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Association of Plasma P-tau217 and P-tau181 with clinical phenotype, neuropathology, and imaging markers in Alzheimer's disease and frontotemporal lobar degeneration: a retrospective diagnostic performance study

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Data Sharing

Data and informed consent form are available upon request after publication (University of California, San Francisco Memory and Aging Center: <http://memory.ucsf.edu/research-trials/professional/open-science> and Advancing Research and Treatment for Frontotemporal Lobar Degeneration consortium: <https://www.allftd.org/data>). Requests will be considered by each study investigators based on the information provided by the requester regarding the study and analysis plan. If the use is appropriate, a data sharing agreement will be put in place before a fully de-identified version of the dataset including the data dictionary used for analysis with individual participant data is made available.

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Abstract

Background—Plasma P-tau217 and P-tau181 are associated with Alzheimer’s disease (AD) tau pathology. P-tau217 is a more novel blood-based biomarker that may be diagnostically superior to P-tau181. We compared the diagnostic value of both biomarkers in a cohort of cognitively normal participants and patients with a clinical diagnosis of Mild Cognitive Impairment, AD or frontotemporal lobar degeneration (FTLD).

Methods—In this multi-cohort diagnostic performance study, we gathered plasma samples from the University of California San Francisco Memory and Aging Center and the Advancing Research and Treatment for Frontotemporal Lobar Degeneration consortium. Plasma Ptau-181 and Ptau-217 were measured using electrochemiluminescence-based assays which only differed in the biotinylated antibody epitope specificity. Receiver operating characteristic analyses were used to determine diagnostic accuracy of both plasma markers using clinical diagnosis, neuropathological findings, and amyloid- and tau-PET measures as gold standards. Difference between two area under the curve (AUC) analyses was tested with the Delong test.

Findings—Data were collected from 593 participants (mean age 64 years [SD 13], 294 [49.6%] females) between July and November 2020. Plasma P-tau217 and P-181 were correlated ($r=0.90$, $p<0.0001$). Both P-tau217 and P-tau181 concentrations were increased in clinical AD (mean age 65.3 years) relative to cognitively normal controls (mean age 60.9 years) (P-tau217 AUC=0.98, 95%CI=[0.95–1.00], P-tau181 AUC=0.97, 95%CI=[0.94–0.99], $p[\text{difference}]=0.31$, $n=75$ vs 118) and in pathology-confirmed AD (mean age 72.8 years) vs FTLN (mean age 67.1 years) (P-tau217 AUC=0.96, 95%CI=[0.92–1.00], P-tau181 AUC=0.91, 95%CI=[0.82–1.00], $p[\text{difference}]=0.22$, $n=15$ vs 68). P-tau217 outperformed P-tau181 in differentiating clinical AD from FTLN spectrum (mean age 66.8 years) (P-tau217 AUC=0.93, 95%CI=[0.91–0.96], P-tau181 AUC=0.91, 95%CI=[0.88–0.94], $p[\text{difference}]=0.007$, $n=75$ vs 274). P-tau217 was a stronger indicator of amyloid-PET positivity (P-tau217: AUC=0.91, 95%CI=[0.88–0.94], P-tau181: AUC=0.89, 95%CI=[0.86–0.93], $p[\text{difference}]=0.049$, $n=146$ vs 214). Tau-PET binding in the temporal cortex was more strongly associated with P-tau217 than P-tau181 ($r=0.79$ vs $r=0.72$, $p[\text{difference}]<0.0001$, $n=230$).

Interpretation—In a direct comparison using matched immunoassays, both P-tau217 and P-tau181 had excellent diagnostic performance for differentiating AD from other groups. There were small, but statistically significant differences in favor of P-tau217 for differential diagnosis of clinical AD, indication of amyloid-PET-positivity and stronger correlations with tau-PET signal. Pending replication in independent, diverse, and older cohorts, plasma P-tau217 and P-tau181 may be useful screening tools to identify individuals with underlying amyloid and AD tau pathology.

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Introduction

Tau pathology plays an essential role in Alzheimer's disease (AD) and in about half of frontotemporal lobar degeneration (FTLD) spectrum diseases.¹ 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 and tau measurements in cerebrospinal fluid (CSF), or amyloid or tau positron emission photography (PET) imaging, have been of limited use for screening because of invasiveness, high costs, and the need for specialized personnel. Blood-based biomarkers generally have lower costs, are less invasive and have the 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 with underlying AD pathology to undergo more established CSF or PET diagnostic testing prior to initiating therapy. This may be particularly valuable in clinical syndromes that are not strongly predictive of a specific neuropathology but where AD pathology or co-pathology is a possibility, such as mild cognitive impairment (MCI),^{2,3} corticobasal syndrome (CBS),⁴ and dementia with Lewy bodies (DLB).⁵

Phosphorylated tau (P-tau) is a key component of neurofibrillary tangles in AD pathology. Plasma P-tau181 is a valuable differential diagnostic marker with concentrations 1.5–3.5 times higher in AD compared to normal controls (NC),^{6–8} and 1.8–3.7 times higher in AD

compared clinical and pathology-confirmed FTLN.⁶ P-tau181 is a strong indicator of both amyloid- (receiver operating characteristic (ROC) area under the curves (AUC): 0.76⁹-0.91)⁶ and tau-PET-positivity (AUC: 0.83⁹-0.93⁸), and can be used for individualized prediction of AD dementia in patients with MCI.^{10,11}

Tau has over forty phosphorylation sites, though little research has been done on their comparative diagnostic value, especially in plasma. A recent autopsy study indicated that P-tau217 was the most important phosphorylation site in the differentiation between AD and control brain tissue, outperforming P-tau181.¹² Several studies have shown an advantage of P-tau217 over P-tau181 when measured in CSF using mass spectrometry^{13,14} or immunoassays.^{15,16} When measured simultaneously in CSF using mass spectrometry, P-tau217 had higher accuracy for differentiating amyloid-PET-positive participants from amyloid-PET-negative (AUC=0.96 versus AUC=0.79, n=82).¹⁴ In an immunoassay study using similar assays for P-tau217 and P-tau181, CSF P-tau217 was consistently more strongly correlated with amyloid- and tau-PET binding than P-tau181. P-tau217 also differentiated AD dementia from other neurodegenerative diseases with somewhat higher accuracy.¹⁵ A comparative mass-spectrometry study (n=92) in plasma also showed that P-tau217 outperformed P-tau181 for detecting amyloid-PET-positivity (AUC=0.92 versus AUC=0.75).¹⁷ Similarly, an immunoassay study in larger cohorts reported that plasma P-tau217 performed similar to CSF P-tau217 and tau-PET and outperformed plasma P-tau181 in identifying AD in a clinical cohort (AUC of 0.96 for P-tau217 vs 0.81 for P-tau181, n=220).⁹ However, the P-tau217 and P-tau181 plasma assays used different platforms, detector antibodies, buffers, and calibrators. As pointed out by the authors,⁹ these important technical differences complicated the interpretation of which P-tau epitope might be superior for differential diagnosis and other purposes. A more recent study using assays with better comparable antibodies and the same technology did not find such large differences in performance.¹⁸ Additional work is therefore needed to confirm the diagnostic superiority of P-tau217 since previously observed differences could be attributable to assay characteristics unrelated to which P-tau residue was measured.

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 relative merits of different epitopes and assays. In this study we compared plasma P-tau217 and P-tau181, measured using a novel P-tau181 assay with the same electrochemiluminescence-based technology, antibody and buffers as the P-tau217 assay. We analyzed a wide range of neurodegenerative diseases including the full spectrum of AD, MCI, FTLN and other dementia patients as well as age matched controls.

Methods

Participants

Data were retrospectively collected between July and November 2020. The cohort consisted of clinically normal controls, patients in the AD or FTLN spectrum, patients with DLB and with traumatic encephalopathy syndrome (TES) (see details in eMethod). Normal controls were healthy elderly with normal neurological examinations, normal neuropsychological

testing and Clinical Dementia Rating (CDR®)¹⁹ scores of 0. 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 participants from the UCSF Memory and Aging Center had a pathology-confirmed diagnosis. AD neuropathological changes, including Thal amyloid phase, CERAD neuritic plaque score and Braak neurofibrillary tangles stage were ascertained as previously described.²⁰ FTLT participants were grouped as FTLT-tau or FTLT-TAR DNA-binding protein 43 (FTLT-TDP) as described.²¹ Lewy body disease and chronic traumatic encephalopathy (CTE) neuropathological changes were also assessed.^{22–24} One AD patient and seven FTLT-TAU patients had Limbic-predominant age-related TDP-43 encephalopathy (LATE) co-pathology.²⁵

Procedures

P-tau217 and P-tau181 assays differed only in their epitope specific capture antibodies. 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 as detector antibody. Assays were performed in technical duplicate on the same sample aliquot and processed together in the same batch on a streptavidin small spot plate using the Meso Scale Discovery platform (MSD, Rockville, Maryland) (see eMethods and eTables 1–3 for details on assays).

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

Amyloid-PET, tau-PET, and MRI image data were acquired across multiple centers; acquisition, processing, and analyses are fully detailed in the eMethod. Amyloid-PET status was derived from visual read²⁶ based on PET acquired with [¹¹C]Pittsburgh Compound B (PIB), [¹⁸F]florbetapir, or [¹⁸F]florbetaben. Tau-PET was acquired using [¹⁸F]Flortaucipir; Standardized Uptake Value Ratio (SUVR) values were extracted from the temporal structures using the inferior cerebellum as a reference, and a threshold of SUVR > 1.27 was used to determine tau-PET positivity.¹ Magnetization Prepared Rapid Gradient Echo (MP-RAGE) T1-weighted images were acquired from various 3 Tesla scanners; total intracranial volume was used as a covariate in volumetric analyses.

Statistical analysis

Raw fluid biomarker concentrations were not normally distributed (skewness: P-tau181=3.47, P-tau217=2.34, kurtosis: P-tau181=20.52, P-tau217=6.32); natural log transformation improved the distributions (skewness: P-tau181=0.69, P-tau217=0.47, kurtosis: P-tau181=0.02, P-tau217=−0.10). To clearly convey the distribution of P-tau concentrations, all figures and plots represent raw P-tau217 and P-tau181 values, although

group comparisons were conducted using ANCOVAs on natural log-transformed values. For each model, the distribution of the residuals was examined using histograms, quantile-quantile, and residuals-predicted plots. All ANCOVAs were adjusted for age and sex; additional covariates were used when applicable: CDRsb when comparing groups of clinically-impaired or pathology-confirmed patients, or time between blood draw and death when analyzing neuropathology data. Partial η^2 (η^2_p) was used as a measure of effect size for group differences, and pairwise post hoc comparisons were conducted using Bonferroni correction. Bivariate correlations between plasma values or plasma and PET values were assessed using both Pearson's (r) and Spearman's (ρ) coefficients. Correlations between biomarkers and cognitive tests, which were not normally distributed, were calculated using Spearman rank tests (ρ). The difference in strength between two correlations was tested using methods described in Diedenhofen et al.²⁷ ROC analyses were used to determine diagnostic accuracy. AUCs were computed from binary logistic regression adjusted for covariates (sex and age for all models; adding CDRsb or time between blood test and death when relevant, see above) and the predicted values were used for ROC analyses. Difference between AUC values was tested with the Delong test.²⁸ Cut-off values maximizing Youden indices were used for sensitivity and specificity.²⁹ Statistical analyses were performed using SPSS (version 26; SPSS/IBM, Chicago, IL) and R (version 3.6.1, packages: Cocor and pROC).

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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.

Results

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 selected cohort included 118 normal controls (NC, detailed in eTable 4) and a broad range of neurodegenerative syndromes: 75 patients in the clinical AD spectrum (58 AD_{clin},³⁰ 15 logopenic variant primary progressive aphasia, [lvPPA] and 2 posterior cortical atrophy, [PCA]), 99 MCI patients³¹, and 274 individuals with clinical diagnoses in the FTLT spectrum (79 CBS³², 74 progressive supranuclear palsy [PSP],³³ 62 behavioral variant FTD [bvFTD],³⁴ 32 nonfluent variant PPA [nfvPPA], and 27 semantic variant PPA [svPPA]³⁵). In addition, 14 participants with DLB and 13 participants with Traumatic Encephalopathy syndrome (TES) were included (Table 1). Eighty-five percent (n=507) of participants were white, and 294 (49.6%) were females. Eighty-three participants had a pathology-confirmed diagnosis, 15 AD, 52 FTLT-TAU, and 16 FTLT-TDP (detailed in eTable 5). The average time between blood draw and death was 33±19 months. The participants with an FTLT clinical diagnosis included 77 carriers of FTLT-causing mutations: 44 microtubule-associated protein tau (*MAPT*) associated with FTLT-tau, 13 progranulin (*GRN*) and 20 chromosome 20 open reading frame (*C9orf72*), associated with FTLT-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).³⁶ Amyloid-PET imaging was available for 360 participants; average time from plasma collection: 5 ± 16 months. Tau-PET was available for 230 participants; average time from plasma collection: 1.6 ± 7 months. MRI was available for 535 participants; average time from plasma collection: 1.7 ± 8 months.

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 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 correlated ($r=0.90$, $p<0.0001$, 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 concentrations were 4.4-fold higher in patients in the clinical AD-spectrum (0.74 ± 0.4 pg/mL, $n=75$) compared to controls (0.17 ± 0.2 pg/mL, $n=118$, $\eta^2p=0.69$, $p<0.0001$). P-tau181 concentrations were 2.8-fold higher in the clinical AD-spectrum (2.4 ± 1 pg/mL, $n=75$) compared to controls (0.87 ± 1 pg/mL, $n=118$, $\eta^2p=0.60$, $p<0.0001$). The AUCs for AD versus controls were comparable for P-tau217 (AUC=0.98, $p<0.0001$) and P-tau181 (AUC=0.97, $p<0.0001$, DeLong test for difference in AUC accuracy $p=0.31$, eTable 6).

P-tau217 concentrations were increased 3.5-fold in the clinical AD-spectrum compared to the clinical FTLN-spectrum (CBS+PSP+bvFTD+nfvPPA+svPPA) (0.21 ± 0.3 pg/mL, $n=274$, $\eta^2p=0.42$, $p<0.0001$). P-tau181 was 2.4-fold higher in the clinical AD-spectrum compared to the clinical FTLN-spectrum (1.0 ± 1 pg/mL, $n=274$, $\eta^2p=0.36$, $p<0.0001$). Results were unchanged when adding CDRsb as a covariate in the ANCOVA ($\eta^2p=0.42$ for P-tau217 and $\eta^2p=0.36$ for P-tau181; with 5 missing values in the FTLN group). The AUC for AD versus FTLN for P-tau217 (0.93, $p<0.0001$) was higher than for P-tau181 (AUC=0.91, $p<0.0001$, DeLong test $p=0.007$, eTable 6). The outliers in the FTLN groups are discussed in the eResults.

Both P-tau217 and P-tau181 concentrations were associated with AD neuropathology in autopsy-confirmed participants. P-tau217 concentrations varied across neuropathological groups ($\eta^2p=0.41$, $p<0.0001$); pairwise comparisons showed that P-tau217 concentration were 4.1-fold higher in AD_{path} (0.62 ± 0.4 pg/mL, $n=15$) compared to FTLN-tau (0.15 ± 0.1 pg/mL, $n=52$, AUC=0.96 $p<0.0001$) and 5.6-fold compared to FTLN-TDP (0.11 ± 0.04 pg/mL, $n=16$, AUC=0.98, $p<0.0001$) (Figure 2A, eTable 6). No difference was observed between FTLN-tau and FTLN-TDP ($p=0.39$). P-tau217 was associated with Thal phase ($\eta^2p=0.60$, $p<0.0001$), Braak stage ($\eta^2p=0.57$, $p<0.0001$), and neuritic plaque CERAD score ($\eta^2p=0.49$, $p<0.0001$). Post-hoc comparisons (eFigures 2–5) showed that Ptau217 values were significantly increased at Thal phases 4–5, Braak stages V–VI, and CERAD frequent scores (eFigure 2–5). P-tau181 concentrations also varied across neuropathological diagnoses ($\eta^2p=0.38$, $p<0.0001$) and were 2.8-fold higher in AD_{path} (2.2 ± 1 pg/mL, $n=15$) compared to FTLN-tau (0.8 ± 0.5 pg/mL, $n=52$, AUC=0.90, $p<0.0001$) and 3.7-fold compared to FTLN-TDP (0.6 ± 0.1 pg/mL, $n=16$, AUC=0.95, $p<0.0001$) (Figure 2B, eTable 6). No difference was observed between FTLN-tau and FTLN-TDP ($p=0.14$). P-tau181 was associated with Thal phase ($\eta^2p=0.55$, $p<0.0001$), Braak stage ($\eta^2p=0.52$, $p<0.0001$), and CERAD score ($\eta^2p=0.42$, $p<0.0001$); see eFigures 2–5 for post-hoc analyses. The AUC for

the differentiation between AD and FTLT (FTLT-tau+FTLT-TDP) was 0.96 for P-tau217 and 0.91 for P-tau181 (Figure 2C); Delong test: $p=0.22$, (eTable6).

Seventy-seven individuals had an FTLT-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 (P-tau217: $\eta^2p=0.03$, $p=0.30$; P-tau181: $\eta^2p=0.01$, $p=0.66$). When studying the *MAPT* mutation carriers in details, 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 , $\eta^2p=0.07$, $p=0.19$; P-tau181: 1.1 ± 0.5 , $\eta^2p=0.04$, $p=0.37$, $n=11$). Since these results differed from our previous finding of increased plasma P-tau181 concentrations in 3R/4R mutation carriers,⁶ 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 in *MAPT* mutation carriers ($r=-0.02$, $p=0.99$), even though the old and new P-tau181 concentrations were correlated in the whole cohort ($r=0.69$, $p<0.0001$, eFigure 6).

Both P-tau217 and P-tau181 showed similar Spearman rank-order correlation coefficients 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 (range absolute ρ : 0.133–0.393, eTable 7). In the AD-spectrum subgroup, P-tau217 remained associated with MMSE, CDRsb, FAQ, trail making test, semantic fluency and D-word fluency (range absolute ρ : 0.247–0.470), whereas P-tau181 was only significant associated with MMSE ($\rho=-0.347$, $p=0.003$, see eTable7 for details). Correlations should be interpreted with caution as they were globally weak ($\rho<0.5$) and only significant when not correcting for multiple tests.

There was no correlation between plasma NfL and P-tau217 ($r=-0.07$, $p=0.25$) or P-tau181 ($r=-0.013$, $p=0.83$, $n=268$) in the whole sample, nor in the AD-spectrum (P-tau217: $r=0.013$, $p=0.95$; P-tau181: $r=0.20$, $p=0.30$, $n=28$). Plasma NfL was inferior to either plasma P-tau in diagnostic accuracy (eTable 6). CSF P-tau181 ($n=199$) was correlated with plasma P-tau217 ($r=0.60$, $p<0.0001$) and P-tau181 ($r=0.50$, $p<0.0001$), even though the CSF and plasma samples were not measured with the same assay. CSF P-tau181 showed similar accuracy to plasma P-tau217 (eTable 6).

Both plasma P-tau217 and P-tau181 were associated with amyloid-PET positivity. P-tau217 was 3.9-fold higher in amyloid-PET-positive (0.58 ± 0.4 pg/mL, $n=146$) compared to amyloid-PET-negative participants (0.15 ± 0.2 pg/mL, $n=214$, $\eta^2p=0.49$, $p<0.0001$, controlling for age and sex Figure 3A and C). P-tau181 was 2.5-fold higher in amyloid-PET-positive (2.0 ± 1 pg/mL, $n=146$, Figure 3B and C) compared to amyloid-PET-negative participants (0.8 ± 0.6 pg/mL, $n=214$, $\eta^2p=0.44$, $p<0.0001$). Group differences were unchanged when including CDRsb as a covariate in the ANCOVA ($\eta^2p=0.47$ for P-tau217 and $\eta^2p=0.42$ for P-tau181; with 5 missing values in the amyloid-negative group). P-tau217 differentiated amyloid-PET positive from negative individuals with a ROC AUC of 0.91,

which was slightly higher than the differentiating accuracy of P-tau181 (AUC=0.89, Delong test $p=0.049$, Figure 3C, eTable 6). We assessed whether P-tau217 and P-tau181 could predict amyloid-PET positivity within clinical diagnoses of MCI, CBS, DLB, or controls, as underlying AD pathology is common in these groups.^{2-4,37} Amyloid-PET positivity was found in 40% of patients with (AUC = 0.93 for P-tau217 and 0.92 for P-tau181, both $p<0.0001$, $n=73$), in 34% of patients with CBS (AUC = 0.90 for both P-tau217 and P-tau181, both $p<0.0001$, $n=50$), and in 17% of controls (AUC of 0.83 for P-tau217 and 0.85 for P-tau181, both $p=0.001$, $n=64$, eTable 6, eFigure 7). There was no significant difference in AUC between the two plasma biomarkers for these subgroups (all $p's>0.62$, eTable 6). Amyloid-PET status was only available of 3 DLB patients, preventing any statistical analyses.

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 ($r=0.80$ vs $r=0.72$ using log-transformed P-tau values, both $p<0.0001$, $n=230$, $r=0.08$, 95%CI=[0.057–0.106], $p<0.0001$, Figure 3A–B). The correlation in the amyloid-PET-positive group ($n=117$) was $r=0.73$, $p<0.0001$ for P-tau217 and $r=0.61$, $p<0.0001$ for P-tau181 ($r=0.12$, 95%CI=[0.067–0.198], $p<0.0001$). In the amyloid-PET-negative group ($n=104$) temporal tau-PET binding was not correlated with P-tau217 ($r=0.14$, $p=0.17$) or P-tau181 ($r=0.07$, $p=0.46$); note that for this subgroup analysis, one amyloid-PET negative participant with high P-tau217 and P-tau181 concentrations was excluded since it was a clear outlier on both PET and plasma variables (right-most data point included in figure 3A–B).

When binarizing temporal tau-PET values into positive and negative groups, P-tau217 concentrations were 4.5-fold higher in tau-PET-positive (0.68 ± 0.4 pg/mL, $n=112$) compared to tau-PET-negative participants (0.15 ± 0.1 pg/mL, $n=118$, $\eta^2p=0.62$, $p<0.0001$, controlling for age and sex). P-tau181 concentrations were 2.9-fold higher in tau-PET-positive (2.2 ± 1 pg/mL, $n=112$) compared to tau-PET-negative participants (0.76 ± 0.6 pg/mL, $n=118$, $\eta^2p=0.55$, $p<0.0001$). Group differences were unchanged when including CDRsb as a covariate in the ANCOVA ($\eta^2p=0.60$ for P-tau217 and $\eta^2p=0.53$ for P-tau181; with 1 missing value in the tau-PET-negative group). P-tau217 differentiated between tau-PET-positive and -negative with an AUC of 0.96, which was slightly higher than P-tau181 (AUC=0.94, Delong test: $p=0.03$, Figure 3D, eTable 6). P-tau217 and P-tau181 could also differentiate between tau-PET-positive and -negative participants within subgroups with a clinical diagnosis of MCI and CBS (all AUC's ≥ 0.88), with no significant difference in discriminability between the two plasma markers (eFigure 8, eTable 6). 46 MCI patients had both tau-PET and amyloid-PET. Nineteen of those were amyloid-PET and tau-PET negative (A–T–), four were A+T–, two were A–T+, and 21 were A+T+. The P-tau217 and P-tau181 concentrations seemed increased in A+T+ MCI patients, compared to A–T– MCI patients, and to A+T– MCI patients, though sample sizes were too small to perform accurate statistical analyses (eFigure 9).

A small number of participants ($n=55$) had plasma biomarkers, tau-PET and CSF P-tau181; correlations in this subgroup are available in eTable8

In the whole tau-PET sample, plasma P-tau concentrations were associated with tau-PET throughout the cortex, with strongest correlations in temporoparietal and lateral frontal areas (Figure 4). The correlation was most pronounced for P-tau217, with correlation 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 participants, after excluding one amyloid-PET-negative outlier described above. In contrast, tau-PET-plasma associations were significant in the amyloid-PET-positive group, with correlation coefficients up to 0.74 for P-tau217 and 0.59 for P-tau181 (eFigure 10). 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 4, eFigure 10). In the whole cohort, the association was maximal in temporoparietal areas, with correlation coefficients 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 participants and patients with clinical diagnoses of MCI or CBS, but not in the amyloid-PET negative subgroup.

Discussion

We compared the diagnostic performance of plasma P-tau217 with a novel P-tau181 assay, designed to match the assay characteristics of the P-tau217 assay, in a large cohort of patients with various neurodegenerative diseases. Both plasma biomarkers could distinguish AD from non-AD disorders defined either clinically or neuropathologically with high accuracy ($AUC's > 0.90$), 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 slightly 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 FTLT, amyloid-PET-positive vs -negative participants, and tau-PET-positive vs -negative participants, although the magnitudes of the performance differences were small ($AUC < 0.02$).

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. Both P-tau217 and P-tau181 were associated with amyloid and tau-PET biomarkers of AD in patients with clinical MCI or CBS diagnoses ($AUCs$ for both > 0.88). Voxelwise analyses showed strong associations between both plasma P-tau species and tau-PET binding in regions affected by AD pathology in MCI and CBS patients. This study and studies by us and others showed that plasma P-tau has better diagnostic accuracy for AD than plasma NFL^{6,7,9} and similar to CSF P-tau181.^{7,9} 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, or exclude them from trials targeting non-AD-tau pathology. Indeed, recent studies demonstrated that plasma P-tau217 increased longitudinally in preclinical AD³⁸ and that plasma P-tau217 or P-tau181 (combined with NfL) provided subject-level prognostic information about cognitive decline due to AD in MCI patients.¹⁰ Recently, a comprehensive study found that the most parsimonious model for predicting AD dementia in a heterogeneous population of individuals with SCD and MCI was a combination of plasma P-tau, APOE genotype and three brief cognitive tests of memory and executive function.¹¹ Importantly, in that study the prognostic algorithm established in BioFINDER, using normalized plasma P-tau217 levels, could be cross-validated in ADNI, using normalized plasma P-tau181 levels, emphasizing the results from the present study that high-performing assays for P-tau217 and P-tau181 can be used interchangeably in clinical practice and trials.

Although CSF studies have suggested that P-tau217 might be an earlier marker of AD pathology than P-tau181,¹³ we found no significant difference between P-tau217 and P-tau181 in indicating amyloid- or tau-PET positivity in MCI patients, or in indicating amyloid-PET positivity in controls. In the whole cohort, P-tau217 was more strongly correlated with cognitive performance, a later occurring feature of AD. This lack of superiority for plasma compared to the CSF assays may be due to the difference in assay technology, a difference in cohort composition, or might be attributable to a high pass filter effect of the blood-brain barrier for P-tau217 fragments which could reduce the relative sensitivity of plasma compared to CSF measurements for detecting AD pathology.

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.⁶ This likely reflected the use of a different P-tau181 assay with a different detector antibody and different sample buffer. Although the P-tau181 concentrations of these new and old assays were moderately correlated in the whole cohort, there was no correlation 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.

To widely implement P-tau as a biomarker for AD, diagnostic cut-off values should be established. Arguably, clinically, the cut-off value that can help to estimate whether dementia syndromes are due to AD or FTLN 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 patients had plasma P-tau217 and P-tau181 concentrations above the cut-off value for AD compared to FTLN. Assay validation in a larger, more diverse community with close attention to pre-analytic sample handling will be critical to determine widely applicable cut-off concentrations.

A strength of this study was its robust approach to comparing plasma P-tau217 and P-tau181, 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 that were unrelated to the targeted epitope, a limitation noted by the authors of the study.⁹ This is particularly an issue for immunoassays and less so for mass spectrometry studies.¹⁷ The cohort covered a broad range of neurodegenerative diseases, including FTLT cases with non-AD tau pathology of considerable sample sizes to AD, allowing us to demonstrate differential diagnostic performance similar to a recent study in a larger cohort.⁹

This study also had several limitations. The P-tau217 and P-tau181 concentrations were very low in the FTLT group, with 79 measurements <LLOQ for P-tau217. This indicates a floor effect that limits the dynamic range of the marker for the low concentrations. A relatively small number of patients in the AD spectrum was included in our cohort, since we focused on differential diagnosis. Further, our cohort was relatively young, and therefore provides little information on how plasma P-tau will perform in older age, when AD is more prevalent. However, in this cohort we found no correlation of plasma P-tau with age. Only three out of 13 DLB patients had amyloid-PET data and therefore we could not determine whether P-tau217 or 181 could successfully identify amyloid co-pathology in DLB. Another limitation was the lack of ethnic and socioeconomic diversity in our cohort, in contrast to a recently published multi-ethnic study with plasma P-tau.¹⁸

In conclusion, in this direct comparison of the diagnostic performance of plasma P-tau217 and P-tau181 we demonstrate that both markers have excellent diagnostic performance for AD. Although the fold difference in concentration between diagnostic groups were higher with P-tau217 than P-tau181, this did not translate to a clinically meaningful difference in diagnostic utility in our highly selected research population. Both plasma P-tau species can aid in evaluation of participants by identifying those with underlying AD tau pathology. Additional studies in larger and more diverse cohorts are needed to establish if differences between these biomarkers are clinically meaningful.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Declaration of interests

E.H.T, R.L.J, A.S., L.I., L.V. declare no conflict of interest. J.L.D. and N.K.P are employees of Eli Lilly and Company who is exploring commercial development opportunities for P-tau assays. J.L.D. is listed as an inventor on a patent related to reagents used in the P-tau assays. HZ 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. OH has acquired research support (for the institution) from AVID Radiopharmaceuticals, Biogen, Eli Lilly, Eisai, GE Healthcare, Pfizer, and Roche. In the past 2 years, he has received consultancy/speaker fees from AC Immune, Alzpath, Biogen, Cerveau and Roche. K.B. served as a consultant, at advisory boards, or at data monitoring committees for Abcam, Axon, Biogen, JOMDD/Shimadzu, Julius Clinical, Lilly, MagQu, Novartis, Roche Diagnostics, and Siemens Healthineers, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program, 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*. H.R. has received research support from Biogen Pharmaceuticals, has consulting agreements with Wave Neuroscience and Ionis Pharmaceuticals, and receives research support from NIH. B.F.B. receives research support from NIH, the Mangurian Foundation and the Little Family Foundation. He is on the Scientific Advisory Board for the Tau Consortium. He has received grant support for clinical trials from Biogen, Alector and EIP Pharma. G.D.R. receives research support from NIH, Alzheimer's Association, American College of Radiology, Rainwater Charitable Foundation, Avid Radiopharmaceuticals, Eli Lilly, GE Healthcare, Life Molecular Imaging. He has served as a consultant for Eisai, Merck, Axon Neurosciences, Johnson & Johnson. 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 the NIH, the Rainwater Charitable Foundation, the Association for Frontotemporal Degeneration, Bluefield Project to Cure Frontotemporal Dementia, the Alzheimer's Drug Discovery Foundation and the Alzheimer's Association. He has served as a consultant for Alector, AGTC, Arkuda, Arvinas, AZTherapies, GSK, Oligomerix, Ono, Regeneron, Roche, Samumed, Stealth, Third Rock, Transposon and Wave, and received research support from Biogen, Eisai and Regeneron.

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Research in context

Evidence before this study:

We searched PubMed for all articles published up to June 1, 2021, 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 Alzheimer’s disease 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 participants from amyloid-PET or tau-PET negative participants. One study using immunoassays showed that P-tau217 was significantly better than P-tau181 when differentiating AD dementia to other dementias, however there were important technical differences between the P-tau217 and P-tau181 assays used. Further, a mass spectrometry-based study has shown better separation between amyloid-PET positive and negative cases using P-tau217 compared to P-tau181.

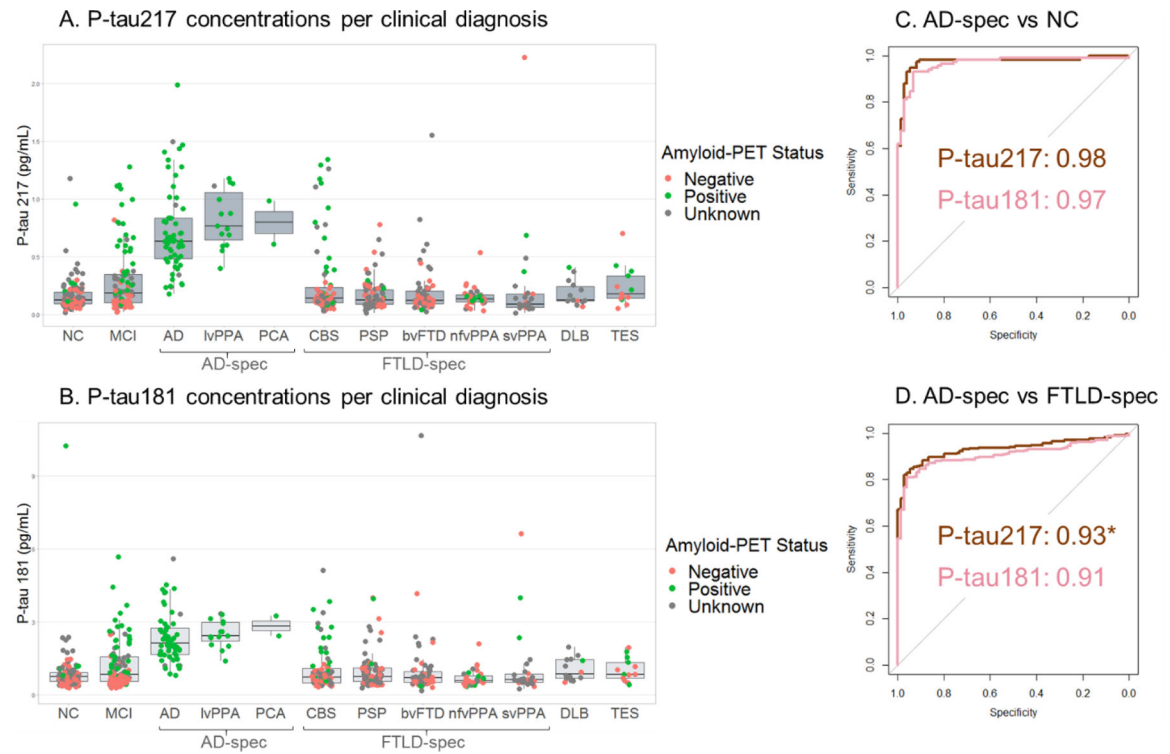
Added value of this study:

This study provides a direct comparison between two plasma P-tau assays for 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 directly comparable. Similar to previous work, both P-tau217 and P-tau181 were increased in AD clinical syndromes compared to healthy controls and a spectrum of FTLN syndromes, Lewy body dementia and Traumatic Encephalopathy Syndrome (TES) patients. Both P-tau species were also increased in pathology-confirmed AD compared to pathology-confirmed FTLN, but neither could differentiate between 3R/4R tau versus 4R tau producing microtubule-associated protein tau (*MAPT*) mutation carriers. Both P-tau species were increased in amyloid-PET-positive compared to amyloid-PET-negative participants, and in tau-PET-positive compared to tau-PET-negative participants 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 when differentiating AD dementia to other dementias and when correlating with amyloid- and tau-PET, but overall, both assays performed very well.

Implications of all the available evidence:

This study shows that 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 scalable and more easily deployed to medically underserved and remote populations. Plasma P-tau217 and P-tau181 are particularly powerful tools 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.



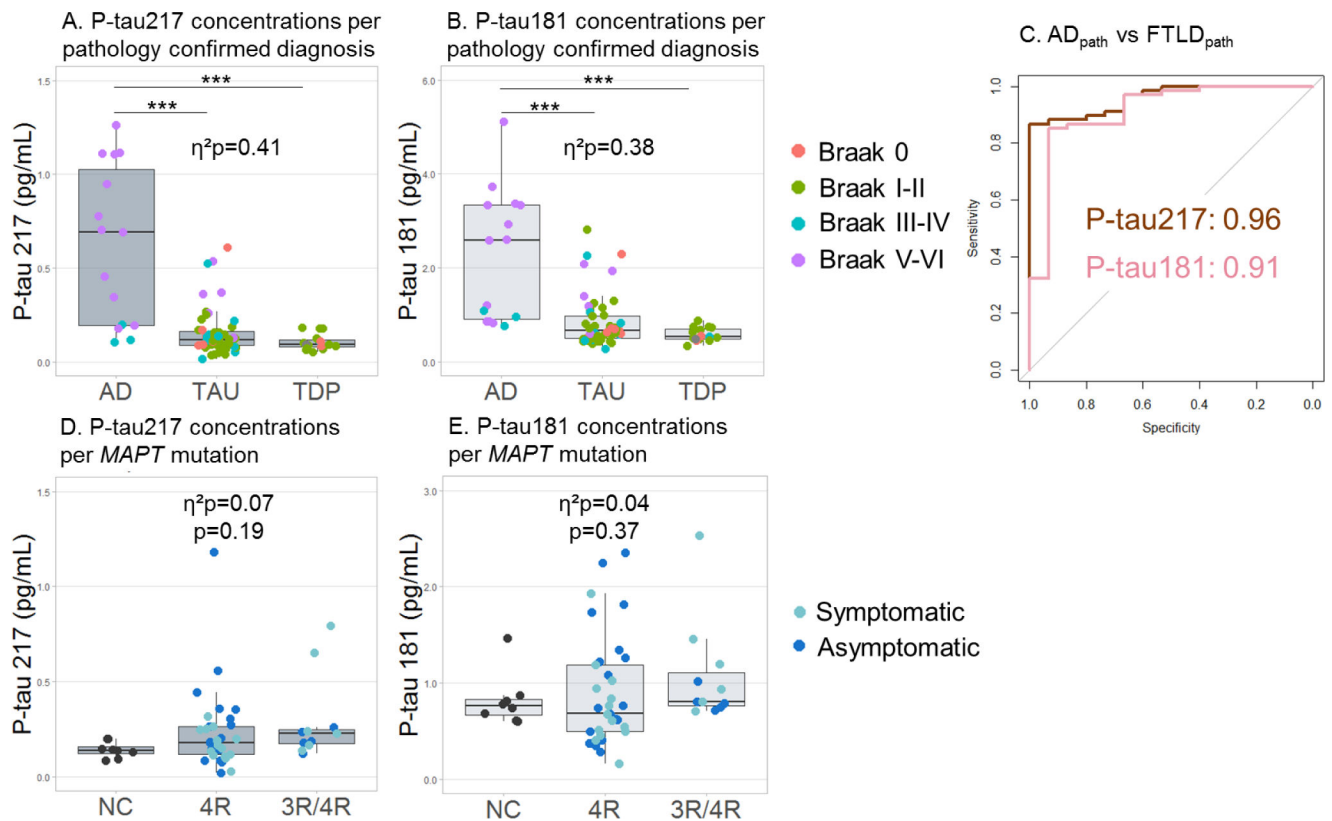


Figure 2. P-tau217 and P-tau181 concentrations per pathology confirmed diagnosis and MAPT genotype.

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 participants. Brown represents P-tau217, pink represents P-tau181. **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). **E.** There was no difference in P-tau181 concentrations between 4R, 3R/4R *MAPT* and age and sex matched controls. Three of the five *MAPT* mutation carriers with the highest P-tau values (P-tau217>0.5 pg/mL or P-tau181>2.0 pg/mL) had a known CSF P-tau concentration which was also high (>30 pg/mL). *** $p<0.0001$, adjusted for sex, age, CDRsb (and time between plasma sample and death for pathology-confirmed participants). For visualization, raw biomarker concentrations were used, although natural log transformed data were used for ANCOVA.

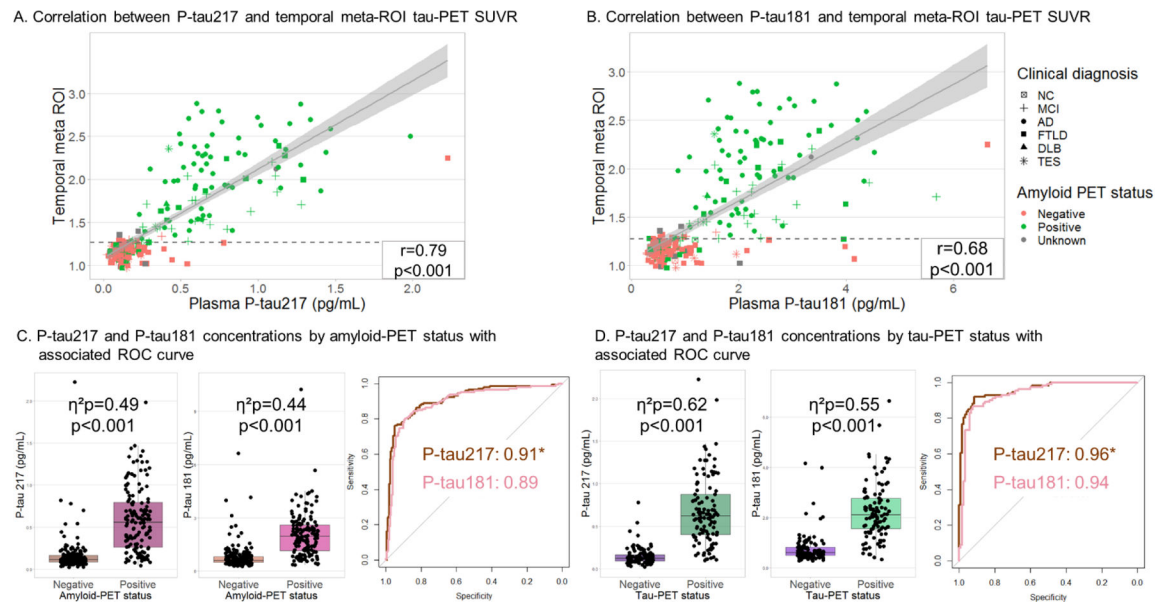


Figure 3. Association between P-tau217, P-tau181, amyloid-PET and tau-PET.

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. Correlation coefficient indicates Pearson's correlation on raw plasma and PET values, but correlation strength was not impacted by log-transformation of P-tau217 values ($r=0.80$) or using rank correlation (Spearman $\rho=0.79$). **B.** The correlation between plasma P-tau181 and temporal tau-PET SUVR. Correlation strength was little impacted by log-transformation of P-tau181 ($r=0.72$) or rank correlation (Spearman $\rho=0.72$). **C.** Differentiation between amyloid-PET-positive and -negative, by boxplot and ROC AUC. Brown represents P-tau217, pink represents P-tau181. **D.** Differentiation between tau-PET-positive and -negative, by boxplot and ROC AUC. Brown represents P-tau217, pink represents P-tau181. *Significant difference in AUC, adjusted for sex, age. For visualization, raw biomarker concentrations were used, although natural log transformed data were used for ANCOVA.

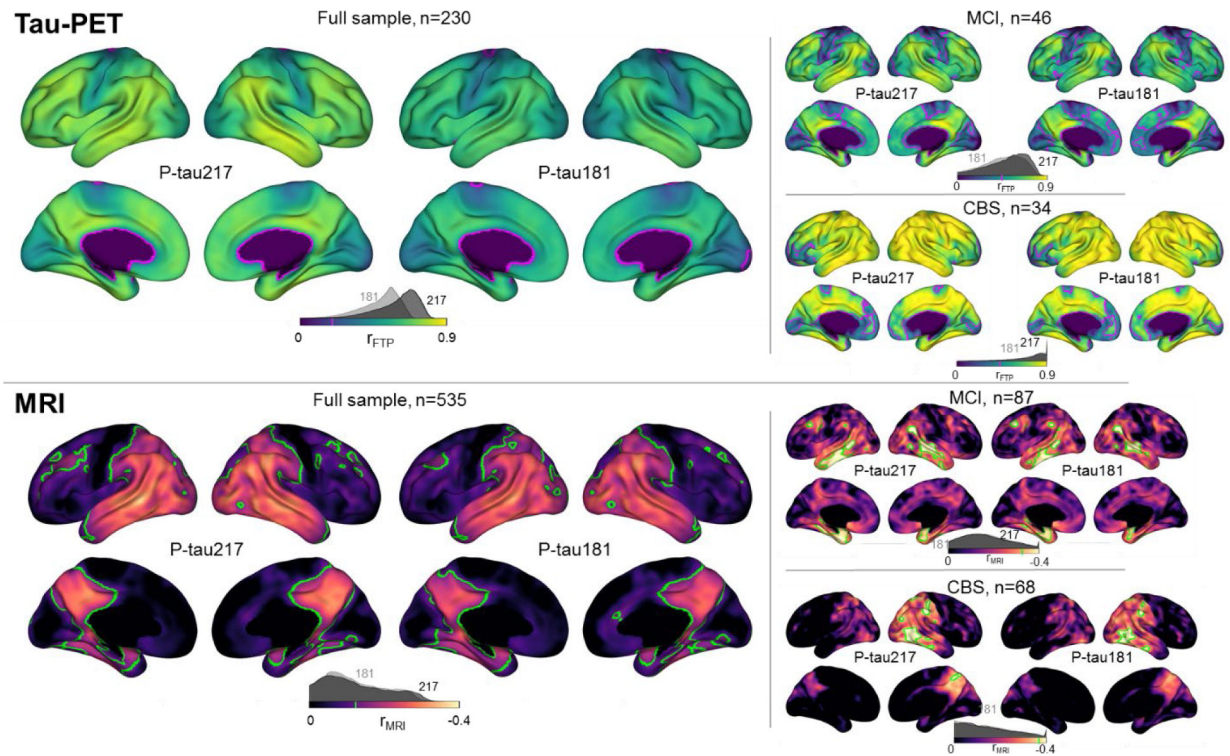


Figure 4. Voxelwise correlation between plasma P-tau217, P-tau181 and Tau-PET tracer binding or grey matter volume.

The density plots on top of the color bars show the distribution of Pearson's r values across all voxels included in the GM cortex mask. The pink lines in the color bar indicate $p < 0.0001$ at the voxel level for tau-PET, green lines in the color bar indicate $p < 0.0001$ for grey matter atrophy, 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/>

Table 1.

Participant characteristics.

	NC (n=118)	MCI (n=99)	AD _{clin} (n=58)	lvPPA (n=15)	PCA (n=2)	CBS (n=79)	PSP (n=74)	bvFTD (n=62)	nvPPA (n=32)	svPPA (n=27)	DLB (n=14)	TES (n=13)	All (n=593)
Sex, M/F	55/63	55/44	25/33	7/8	0/2	36/43	34/40	37/25	17/15	11/16	9/5	13/0	299/294
Age, y	60.9 (18)	65.5 (13)	65.3 (10)	63.1 (9)	57.6 (11)	67.3 (8) ^a	68.5 (7) ^a	61.2 (10)	69.8 (7) ^a	70.0 (7) ^a	69.3 (6)	63.2 (13)	64.3 (13)
APOE E4 positivity N/total N (%)	31/109 (28%)	31/92 (34%)	37/58 (64%)	7/14 (50%)	2/2 (0%)	11/42 (26%)	11/53 (21%)	7/40 (18%)	5/28 (18%)	8/27 (30%)	4/13 (31%)	3/13 (23%)	155/491 (32%)
Disease severity													
CDR sum of boxes	0 (0) ^{b,c}	2 (1) ^{a,c}	6 (3) ^{a,b}	3 (2) ^a	2 (0)	4 (3) ^{a,b,c}	4 (3) ^{a,b}	7 (4) ^{a,b}	3 (3) ^{a,c}	6 (3) ^{a,b}	5 (2) ^{a,b}	4 (2) ^a	3 (3)
N	113	47	58	15	2	79	70	62	31	27	13	13	582
SEADL, %	100 (1)	89 (18)	73 (21)	78 (13)	-	55 (24) ^{a,b}	49 (26) ^{a,b}	50 (24) ^{a,b}	67 (25) ^a	58 (19) ^{a,b}	55 (7)	-	65 (29)
N	55	20	3	4	-	65	64	48	22	17	2	-	300
FAQ	0 (0) ^b	5 (4) ^a	16 (8) ^{a,b}	10 (7) ^a	9 (8)	12 (7) ^{a,b}	14 (6) ^{a,b}	18 (7) ^{a,b}	11 (7) ^a	16.0 (8) ^{a,b}	15 (7) ^{a,b}	10 (7) ^a	10 (9)
N	83	85	56	14	2	42	49	37	11	27	14	13	433
GDS	3 (3)	8 (10)	8 (5)	14 (23) ^a	4 (1)	9 (11) ^a	10 (7) ^a	9 (17)	13 (26) ^a	7 (4)	9 (7)	10 (9)	8 (11)
N	103	87	49	13	2	65	60	52	20	17	8	10	486
Neuropsychology													
MMSE	29 (1) ^c	27 (2) ^c	19 (7) ^{a,b}	22 (6) ^{a,b}	25 (1)	23 (6) ^{a,b,c}	25 (4) ^{a,c}	22 (8) ^{a,b}	22 (6) ^a	20 (8) ^{a,b}	24 (2) ^c	24 (7) ^a	24 (6)
N	82	86	54	14	2	40	49	37	11	27	13	12	427
Imaging													
Total intracranial volume, L	1.5 (0.2)	1.5 (0.2)	1.5 (0.2)	1.5 (0.2)	1.4 (0)	1.5 (0.2)	1.5 (0.2)	1.5 (0.2)	1.5 (0.2)	1.5 (0.2)	1.6 (0.2)	1.7 (0.1) ^{a,b,c}	1.5 (0.2)
N	118	87	55	15	2	68	65	51	31	26	7	11	536
Bilateral hippocampal volume, mm ³ *	5668 (606) ^{b,c}	5292 (825) ^{a,c}	4834 (698) ^{a,b}	5014 (688) ^a	5385 (478)	5330 (717) ^{a,c}	5287 (608) ^{a,c}	4816 (763) ^{a,b}	5341 (635) ^c	4166 (760) ^{a,b,c}	5802 (483) ^c	5349 (1114) ^a	5235 (793)
N	118	87	55	15	2	68	65	51	31	26	7	11	536
Amyloid-PET read, neg/pos (%positive)	53/11 (17%)	44/29 (40%)	0/54 (100%)	0/14 (100%)	0/2 (100%)	33/17 (34%)	24/3 (11%)	23/3 (12%)	19/5 (21%)	8/2 (20%)	2/1 (33%)	8/5 (38%)	214/146 (41%)

	NC (n=118)	MCI (n=99)	AD _{clin} (n=58)	lvPPA (n=15)	PCA (n=2)	CBS (n=79)	PSP (n=74)	bvFTD (n=62)	nvPPA (n=32)	svPPA (n=27)	DLB (n=14)	TES (n=13)	All (n=593)
N	64	73	54	14	2	50	27	26	24	10	3	13	360
FTP-PET temporal meta ROI SUVR	1.1 (0.1) ^c	1.4 (0.3) ^c	2.0 (0.4) ^{a,b}	2.3 (0.4) ^{a,b}	-	1.4 (0.4) ^c	1.2 (0.1) ^c	1.2 (0.1) ^c	1.1 (0.1) ^c	1.7 (0.5)	1.7	1.3 (0.4)	1.5 (0.5)
N	8	46	50	15	-	34	24	19	21	3	1	9	230
FTP-PET temporal meta ROI neg/ pos(% positive)	8/0 (0%)	23/23 (50%)	0/50 (100%)	0/15 (100%)	-	24/10 (29%)	21/3 (13%)	16/3 (16%)	18/3 (14%)	1/2 (67%)	0/1 (100%)	7/2 (22%)	118/112 (49%)
N	8	46	50	15	-	34	24	19	21	3	1	9	230
Fluid biomarkers													
Plasma P-tau217, pg/ mL ^{**}	0.17 (0.1) ^{b,c}	0.29 (0.3) ^{a,c}	0.72 (0.4) ^{a,b}	0.82 (0.2) ^{a,b}	0.80 (0.3)	0.27 (0.3) ^c	0.17 (0.1) ^c	0.20 (0.2) ^c	0.15 (0.1) ^c	0.23 (0.4) ^c	0.18 (0.1) ^c	0.25 (0.2)	0.28 (0.3)
N	118	99	58	15	2	79	74	62	32	27	14	13	593
Plasma P-tau181, pg/ mL ^{**}	0.9 (1) ^{b,c}	1.2 (1) ^{a,c}	2.3 (1) ^{a,b}	2.5 (1) ^{a,b}	2.8 (1)	1.1 (1) ^c	1.0 (1) ^c	1.0 (1) ^c	0.7 (0) ^c	1.1 (1) ^c	1.0 (0) ^c	1.0 (0)	1.2 (1)
N	118	99	58	15	2	79	74	62	32	27	14	13	593
Plasma NfL, pg/ mL ^{**}	20.3 (26)	19.3 (13)	24.2 (12)	-	-	51.2 (26) ^{a,b,c}	41.4 (24) ^{a,b}	55.9 (45) ^{a,b,c}	46.4 (27) ^a	59.9 (29) ^{a,b,c}	-	-	34.3 (30)
N	73	37	28	-	-	34	50	28	10	8	-	-	268
CSF P-tau181, pg/ mL ^{**}	20.9 (8) ^{b,c}	28.7 (12) ^a	39.9 (18) ^a	-	-	22.5 (15) ^c	14.5 (5) ^{b,c}	20.2 (11) ^{b,c}	24.1 (7) ^c	16.8 (3)	-	-	23.4 (13)
N	48	23	24	-	-	36	30	28	8	2	-	-	199

Values shown as mean (standard deviation). Abbreviations: AD_{clin}: Clinical Alzheimer's disease; APOE, apolipoprotein E; BNT, Boston Naming Test; bvFTD, behavioral variant FTD; CDR, Clinical Dementia Rating; CBD, corticobasal degeneration; FAQ, Functional Activities Questionnaire; FTP-PET, 18F-Flortaucipir; GDS, Geriatric depression scale; MCI, mild cognitive impairment; MMSE, Mini-Mental State Test; NfL, neurofilament light chain; NC, Normal control; SEADL, Schwab and England Activities of Daily Living; SUVR, Standardized Uptake Value Ratio; PET, positron emission tomography; P-tau, phosphorylated tau; PPA, primary progressive aphasia; PSP, progressive supranuclear palsy; TES, traumatic encephalopathy syndrome. Amyloid status was based on visual read.

* ANCOVA corrected for TIV,

** ANCOVA corrected for age and sex

^a Indicates a statistically significant difference between groups ($p < 0.05$) with NC in ANCOVA with post hoc pairwise comparisons (Bonferroni)

^b $p < 0.05$ vs MCI

^c $p < 0.05$ vs AD_{clin}