Received: 21 June 2022 | Revised: 5 July 2022 | Accepted: 7 July 2022 | Published online: 5 August 2022

DOI: 10.1002/dad2.12348

Alzheimer's & Dementia
Diagnosis, Assessment & Disease Monitoring

EDITORIAL

Uncertainties in the PET defined A-/$T_{Neocortical}$+ subtype

In the 15+ years following the advent of the first promising PET ligand to robustly measure amyloid plaques in vivo,[1] the Alzheimer's disease (AD) research field has amassed large cohorts of research participants thoroughly characterized with biomarkers of key AD hallmarks (such as AIBL, ADNI, etc.). These transformative studies address the timely need to evaluate the utility of quantitative biomarkers of amyloid and tau throughout the long disease continuum of AD. An important opportunity that has emerged from these large cohorts of deeply phenotyped research participants is the ability to detect "outliers," individuals that do not match an expected typical pattern of disease progression. You need hundreds if not thousands of datapoints to find outliers and probe into their meaning. The detection of an outlier oftentimes depends on joint status across modalities, as seen in Krishnadas et al.[2] These outlier exceptions give researchers the opportunity to challenge dominant hypotheses, discover additional mechanisms, and assess limitations and nuance in our measurement tools.

The work presented by Krishnadas et al.[2] leverages the team's extensive dataset of over 450 individuals characterized with both amyloid and tau PET to identify a small set of outliers defined across two key PET modalities. Specifically, the manuscript discusses individuals that are amyloid PET-negative (via 18F-NAV4694) but have clear tau PET signal elevations with 18F-MK6240 throughout neocortex. Of the 452 research participants that contributed to this effort, 276 were Aβ- using a centiloid (CL) threshold of 25. Of the 276 Amyloid- participants, 12 were flagged using quantitative tau PET values in neocortex. Upon a second step that involved a qualitative read by a blinded expert, four of the 12 were confirmed to have unequivocal neocortical tau PET elevations. These four A-/T+ outliers, which reflect 1.4% of the Amyloid- group, form the basis of the manuscript.

The possibility of A-/T+ individuals is included in the framework that describes a biological research definition of AD.[3] The anticipated likelihood of this combination depends on how T-positivity is defined. It is well established that tau deposition occurs in the brainstem and entorhinal cortex before amyloid-abnormalities,[4,5] and there are also cases described in the postmortem literature that have tangles in hippocampus (Braak III/IV) without evidence of amyloid (a combination that has been labeled "Primary Age-Related Tauopathy," PART).[6] It is generally appreciated that involvement of neocortex (Braak stages V-VI) occurs exclusively in the presence of abnormal amyloid, which is not the case for the four research participants described by Krishnadas. Whereas PART could be more accurately labeled as A-/T_{MTL}+, Krishnadas's four cases could be labeled A-/$T_{Neocortical}$+.

For any approach that involves dichotomization of continuous values, there is always the possibility that the resulting A- classification is merely an artifact of arbitrary threshold selections and measurement error. To this end, we have seen that amyloid PET CL values of 12 align with cerebrospinal fluid (CSF) derived measures of amyloid,[7] and CL values of 5–10 predict future longitudinal amyloid accumulation.[8] Although, it remains unclear how these CL thresholds vary by amyloid ligand and other methodological details that may influence the precision of these values especially in the low CL range, the overall pattern from the literature highlights that CL values lower than 25 can be an indicator of abnormal amyloid processes and be associated with downstream effects. With that being said, three of the four cases from Krishnadas et al. had CL values strongly indicating Amyloid PET-negativity (CL values between -3 and 2). Participant 4 is an exception with a CL value of 18, so one might wonder if measurement error in that case's PET scan caused it to dip below the positive threshold and should in fact be A+/T+. Further, some studies have reported negative slopes of amyloid accumulation in some A+ participants, a phenomenon that is poorly understood and may reflect a loss of plaque as a function of neuronal loss. Participant 4 with the CL value of 18 was the most advanced case of the four A-/$T_{Neocortical}$+ cases (Participant 4 has AD dementia, whereas Participants 1-3 are either clinically unimpaired or have mild cognitive impairment). It is possible that Participant 4 may behave similarly to one of the negative slope data points shown in Figure 2A of Villemagne et al.[9]

To further understand these outliers, the authors additionally examined biofluid measures (CSF, N = 1 or plasma, N = 3, see Table 1). The exact biofluid measurement was inconsistently available across the four A-/T+ cases, but the two participants that had an additional biofluid amyloid measurement were both biofluid-positive for amyloid (one via CSF, and one via a previously published plasma amyloid composite measure[10]), providing some evidence for a lack of sensitivity from amyloid PET. All four participants had a positive tau biofluid measure (one via CSF, and three via plasma ptau). For the three cases with tau measured in the plasma, the authors evaluated plasma "p217+tau," a measurement that captures tau when phosphorylated at the 217 amino acid location, but also integrates phosphorylation at other sites such as 212.[11] Similar to other studies evaluating plasma p217 alone,[12]

TABLE 1 Biomarker synthesis

	A-/$T_{Neocortical}$+ participants			
	#1	#2	#3	#4
Amyloid CL	−3	1	2	18
Positive tau PET ROIs	Te	Me, Te, R	Me, Te, R	Te, R
Biofluid amyloid	+	+	NA	NA
Biofluid tau	+	+	+	+

Note: Green indicates values clearly within the negative range, yellow suggests more intermediate values, and red indicates clear positivity across biomarkers. NA indicates not assessed. *PET synthesis*: Participant 1 showed the most restrictive neocortical tau uptake. This participant was positive only in temporoparietal (Te) with an SUVR of 1.37, just slightly above the SUVR threshold of 1.33 for that region. Participant 4 was showed the most elevated amyloid centiloid value among the four cases, which could reflect some underlying amyloid pathology. Participant 2 & 3 show the most discordance between amyloid and tau PET measures (low amyloid centiloid values with clearly elevated neocortical tau PET signal). *Biofluid synthesis*: The plasma amyloid composite value of 1.69 reported for participant 1 was deemed clearly positive in comparison to the previously published[10] distribution of this variable (Figure 1 and Extended Data Table 1 in ref.[10]). Cerebrospinal fluid values and thresholds were reported in the manuscript for Participant 2, with values clearly abnormal relative to the reported thresholds. Plasma p217+tau values were not provided for the three A-/$T_{Neocortical}$+ participants that had this measure in the current manuscript (participant 1, 3, and 4), so it is unclear whether these values are peri-threshold or clearly positive (for these values, table cells below are unfilled, with a positive symbol indicating author reported positive status). For reference, the association between p217+tau and tau PET from the larger Australian cohort is shown in Figure 4 in Dore et al.[11] Overall, the accompanying biofluid data supports underlying abnormalities in amyloid and/or tau in all four participants.
Abreviation: ROI, region of interest.

the plasma p217+tau measure tracks strongly with both amyloid and tau PET positivity.[11] Elevated plasma p217+tau in the three A-/T+ cases is consistent with their neocortical tau PET positivity, providing confidence that the tau PET signal is not artifactual. Overall, the biofluid-tau PET biomarker profile is consistent with abnormal levels of both amyloid and tau in these cases. The negative amyloid PET status stands out as inconsistent with this AD-like profile, and implies either a lack of sensitivity of the amyloid PET scan, a distinct biological subtype that is less prone to amyloid plaque formation (but may have other amyloid abnormalities such as oligomerization), or mechanisms that promote an exaggerated tau response relative to minimal underlying amyloid burden. These potential explanations are discussed below.

A primary hypothesis from the authors is that these cases do in fact have abnormalities in amyloid processing, but that these abnormalities were not detected by the amyloid PET scan. This explanation is consistent with the positive plasma/CSF biofluid measures for these cases, and is in line with various studies showing the possibility of abnormal CSF amyloid levels in the context of an amyloid PET negative scan. Using a large multi-site PIB imaging-postmortem cohort, La Joie et al. demonstrated that amyloid Thal stages of 4 and 5 were generally associated with CL values above 25, whereas all the Thal 0 and 1 cases were below 12.[13] However, the range of CL values for Thal 2 and 3 spanned approximately -10 to +130 CLs (see Figure 2A in La Joie et al.[13]), suggesting poor correspondence between Thal staging and amyloid PET imaging at intermediate levels of amyloid plaques. Another explanation for the lack of elevated amyloid PET signal in these cases is the possibility that disease subtypes exist that result in a predilection toward non-plaque forms of amyloid accumulation, such as oligomerization. Even among individuals that have autosomal dominant mutations that result in high production of the amyloid-42 protein, varying levels of PET-positivity have been demonstrated despite consistent abnormalities in CSF-amyloid levels (see Figure 6D and J in Chhatwal et al.[14]). Interestingly, different mutations can result in a globally elevated amyloid PET pattern, a striatal dominant PET pattern, or limited amyloid PET elevations in cortex and striatum altogether. Examples of the disconnect between amyloid measured by PET and by CSF in autosomal dominant mutation carriers highlights that amyloid abnormalities (such as amyloid oligomers) can still exist in the absence of detectable amyloid plaques, which may account for the PET-defined A-/$T_{Neocortical}$+ profile.

Another explanation for the appearance of this unexpected A-/$T_{Neocortical}$+ profile is that the elevations observed on the tau PET side were false positives. Tau thresholds are less established and likely cannot be summarized by a single global threshold, as is routinely done with amyloid PET. Nevertheless, the approach used by Krishnadas was quite conservative, using a threshold defined by the 99th percentile of tau PET values from the Amyloid- clinically unimpaired group, and also evaluated in three sets of regions, medial temporal ("Me"), temporoparietal ("Te"), and the rest of neocortex ("R"; frontal, superior temporal, anterior cingulate). Furthermore, in a second step, all cases identified with these conservative quantitative thresholds were further confirmed via a visual read. This procedure identified 12 A- participants that surpassed the quantitative tau PET threshold, and only four of these were confirmed to have neocortical tau on visual inspection. Although, no further detail was provided for the eight that were found qualitatively unclear, the authors were confident that the remaining four A-/$T_{Neocortical}$+ reflect "real" tau PET signal. However, we have seen strange levels of uptake with other tau ligands such as 18F-Flortaucipir in the temporal poles of semantic dementia cases that likely have underlying TDP-43 pathology,[15] as well as tau PET update that is co-localized with infarcts and other incidental findings that are associated with iron and mineralization.[16] However, these tau PET off-target examples from the literature tend to involve focal elevations, whereas the 18F-MK6240 elevations from Krishnadas' A-/$T_{Neocortical}$+ cases were broad throughout neocortex and importantly, followed a cortical gray matter pattern (the one exception may be what appears as a focal asymmetrical hotspot in the lateral parietal/occipital for Participant 1). Nevertheless, given that 18F-MK6240 is a new tau PET ligand it is possible that there are yet to be discovered sources of off-target binding.

The main importance of this paper is drawing attention to a specific group of outliers that are amyloid-PET negative and have pronounced neocortical tau PET uptake reminiscent of Braak V-VI. This profile is unexpected and is distinct from other known A-/T+ profiles that involve focally restricted tangle deposition to the medial temporal lobe (PART). The identification of this subgroup depended on a large

cohort, and further required visual examination to distinguish from cases that had presumed artifacts driving high quantitative values. Thus, it is easy to see how these cases could be dismissed, or averaged out using standard group level approaches. By isolating these outlier cases, Krishnadas provides an opportunity to better understand our PET measurements and hypothesize potential explanations. It remains unclear whether mechanisms driving this profile are the same or different across the four individuals. Although the authors hypothesize that amyloid-abnormalities are present albeit undetected by amyloid PET, it is also possible that there are yet to be discovered (uncommon) mechanisms leading to neocortical tau deposition that are not mediated by amyloid-abnormalities, or along those lines, there could be subgroups of individuals prone to aggressive tau deposition with only minimal amyloid burden (below the threshold detected with amyloid PET imaging). The clinical implications of the A-/$T_{Neocortical}$+ group are unknown and it is interesting that three of the four cases had normal cognition or only mild cognitive impairment, providing an opportunity to observe clinical progression and longitudinal biomarker trajectories. Post-hoc leveraging across multiple cohorts to build larger cohorts of A-/$T_{Neocortical}$+ cases, along with longitudinal follow up of these cases should improve our understanding of this unexpected profile.

CONFLICT OF INTEREST

Dr. Mormino receives research funding from NIH (R01AG074339, P30AG06615, U24AG067418, U24AG074855). Dr. Mormino is a paid consultant to Eli Lilly, Roche, and Neurotrack. Dr. Insel is a paid consultant to Roche.

Elizabeth C. Mormino PhD[1,2]
Philip S. Insel PhD[3]

[1]*Department of Neurology and Neurological Sciences, Stanford, Palo Alto, California, USA*
[2]*Wu Tsai Neuroscience Institute, Stanford, Palo Alto, California, USA*
[3]*Department of Psychiatry and Behavioral Sciences, University of San Francisco, San Francisco, California, USA*

Correspondence
Elizabeth C. Mormino, PhD, Department of Neurology and Neurological Sciences, Stanford, Palo Alto, CA 94305, USA.
Email: bmormino@stanford.edu

REFERENCES

1. Klunk WE, Engler H, Nordberg A, et al. Imaging brain amyloid in Alzheimer's disease with Pittsburgh Compound-B. *Ann Neurol*. 2004;55(3):306-319.
2. Krishnada N, Doré V, Laws SM, et al. Exploring discordant low amyloid-β and high neocortical tau PET cases. *Alzheimer's Dement*. 2022;14:e12326.
3. Jack CR, Jr, Bennett DA, Blennow K, et al. NIA-AA Research Framework: toward a biological definition of Alzheimer's disease. *Alzheimers Dement*. 2018;14(4):535-562.
4. Braak H, Thal DR, Ghebremedhin E, Del Tredici K. Stages of the pathologic process in Alzheimer disease: age categories from 1 to 100 years. *J Neuropathol Exp Neurol*. 2011;70(11):960-969.
5. Nelson PT, Alafuzoff I, Bigio EH, et al. Correlation of Alzheimer disease neuropathologic changes with cognitive status: a review of the literature. *J Neuropathol Exp Neurol*. 2012;71(5):362-381.
6. Crary JF, Trojanowski JQ, Schneider JA, et al. Primary age-related tauopathy (PART): a common pathology associated with human aging. *Acta Neuropathol*. 2014;128(6):755-766.
7. Salvado G, Molinuevo JL, Brugulat-Serrat A, et al. Centiloid cut-off values for optimal agreement between PET and CSF core AD biomarkers. *Alzheimers Res Ther*. 2019;11(1):27.
8. Jagust WJ, Landau SM. Alzheimer's disease neuroimaging I. Temporal dynamics of beta-amyloid accumulation in aging and Alzheimer disease. *Neurology*. 2021;96(9):e1347-e1357.
9. Villemagne VL, Burnham S, Bourgeat P, et al. Amyloid beta deposition, neurodegeneration, and cognitive decline in sporadic Alzheimer's disease: a prospective cohort study. *Lancet Neurol*. 2013;12(4):357-367.
10. Nakamura A, Kaneko N, Villemagne VL, et al. High performance plasma amyloid-beta biomarkers for Alzheimer's disease. *Nature*. 2018;554(7691):249-254.
11. Dore V, Doecke JD, Saad ZS, et al. Plasma p217+tau versus NAV4694 amyloid and MK6240 tau PET across the Alzheimer's continuum. *Alzheimers Dement (Amst)*. 2022;14(1):e12307.
12. Thijssen EH, La Joie R, Strom A, et al. Plasma phosphorylated tau 217 and phosphorylated tau 181 as biomarkers in Alzheimer's disease and frontotemporal lobar degeneration: a retrospective diagnostic performance study. *Lancet Neurol*. 2021;20(9):739-752.
13. La Joie R, Ayakta N, Seeley WW, et al. Multisite study of the relationships between antemortem [(11)C]PIB-PET Centiloid values and postmortem measures of Alzheimer's disease neuropathology. *Alzheimers Dement*. 2019;15(2):205-216.
14. Chhatwal JP, Schultz SA, McDade E, et al. Variant-dependent heterogeneity in amyloid beta burden in autosomal dominant Alzheimer's disease: cross-sectional and longitudinal analyses of an observational study. *Lancet Neurol*. 2022;21(2):140-152.
15. Makaretz SJ, Quimby M, Collins J, et al. Flortaucipir tau PET imaging in semantic variant primary progressive aphasia. *J Neurol Neurosurg Psychiatry*. 2018;89(10):1024-1031.
16. Lockhart SN, Ayakta N, Winer JR, La Joie R, Rabinovici GD, Jagust WJ. Elevated (18)F-AV-1451 PET tracer uptake detected in incidental imaging findings. *Neurology*. 2017;88(11):1095-1097.