

CUREPSP

Final Scientific Progress Report

Tau in Peripheral Tissues of PSP and CBD. Brittany Dugger, PhD; University of California San Francisco

Specific Aim: Using immunohistochemical methods on autopsy tissue, we surveyed the presence of tau deposits in biopsy accessible peripheral sites (abdominal skin, colon, and submandibular gland) of subjects with PSP and CBD compared to clinically normal elderly individuals (controls).

Background and Significance:

Determining if tau deposits can be detected in peripheral tissues in PSP and CBD will provide the groundwork for a biomarker to improve the clinical diagnostic accuracy of PSP and CBD, as well as providing new insights into disease manifestations. This is critical since many PSP and CBD cases can be underreported and/or misdiagnosed during life. Our group and others have demonstrated that many elderly individuals who have had autopsy confirmed PSP do not present with the characteristic PSP clinical signs and symptoms- many of which can be misdiagnosed as Parkinson's disease (PD) during life.^{1,2} This comes as no surprise since reports of the diagnostic accuracy of PSP have ranged from 41% to 88%.³⁻⁶ With CBD, even at the last visit before death the sensitivity of a clinical diagnosis is 48%.⁷ Although there is ongoing work done in other neurodegenerative diseases with respect to peripheral biomarkers to improve clinical diagnostic accuracy, such as the extent of α -synuclein in peripheral organs of PD,^{8,9} no one to date has pursued similar techniques to investigate the potential of a biomarker for PSP and CBD through examination of tau pathology in peripheral tissues.

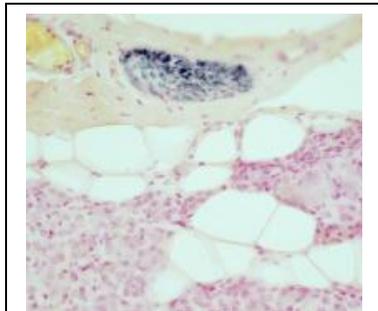


Fig. 1. T231 immunoreactive nerve fibers in a stromal nerve fascicle in the submandibular gland of a CBD case at 20x.

A handful of studies have examined tau deposition outside the brain in PSP and CBD, but these have mainly been limited to the spinal cord.¹⁰⁻¹³ We are aware of no published studies examining tau species in peripheral tissues such as skin, submandibular gland, and colon of PSP and CBD cases. One extensive study having 8 CBD spinal cords, revealed widespread and extensive neuropil threads in the grey matter with a rostral-caudal density gradient.¹¹ With respect to PSP, an examination of 10 spinal cords revealed neuropil threads and cytoplasmic inclusions particularly in the cervical gray matter; also shown was tract degeneration, and atrophy and myelin pallor of the anterior and anterolateral funiculus.¹³ In both CBD and PSP spinal cords, tau

deposits were shown with Gallyas and AT8 tau immunostaining, and were not easily detected by Bodian silver staining, suggesting the main composition of spinal cord cytoplasmic inclusions are immature pre-tangles. We recently conducted a small pilot study examining the distribution of tau deposits in spinal cords of 3 CBD, 10 PSP, and 37 controls, utilizing the AT8 antibody. We found similar results to published studies. All PSP cases examined contained spinal cord tau deposition, mainly in ventral regions having phosphorylated tau deposits in neuropil threads and neurofibrillary tangles within motor neurons; this was a significantly greater frequency than that found within controls. With respect to CBD, phosphorylated tau deposits in the form of neuropil threads, and neurofibrillary tangles were diffusely scattered within the grey matter of all regions of the spinal cord- with densities ranging from moderate to severe but all 3 cases had

far greater densities than that found in PSP and in controls. As the spinal cord innervates all peripheral organs through autonomic and somatosensory nerve fibers, and previous studies, including ones from our laboratory have demonstrated other such aggregated proteins in organs other than brain,^{8, 9, 14, 15} **we hypothesize that tau deposits exist within PSP and CBD peripheral tissues.**

Table 1. Demographics of series analyzed. PMI=postmortem interval. Age at death and PMI are listed as mean \pm standard deviation.

	NC	PSP	CBD
N	19	26	3
M:F	12:7	18:8	2:1
age at death (yrs)	77 \pm 15.8	84 \pm 10.2	73 \pm 0.58
PMI (hrs)	3 \pm 2.3	3 \pm 0.87	4 \pm 1.1
concomitant AD, N	n/a	8	1
concomitant PD, N	n/a	5	1

Additionally, studies indicate that such peripheral nervous system spread is possible with pathological protein deposits, including tau.¹⁵ Also, given there are differences in spinal cord pathologies between PSP and CBD, we suspect that this difference may be translatable to peripheral deposits of tau. Preliminary IHC staining for T231 (detecting tau phosphorylated on threonine 231) in the submandibular gland of a CBD case reveals phosphorylated tau can be detected in nerve elements located within peripheral tissues (Fig. 1). In this study, we extended these preliminary findings and identified different tau species in peripheral areas.

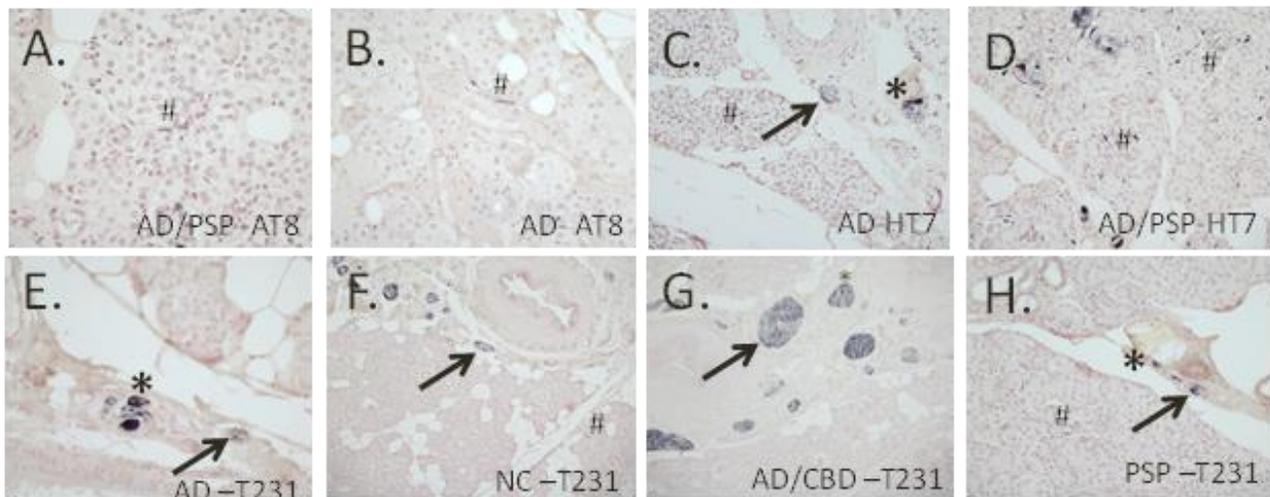


Figure 2. Representative tau staining of submandibular glands of a variety of cases (AD=Alzheimer's disease, PSP= progressive supranuclear palsy ; AD/PSP= AD with a concomitant diagnosis of PSP; NC= normal control; AD/CBD= AD with a concomitant diagnosis of CBD. Stromal nerve fascicles (arrows) ganglion cells (*) and fine threads interspersed among acini (#) were immunoreactive for select tau antibodies- AT8, HT7, and T231.

Summary of Progress:

We have completed tissue processing, staining, and initial analysis of abdominal skin, submandibular gland, and colon on 26 PSP, 3 CBD and 19 normal control cases (Table 1) as well as 8 AD/PSP cases. These tissues were subject to immunohistochemistry for certain tau species: AT8 (recognizing tau phosphorylated at serine residues 202 and 205), T231 (recognizing tau phosphorylated at threonine residue 231) and HT7 (recognizing human tau between residues 159 and 163).

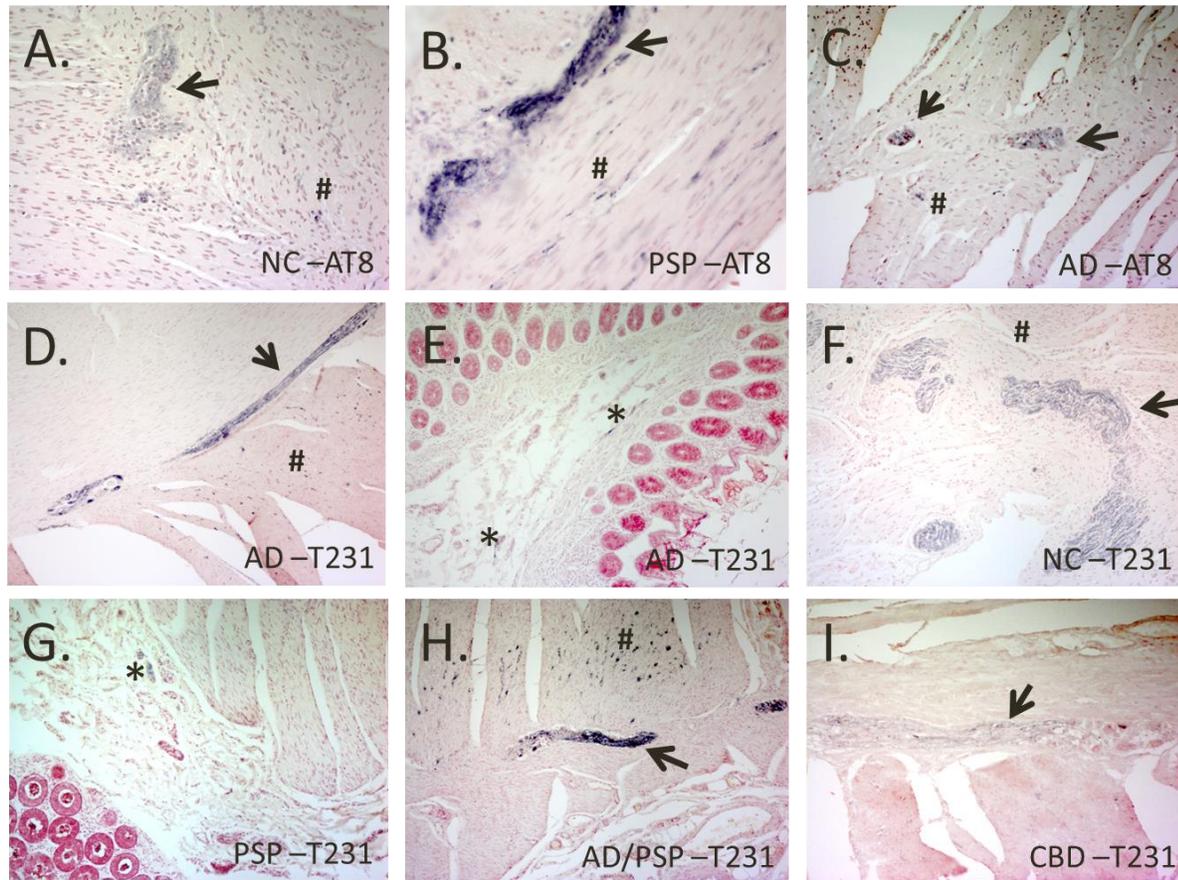
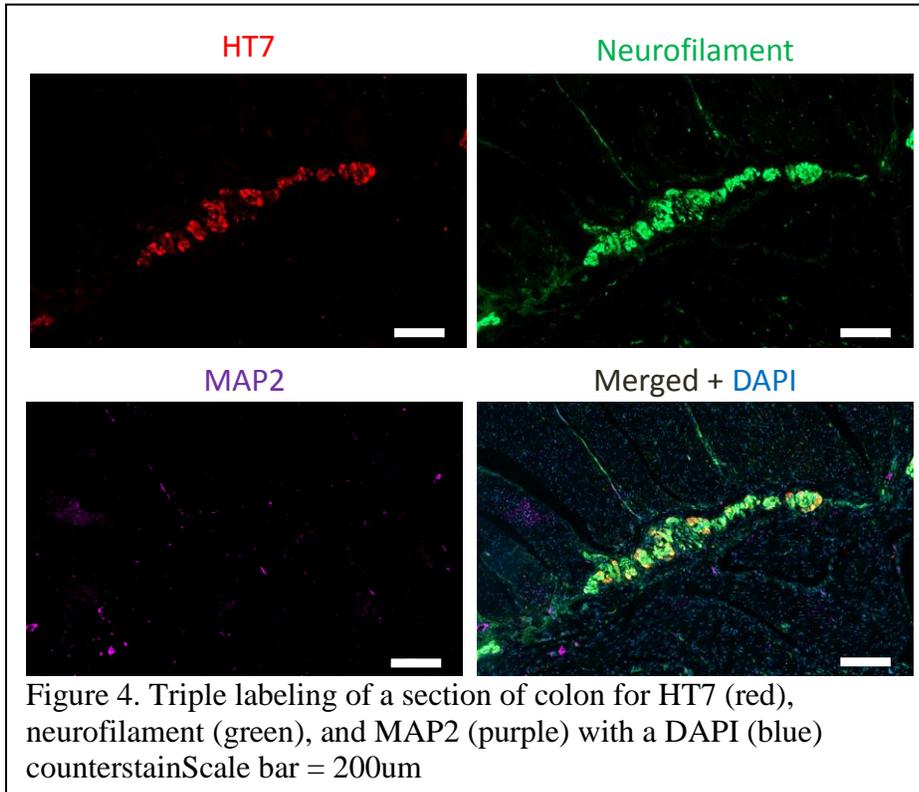


Figure 3 . Phosphorylated tau immunoreactivity within the sigmoid colon. Photos A,C taken at 20x, D-H taken at 10x and B and I taken at 40x magnification. Tau immunoreactivity was found within the myenteric plexus (arrows), submucosa (*) and Muscularis (#) among all groups.

HT7 immunohistochemistry of abdominal skin revealed immunoreactivity of potential nerve elements in 7% of PSP cases, 13% AD/PSP, 0% NC, and only 1 CBD case; skin sections lacked AT8 and pT231 immunoreactive nerve elements. Submandibular glands (representative images in Fig. 2) from all cases were immunoreactive for HT7; while pT231 was present in 86% of PSP, 100% of AD/PSP, NC, and CBD cases. AT8 was present within 53% of NC, 33% of PSP, and 100% within AD/PSP and CBD (there were no statistical significant difference among groups). In sigmoid colon (representative images in Fig. 3), HT7 immunoreactivity was present in disease cases and all but 2 NC cases, pT231 44% of NC, 69% of PSP, and 63% of AD/PSP. Only 1 CBD case was analyzed and it contained HT7 immunoreactivity. AT8 was present within the sigmoid colon of 17% of NC, 14% of PSP, and 13% of AD/PSP. No particular tissue contained tau immunoreactivity in one disease.

Co-localization studies completed on samples which were positive for the above tau antibodies with select neuronal markers (MAP2 and Neurofilament) and certain neurotransmitter markers (tyrosine hydroxylase and choline acetyl transferase) provided further evidence of the neuronal origin of tau within these peripheral tissues. Figure 4 shows an example of MAP2 (Cat # ab7795 from abcam; in purple), neurofilament (Cat # n4142 from sigma, in green) and HT7 (red) within the sigmoid colon.



These data support a concept of tau species anatomic variability and a notion that certain tau species, known to be pathogenic within brain, may not be pathogenic within the periphery. Determining the presence of tau species in peripheral tissues is critical since it can provide new insights into disease manifestations, serve to aid in development of better model systems, and potentially provide insights into adverse peripheral effect of tau therapeutics.

We have obtained additional funding with an Alzheimer's Association Research Grant to further examining these axonal, dendritic, and synaptic proteins within peripheral tissues performing both biochemical and immunohistochemical analysis. Dr. Dugger has also obtained a prestigious "tenure" track faculty position at the University of California- Davis where she will start her own laboratory focusing on heterogeneity and peripheral aspects of neurodegenerative diseases.

Main conclusions

Given the current results there are two main conclusions that can be drawn thus far for HT7, T231, and AT8 staining in the submandibular gland, abdominal skin, and colon:

- 1) Tau staining of the submandibular gland, abdominal skin, and colon do not segregate disease type since there is no particular anatomic area of these tissues that was only stained in one group (NC, PSP, AD/PSP, and/or CBD). Suggesting these specific tau species in these areas are physiologic and not pathologic in nature.
- 2) Specific tau species within the periphery appear to be neuronal in origin given evidence of co-localization with neuronal specific markers including MAP2 and neurofilament as well as neurotransmitter markers- tyrosine hydroxylase and choline acetyl transferase.

Publications and Manuscripts

Dugger BN, Hoffman BR, Scroggins A, Serrano G, Adler CH, Shill HA, Belden CM, Sabbagh MN, Caviness JN, Driver Dunckley E, Beach TG. Tau Immunoreactivity in Human Peripheral Tissues of Tauopathies and Aged Individuals. *In preparation*.

Dugger BN, Hoffman BR, Scroggins A, Serrano G, Adler CH, Shill HA, Belden B, Sabbagh MN, Caviness JN, Driver Dunckley E, Beach TG. The Presence of Tau in Human Sigmoid Colon. CurePSP International Research Symposium 2016.

Dugger BN, Hoffman BR, Scroggins A, Serrano G, Beach TG. Tau Immunohistochemistry in Peripheral Tissues of Progressive Supranuclear Palsy, Corticobasal Degeneration, and Alzheimer's Disease. 92nd Annual Meeting of the American Association of Neuropathologists 2016.

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