher magnification, electron dense immunoperoxidase reaction products were attached to granular or tubular structures of different sizes, which were approximately 250 to 500nm away from lysosomes, and no immunoperoxidase products were found in the lysosomes. One group of TDP-O–labeled structures appeared to be granular and measured approximately 200nm. Other TDP-O–immunolabeled elements appeared to also be tubular in different sizes with fiber tracts of tubules likely connecting to lysosomes. More diffusely labeled elements were also found in the cytoplasm.

Discussion

Using TDP-O, a well-characterized TDP-43 oligomer-specific antibody,[12] we provided the first line of evidence that TDP-43 forms oligomers in the human brain, constituting a part of abnormal TDP-43 inclusions identified in the brains of patients with FTLD-TDP and ALD/MND. This observation, together with our previous finding that TDP-O–immunoprecipitated spherical oligomers from human FTLD-TDP brains could be cross-recognized by anti–

TDP-43,[12] provides clear evidence supporting formation of amyloid oligomers by TDP-43 in the human brain. TDP-43 oligomers were preferentially present in FTLD-TDP, minimally present in brains of neurologically normal subjects, and absent in HS hippocampi despite concurrent abundance of TDP-43 inclusions. TDP-43 oligomers were particularly abundant in type C, suggesting a predilection toward oligomer formation in this FTLD-TDP subtype.

Currently the histomorphology-based neuropathological subtyping of FTLD-TDP affords relatively specific but not entirely consistent clinicopathological correlations. Type A is associated with progressive nonfluent aphasia and corticobasal syndrome, type B with MND, and type C with semantic dementia; behavioral variant FTD, however, can be associated with any pathological subtype.[20] Recently it was proposed that conformational changes of TDP-43 lead to the formation of multiple “strains” that could determine the clinicopathological phenotypes of FTLD-TDP and therefore conformational classification could be useful for more specific subtyping.[38] Our consistent findings of abundant TDP-O inclusions in FTLD-TDP type C suggest that a relatively uniform TDP-43 conformational strain determines the pathological manifestations of this subtype. In our series, all type C cases were sporadic, whereas 8 of 13 cases of other FTLD subtypes had a mutation (see Table 1). Therefore, it appears that TDP-O is a shared feature across sporadic and genetic forms of FTLD-TDP.

A sharp contrast can be made with HS, cases of which consistently showed no TDP-O despite having numerous concurrent TDP-43 inclusions. These results suggest a distinct, nonamyloid conformation of TDP-43 aggregates in HS, and perhaps a different pathophysiology. Therefore, our results could aid in further refinement of the classification scheme, which is instrumental for effective design of targeted therapy. Furthermore, it would be interesting to explore whether TDP-O in cerebrospinal fluid or blood samples might serve as a biomarker for FTLD-TDP, especially type C, in prospective studies.

Our results could also help address a long-standing concern about the rather frequent presence of TDP-43 inclusions, sometimes large in quantity, in many non–FTLD-TDP neurodegenerative disorders, such as HS, Alzheimer disease, dementia with Lewy bodies, and even diseases in the FTLD-tau group such as Pick disease, corticobasal degeneration, and progressive supranuclear palsy.[38] Because of the frequent “nonspecific” co-occurrence, one may question whether TDP-43 inclusions simply represent a nonspecific marker of neurode-generation. Although a comprehensive survey of all these disorders was not the goal of this study, our finding of the absence of TDP-O inclusions in HS samples supports the conformational heterogeneity of TDP-43 inclusions in different disorders, despite their morphological similarity. Moreover, our results suggest that different “strains” of TDP-43 aggregates may contribute to different neurodegenerative entities. For example, the NCIs seen in HS cases were found to be enriched in phosphorylated TDP-43,[37] and it could be speculated that the specific phosphorylation “strain” of TDP-43 in HS brains could prevent oligomerization and assume other conformations. Future studies should address whether the TDP-43 aggregates seen in other non–FTLD-TDP histopathological entities also lack oligomeric TDP-43.

Ultrastructurally, the TDP-O-NCIs appear to be composed of granular and tubular structures. A prior ultrastructural study using postembedding staining by anti-TDP-43 showed morphologically heterogeneous structures, mainly composed of packed straight filaments of slightly different diameters, variably associated with electron-dense granular material.[39] In contrast, our study did not find any TDP-O–decorated straight filaments, suggesting that the straight filaments in NCIs are not composed of TDP-43 oligomers or that they may mask the TDP-O epitopes. The ultrastructural localization of TDP-O–immunoreactive structures in the close vicinity of but not within lysosomal structures is somewhat similar to that reported for phosphorylated TDP-43–immunoreactive inclusions found in motor neurons.[40] This proximate association with neuronal lysosomes was consistent between individual neurons and rather frequent (see Fig 5); however, its significance awaits further studies. One intriguing possibility is that the TDP-43 oligomers may reside in lysosome-associated autophagosomal elements that are abnormal, showing ill-defined boundaries and seemingly failing to fuse with lysosomes.

The mechanism through which TDP-43 is involved in neuronal degeneration in the frontal, insular, and temporal lobes in FTLD-TDP remains unclear. Perhaps the most significant implication of our results is that FTLD-TDP may be characterized by TDP-43 oligomer formation-dependent neurotoxicity, a mechanism that has been well studied and conceived as a high-priority therapeutic target in several other neurodegenerative disorders.[19] Amyloid oligomers made of various amyloidogenic proteins were shown to be highly toxic, much more so than amyloid fibrils. Because TDP-43 oligomers are immunoreactive to A11,[12] an antibody recognizing common amyloid oligomer structures,[18] current experimental therapies targeting such structures could prove to be useful. Such therapies include active and passive immunotherapies and several small molecules.[41] Our results support pursuit of such therapies for types of TDP-43 proteinopathy that are enriched in TDP-43 oligomers, such as FTLD-TDP type C.

Acknowledgment

This work was supported by the NIH/NIA (AG 010129, P.F.K., C.D., L.-W.J.; AG 023501, W.W.S.; AG 19724, W.W.S.) and Consortium for Frontotemporal Dementia Research.

References

1. Arai T, Hasegawa M, Akiyama H, et al. TDP-43 is a component of ubiquitin-positive tau-negative inclusions in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. Biochem Biophys Res Commun. 2006; 351:602–611. [PubMed: 17084815]
2. Chen-Plotkin AS, Lee VM, Trojanowski JQ. TAR DNA-binding protein 43 in neurodegenerative disease. Nat Rev Neurol. 2010; 6:211–220. [PubMed: 20234357]
3. Arnold SE, Toledo JB, Appleby DH, et al. Comparative survey of the topographical distribution of signature molecular lesions in major neurodegenerative diseases. J Comp Neurol. 2013; 521:4339–4355. [PubMed: 23881776]
4. Neumann M, Sampathu DM, Kwong LK, et al. Ubiquitinated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. Science. 2006; 314:130–133. [PubMed: 17023659]
5. Wang IF, Wu LS, Chang HY, Shen CK. TDP-43, the signature protein of FTLD-U, is a neuronal activity-responsive factor. J Neurochem. 2008; 105:797–806. [PubMed: 18088371]
6. Wang IF, Wu LS, Shen CK. TDP-43: an emerging new player in neurodegenerative diseases. Trends Mol Med. 2008; 14:479–485. [PubMed: 18929508]

7. Buratti E, Baralle FE. Multiple roles of TDP-43 in gene expression, splicing regulation, and human disease. Front Biosci. 2008; 13:867–878. [PubMed: 17981595]
8. Fiesel FC, Kahle PJ. TDP-43 and FUS/TLS: cellular functions and implications for neurodegeneration. FEBS J. 2011; 278:3550–3568. [PubMed: 21777389]
9. Johnson BS, McCaffery JM, Lindquist S, Gitler AD. A yeast TDP-43 proteinopathy model: exploring the molecular determinants of TDP-43 aggregation and cellular toxicity. Proc Natl Acad Sci U S A. 2008; 105:6439–6444. [PubMed: 18434538]
10. Zhang YJ, Xu YF, Cook C, et al. Aberrant cleavage of TDP-43 enhances aggregation and cellular toxicity. Proc Natl Acad Sci U S A. 2009; 106:7607–7612. [PubMed: 19383787]
11. Lee EB, Lee VM, Trojanowski JQ. Gains or losses: molecular mechanisms of TDP43-mediated neurodegeneration. Nat Rev Neurosci. 2012; 13:38–50. [PubMed: 22127299]
12. Fang YS, Tsai KJ, Chang YJ, et al. Full-length TDP-43 forms toxic amyloid oligomers that are present in frontotemporal lobar dementia-TDP patients. Nat Commun. 2014; 5:4824. [PubMed: 25215604]
13. Shodai A, Morimura T, Ido A, et al. Aberrant assembly of RNA recognition motif 1 links to pathogenic conversion of TAR DNA-binding protein of 43 kDa (TDP-43). J Biol Chem. 2013; 288:14886–14905. [PubMed: 23558684]
14. Chang CK, Chiang MH, Toh EK, et al. Molecular mechanism of oxidation-induced TDP-43 RRM1 aggregation and loss of function. FEBS Lett. 2013; 587:575–582. [PubMed: 23384725]
15. Wang YT, Kuo PH, Chiang CH, et al. The truncated C-terminal RNA recognition motif of TDP-43 protein plays a key role in forming proteinaceous aggregates. J Biol Chem. 2013; 288:9049–9057. [PubMed: 23372158]
16. Kuo PH, Doudeva LG, Wang YT, et al. Structural insights into TDP-43 in nucleic-acid binding and domain interactions. Nucleic Acids Res. 2009; 37:1799–1808. [PubMed: 19174564]
17. Jiang LL, Che MX, Zhao J, et al. Structural transformation of the amyloidogenic core region of TDP-43 protein initiates its aggregation and cytoplasmic inclusion. J Biol Chem. 2013; 288:19614–19624. [PubMed: 23689371]
18. Kayed R, Head E, Thompson JL, et al. Common structure of soluble amyloid oligomers implies common mechanism of pathogenesis. Science. 2003; 300:486–489. [PubMed: 12702875]
19. Glabe CG. Common mechanisms of amyloid oligomer pathogenesis in degenerative disease. Neurobiol Aging. 2006; 27:570–575. [PubMed: 16481071]
20. Mackenzie IR, Neumann M, Baborie A, et al. A harmonized classification system for FTLD-TDP pathology. Acta Neuropathol. 2011; 122:111–113. [PubMed: 21644037]
21. Montine TJ, Phelps CH, Beach TG, et al. National Institute on Aging-Alzheimer's Association guidelines for the neuropathologic assessment of Alzheimer's disease: a practical approach. Acta Neuropathol. 2012; 123:1–11. [PubMed: 22101365]
22. Jagust WJ, Zheng L, Harvey DJ, et al. Neuropathological basis of magnetic resonance images in aging and dementia. Ann Neurol. 2008; 63:72–80. [PubMed: 18157909]
23. Mackenzie IR, Baborie A, Pickering-Brown S, et al. Heterogeneity of ubiquitin pathology in frontotemporal lobar degeneration: classification and relation to clinical phenotype. Acta Neuropathol. 2006; 112:539–549. [PubMed: 17021754]
24. Sampathu DM, Neumann M, Kwong LK, et al. Pathological heterogeneity of frontotemporal lobar degeneration with ubiquitin-positive inclusions delineated by ubiquitin immunohistochemistry and novel monoclonal antibodies. Am J Pathol. 2006; 169:1343–1352. [PubMed: 17003490]
25. Pletnikova O, Sloane KL, Renton AE, et al. Hippocampal sclerosis dementia with the C9ORF72 hexanucleotide repeat expansion. Neurobiol Aging. 2014; 35:2419.e17–2419.e21. [PubMed: 24819148]
26. Al-Sarraj S, King A, Troakes C, et al. p62 positive, TDP-43 negative, neuronal cytoplasmic and intranuclear inclusions in the cerebellum and hippocampus define the pathology of C9orf72-linked FTLD and MND/ALS. Acta Neuropathol. 2011; 122:691–702. [PubMed: 22101323]
27. de Carvalho M, Dengler R, Eisen A, et al. Electrodiagnostic criteria for diagnosis of ALS. Clin Neurophysiol. 2008; 119:497–503. [PubMed: 18164242]

28. Liu XB, Jones EG. Localization of alpha type II calcium calmodulin-dependent protein kinase at glutamatergic but not gamma-aminobutyric acid (GABAergic) synapses in thalamus and cerebral cortex. Proc Natl Acad Sci U S A. 1996; 93:7332–7336. [PubMed: 8692993]

29. Liu X, Jones EG. Alpha isoform of calcium-calmodulin dependent protein kinase II (CAM II kinase-alpha) restricted to excitatory synapses in the CA1 region of rat hippocampus. Neuroreport. 1997; 8:1475–1479. [PubMed: 9172157]

30. Josephs KA, Stroh A, Dugger B, Dickson DW. Evaluation of sub-cortical pathology and clinical correlations in FTLD-U subtypes. Acta Neuropathol. 2009; 118:349–358. [PubMed: 19455346]

31. Oskarsson B, Wheelock V, Benatar M, et al. A case of familial ALS due to multi-system proteinopathy 1 and Huntington disease. Amyotroph Lateral Scler Frontotemporal Degener. 2015; 16:124–126. [PubMed: 25205077]

32. Nakashima-Yasuda H, Uryu K, Robinson J, et al. Co-morbidity of TDP-43 proteinopathy in Lewy body related diseases. Acta Neuropathol. 2007; 114:221–229. [PubMed: 17653732]

33. Arnold SJ, Dugger BN, Beach TG. TDP-43 deposition in prospectively followed, cognitively normal elderly individuals: correlation with argyrophilic grains but not other concomitant pathologies. Acta Neuropathol. 2013; 126:51–57. [PubMed: 23604587]

34. Amador-Ortiz C, Lin WL, Ahmed Z, et al. TDP-43 immunoreactivity in hippocampal sclerosis and Alzheimer's disease. Ann Neurol. 2007; 61:435–445. [PubMed: 17469117]

35. Josephs KA, Murray ME, Whitwell JL, et al. Staging TDP-43 pathology in Alzheimer's disease. Acta Neuropathol. 2014; 127:441–450. [PubMed: 24240737]

36. Liu R, Yang G, Nonaka T, et al. Reducing TDP-43 aggregation does not prevent its cytotoxicity. Acta Neuropathol Commun. 2013; 1:49. [PubMed: 24252504]

37. Yokota O, Davidson Y, Bigio EH, et al. Phosphorylated TDP-43 pathology and hippocampal sclerosis in progressive supranuclear palsy. Acta Neuropathol. 2010; 120:55–66. [PubMed: 20512649]

38. Arai T. Significance and limitation of the pathological classification of TDP-43 proteinopathy. Neuropathology. 2014; 34:578–588. [PubMed: 25196969]

39. Lin WL, Dickson DW. Ultrastructural localization of TDP-43 in filamentous neuronal inclusions in various neurodegenerative diseases. Acta Neuropathol. 2008; 116:205–213. [PubMed: 18607609]

40. Matej R, Botond G, Laszlo L, et al. Increased neuronal Rab5 immunoreactive endosomes do not colocalize with TDP-43 in motor neuron disease. Exp Neurol. 2010; 225:133–139. [PubMed: 20558162]

41. Guerrero-Munoz MJ, Castillo-Carranza DL, Kayed R. Therapeutic approaches against common structural features of toxic oligomers shared by multiple amyloidogenic proteins. Biochem Pharmacol. 2014; 88:468–478. [PubMed: 24406245]

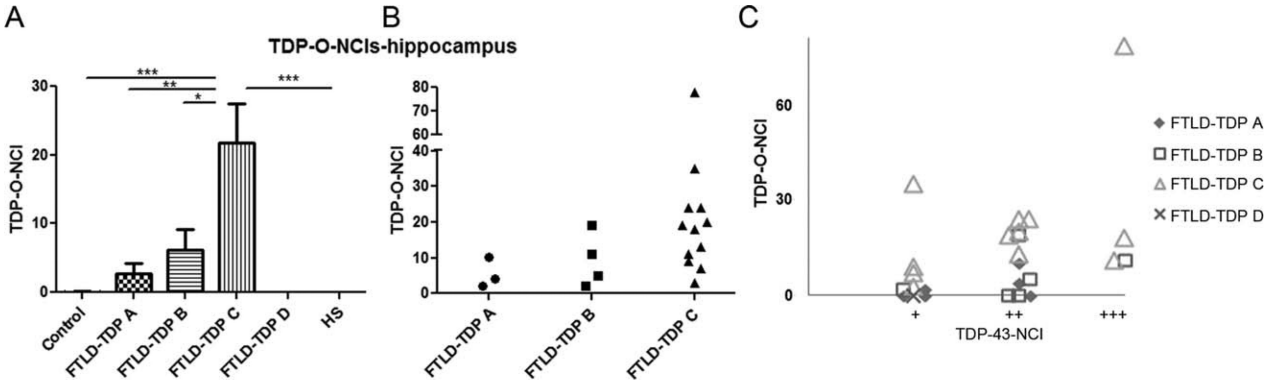

FIGURE 1.

Number of TDP-O neuronal cytoplasmic inclusions (NCIs) in the hippocampal dentate granule cells. Frontotemporal lobar degeneration (FTLD)-TDP type C cases had significantly more TDP-O-NCIs as compared to controls, FTLD-TDP type A, and FTLD-TDP type B (A). Of the 15 control cases analyzed, only 1 case had a rare finding of an NCI in the dentate layer. The single FTLD-TDP type D case had no TDP-O-NCIs and was excluded from the statistical analysis because n = 1. FTLD-TDP type C cases had significantly more NCIs than hippocampal sclerosis (HS) cases where no TDP-O-NCIs were found (A). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. (B) Number of TDO-O-NCIs in subtypes A to C for cases that showed presence of TDP-O-NCIs. (C) Distribution of TDP-O-NCIs counts stratified to the frequency of TDP-43-NCIs, where + indicates rare, ++ indicates moderate, and +++ indicates frequent TDP-43-NCIs in the dentate gyrus. The number of TDP-43-NCIs had no significant relationship to the number of TDP-O-NCIs.

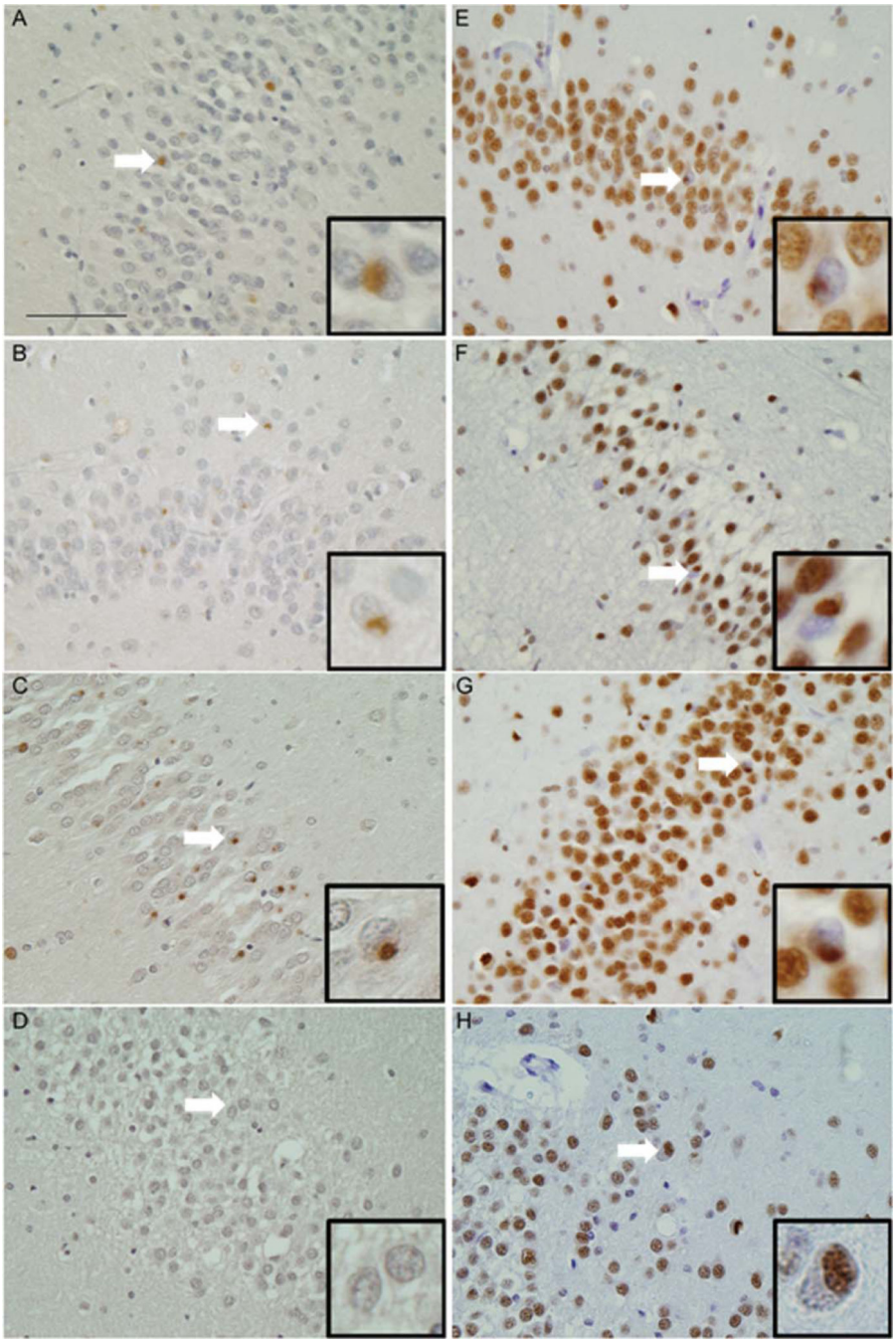

FIGURE 2.

Representative TDP-O and anti–TDP-43 images from hippocampal dentate gyrus of frontotemporal lobar degeneration (FTLD)-TDP type A, B, C, and D cases. Few TDP-O neuronal cytoplasmic inclusions (NCIs) were found in FTLD-TDP type A (A) and B (B) cases, and frequent inclusions were found in the dentate granule cells of FTLD-TDP type C (C) cases. The FTLDTDP type D case had no TDP-O-NCIs (D). The TDP-43 antibody stained native monomeric TDP-43 normally present in the nucleus and highlighted NCIs in FTLD-TDP types A, B, C, and D (E–H, respectively; see *insets*). In neurons with TDP-43

NCIs, nuclear staining was lost. Arrows point to cells that are highlighted in the magnified insets. The scale bar indicates 100μm for all photomicrographs.

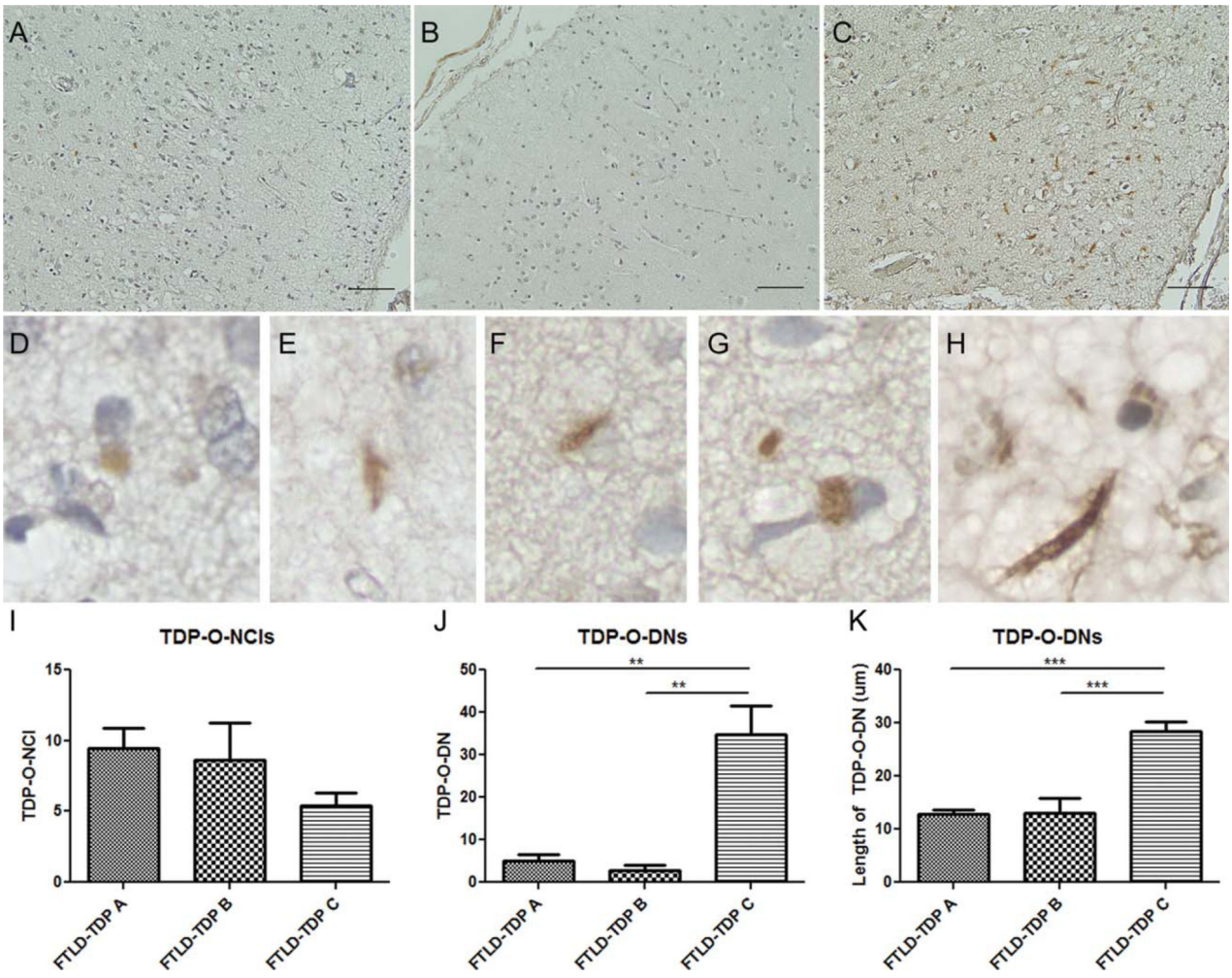

FIGURE 3.

Representative images from anterior orbital cortex of frontotemporal lobar degeneration (FTLD)-TDP type A, B, and C show few TDP-O neuronal cytoplasmic inclusions (NCIs) in all 3 types at low magnification (A–C). Occasional NCIs and short dystrophic neurites (DNs) can be found in cortical layers of FTLD-TDP type A (D, E) and FTLD-TDP type B (F, G) cases. Few NCIs and frequent, long DNs are found in FTLD-TDP type C (H). Scale bars indicate 100μm. The number of TDP-O-NCIs did not differ significantly between FTLD-TDP types A, B, and C (I), but FTLD-TDP C had significantly more (J) and longer (K) TDP-O-DNs as compared to FTLD-TDP type A and B cases. $^{**}p \leq 0.01$, $^{***}p \leq 0.001$.

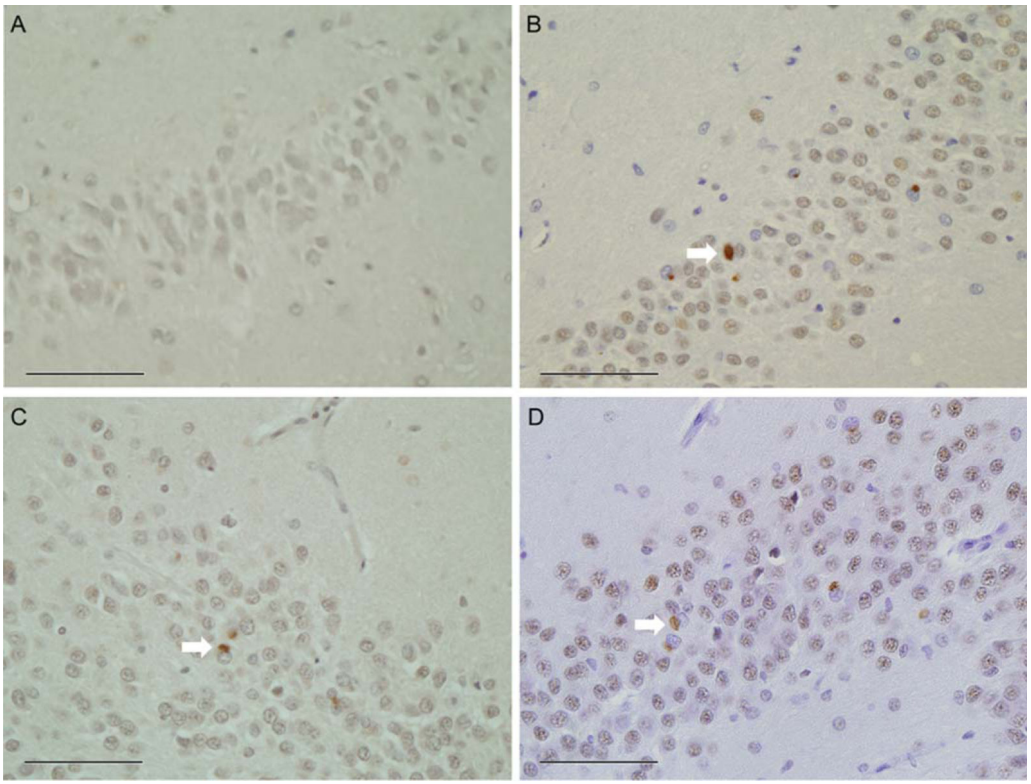

FIGURE 4.

Representative images of TDP-O (A and C) and TDP-43 (B and D) immunoreactive inclusions from the hippocampus dentate gyrus of hippocampal sclerosis (HS) and amyotrophic lateral sclerosis (ALS) cases. No TDP-O neuronal cytoplasmic inclusions (NCIs) were found in the dentate gyrus of HS cases (A), although NCIs were found with TDP-43 antibody (B; see *arrows*). Two of 5 ALS cases without dementia had TDP-O-NCIs (C) and TDP-43-NCIs (D; see *arrows*). Scale bars indicate 100 μm.

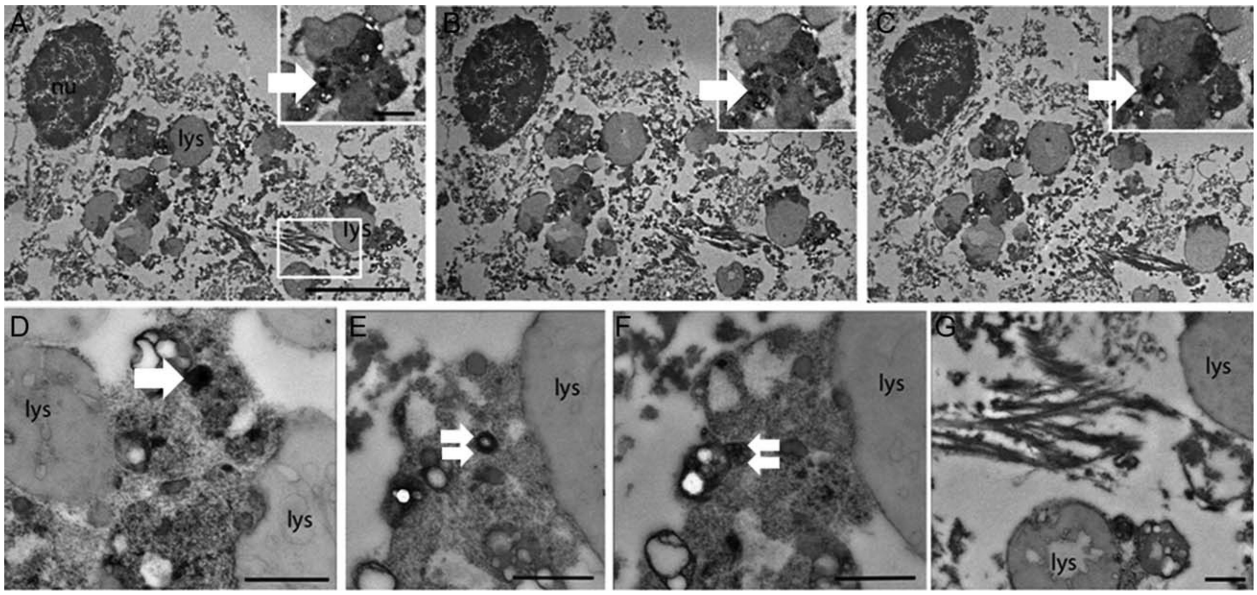

FIGURE 5.

Ultrastructural analysis of TDP-O immunoperoxidase labeled structures in frontotemporal lobar degeneration–TDP type C. Neuronal profiles were considered immunopositive when electron-dense immunoperoxidase reaction product could be detected in at least 3 serial sections, as shown in the insets at higher magnification (arrows in A–C). TDP-O–immunolabeled neurons show oblong nuclei (A–C). Higher magnification electron microscopic images show dense immunolabeled granular (*arrow* in D) and tubular structures (*double arrows* in E), localized in the cytoplasm and outside of lysosomes (D–G). Granular and tubular immunopositive structures are approximately 200nm in diameter and 250 to 500nm outside lysosomes (*double arrows* in E and F). (G) Enlargement of the outlined region in A, showing a longitudinal cut of similar tubular structures seen as a cross section in F (*double arrows*). Scale bars indicate 5μm (A–C), 1μm (*insets*, A–C), and 500nm (D–G). lys 5 lysosome; nu 5 nucleus.

TABLE 1

Summary of FTLD-TDP and ALS/MND-TDP Cases

Case ID	Sex	Age	PMI, h	Gene	TDP-43 DG NCIs	TDP-43 DG NIIs	Neuropath Diagnosis
F1	M	74	30.9	*GRN*	+	0	FTLD-TDP-A
F2	F	73	20.7	None	+	0	FTLD-TDP-A
F3	M	64	2.3	*C9ORF72*	++	0	FTLD-TDP-A; argyrophilic grain disease
F4	M	72	23.8	None	++	1	FTLD-TDP-A
F5	M	70	34.4	*C9ORF72*	++	0	FTLD-TDP-A
F6	F	66	7.4	*GRN*	+	0	FTLD-TDP-A
F7	M	68	7.5	*C9ORF72*	++	0	FTLD-TDP-B; ALS/MND
F8	M	71	11.5	*C9ORF72*	++	0	FTLD-TDP-B; ALS/MND
F9	F	62	42.3	None	+++	0	FTLD-TDP-B; ALS/MND
F10	M	66	12.1	None	++	0	FTLD-TDP-B; ALS/MND
F11	M	66	18.5	None	+	0	FTLD-TDP-B; ALS/MND
F12	M	48	21	*C9ORF72*	++	0	FTLD-TDP-B
F13	M	62	28	None	+	0	FTLD-TDP-C
F14	F	72	6.1	None	+++	0	FTLD-TDP-C
F15	F	76	17.1	None	+	0	FTLD-TDP-C
F16	M	65	14.3	None	++	0	FTLD-TDP-C
F17	F	69	10.7	None	++	0	FTLD-TDP-C
F18	M	74	3.3	None	++	0	FTLD-TDP-C
F19	M	70	13.2	None	++	0	FTLD-TDP-C
F20	M	66	10	None	+	0	FTLD-TDP-C
F21	F	71	7.3	None	++	0	FTLD-TDP-C
F22	F	72	13.5	None	+++	0	FTLD-TDP-C
F23	F	52	20	None	+	0	FTLD-TDP-C
F24	F	83	NA	None	+++	0	FTLD-TDP-C
F25	M	47	NA	*VCP*	+	0	FTLD-TDP-D; ALS/MND
A1	M	69	51	NA	+	0	ALS/MND-TDP subtype unspecified
A2	M	62	NA	NA	+	0	ALS/MND-TDP subtype unspecified

0 = none; + = rare; ++ = moderate; +++ = frequent.

ALS = amyotrophic lateral sclerosis; DG = dentate gyrus; F = female; FTLD = frontotemporal lobar degeneration; M = male; MND = motor neuron disease; NA = not available; NCI = neuronal cytoplasmic inclusion; NII = neuronal intranuclear inclusion; PMI = postmortem interval.

TABLE 2

Demographics and Hippocampal Dentate Gyrus Pathology

Characteristic/Pathology	Controls	FTLD-TDP A	FTLD-TDP B	FTLD-TDP C	FTLD-TDP D	HS	ALS/MND without Dementia
Mean age at death ± SD, yr	81 ± 9	70 ± 4	66 ± 3	69 ± 8	47	88 ± 6	61 ± 14
Sex, M:F	6:9	4:2	5:1	5:7	1:0	5:4	4:1
TDP-43-immunoreactive lesion cases, No. (%)	2/15 (13%)	6/6 (100%)	6/6 (100%)	12/12 (100%)	1/1 (100%)	8/9 (89%)	2/5 (40%)
TDP-43 oligomer immunoreactive lesion cases, No. (%)	1/15 (7%)	3/6 (50%)	4/6 (67%)	12/12 (100%)	0/1 (0%)	0/9 (0%)	2/5 (40%)

ALS = amyotrophic lateral sclerosis; F = female; FTLD = frontotemporal lobar degeneration; HS = hippocampal sclerosis; M = male; MND = motor neuron disease; SD = standard deviation.