Selective tau seeding assays and isoform-specific antibodies define neuroanatomic distribution of progressive supranuclear palsy pathology arising in Alzheimer’s disease

David G. Coughlin¹ · Vanessa S. Goodwill² · Heidi G. Standke³ · Yongya Kim² · Nicolas Coley² · Donald P. Pizzo² · Douglas Galasko¹ · Allison Kraus³ · Annie Hiniker²

Received: 5 May 2022 / Revised: 19 July 2022 / Accepted: 3 August 2022 / Published online: 18 August 2022
© The Author(s) 2022

Incipient progressive supranuclear palsy (PSP) pathology in the setting of Alzheimer’s disease neuropathologic change (ADNC) has only rarely been reported (see Supplemental Table 1 for key prior works) [1]. These works suggest that the stereotyped neuroanatomic distribution of AD-related 3R/4R tauopathy and PSP-related 4R tauopathy are preserved in this setting; however, this has not been rigorously examined. Recently, isoform-specific tau antibodies (RD3, RD4, and AD-specific GT38) and isoform-selective tau seeding assays (3R/4R and 4R real-time quaking-induced conversion [RT-QuIC]) have been developed by us and others (methods and references in Supplement) that we hypothesized should delineate the neuroanatomic localization of co-occurring 3R/4R and 4R-tau pathology. We tested this hypothesis in five cases of high ADNC with incidental PSP (“ADNC + PSPi”) pathology and found, first, that neuroanatomic distributions of 4R PSP and 3R/4R AD pathology are conserved in ADNC + PSPi and, second, that RT-QuIC can robustly distinguish the neuroanatomic distribution of 3R/4R versus 4R pathology in cases with mixed tau pathology, suggesting its utility as a tool to interrogate pathophysiology.

We reviewed 1296 autopsy cases with intermediate or high ADNC and identified 5 cases (0.3%) with incidental co-occurring PSP pathology (Table 1 for additional clinical and pathologic information). Age of onset was 72 ± 3.8 years and disease duration was 11 ± 2.1 years (mean ± SD). Two cases had mild parkinsonism; one had gait disorder 5 years after symptom onset. No cases met NINDS-SPSP or MDS criteria for possible or probable PSP [2]. Case 3 had extensive aging-related tau astrogliopathy (ARTAG, 4R tauopathy) throughout the brain.

Immunohistochemistry and immunofluorescence for phospho-tau (AT8), 3R-tau (RD3), 4R-tau (RD4), and AD-specific tau (GT38) was performed on pons, midbrain, hippocampus, basal ganglia, temporal cortex, midfrontal cortex, and occipital cortex (see Supplement for methods). In ADNC + PSPi, we could distinguish AD pathology from PSP pathology by staining characteristics and morphology of inclusions: all pathological phospho-tau inclusions stained with AT8; AD neurofibrillary tangles (NFTs) but not PSP pathology stained with GT38 and RD3; PSP pathology stained with RD4 but not GT38. Consistent with the neuroanatomic distributions of each pathology in isolation, GT38-positive NFTs were abundant in midfrontal cortex and hippocampus, while RD4-positive astrocytes and oligodendroglia were prominent in midfrontal white matter and basal ganglia (Fig. 1a, S1). Semi-quantitative regional scoring demonstrated similar neuroanatomic distribution of AD versus PSP pathology in the five cases (Fig. 1b and Supplemental Results).

Vanessa S. Goodwill and Heidi G. Standke have contributed equally to this work.

Allison Kraus and Annie Hiniker have contributed equally to this work.

1 Departments of Neurosciences, University of California, San Diego, USA
2 Departments of Pathology, University of California San Diego, 9500 Gilman Drive, La Jolla, San Diego, CA 92093-0612, USA
3 Department of Pathology, Case Western Reserve University School of Medicine, 2103 Cornell Road, Cleveland, OH 44106, USA

* Allison Kraus
Allison.kraus@case.edu

* Annie Hiniker
ahiniker@health.ucsd.edu

© Springer
To further discriminate neuroanatomic patterns of AD and PSP disease activity in these mixed cases, we performed 3R/4R and 4R RT-QuIC on regions with distinct tau isoform immunostaining: globus pallidus (high PSP, low AD pathology), hippocampus (low PSP, high AD pathology), and frontal lobe (high PSP, high AD pathology). Our published RT-QuIC methodology allowed us to quantify seeding activity over a large (10⁹-fold) dynamic range with regional seeding doses for 3R/4R-tau and 4R-tau consistent with semiquantitative immunostaining (Fig. 1c, S2-S4, Supplemental Results). Because Case 3 showed extensive ARTAG (4R-tau), it was excluded from RT-QuIC analysis. Importantly, seeding activity favored 4R-tau in the globus pallidus, 3R/4R-tau in the hippocampus, and was variable in the midfrontal cortex (p < 0.0001, see Supplement). Two cases of cognitively normal older adults (age of death: 75 and 84 years, both Braak stage I/VI) and two cases of Huntington’s disease (age of death: 35 and 37 years, both Braak stage 0/VI) were selected as controls. All four controls showed markedly lower 3R/4R and absent 4R seeding from all regions (Figure S2 and Supplement).

In AD, 3R/4R NFTs arise in medial temporal lobe and locus coeruleus prior to apparent spread to other regions. In PSP, 4R-tau pathology likely begins in globus pallidus and brainstem and spreads via glial and neuronal elements. Whether AD-related 3R/4R-tau species can interact with PSP-related 4R-tau species is unknown [1]. Using immunostaining and RT-QuIC, we observed distributions of PSP-related tauopathy in ADNC + PSPi similar to those reported in primary PSP, consistent with prior case reports. We did not observe alterations in pathological distribution of AD or PSP-specific tau species that would indicate a direct interaction, consistent with other work suggesting that AD and PSP-specific tau species are largely distinct [1]. Our findings are also consistent with prior work showing selectivity of RT-QuIC assays for 3R/4R-tau versus 4R-tau in midfrontal lobe of various tauopathies (see references in Supplement).

Many seeding assays qualitatively indicate seed occurrence and are not isoform-specific, whereas RT-QuIC allows quantification of isoform-specific regional seeding activity over a billion-fold dynamic range. Our work provides the first evidence that tau RT-QuIC can selectively indicate pathologic seed burden in a neuroanatomic fashion, highlighting its potential to interrogate pathophysiology in neurodegenerative diseases. This study is limited by small case numbers, indicative of the rarity of this pathological combination. We also cannot fully differentiate incipient PSP from ‘form-fruste’ PSP: Although cases had yearly structured clinical evaluations and PSP was not clinically evident, emergence of diagnostic symptoms at the end of life cannot be fully excluded (interval from last evaluation to death was 13 years). A list of clinical and neuropathologic characteristics of ADNC-PSPi cases is shown in Table 1.

### Table 1 Clinical and neuropathologic characteristics of ADNC-PSPi cases

| Case # | 1 | 2 | 3 | 4 | 5 |
|--------|---|---|---|---|---|
| **Clinical characteristics** | | | | | |
| Sex | Female | Male | Male | Male | Female |
| Age of onset (y) | 67 | 70 | 76 | 74 | 75 |
| Disease duration (y) | 13 | 8 | 10 | 11 | 13 |
| Clinical diagnosis | Probable AD | Probable DLB | Probable AD | Probable AD | Probable AD |
| NINDS-SPSP criteria | Not met | Not met | Not met | Not met | Not met |
| MDS-PSP criteria | a0, p0, a0, c0 | a0, p0, a2, c2 | a0, p0, a2, c0 | a0, p0, a0, c0 | a0, p0, a0, c0 |
| APOE | 3,4 | 3,4 | 3,3 | 3,4 | 3,3 |
| **Neuropathology** | | | | | |
| Brain weight (g) | 980 | 1236 | 1248 | 1158 | 1050 |
| Thal phase | A2 | A2 | A2 | A3 | A3 |
| Braak tau stage | VI, B3 | VI, B3 | V, B3 | VI, B3 | III, B2 |
| CERAD stage | 3 | 3 | 3 | 2 | 2 |
| ADNC | Intermediate | Intermediate | Intermediate | High | Intermediate |
| LBD stage | None | None | None | None | Amygdala Predominant |
| Other | None | LATE Stage 2 with hippocampal sclerosis | ARTAG, LATE Stage 2 with hippocampal sclerosis | LATE Stage 2 with hippocampal sclerosis | LATE Stage 1 without hippocampal sclerosis |
death: 0.5–6 years). Future directions include studying larger cohorts of tauopathies, including ‘pure’ AD, ‘pure’ PSP, and PSP with AD co-pathology, to more precisely delineate the extent to which RT-QuIC can measure isoform-specific tau burden in a neuroanatomic manner.

**Supplementary Information** The online version contains supplementary material available at https://doi.org/10.1007/s00401-022-02480-x.

**Funding** This work was made possible with support from the NIH including NINDS NS120038 (DC), NINDS R01NS118760 (AK), NIA AG062429 (AH), and NIA R01AG067607 (AK) and the Alzheimer’s Association (AACSFP 684991 to AH).

**Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.

**References**

1. Ebashi M et al (2019) How to demix Alzheimer-type and PSP-type tau lesions out of their mixture-hybrid approach to dissect comorbidity. Acta Neuropathol Commun. https://doi.org/10.1186/s40478-019-0708-4

2. Höglinger GU et al (2017) Clinical diagnosis of progressive supranuclear palsy: The movement disorder society criteria. Mov Disord 32:853–864. https://doi.org/10.1002/MDS.26987

**Publisher’s Note** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.