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Inclusions in frontotemporal lobar degeneration with TDP-43 proteinopathy (FTLD-TDP) and amyotrophic lateral sclerosis (ALS), but not FTLD with FUS proteinopathy (FTLD-FUS), have properties of amyloid

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TDP-43 and FUS normal cytoplasmic functions are thought to involve regulated aggregation and disaggregation [1, 5, 8], similar to that of prion proteins, where aggregation occurs by a self-templating process, likely involving properties of amyloid, or beta pleated sheet structure. Both thioflavin-T and thioflavin-S fluoresce when bound to amyloid fibrils [7]. We previously showed that TDP-43 peptides form amyloidogenic fibrils, binding to thioflavin-T [3]. Recently, TDP-43-positive lower motor neuron (LMN) inclusions in 28% of 47 cases of ALS, but no inclusions in 22 FTLD-TDP cases, were shown to be positive with thioflavin-S [6]. Using thioflavin-S, we surveyed brain tissues from 44 cases, including FTLD-TDP type A, (17 cases), type B (14 cases), type C (3 cases), sporadic ALS (2 cases) familial ALS (FALS) (2 each with *SOD1* and *C9orf72* mutations), and FTLD-FUS, including atypical FTLD-U (aFTLD-U, 2 cases) and basophilic inclusion body disease (BIBD, 2 cases). Our routine modified thioflavin-S stain includes pretreatment of tissue sections with potassium permanganate and bleaching with potassium metabisulfite and oxalic acid followed by treatment with sodium hydroxide and hydrogen peroxide, removing lipid autofluorescence and resulting in improved definition of pathological lesions. Slides were viewed using a Nikon BV-2A filter cube. Confocal microscopy was performed using thioflavin-S staining as above and phospho TDP43 (pS409/410-1). Inclusions in most cases of FTLD-TDP and ALS were thioflavin-S positive. The density and distribution of thioflavin-S positive inclusions was similar to that seen with ubiquitin and fewer than with TDP-43 immunohistochemistry. In FTLD-TDP type A, neuronal intranuclear inclusions

(NIIs), neuronal cytoplasmic inclusions (NCIs) and dystrophic neurites (DNs) were strongly fluorescent (Fig. 1a). Fluorescent confocal microscopy showed co-localization of thioflavin-S with TDP-43 positive inclusions (Fig. 1b). In FTLD-TDP type B, rare NCIs in all layers of cortex were positive but dentate gyrus inclusions were easily seen (Fig. 1c). In FTLD-TDP type C, long DNs in cortex were strongly thioflavin-S positive (Fig. 1d). Inclusions in FTLD-FUS were negative with thioflavin-S; while rare hippocampal dentate gyrus neurons had fluorescent granular inclusions, large cytoplasmic inclusions and intranuclear vermiform inclusions were not seen with thioflavin-S (Fig. 2). Skein-like inclusions (SLI) and Lewy-like bodies (LLB) in LMNs of ALS cases were positive with thioflavin-S (Fig. 3). The two cases of FALS with *SOD1* mutations were not thioflavin-S positive. Additionally, eight of the 17 cases of FTLD-TDP type A had exuberant thioflavin-S positive astrocytosis, making identification of TDP-43 pathology problematic in five of these (Fig. 4).

There are at least three possible reasons that these results differ from those of Robinson et al. The modified thioflavin-S protocol used in the current study provides results that are superior to other thioflavin-S protocols, the BV-2A filter produces the brightest images, and the brain tissue is briefly paraformaldehyde-fixed (30 hours). Fixation does not likely impact results, but we cannot exclude that possibility.

Clearly more cases need to be analyzed with thioflavin-S, and that work is in progress. Nonetheless, it is important that thus far, inclusions in most cases of FTLD-TDP, but not FTLD-FUS or *SOD1* FALS, are thioflavin-S positive, suggesting that the prion-like property of TDP-43 may be involved in FTLD-TDP and sporadic ALS and non-*SOD1* FALS, but that this may not be the case in FTLD-FUS. The significance of the thioflavin-S positive astrocytosis in some cases of FTLD-TDP type A is currently under investigation. In addition, the fact that TDP-43 in FTLD-TDP has properties of amyloid has implications for the interpretation of amyloid imaging studies [2], as Pittsburgh Compound-B is a modified thioflavin-T derivative [4].

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Fig. 1. Superficial frontal cortex, FTLD-TDP type A (1a). Numerous thioflavin-S positive c-shaped and annular NCIs and a few DNs. Inset: thioflavin-S positive NII. Thioflavin-S, 400x. Confocal microscopy (1b). Same case as Fig. 1a, with thioflavin-S (green, left), TDP-43 (red, middle), and overlay (yellow, right) showing co-localization in NCIs (arrows). Fluorescent confocal microscopy, thioflavin-S and TDP-43 immunohistochemistry. Frontal cortex (left) and dentate gyrus, FTLD-MND type B (1c). Granular thioflavin-S positive cortical CIs. Dentate gyrus CIs are both granular (top) and dense, c-shaped/perinuclear (middle). Rare NIIs were seen in type B cases (bottom) in only one case. Thioflavin-S, 600x. Frontal cortex, FTLD-TDP type C (1d). Numerous thioflavin-S positive thick, long DNs in cortex. Inset: several dense CIs, dentate gyrus. Thioflavin-S, 400x.

Fig. 2. Motor cortex, FTLD-FUS (BIBD) (2a). Large cytoplasmic upper motor neuron inclusion on left, consistent with basophilic inclusion body (BIB), negative with thioflavin-S. BIB on right immunolabeled with FUS for comparison. Thioflavin-S stain and FUS immunohistochemistry, 600x. Granular NCI in dentate gyrus, FTLD-FUS (aFTLD-U) (2b). Thioflavin-S, 600x.

Fig. 3. Lumbar anterior horn, ALS. Lower motor neurons with LLB (left) and SLIs (middle and right). Scattered shrunken neurons nearby (middle and right images) contain compressed SLIs. Thioflavin-S, 600x.

Fig. 4.
Superficial frontal cortex, FTL-D-TDP type A. Exuberant thioflavin-S positive astrocytosis.
Thioflavin-S, 200x.