Plasma phosphorylated tau 217 and phosphorylated tau 181 as biomarkers in Alzheimer's disease and frontotemporal lobar degeneration: a retrospective diagnostic performance study

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Abstract

Background

Plasma P-tau181 has been shown to be associated with Alzheimer's disease (AD) tau pathology. P-tau217 is a novel blood-based biomarker that may be diagnostically superior to P-tau181. We compared the diagnostic value of plasma P-tau217 and P-tau181 in the same cohort of patients across the AD and frontotemporal lobar degeneration (FTLD) spectrum with matched immunological assays.

Methods

Retrospective study of 593 cases (64±13 y, 50% female) with both plasma P-tau217 and P-tau181 concentrations. The cohort included 15 AD and 68 FTLD pathology-confirmed cases, 360 cases with amyloid-PET (41% positive) and 230 cases with flortaucipir (FTP)-PET (49% positive). P-tau was measured using custom electrochemiluminiscence-based assays at Lilly.

Findings

Plasma P-tau217 and 181 were strongly correlated (r=0.88, p<0.001). P-tau217 and P-tau181 concentrations were increased in clinical AD relative to cognitively normal controls (P-tau217 AUC=0.98, P-tau181 AUC=0.97, p(difference)=0.31). P-tau217 slightly outperformed P-tau181 in differentiating between clinically diagnosed AD and FTLD (P-tau217 AUC=0.93, P-tau181 AUC=0.91, p=0.007), but not in differentiating between pathology-confirmed AD vs FTLD (P-tau217 AUC=0.96, P-tau181 AUC=0.90, p =0.22). P-tau217 was 3.9-fold and P-tau181 was 2.5-fold increased in amyloid-PET-positive compared to amyloid-PET-negative cases (P-tau217 AUC=0.91, P-tau181 AUC=0.89, p=0.049). Tau-PET binding in the temporal cortex was more strongly associated with P-tau217 than P-tau181 (r=0.80 vs r=0.72, p difference <0.001).

Interpretation

In a direct comparison using matched immunoassays, both P-tau217 and P-tau181 had good diagnostic performance for differentiating AD from other groups. There were small, but statistically significant differences in favor of P-tau217 for differential diagnosis of neuropathologically confirmed AD and in correlations with FTP-PET uptake. Plasma P-tau181 and 217 may be useful screening tools to identify individuals with underlying amyloid and AD tau pathology.

Funding: US National Institutes of Health, State of California Department of Health Services, Tau Consortium, Michael J Fox foundation, Alzheimer's Association.

Research in context

Evidence before this study:

We searched PubMed for all articles published up to October 27, 2020, with no language limitations. Keywords included: "plasma phosphorylated tau", "CSF phosphorylated tau", "tau-PET", "amyloid-PET", "MRI", "*MAPT* mutation carriers, "Alzheimer's disease", "Alzheimer's pathology", and "frontotemporal lobar degeneration". CSF P-tau181 is one of the core biomarkers incorporated into the NIA-AA Research Framework to define Alzheimer's disease. Recent studies suggested that CSF p-tau217 is consistently more strongly related to the AD pathological process and might be more useful than P-tau181. To make testing for AD pathology more widely accessible, assays were developed that are sensitive enough to measure P-tau in blood. Previous cross-sectional and longitudinal studies covering the clinical AD continuum have indicated the value of plasma P-tau217 and P-tau181 for differentiation of clinical and pathology-confirmed AD, and differentiation of amyloid-PET or tau-PET positive cases from amyloid-PET or tau-PET negative cases. One study suggested that P-tau217 outperformed P-tau181, however there were important technical differences between the P-tau217 and P-tau181 assays used.

Added value of this study:

This study provides a direct comparison between two novel plasma P-tau assays, either measuring P-tau217 or P-tau181 in a broad range of diagnostic and correlative neuroimaging analyses. The assay conditions were optimized to ensure that the immunochemical properties of the P-tau217 and P-tau181 assays were essentially the same. Similar to previous work, both P-tau217 and 181 were increased in AD clinical syndromes compared to a spectrum of FTLD syndromes, Lewy body dementia and Traumatic Encephalopathy Syndrome (TES) cases. Both P-tau species were also increased in pathology-confirmed AD compared to pathology-confirmed FTLD, but neither could differentiate between 3R/4R tau versus 4R tau producing *MAPT* mutation carriers. Both P-tau species were increased in amyloid-PET-positive cases compared with amyloid-PET-negative cases, and in tau-PET-positive cases compared with tau-PET-negative cases, and correlated with regional tracer uptake on the voxel level. In addition, both plasma P-tau measures strongly reflected underlying AD pathology in patients with clinical diagnoses of either mild cognitive impairment (MCI) or corticobasal syndrome (CBS), for which underlying AD pathology is a possible but uncertain etiology. We show that plasma P-tau217 is slightly superior to P-tau181 in some analyses such as association with amyloid- and tau-PET, but overall, measurement of either P-tau epitope produced very similar results.

Implications of all the available evidence:

This study shows that careful consideration of assay characteristics is critical to understanding the relative performance and utility of plasma P-tau biomarkers. These data, together with other recent reports, suggest that both P-tau217 and P-tau181 are useful biomarkers of AD pathology. Plasma biomarkers are likely to be less expensive and more accessible than CSF or PET, allowing them to be more highly scalable and more easily deployed to medically underserved and remote populations. Plasma P-tau is a particularly powerful tool for differential diagnosis between AD and FTLD pathologies, which may further improve the ability to identify suitable participants for clinical trials and large-scale epidemiological studies.

Main manuscript

Introduction

Tau pathology plays an essential role in Alzheimer's disease (AD) and about half of the frontotemporal lobar degeneration (FTLD) spectrum diseases.[1,2] The clinical syndromes associated with AD or FTLD pathology are heterogeneous and frequently overlap, particularly in younger individuals. Established biomarkers for AD including beta amyloid measurements in cerebrospinal fluid (CSF), or amyloid or tau positron emission photography (PET) imaging, have been of limited use for screening because of the invasiveness of the lumbar puncture and the high costs of PET. Blood-based biomarkers may have advantages due to their lower costs, less invasive nature and potential to be deployed widely throughout the community, allowing for early and repeated testing. With the development of disease-modifying AD therapies, a blood test could be employed to identify patients who are likely to harbor AD pathology to undergo more established CSF or PET diagnostic testing prior to initiating therapy. When combined with simple clinical assessment tools, results of screening blood tests could also be used to guide referrals to specialized memory clinics for management and research.

We and other groups have shown the value of plasma P-tau181 for differential diagnosis of clinically defined dementia syndromes, with P-tau181 concentrations being 2.5-3.5 times higher in AD compared to normal controls (NC).[3-5] P-tau181 was not increased in FTLD and could differentiate FTLD from AD with receiver operating curve (ROC) area under the curves (AUC) ranging from 0.81[6] to 1.[5] P-tau181 was increased in pathology-confirmed AD compared to pathology-confirmed FTLD.[3] P-tau181 was a strong indicator of amyloid-PET positivity (AUC: 0.76[6]-0.91)[3] and correlated with tau-PET binding,[3-5] successfully predicting tau-PET positivity (AUC: 0.83[6]-0.93[5]).

More recently, plasma P-tau217 has been shown to outperform plasma P-tau181 in identifying AD in an autopsy-confirmed cohort (AUC of 0.89 for P-tau217 vs 0.72 for P-tau181) and in a clinical cohort (AUC of 0.96 for P-tau217 vs 0.81 for P-tau181). However, there were important technical differences between the P-tau217 and P-tau181 plasma assays used, complicating the interpretation of which P-tau epitope might be superior for differential diagnosis and other purposes.[6] Observed differences could be attributable to assay characteristics unrelated to the P-tau epitope.

When measured simultaneously in CSF using mass spectrometry, P-tau217 showed higher accuracy in differentiating AD from FTLD and controls (AUC=0.99) compared to P-tau181 (AUC=0.86),[7 8 9] though no direct one-on-one comparison has been made between plasma measures of P-tau217 and P-tau181 to date.

As plasma P-tau measurements become more widely available and are increasingly planned for use in research and clinical care, it will be important to understand the relatively merits of different assays. In this study we compared plasma P-tau217 and P-tau181 measured using MSD technology and with the same immunochemical set-up for both markers across a wide range of neurodegenerative diseases including the full spectrum of AD, mild cognitive impairment (MCI), FTLD and other dementia cases as well as age matched controls.

Methods

Participants

This clinical study included 593 participants from two cohorts; 443 from the University of California San Francisco (UCSF) Memory and Aging Center, and 150 from the Advancing Research and Treatment for Frontotemporal Lobar Degeneration (ARTFL) consortium. The cohort consisted of clinically normal controls, patients in the AD or FTLD spectrum, patients with dementia with Lewy bodies (DLB) and patients with traumatic encephalopathy syndrome (TES), detailed in **Supplement**. Five hundred and seven participants were white. Participants were included when both P-tau217 and P-tau181 were successfully measured in plasma.

Participants provided written informed consent at the time of recruitment. The study was approved by the institutional review board of each research center from which the individual was recruited.

Pathology diagnosis

Eighty-three cases had a pathology-confirmed diagnosis. The average time between blood draw and death was 32±20 months. AD neuropathological changes, including Thal phase, CERAD neuritic plaques and Braak neurofibrillary tangles were ascertained as described.[10] FTLD cases were grouped as FTLD-tau or FTLD-TAR DNA-binding protein 43 (FTLD-TDP) as described.[11,12] Lewy body disease and chronic traumatic encephalopathy (CTE) neuropathological changes were also assessed.[13-16]

Plasma P-tau methods for optimal comparison

P-tau217 and P-tau181 assays were performed in duplicate on the same sample aliquot and processed together in the same batch on a streptavidin small spot plate using the MSD platform. Biotinylated-IBA493 was used as a capture antibody for the P-tau217 assay and biotinylated-AT270 for the P-tau181 assay. In both assays, SULFO-TAG-Ru-4G10-E2 (anti-tau monoclonal antibody) was used for the detector antibody (detailed in **Supplement**).

Plasma NfL method

Plasma NfL was measured using Simoa technology with either a homebrew kit or commercial kit on a Quanterix HD-1 analyzer (Quanterix, Billerica, MA).

Imaging methods

Image data was acquired across multiple centers; acquisition, processing, and analyses are fully detailed in the supplementary analyses and briefly summarized below. Voxelwise analyses and result rendering are detailed in the **supplement**.

Amyloid-PET

Amyloid-PET status was available for 360 participants and derived from visual read[17,18] based on PET acquired with [11C]Pittsburg Compound B (PIB), [18F]florbetapir, or [18F]florbetaben. The average time between plasma sample and amyloid-PET was 5±16 months.

Tau-PET

Tau-PET was acquired using [18F]flortaucipir in 230 participants (**Table 1**). The average time between plasma sample and Tau-PET was 1.6±7 months. Standardized Uptake Value Ratio (SUVR) values were extracted from the temporal cortex and a threshold of 1.27 was used to determine tau-PET positivity.[1]

MRI

Magnetization Prepared Rapid Gradient Echo (MP-RAGE) images were available for 535 participants and acquired from various 3 Tesla scanners. The average time between plasma and MRI imaging was 1.7±8 months. Total intracranial volume was used as a covariate in analyses.[19]

Statistical analysis

All measures were adjusted for age, sex (and CDRsb for pathology-confirmed cases) and corrected for multiple-comparisons with Bonferroni; η^2 was used to measure effect size. For visualization, raw biomarker concentrations were used. Differences in biomarker values were assessed with univariate linear models. P-tau217 and P-tau181 were not normally distributed, therefore Spearman's correlations were used. The difference in strength between two correlations was tested using methods described in Diedenhofen et al.[20] ROC analyses determined differentiation accuracy. Difference between two AUC curves was tested with the Delong test.[21] Youden cut-off values were used for sensitivity and specificity. A two-sided $p<0.05$ was considered significant. Statistical analyses were performed using SPSS (version 26; SPSS/IBM, Chicago, IL) and R (version 3.6.1).

Results

Participant characteristics

The cohort included 118 normal controls (NC) and a broad range of neurodegenerative syndromes:174 cases in the clinical AD spectrum (58 AD_{clin},[22] 15 logopenic variant primary progressive aphasia, lvPPA, and 2 posterior cortical atrophy, PCA), 99 MCI patients[23], and 273 individuals with clinical diagnoses in the FTLD spectrum (79 corticobasal syndrome, CBS[24], 74 progressive supranuclear palsy, PSP,[25] 62 behavioral variant FTD, bvFTD,[26] 32 nonfluent variant PPA, nfvPPA, and 27 semantic variant PPA (svPPA)[27]). In addition, 14 cases with dementia with Lewy bodies (DLB) and 13 cases with traumatic encephalopathy syndrome (TES) were included (**Table 1**). The FTLD cases included 76 carriers of FTLD-causing mutations: 44 *MAPT* associated with FTLD-tau, 13 progranulin (*GRN*) and 20 chromosome 20 open reading frame (*C9orf72*), associated with FTLD-TDP. The *MAPT* group included 11 individuals with mutations that produce 3R/4R tau (6 V337M and 5 R406W), and 33 with mutations that produce 4R tau (20 P301L, 7 N279K, 6 IVS10+16C>T).[28]

Table 1 goes here

Differentiation based on clinical diagnosis

There was no correlation between age and P-tau217 (R=-0.02, p=0.61) or P-tau181 (R=0.04, p=0.37) and no difference in P-tau217 and P-tau181 concentrations between male (P-tau217: 0.26±0.3 pg/mL; P-tau181: 1.2±1 pg/mL) and female participants (P-tau217:0.31±0.4 pg/mL, p=0.98; P-tau181: 1.2±1 pg/mL, p=0.94). P-tau217 and P-tau181 concentrations were strongly correlated (r=0.88, p<0.001, **eFigure 1**). Both P-tau217 and P-tau181 were increased in the clinical AD spectrum (AD_{clin}+lvPPA+PCA) compared to all other diagnostic groups (**Figure 1**). P-tau217 was 4.4-fold increased in patients in the clinical AD spectrum (0.74±0.4 pg/mL, n=75) compared to controls (0.17±0.2 pg/mL, n=118, η^2=0.63, p<0.001). P-tau181 was 2.8-fold increased in the clinical AD-spectrum (2.4±1 pg/mL, n=75) compared to controls (0.87±1 pg/mL, n=118, η^2=0.54, p<0.001). The associated AUCs were comparable for P-tau217 (0.98, p<0.001) and P-tau181 (AUC=0.97, p<0.001, DeLong test p=0.31, **Table 2**). In MCI patients, P-tau217 was increased compared to controls and lower than AD, whereas P-tau181 was not increased compared to controls (**Table 1**).

P-tau217 was increased by 3.5 fold in the clinical AD-spectrum compared to the clinical FTLD-spectrum (CBS+PSP+bvFTD+nfvPPA+svPPA) (0.21±0.3 pg/mL, n=274, η^2=0.38, p<0.001). P-tau181 was 2.4-fold increased in the clinical AD-spectrum compared to the clinical FTLD-spectrum (1.0±1 pg/mL, n=274, η^2=0.32 p<0.001). The associated AUC for P-tau217 (0.93, p<0.001) was higher than for P-tau181 (AUC=0.91, p<0.001, DeLong test p=0.007).

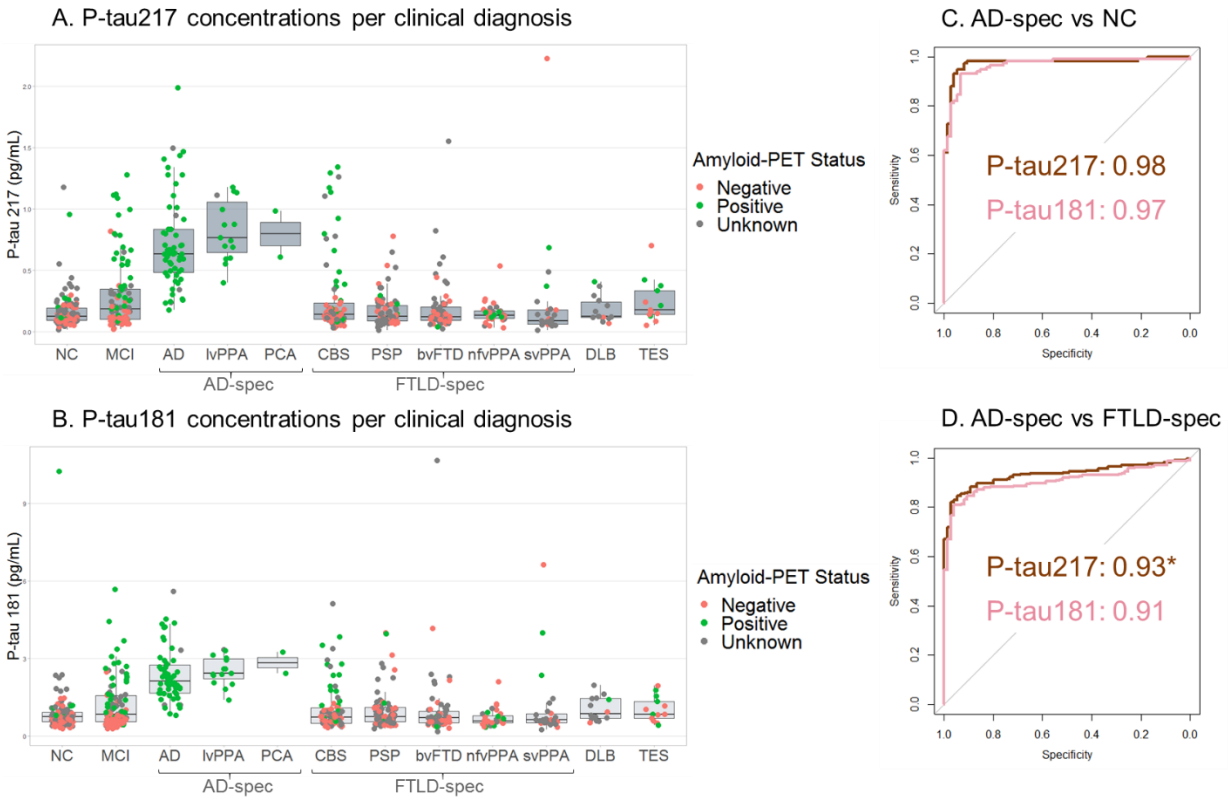

Figure 1. P-tau217 and P-tau181 concentrations per clinical diagnosis. A. P-tau217 was increased in the AD spectrum compared to all other diagnoses. **B.** P-tau18 was increased in the AD spectrum compared to all other diagnoses. **C**. ROC curve analyses of the differentiation between the cases in the AD spectrum and the normal controls. **D.** ROC curve analyses of the differentiation between the cases in the AD spectrum and cases in the FTLD spectrum. *Significant difference in AUC.

Differentiation based on pathology confirmed diagnosis and *MAPT* mutation status

Both P-tau217 and P-tau181 were associated with AD in pathology confirmed cases. P-tau217 concentrations were 4.1 fold increased in AD_{path} (0.62±0.4 pg/mL, n=15) compared to FTLD-tau (0.15±0.1 pg/mL, n=52, η^2=0.15, p=0.006) and 5.6 fold compared to FTLD-TDP (0.11±0.04 pg/mL, n=16, η^2=0.23, p=0.025) (**Figure 2A**). P-tau217 concentrations were associated with Braak stage as a continuous variable (β=0.69, p<0.001) and were higher in Braak V-VI compared to all earlier Braak stages (p<0.02, **eFigure 2A**). P-tau217 concentrations were associated with neuritic plaque CERAD score (β=0.67, p<0.001) and were higher in CERAD frequent compared to CERAD

none and moderate (p<0.01, **eFigure 3A, eFigure 4A**). P-tau181 concentrations were 2.8 fold increased in AD_{path} (2.2±1 pg/mL, n=15) compared to FTLD-tau (0.8±0.5 pg/mL, n=52, η^2=0.10, p=0.034) and 3.7 fold compared to FTLD-TDP (0.6±0.1 pg/mL, n=16, η^2=0.23, p=0.026) (**Figure 2B**). P-tau181 concentrations were associated with Braak stage (β=0.63, p<0.001) and were higher in Braak V-VI compared to Braak I-II, and Braak III-IV (p<0.001) (**eFigure 2B**). P-tau181 concentrations were associated with CERAD score (β=0.60, p<0.001) and were higher in CERAD frequent compared to CERAD none and moderate (p<0.04, **eFigure 3B, eFigure 4B**). The AUC for the differentiation between AD and FTLD (FTLD-tau+FTLD-TDP) was 0.96 with P-tau217 and 0.90 with P-tau181 (**Figure 2C**), which was not statistically different (Delong test: p=0.22, **Table 2**).

Seventy-seven individuals had an FTLD-causing mutation. There was no difference in P-tau217 or P-tau181 concentrations between *MAPT*, *GRN*, or *C9orf72* mutation carriers or between the mutation carrier groups and normal controls. When studying only the *MAPT* mutation carriers, there was no difference in P-tau217 or P-tau181 concentrations between age- and sex-matched amyloid-PET negative NC (P-tau217:0.14±0.04 pg/mL, P-tau181:0.82±0.3 pg/mL, n=8), 4R (P-tau217: 0.23±0.21 pg/mL, P-tau181: 0.9±0.6 pg/mL, n=33), or AD-like 3R/4R tau pathology mutation carriers (P-tau217: 0.29±0.2, P-tau181: 1.1±0.5, n=11, η^2=0.05, p=0.32 for P-tau217, η^2=0.05, p=0.30 for P-tau181). Since these results differed from our previous finding of increased plasma P-tau181 concentrations in 3R/4R mutation carriers,[3] we directly compared the results from 39 *MAPT* individuals who were measured with both the previous assay and the new P-tau181 assay designed to be more comparable to the P-tau217 assay. P-tau181 concentrations with both assays did not correlate (R=-0.002, p=0.99) in *MAPT* mutation carriers, even though the old and new P-tau181 concentrations were correlated in the whole cohort (R=0.69, p<0.001, **eFigure 5**).

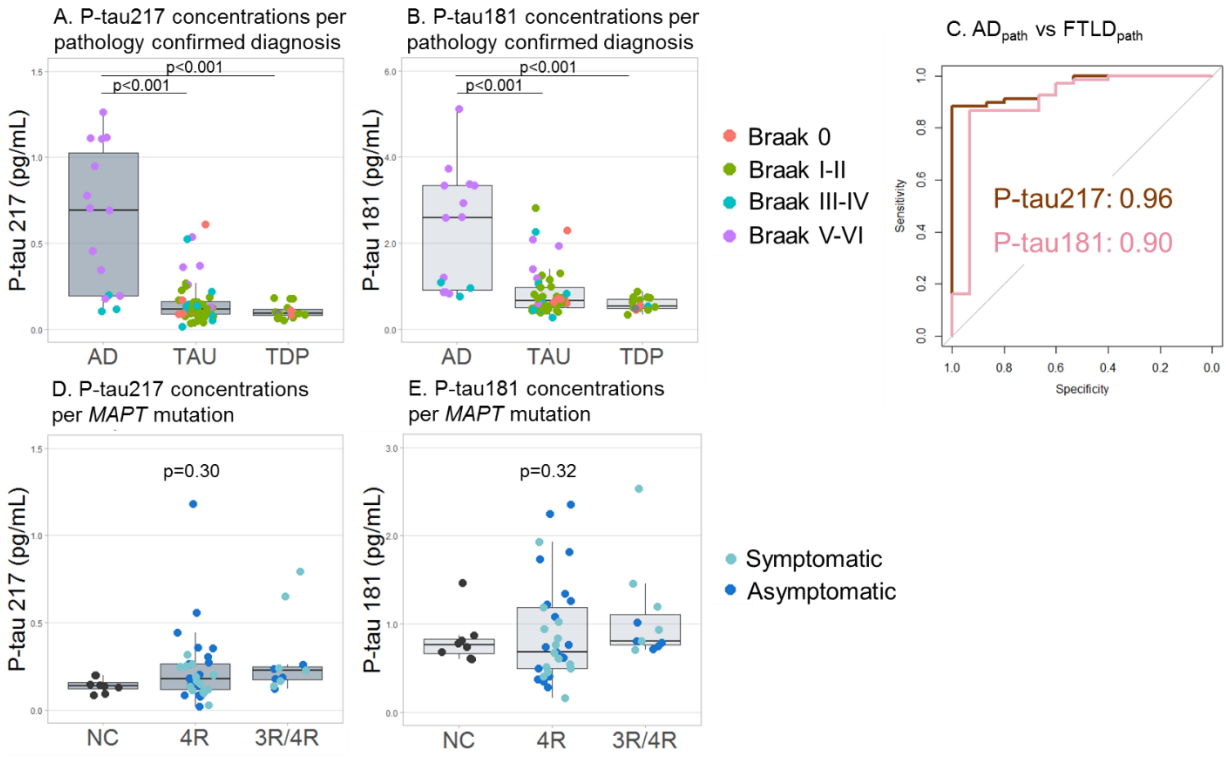

Figure 2. P-tau217 and P-tau181 concentrations per pathology-confirmed diagnosis and *MAPT* genotype. **A.** P-tau217 was increased in the pathology-confirmed AD group compared to the FTLD-TDP and FTLD-tau groups. **B.** P-tau181 was increased in the pathology confirmed AD group compared to the FTLD-TDP and FTLD-tau groups. **C.** ROC curve analyses of the differentiation between the pathology-confirmed AD and FTLD-tau combined with the FTLD-TDP cases. **D.** There was no difference in P-tau217 concentrations between 4R (n=33, 58% female, mean age=41.8), 3R/4R (n=11, 45% female, mean age=45.3) *MAPT* and age- and sex-matched amyloid-PET-negative controls **(**n=8, 50% female, mean age=50.7). There was no difference in P-tau181 concentrations between 4R (n=33), 3R/4R (n=11) *MAPT* and age- and sex-matched controls **(**n=19). ***p<0.001, adjusted for sex, age, and CDRsb.

Associations between P-tau217, P-tau181 with clinical and plasma biomarkers of disease status

Both P-tau217 and P-tau181 similarly correlated with MMSE, CDRsb, functional activities questionnaire (FAQ), modified Rey figure recall, trail making test, Stroop color-naming, semantic fluency, and D-word fluency in the whole cohort (**eTable 3**). In the AD spectrum subgroup, P-tau217 remained associated with MMSE, CDRsb, FAQ, trail making test, semantic fluency and D-word fluency, whereas P-tau181 was only associated with MMSE. In the FTLD spectrum subgroup, both P-tau217 and P-tau181 only correlated with Stroop color-naming and D-word fluency.

There was no correlation between plasma NfL and P-tau217 (R=-0.06, p=0.32, n=268) or P-tau181 (R=0.008, p=0.90, n=268), nor in the AD spectrum (P-tau217: R=0.06, p=0.75; P-tau181: R=0.26, p=0.18, n=28) or FTLD spectrum (P-tau217: R=-0.05, p=0.61; P-tau181: R=0.003, p=0.97, n=130) subgroup analyses. Plasma NfL was not superior to either plasma P-tau for most diagnostic comparisons but performed with similar accuracy in differentiating AD$_{path}$ from FTLD$_{path}$ (**Table 2**). CSF P-tau181 was correlation with plasma P-tau217 (R=0.57,

p<0.001, n=199) and P-tau181 (R=0.48, p<0.001, n=199). CSF P-tau181 showed similar accuracy to plasma P-tau217 in all diagnostic comparisons performed (**Table 2**).

Association between P-tau217, P-tau181 and amyloid-PET status

Both plasma P-tau217 and P-tau181 were associated with amyloid-PET positivity. P-tau217 was 3.9-fold increased in amyloid-PET-positive (0.58±0.4 pg/mL, n=146) compared to amyloid-PET-negative cases (0.15±0.2 pg/mL, n=214, η^2=0.33, p<0.001, **Figure 3B**) across clinical diagnoses. P-tau181 was 2.5-fold increased in amyloid-PET-positive (2.0±1 pg/mL, n=146, **Figure 3B**) compared to amyloid-PET-negative cases (0.8±0.6 pg/mL, n=214, η^2=0.28, p<0.001). P-tau217 differentiated amyloid-PET-positive from negative individuals with an AUC of 0.91, which was slightly higher than the differentiating accuracy of P-tau181 (AUC=0.89, Delong test p=0.049, **Figure 3B, Table 2**). We assessed whether P-tau217 and P-tau181 could predict amyloid-PET positivity within clinical diagnoses of MCI, CBS, or controls, as these clinical groups often have underlying AD pathology.[29-33] 40% of the MCI cases were amyloid-PET-positive (AUC of 0.93 for P-tau217 and 0.92 for P-tau181, both p<0.001, n=73), 34% of the CBS cases were amyloid-PET-positive (AUC of 0.90 for both P-tau217 and P-tau181, both p<0.001, n=50), and 17% of the controls were amyloid-PET-positive (AUC of 0.83 for P-tau217 and 0.85 for P-tau181, both p≤0.001, n=64, **Table 2, eFigure 6**). There was no significant difference in AUC between the two plasma biomarkers.

Association between P-tau217, P-tau181 and tau-PET

Plasma P-tau217 was more strongly correlated with temporal tau-PET binding (combining bilateral entorhinal, amygdala, fusiform, inferior and middle temporal cortices)[1] than P-tau181 (R=0.79 vs R=0.72, both p<0.001, n=230, ΔR=0.07, 95%CI=[0.030-0.115], p<0.001, **Figure 3A-B**). The correlation in the amyloid-PET-positive group (n=117) was R=0.73, p<0.001 for P-tau217 and R=0.59, p<0.001 for P-tau181 (ΔR= 0.14, 95%CI=[0.081-0.216], p<0.001). There was no correlation in the amyloid-PET-negative group (n=105) with P-tau217 (R=0.17, p=0.08) or P-tau181 (R=0.10, p=0.30). P-tau217 was 4.5-fold increased in tau-PET positive cases (0.68±0.4 pg/mL, n=112) compared to tau-PET-negative cases (0.15±0.1 pg/mL, n=118, η^2=0.41, p<0.001) across clinical diagnoses. P-tau181 was 2.9-fold increased in tau-PET-positive cases (2.2±1 pg/mL, n=112) compared to tau-PET-negative cases (0.76±0.6 pg/mL, n=118, η^2=0.37, p<0.001). P-tau217 differentiated between tau-PET-positive and -negative with an AUC of 0.96, which was better than P-tau181 (AUC=0.94, Delong test: p=0.03, **Figure 3C, Table 2**). P-tau217 and P-tau181 could also differentiate between tau-PET-positive and -negative cases

within clinical diagnoses of MCI and CBS, with no significant difference in discriminability between the two plasma markers (**eFigure 7**).

The optimal plasma P-tau cut-off concentrations for Tau-PET positivity were similar to those for differentiation between controls and AD patients **(Table 2**). The cut-off concentrations to differentiate between clinical AD and FTLD were similar to those for differentiation between pathology-confirmed AD and FTLD.

Table 2 goes here

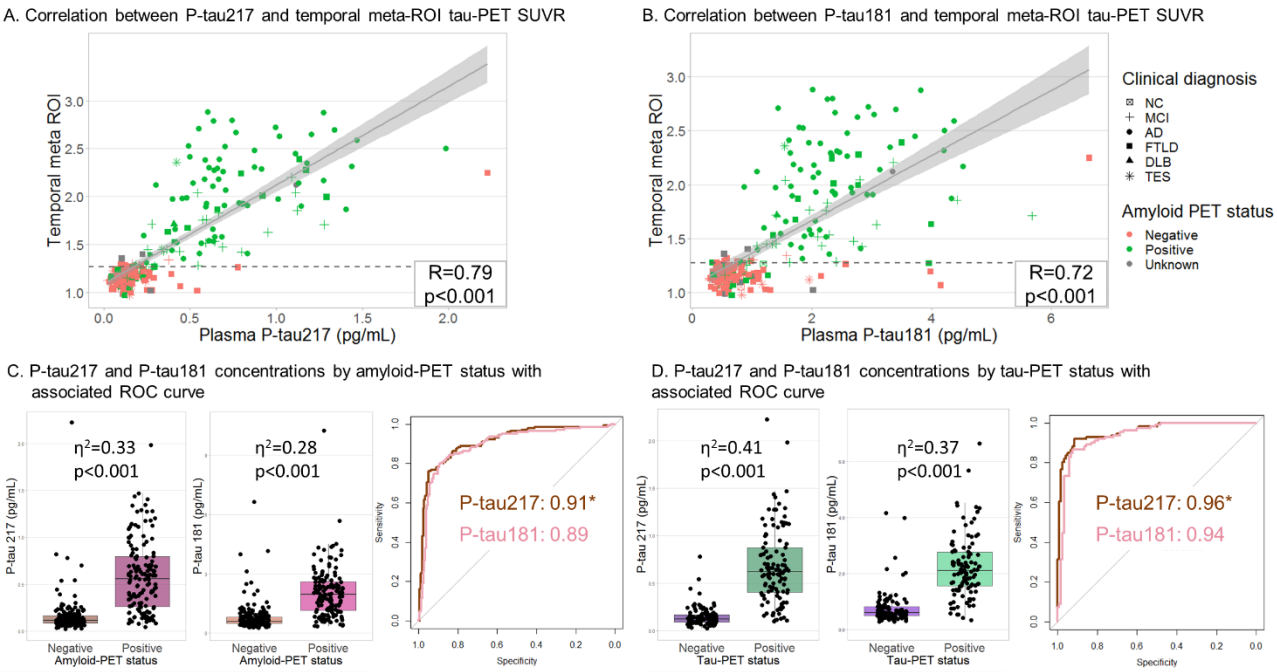

Figure 3. Association between P-tau217, P-tau181, amyloid-PET and tau-PET. A. The correlation between plasma P-tau217 and temporal tau-PET SUVR, shapes show clinical diagnosis and color shows amyloid-PET status. The dotted line is the cut-off value for tau-PET positivity of 1.27 SUVR. **B**. The correlation between plasma P-tau181 and temporal tau-PET SUVR, shapes show clinical diagnosis and color shows amyloid-PET status. The dotted line is the cut-off value for tau-PET positivity of 1.27 SUVR. **C.** Differentiation between amyloid PET-positive and -negative, by boxplot and ROC AUC. **D**. Differentiation between tau-PET-positive and -negative, by boxplot and ROC AUC. *Significant difference in AUC, adjusted for sex, age.

Voxelwise correlation between P-tau217, P-tau181, tau-PET binding, and MRI.

In the whole tau-PET sample, plasma P-tau concentrations were associated with tau-PET throughout the cortex, with strongest correlations in the temporoparietal areas, and lateral frontal regions (**Figure 4**). The correlation was most pronounced for P-tau217, with coefficients of up to 0.81 compared to 0.70 for P-tau181, both peaking in the right middle temporal lobe. There was no significant association between P-tau concentration and tau-PET in

amyloid-PET negative cases, after excluding one amyloid-PET negative outlier with high plasma values and high tau-PET SUVR. In contrast, tau-PET-plasma associations were significant in the amyloid-PET-positive group, with correlations up to 0.74 for P-tau217 and 0.59 for P-tau181. The pattern of voxelwise correlations was very similar in patients with a clinical diagnosis of MCI, and even stronger in CBS, with correlations exceeding r=0.9 in frontal, parietal, and temporal areas.

Higher plasma P-tau concentration was associated with lower GM volume in a pattern that resembled but was more restricted than the pattern seen with tau-PET (**Figure 5**). In the whole cohort, the association was maximal in temporoparietal areas, with correlations reaching -0.36 for P-tau217 and -0.33 for P-tau181. Strong associations between plasma P-tau markers and temporo-parietal volume were seen in amyloid-PET-positive cases and patients with clinical diagnoses of MCI or CBS, but not in the amyloid-PET-negative subgroup.

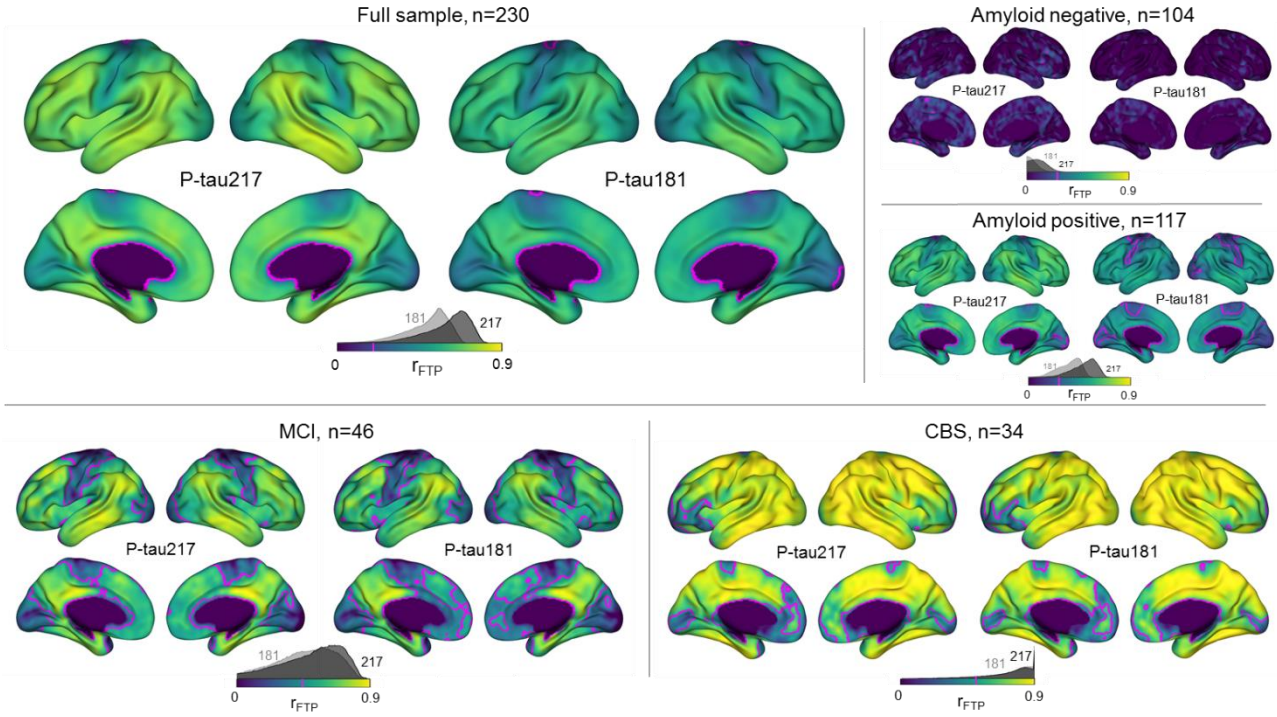

Figure 4. Voxelwise correlation between plasma P-tau217, P-tau181, and Tau-PET tracer binding. The density plots on top of the color bars show the distribution of R values across all voxels included in the GM cortex mask. The pink lines in the color bar indicate p<0.001 at the voxel level and correspond to various R values depending on each subsample size. The voxelwise correlation brain maps are available on Neurovault: https://neurovault.org/collections/SDXLLPNR/

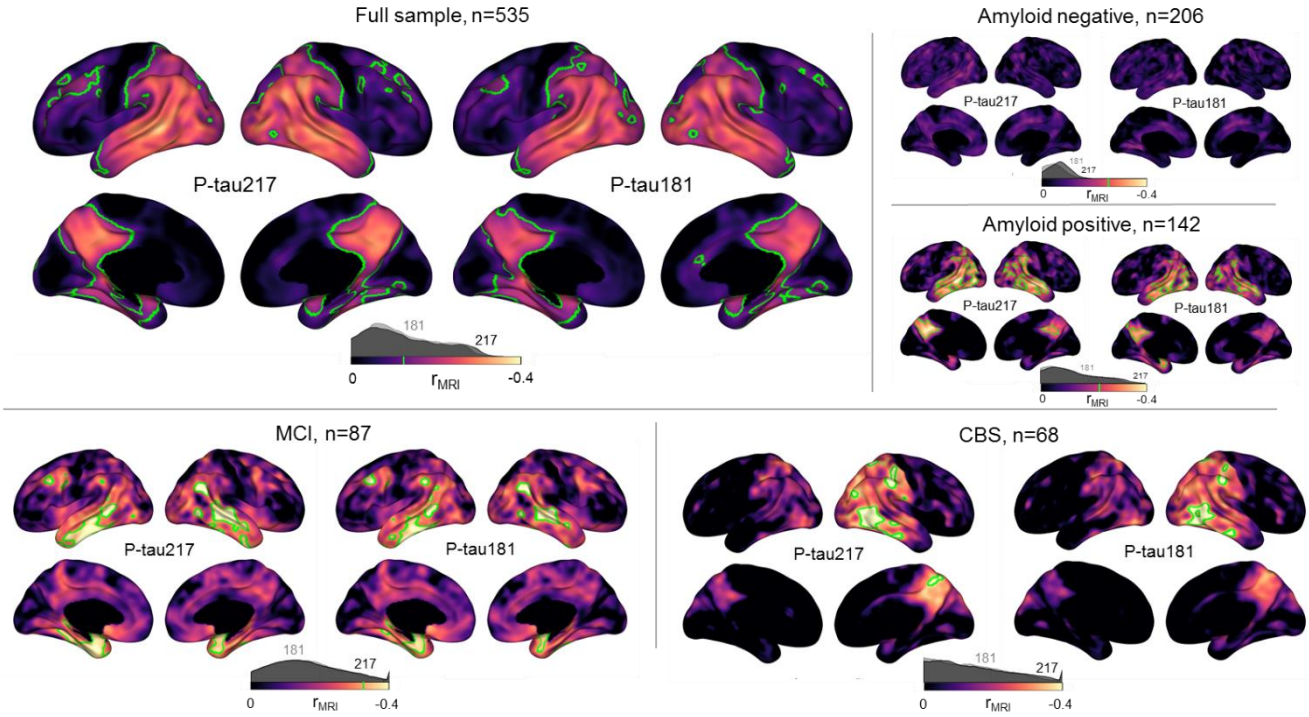

Figure 5. Voxelwise correlation between plasma P-tau217, P-tau181, and grey matter volume. The density plots on top of the color bars show the distribution of R values across all voxels included in the GM cortex mask. The green lines in the color bar indicate p<0.001 at the voxel level and correspond to various R values depending on each subsample size. The voxelwise correlation brain maps are available on Neurovault: https://neurovault.org/collections/SDXLLPNR/

Discussion

We compared the diagnostic performance of plasma P-tau217 or P-tau181 in a large cohort of patients with various neurodegenerative diseases using assays matched for technical conditions. Both plasma biomarkers could distinguish AD from non-AD disorders defined either clinically or neuropathologically (AUCs>0.90), and both were associated with *in vivo* presence of amyloid and tau pathology as measured with PET. Higher P-tau concentrations were also associated with greater brain atrophy measured on MRI scans in brain regions typically affected by AD pathology, and with cognitive impairment. Across multiple contrasts of interest, plasma P-tau217 tended to perform better than P-tau181: the diagnostic accuracy of P-tau217 as measured with the AUC curve was higher for differentiation of clinical AD vs clinical FTLD, amyloid-PET-positive vs -negative cases, and tau-PET-positive vs -negative cases, although the differences in performance were modest (ΔAUC<0.02).

The data collected from this cohort including both primary (FTLD-tau) and secondary tauopathies (AD) support the use of either plasma P-tau217 or P-tau181 as screening tools to help target therapies to individuals with underlying AD tau pathology.[34] These biomarkers may be particularly valuable in clinical syndromes that are poorly predictive of an underlying pathology but where AD is a possibility. For example, only 50% of MCI patients have AD pathology at autopsy[29,30] or an CSF or PET biomarker profile.[31] Likewise, 25%-35% of patients with CBS, classically associated with FTLD-tau pathology (CBD or PSP),[24,32] have underlying AD.[29,32,33] AD co-pathology is also common in clinically diagnosed DLB patients who come to autopsy.[35] In our study, both P-tau217 and P-tau181 were associated with visual reads of amyloid and tau-PET biomarkers of AD in patients with clinical MCI or CBS diagnoses (AUCs for both ≥0.90); and in the small number of DLB patients studied, the highest plasma P-tau concentrations were in an amyloid-PET-positive individual. Further, voxelwise analyses showed strong associations between the concentrations of both plasma P-tau species and tau-PET binding in regions affected by AD pathology in MCI and CBS due to AD. This study and studies by us and others showed that plasma P-tau has better diagnostic accuracy for AD than plasma NfL[3,4,6] and similar to CSF P-tau181.[4,6] Our data suggest that plasma P-tau may be a particularly useful biomarker to screen MCI or CBS patients for underlying AD pathology to allow them to participate in clinical trials of AD-tau-targeted therapies.

Although CSF studies have suggested that P-tau217 might be an earlier marker of AD pathology than P-tau181,[37] we found no significant difference between P-tau217 and P-tau181 in predicting amyloid- or tau-PET positivity in MCI patients, or in predicting amyloid-PET positivity in controls. P-tau217 was more strongly correlated with cognitive performance, a later occurring feature of AD. The difference with the CSF studies may be due to reduced sensitivity of plasma biomarkers to brain pathology, methodological differences related to the use of immuno-

based as opposed to MS based assays or to a difference in the composition of the cohort we studied. Similar to our results, a recent study of plasma P-tau217 and P-tau181 measured using MS in a smaller cohort that did not include FTLD-tau patients suggested small numerical advantages in favor of P-tau217.[38] Another study has shown that plasma P-tau217 concentration increases before tau-PET tracer binding in preclinical AD.[39]

In this study, we did not replicate the relative increase in plasma P-tau181 concentrations in 3R/4R tau-producing compared to 4R tau-producing *MAPT* mutation carriers that we found in our previous study.[3] This likely reflected the use of a different P-tau181 assay with a different detector antibody and different sample buffer. Although plasma P-tau181 concentrations measured using the new assay were moderately correlated with concentrations measured with the old assay across all participants, there was no correlation between the results of the two P-tau181 assays in *MAPT* carriers alone. One potential explanation for this difference is that the physicochemical properties of *MAPT* 3R/4R soluble tau species might differ from AD 3R/4R soluble tau species in sensitivity to the assay conditions. This difference could expose a binding region with more affinity for the capture antibody from the older assay. If true, this phenomenon suggests that careful examination of AD 3R/4R tau protein antibody interactions might reveal therapeutically relevant differences in *MAPT* mutation carrier 3R/4R tau structure that could affect the binding of anti-tau monoclonal antibodies designed to treat AD-tau.

To widely implement P-tau as a biomarker for AD, diagnostic cut-off values should be established. Arguably, for clinical diagnosis, the cut-off value that can help to estimate whether dementia syndromes are due to AD or FTLD might be most useful. For example, our pathology-confirmed cohort included three patients who had a clinical diagnosis of CBS but had AD pathology at autopsy. These cases had plasma P-tau217 and P-tau181 concentrations above the cut-off value for AD compared to FTLD. Assay validation in a larger, more diverse community, as opposed to highly selected research populations will be critical to determine widely applicable cut-off concentrations.

A strength of this study was its robust approach to comparing both plasma P-tau217 and P-tau181 markers in an optimized way, using matched immunochemical assays. This is most likely the reason why the difference in diagnostic accuracy was not as large as previously reported, since the previous study could be confounded by differences in the types of assays used in some of the analyses that were unrelated to the targeted epitope.[6]

This study also had several limitations. Only three out of 13 DLB patients had amyloid-PET data and therefore we were unable to address the question of whether P-tau217 or 181 could successfully identify amyloid co-pathology

in DLB. Plasma NfL and CSF P-tau181 measurements were only available for subsets of the whole cohort. Another limitation of this study was the lack of ethnic and socioeconomic diversity in our research cohort.

In conclusion, in this direct one-to-one comparison of plasma P-tau217 and P-tau181 we demonstrate that both markers have good diagnostic performance for AD. Although the fold change in concentration between diagnostic groups are higher with P-tau217 than P-tau181, this does not translate to a large difference in diagnostic utility. Both plasma P-tau species can aid in evaluation of subjects by testing for underlying AD related tau pathology.

Acknowledgements

Data collection and dissemination of the data presented in this manuscript was supported by the ALLFTD Consortium (U19: AG063911, funded by the National Institute on Aging and the National Institute of Neurological Diseases and Stroke) and the former ARTFL & LEFFTDS Consortia (ARTFL: U54 NS092089, funded by the National Institute of Neurological Diseases and Stroke and National Center for Advancing Translational Sciences; LEFFTDS: U01 AG045390, funded by the National Institute on Aging and the National Institute of Neurological Diseases and Stroke). The authors acknowledge the invaluable contributions of the study participants and families as well as the assistance of the support staffs at each of the participating sites.

Funding details

US National Institutes of Health (U19AG063911, U01AG045390, U54NS092089, R01AG038791, P01AG019724, U24AG21886, R01AG045611, P50AG023501, P50AG016574, K24AG053435); State of California Department of Health Services (04-33516); Tau Consortium, Michael J Fox foundation, Alzheimer's Association (AARF-16-443577). Avid Radiopharmaceuticals enabled use of the [18F]AV1451 tracer by providing precursor, but did not provide direct funding and was not involved in data analysis or interpretation. Plasma P-tau assay results were donated by Lilly Research laboratories. HZ is a Wallenberg Scholar supported by grants from the Swedish Research Council (#2018-02532), the European Research Council (#681712), Swedish State Support for Clinical Research (#ALFGBG-720931), the Alzheimer Drug Discovery Foundation (ADDF), USA (#201809-2016862), the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 860197 (MIRIADE), and the UK Dementia Research Institute at UCL.

Author contributions:

E.H.T., R.L.J., and J.C.R. had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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Acquisition, analysis, or interpretation of data: All authors.

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Obtained funding: A.L.B., B.F.B., H.R., B.L.M., G.D.R., J.H.K., J.L.D.

Supervision: J.L.D., J.C.R., A.L.B.

All authors contributed to revision and editing of the manuscript.

Role of funder/sponsor:

The funding agencies had no role in the design and conduct of the study, collection, management, analysis or interpretation of the data, preparation, review or approval of the manuscript or decision to submit the manuscript for publication.

Declaration of interests

E.H.T, declare no conflict of interest. J.L.D. and N.K.P are employees of Eli Lilly and Company. H.Z. has served at scientific advisory boards for Denali, Roche Diagnostics, Wave, Samumed, Siemens Healthineers, Pinteon Therapeutics and CogRx, has given lectures in symposia sponsored by Fujirebio, Alzecure and Biogen, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program. K.B. served as a consultant or at advisory boards for Alector, Biogen, CogRx, Lilly, MagQu, Novartis and Roche Diagnostics, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB, a GU Venture-based platform company at the University of Gothenburg, all unrelated to the work presented in this paper. L.T.G. receives research support from Avid Radiopharmaceuticals, Eli Lilly. She has received consulting fees from the Simon Foundation and Cura Sen, Inc. She serves as associate editor for Frontiers in Aging Neurosciences, Frontiers

in Dementia and the Journal of Alzheimer Disease. G.D.R. receives research support from NIH, Alzheimer's Association, American College of Radiology, Tau Research Consortium, Avid Radiopharmaceuticals, Eli Lilly, GE Healthcare, Life Molecular Imaging. He has served as a consultant for Eisai, Merck, Axon Neurosciences. He received speaking honoraria from GE Healthcare. He serves as Associate Editor for JAMA Neurology. J.C.R. is a site PI for clinical trials supported by Eli Lilly and receives support from NIH. A.L.B. receives research support from NIH, the Tau Research Consortium, the Association for Frontotemporal Degeneration, Bluefield Project to Cure Frontotemporal Dementia, Corticobasal Degeneration Solutions, the Alzheimer's Drug Discovery Foundation and the Alzheimer's Association. He has served as a consultant for Aeton, Abbvie, Alector, AGTC, Amgen, Arkuda, Arvinas, Asceneuron, Ionis, Lundbeck, Novartis, Passage BIO, Sangamo, Samumed, Third Rock, Toyama and UCB, and received research support from Avid, Biogen, BMS, C2N, Cortice, Eli Lilly, Forum, Genentech, Janssen, Novartis, Pfizer, Roche and TauRx.

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