Biochemical Evidence for a Pathological Variant of Progressive Supranuclear Palsy

Michael DeTure PhD, Shanu Roemer MD PhD, Bailey Rawlinson BS, Melissa E Murray PhD and Dennis W Dickson MD

Department of Molecular Neuroscience, Mayo Clinic College of Medicine, Jacksonville, FL

Abstract

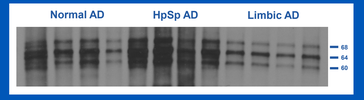
Background: Tau proteins aggregate into morphologically distinct lesions in primary and secondary tauopathies[1]. These signature lesions result from deposition of tau proteins that are uniquely modified via missense mutation and/or post-translational modification for each disease strain[2]. Identifying the unique tau protein abnormalities that lead to specific neurodegenerative diseases would be useful in their early diagnosis and treatment[3].

Objective: Biochemical and immunohistochemical analyses with cryo-EM of tau aggregates and lesions in AD, PSP, CBD, Pick's, GGT and FTDP-17 can be used to demonstrate that the tau protein modifications observed in each disease result from tau that is uniquely modified and thereby forms the basis of the distinct morphological aggregates and disease strains observed. Here data collected using the sarkosyl-insoluble tau fractions from tissue samples of pathologically diagnosed patients with either sporadic or familial primary tauopathies allows for the direct observation of the modifications that lead to accumulation in each disease and the resulting structural implications. Most interesting is the variability of the tau proteins that actually collect within like diseases opening up the possibility that tau strains may exist within any given tauopathy even as the clinical manifestation appears similar. This indicates that early and specific identification and detection of these strains is critical for the development of therapies for these diseases.

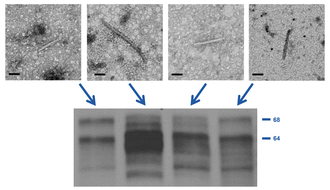
Results: Structural analyses of tau filaments from each primary and secondary tauopathy using electron microscopy and IHC or amyloid staining methods suggest that the tau protein modifications have a limited effect on the tau filament morphology or tau protein isoforms incorporated into the aggregates even as the truncation and phosphorylation events can vary widely as observed by western blotting of these insoluble samples.

Conclusions: Analysis of insoluble tau from pure PSP cases suggests two potential strains exist including a newly identified variant with earlier disease onset and increased tau deposition.

Insoluble Tau in AD Subtypes

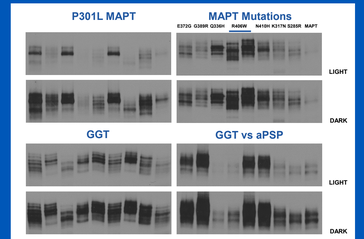


Tau Variability in 4R GGT



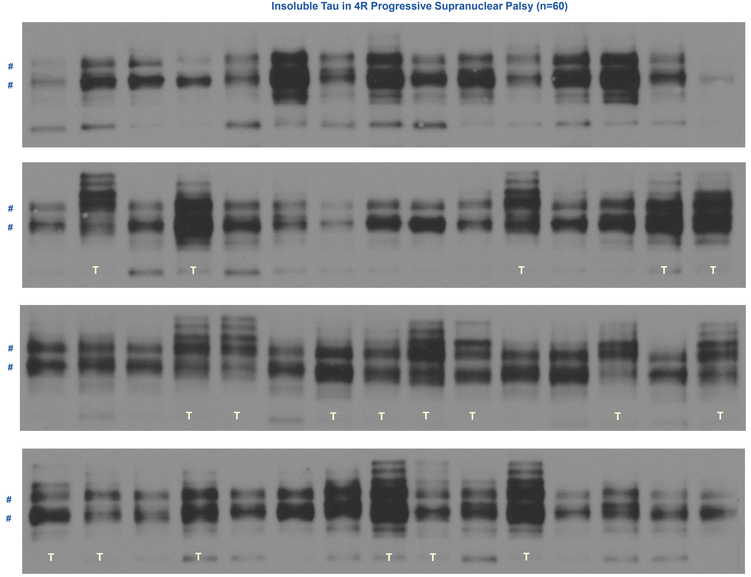
Sarksosyl Insoluble P3 Tau in GGT. Mature tau aggregates were purified from frontal tissues in idiopathic and familial GGT cases. EM demonstrates similar filament morphology while WB provides evidence of subunit variability. Scale bar = 50 nm. Antibody = E1.

Tau Aggregates in MAPT and GGT



Insoluble P3 Tau in MAPT and GGT Series. P301L samples with variability in tau filament subunits. This is also observed in matching R406W cases and within both the GGT and GGT vs aPSP series.

Two Classes of 4R tau in 4R PSP



Insoluble Tau in PSP. Aggregated tau from 4R PSP falls broadly into two classes including those with the classic 64/68 kDa doublet[#] and a large number with this doublet plus a heavier triplet.

Methods and Study Design

Model:

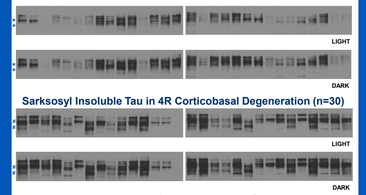
Chemical Structure ⇄ **Biochemical Strain** ⇄ **Cellular Morphology** ⇄ **Disease Pathogenesis**

Study Design: Modified tau proteins form the building blocks for tau filament assembly in neurodegenerative diseases, and variation in these subunits form the basis of several tauopathy strains including PSP and CBD. Here the existence of tau strains within a specific tauopathy without co-pathology is examined by WB of the monomers.

Cases for Study: Pure MAPT=20 (excluding intronic mutations, N279K and A152T), GGT=20, Pick's=30, CBD=30, PSP=60.

Methods: Frontal tissues were homogenized in 1X TBS with protease and phosphatase inhibitors. Homogenates were spun at 20,000 x g for 20 minutes. Pellets were suspended in high salt buffer containing 800 mM NaCl and 10% sucrose and clarified at 20,000 x g for 20 minutes. The resulting supernatant was supplemented with 1% sarkosyl for 1 hour at 37 C and the P3 fraction was collected at 100,000 x g for 75 minutes. Additionally, the original low speed supernatant was clarified at 100,000 x g for 75 minutes to obtain high speed S1 and oligomeric S1P fractions for future studies. Ab= E1.

Insoluble 3R Pick's and 4R CBD Tau



Insoluble Tau in Pick's and CBD. Insoluble tau from 3R Pick's and 4R CBD both exhibit significant variability in subunit composition. In each, numerous bands are visible in addition to the expected 3-repeat 60/64 kDa doublet* in Pick's and 4-repeat 64/68 kDa doublet[#] in CBD.

Conclusions and Future Directions

Conclusions: The purpose of these studies was to examine the existence of strains within primary tauopathies. The compositional subunits of the aggregated filamentous tau varied for MAPT, GGT, Pick's and CBD, but no tau bands were observed above 68 kDa. This however was not true for PSP where 19/60 cases had an additional higher MW triplet of bands. These cases with the triplet had a younger age of death compared to regular PSP (67.7 vs 71.3 and $p < 0.03$), and they had increased tau burden in cardinal regions need for the pathological diagnosis of PSP[4]. The substantia nigra and subthalamic nucleus had increased NFTs by IHC ($p < 0.10$) while the frontal and motor cortices had increased tufted astrocytes ($p < 0.10$). Further studies to demonstrate changes in protease resistance, templating and propagation are required.

Future Directions: It is tempting to believe that structural differences in the monomer composition and filament morphology might be exploited to generate strain specific antibodies that can be used for earlier clinical diagnosis and treatment of these diseases.

Brain Bank: Potential collaborators should contact Dr. Dickson at the Mayo Clinic Florida Brain Bank: *dickson.dennis@mayo.edu*.

References

1) **Ferrer I, López-González I, Carmona M, Arregui L, Dalfó E, Torrejón-Escribano B, Diehl R, Kovacs GG (2014) Glial and Neuronal Tau Pathology in Tauopathies.** *J Neuropathol Exp Neurol.* **73(1):81-97.**

2) **Taniguchi-Watanabe S, Arai T, Kametani F, Nonaka T, Masuda-Suzukake M, Tarutani A, Murayama S, Saito Y, Arima K, Yoshida M, Akiyama H, Robinson A, Mann D, Iwatsubo T, Hasegawa M. (2016) Biochemical Classification of Tauopathies.** *Acta Neuropath* **131(2):267-280.**

3) **Clavaguera F1, Akatsu H, Fraser G, Crowther RA, Frank S, Hench J, Probst A, Winkler DT, Reichwald J, Staufenbiel M, Ghetti B, Goedert M, Tolnay M. (2013) Brain Homogenates from Human Tauopathies Induce tau Inclusions in Mouse Brain.** *PNAS* **110(23):9535-40.**

4) **Roemer SF, Grinberg LT, Crary JF, Seeley WW, McKee AC, Kovacs GG, Beach TG, Duyckaerts C, Ferrer IA, Gelpi E, Lee EB, Revesz T, White CL 3rd, Yoshida M, Pereira FL, Whitney K, Ghayal NB, Dickson DW (2022) Rainwater Charitable Foundation criteria for the neuropathologic diagnosis of progressive supranuclear palsy** *Acta Neuropath* **144(4):603-614.**

Acknowledgements

Dickson: U54 NS100693 and UG3/UH3 NS104095

Correspondence

Michael DeTure PhD: *deture.michael@mayo.edu*