COMPARISON OF PENTAMERIC FORMYL THIOPHENE ACETIC ACID (pFTAA) STAINING IN HUMANS AND ANIMAL MODELS OF DISEASE

PsychoGenics
Transforming CNS Discovery

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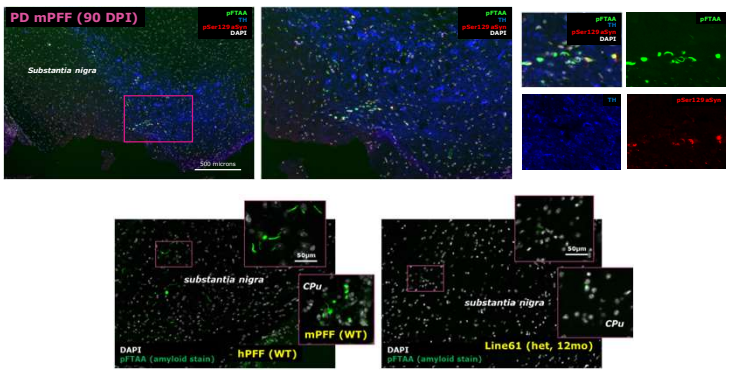
ABSTRACT

Histological detection of amyloids in human and mouse brain tissue is complex. Fibril structures and aggregation lead to a reduction of relevant epitopes for primary antibody detection and some amyloids remain undetected in histology samples because of the lack of antibody binding. Binding sites may become internalized within fibrillary aggregates, others become truncated, modified (e.g. phosphorylation, acetylation), and standard histological tissue processing affect binding sites, especially fixation procedures. pFTAA was published to bind to a plethora of diverse amyloids and prions. While commercially available, pFTAA is sparsely used in clinical or preclinical detection. It has many advantages compared to Thioflavin S or Congo including no binding on natural beta-sheet structures, higher sensitivity and specificity, stronger signal, high stability and very low toxicity.

METHODS

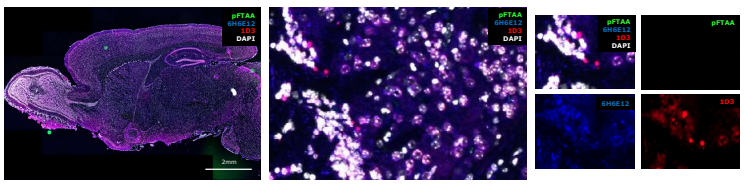
Histological sections from standard blocks (FFPE and Fixed or Fresh Frozen) in human Frontotemporal Dementia (FTD) patients thankfully provided by VectorY Therapeutics and mouse models to AD, PD, Tauopathy and ALS (SODG93A, rNLS8, APP/PS1, rTg4510, hPFF and mPFF, Line61) were stained with pFTAA in combination with typical co-markers of diverse pathologies and imaged on a Zeiss Axio.Scan Z1 slide scanner.

Parkinson's Disease Models (PD)

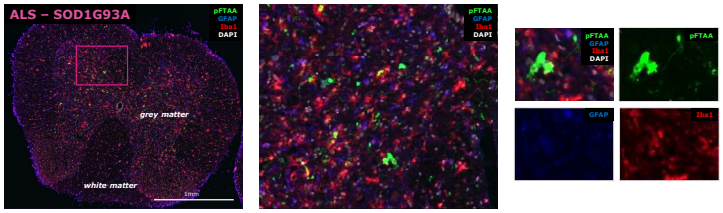


Upper panel: pFTAA staining (green) together with Tyrosine hydroxylase (TH) and pSer129 alpha-synuclein (pSer129 αSyn) immunolabeling on the substantia nigra of mPFF inoculation mouse model. pFTAA proves amyloid conformation of aggregates spreading after PFF exposure, as well as that not all of the amyloids are pSer129 αSyn positive. Lower panel compares pFTAA staining after hPFF inoculation with a standard PD model, the Line61 mice (Thy1-haSyn). While PFF inoculation clearly leads to amyloid formation, the known aggregates in Line 61 are vastly not amyloid.

Amyotrophic lateral Sclerosis (ALS) – pTDP-43 rNLS8 model



Amyotrophic lateral Sclerosis (ALS) – SOD1 (G93A HI) model

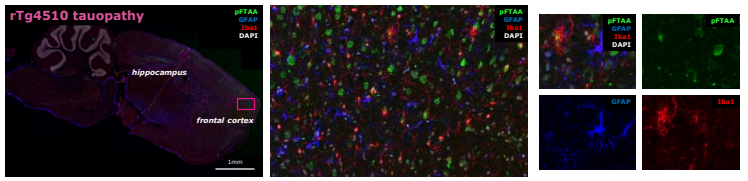


pFTAA staining in animal models of ALS, upper panel in rNLS8 mice (4 weeks off Dox) with pTDP-43 positive inclusions in the striatum, lower panel SOD1G93A mice (16 weeks) with significant SOD1 amyloid aggregates within diverse cell populations, including GFAP+ astrocytes and Iba1 microglia in the lumbar spinal cord. Notably pFTAA is the most reliable marker for SOD1 aggregates in fixed tissues, since commercially available standard antibodies do not bind well or at all to the aggregate formations (exception C4F6 in unfixed tissue).

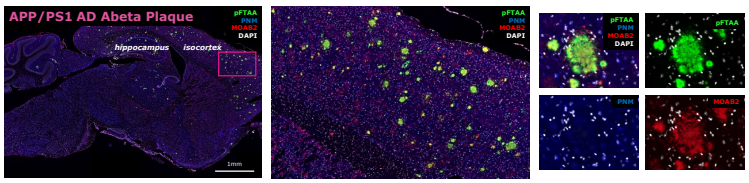
REFERENCES:

- In vivo detection of tau fibrils and amyloid β aggregates with luminescent conjugated oligothiophenes and multiphoton microscopy (Calvo-Rodriguez et al, 2019)
- pFTAA is a high affinity fluorescent oligothiophene probe that labels filamentous tau in neurons (Brelstaff et al, 2015)
- Prominent microglial inclusions in transgenic mouse models of α-synucleinopathy that are distinct from neuronal lesions (Tanrioever et al, 2020)

Alzheimer's Disease (AD) – Tauopathy rTg4510 model

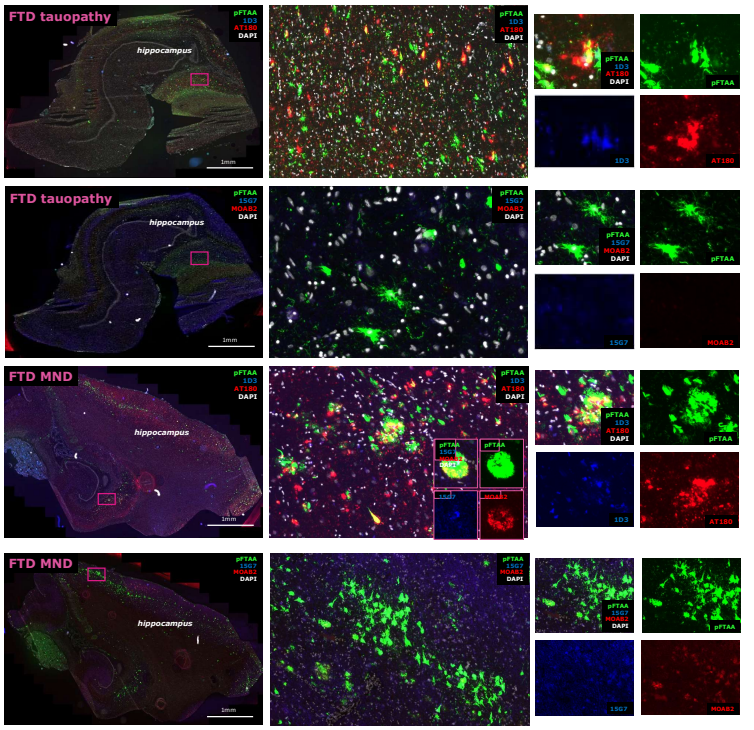


Alzheimer's Disease (AD) – Abeta APP/PS1 model



pFTAA staining in AD mouse models, upper panel a model of Tauopathy (rTg4510), lower panel and Abeta amyloid model (APP/PS1). pFTAA very well depicts neurofibrillary tangle formation with the advantage over ThioS to additionally show amyloid neuropil threads. Abeta amyloids are bound in the vast majority of all forms, thus pFTAA reliably stain diffuse and mature plaque formations, whereas there is still some non-amyloid component in new deposited Abeta in the penumbra of plaques.

Human Mixed Pathologies - FTD (Tau and MND)



pFTAA staining in samples of human FTD with Tauopathy and FTD with motor neuron disease (MND): Images show high load of diverse amyloids. Counterstained for pTau (AT180), Abeta (MOAB2), human alpha-synuclein (15G7) and pTDP-43 (1D3). Both cases show mixed amyloids pathologies in the hippocampus and above all additional amyloids that were not overlapping with classical disease markers.

SUMMARY

- pFTAA reliably detects Abeta plaques, neurofibrillary tangles, pTDP-43 inclusions and SOD1 aggregates, as well as human and mouse fibrillary alpha-synuclein inclusions in animal model and human tissue. The amount of specific amyloids varies across the models with some models showing little to no formation of significant amounts of mature amyloids, such as the Line61 or rNLS8 mice.
- pFTAA has the potential to be the easiest to use and most universal detector for the majority of fibrillary amyloid aggregates. Staining is clear and distinct in samples prepared using standard methods. Here we show the detection both of mixed human pathologies and compare this to prominent preclinical models of amyloid diseases. Notably especially in human it detects additional amyloids that are not identified by diverse standard antibodies for disease classification.