CORRESPONDENCE

Revisiting the utility of TDP-43 immunoreactive (TDP-43-ir) pathology to classify FTLD-TDP subtypes

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Until 2006, the term "frontotemporal lobar degeneration with ubiquitinated inclusions (FTLD-U)" referred to a classification system with subtypes based on the distribution of neuronal cytoplasmic inclusions (NCIs), dystrophic neurites (DNs), and neuronal intranuclear inclusions (NIIs), which were immunoreactive to ubiquitin, and tau- and alpha-synuclein negative [1]. In 2006, the 43-kDa TAR DNA-binding protein (TDP-43) was identified as the elemental ubiquitinated protein in FTLD-U, and soon thereafter, the harmonized classification of FTLD-TDP was derived, giving rise to four subtypes (A, B, C, and D) [3, 4]. The term "FTLD-U" was thus replaced with "FTLD-TDP" because cases positive for TDP-43 could be identified based on TDP-43 immunoreactivity (TDP-43-ir). The most obvious challenge to the present FTLD-TDP-based vs. the past FTLD-U-based classification system is that immunohistochemistry for TDP-43 identifies pathologic patterns that are somewhat different

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from immunohistochemistry for ubiquitin. In an effort to refine the pathologic criteria for subtyping FTLD cases, Mackenzie and Neumann recently re-evaluated previously classified FTLD-U cases in a series of 78 cases according to TDP-43 immunochemistry [2]. They found that 81% of their cases fit into either the FTLD-U A, B, or C subtype, while the remaining 19% had features of combined subtypes. The authors concluded with proposed refinements to the subtyping criteria.

Using the methodology described in Mackenzie and Neumann [2], we re-evaluated 89 cases from the Northwestern Alzheimer's Disease Center autopsy collection with a primary pathologic diagnosis of FTLD-TDP. The major differences to methodology include the evaluation of the dentate gyrus of the hippocampus in FTLD-TDP subtype B, and white matter pathology in the middle frontal region in all cases. All cases were initially subtyped using TDP-43 staining. The goal, like Mackenzie and Neumann [2], was to determine whether using TDP-43-ir immunohistochemistry could help assign a subtype to "difficult-to-classify" cases, thus honing pathologic diagnosis. We also aimed to clarify the relationship between subtypes and genetic mutations leading to FTLD. Our cohort included 51 cases that fulfilled pathologic criteria for amyotrophic lateral sclerosis (ALS) or primary lateral sclerosis, and 36 with a pathogenic mutation or variant in a known FTLD-TDP-causing gene (18 *C9orf72*, 12 *GRN*, 2 *TMEM106B* variants, 1 *TIA1* mutation, and 1 *TBK1* mutation). Scoring of NCIs were compared between frontal cortex and hippocampal dentate using Fisher's exact test. Upper versus deeper layers were compared using the Wilcoxon signed rank test. Subgroups by mutation (*GRN*, *C9orf72*) were compared independently using the Mann–Whitney test.

In our series of 89 cases, 96% were easily classified as having one of the FTLD-TDP subtypes, with 33% ($N=29$) being type A, 52% type B ($N=46$), and 10% type C ($N=9$).

The remaining five cases were interpreted as having combined pathologic features of two FTLD-TDP subtypes or a variant of a particular subtype. No subtype shift occurred in our cases using the refined criteria from Mackenzie and Neumann [2]. Out of 51 ALS cases, 4 showed type A, 41 showed type B, 1 showed type C, and 5 were considered to be not easily classifiable variants (one type B variant with NIIs, one a combination of types B and C pathology, one type C variant with NIIs, and two type B variants with long DNs).

FTLD-TDP type A. According to Mackenzie and Neumann [2], the features of type A cases were: "(1) concentration of pathology in layer II, with (2) moderate or abundant compact NCI in layer II, (3) moderate or abundant short DN, (4) at least some NII". Predominant thread pathology in white matter was also apparent in a number of our cases. Compared to the Mackenzie and Neumann [2] study, our cases showed fewer delicate wispy threads and dots in layer II and deep cortical layers. Cases with *GRN* mutations ($N = 12$) showed FTLD-TDP type A. Compact NCI and short DN were more abundant in upper versus deeper layers ($p < 0.05$) in cases with *GRN* ($N = 12$), and granular NCI were more prominent in the upper versus deeper cortical layers ($p < 0.05$) in cases without *GRN* ($N = 18$). Cases with *C9orf72* ($N = 5$) showed more abundant short DN in upper and deeper layers ($p < 0.05$) (Fig. 1a, b, b1). In addition, cases with mutations of *TBK1* or *TMEM106B* all showed FTLD-TDP type A pathology.

FTLD-TDP type B. Our evaluation distinguished between two "variants" of type B pathology ($N = 46$). The typical pattern of "type B1" cases included (1) NCI that were equally compact and granular, (2) no DN, and (3) prominent oligodendrocyte TDP-43 pathology in white matter as a supportive feature. "Type B2" cases showed (1) moderate to frequent NCI that was predominantly granular, (2) rare/few short DN, and (3) the presence of delicate wispy threads and dots, and prominent oligodendrocyte pathology in white matter as a supportive feature. Type B2 cases with "high" cortical NCI tended to show moderate to numerous TDP-43-ir NCI in the hippocampal dentate gyrus ($p < 0.01$), a unique pathologic feature that was not apparent in other FTLD-TDP subtypes. See Fig. 1c–f. There were no apparent

Fig. 1 *TDP-43 immmunoreactive pathology in FTLD-TDP subtypes.* FTLD-TDP Type A cases demonstrated prominent pathology in upper layers with moderate to frequent neuronal cytoplasmic inclusions (NCI) and short dystrophic neurites (DN), and scattered lentiform neuronal intranuclear inclusions (inset) (**a**, **b**), in addition to apparent thread pathology in white matter (**b1**). Type B1 had rare/few granular NCI in the cortical layers (**c**) and in hippocampal dentate gyrus (**d**). Type B2 had moderate to frequent granular NCI in cortical layers (**e**) and in hippocampal dentate gyrus (**f**). Type C cases showed moderate to frequent long thick DN involving all the layers of the cortex with no obvious NCI (**g**, **h**). **a–h** Immunohistochemistry with anti-phospho TDP-43 antibody. Scale bar **a–f** 100 μm, **g**, **h** 50 μm, **a** inset 13 μm
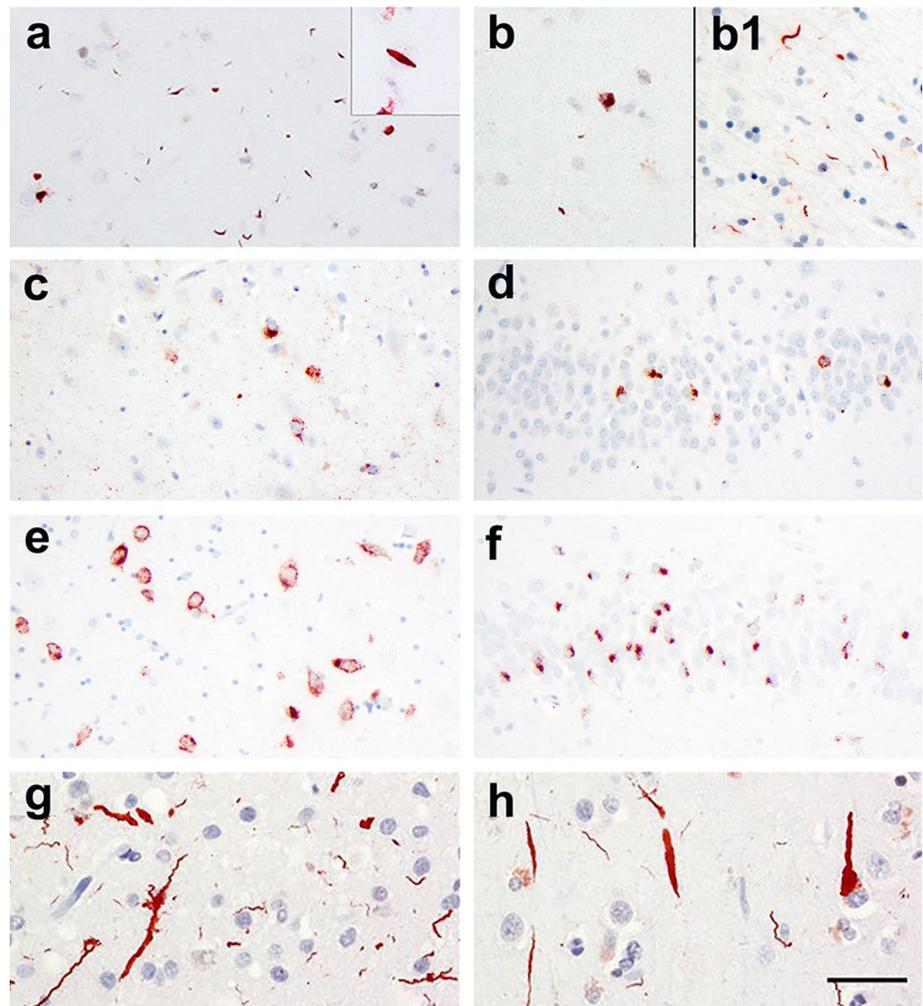
differences between patterns of TDP-43 pathology in cases with and without *C9orf72* in either type B variants. Sub-classifying type B cases into those with rare/few (B1) or moderate/numerous (B2) TDP-43-positive NCI reflects an interesting neuropathologic finding, warranting future clinicopathologic investigation.

FTLD-TDP type C. The type C cases in our cohort showed virtually the same pathology reported in Mackenzie and Neumann [2]. The features of type C cases with pTDP-43 IHC were: (1) moderate or numerous short DN and long DN throughout the cortex, and (2) few NCI that were predominantly compact. See Fig. 1g, h.

Cases with C9orf72 mutations. Eighty percent of Mackenzie and Neumann's [2] unclassifiable cases had *C9orf72* mutations, and these showed combined types A and B. Of the 18 cases in our cohort with a *C9orf72* mutation, all but two showed predominantly typical type A ($N = 5$) or typical type B ($N = 11$) histopathology. One case with *C9orf72* appeared to be a variant of type B1 with NII, which some might consider to be combined types A and B1, and the other showed features of combined types B and C.

Our findings suggest that the majority of cases previously classified under the old ubiquitin-based criteria (FTLD-U) can be readily re-subtyped with criteria that are based on TDP-43-ir. We emphasize that only a minority (5 of 89 cases) still remain difficult to classify, and that these cases are not necessarily ones that show genetic abnormalities.

Our findings support Mackenzie and Neumann's [2] reappraisal of FTLD-TDP pathology. Collectively, these studies can enhance diagnostic accuracy, and can ultimately lead to a consensus decision on the classification of cases with FTLD-TDP.

References

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