nature aging

Letter

https://doi.org/10.1038/s43587-023-00380-7

CSF tau phosphorylation occupancies at T217 and T205 represent improved biomarkers of amyloid and tau pathology in Alzheimer's disease

Received: 17 October 2022

Accepted: 3 February 2023

Published online: 13 March 2023

Check for updates

Nicolas R. Barthélemy[1,2,8] ✉, Benjamin Saef[1], Yan Li[1], Brian A. Gordon[3], Yingxin He[1,2], Kanta Horie[1,2], Erik Stomrud[4,5], Gemma Salvadó[4], Shorena Janelidze[4], Chihiro Sato[1,2], Vitaliy Ovod[1,2], Rachel L. Henson[1], Anne M. Fagan[1,6], Tammie L. S. Benzinger[3,6], Chengjie Xiong[6,7], John C. Morris[1,6], Oskar Hansson[4,5], Randall J. Bateman[1,2,6] & Suzanne E. Schindler[1,6,8] ✉

Cerebrospinal fluid (CSF) amyloid-β peptide (Aβ)42/Aβ40 and the concentration of tau phosphorylated at site 181 (p-tau181) are well-established biomarkers of Alzheimer's disease (AD). The present study used mass spectrometry to measure concentrations of nine phosphorylated and five nonphosphorylated tau species and phosphorylation occupancies (percentage phosphorylated/nonphosphorylated) at ten sites. In the present study we show that, in 750 individuals with a median age of 71.2 years, CSF pT217/T217 predicted the presence of brain amyloid by positron emission tomography (PET) slightly better than Aβ42/Aβ40 ($P = 0.02$). Furthermore, for individuals with positive brain amyloid by PET ($n = 263$), CSF pT217/T217 was more strongly correlated with the amount of amyloid (Spearman's $\rho = 0.69$) than Aβ42/Aβ40 ($\rho = -0.42$, $P < 0.0001$). In two independent cohorts of participants with symptoms of AD dementia ($n = 55$ and $n = 90$), CSF pT217/T217 and pT205/T205 were better correlated with tau PET measures than CSF p-tau181 concentration. These findings suggest that CSF pT217/T217 and pT205/T205 represent improved CSF biomarkers of amyloid and tau pathology in AD.

AD is characterized by the aggregation of Aβ into amyloid plaques and the hyperphosphorylation and accumulation of tau into neurofibrillary tangles, which begins a decade or more before the onset of dementia symptoms. These neuropathological features can be visualized and quantified in living individuals using PET with radiotracers binding to amyloid and tau[1,2]. AD brain pathology is associated with a lower concentration of Aβ42 and a lower ratio of Aβ42:Aβ40 in the CSF, probably due to sequestration of Aβ42 into amyloid plaques[3], but higher concentrations of CSF total tau (t-tau) and p-tau181 (refs. [4,5]). Importantly, CSF t-tau and p-tau181 concentrations increase around the time of amyloid plaque deposition, when no neurofibrillary tangles are detected via tau PET, suggesting that elevated CSF t-tau and p-tau181 concentrations may reflect a response to amyloid plaques rather than neurofibrillary tangle burden[6,7].

Most studies of CSF p-tau have examined only p-tau181, but tau is phosphorylated at many different sites and is truncated in the CSF

A full list of affiliations appears at the end of the paper. ✉e-mail: barthelemy.nicolas@wustl.edu; schindler.s.e@wustl.edu

and plasma[8–11]. Several studies have demonstrated that CSF and/or plasma p-tau217 and p-tau231 concentrations are strongly associated with imaging and clinical measures of AD[12–20]. Immunoassays are widely used to measure concentrations of different p-tau species including p-tau181, p-tau217 and p-tau231, but they employ different antibodies and assay conditions, so in comparative studies it is often unclear whether differences in biomarker associations are related to differences in the analyte or the assay[15,17,19,21,22]. In contrast, mass spectrometry (MS) enables simultaneous measurement of nonphosphorylated and phosphorylated tau species and has high specificity. MS has identified p-tau217, p-tau205 and a species of the microtubule-binding region of tau (MTBR-tau243) as candidate AD biomarkers[23,24], revealed the degree of phosphorylation at different tau sites in response to AD[23,25] and enabled comparisons between CSF p-tau species that demonstrate the order in which sites are phosphorylated over the course of AD[26].

In the present study of older individuals with and without cognitive impairment, MS was used to evaluate 24 different measures of CSF tau: the concentrations of nine phosphorylated and five nonphosphorylated tau peptides, and the phosphorylation occupancy (percentage phosphorylated:nonphosphorylated) at ten sites. This comprehensive evaluation allowed analysis of which CSF tau measures are most strongly associated with amyloid plaques and neurofibrillary tangles as measured by PET, regional brain volumes, clinical status (cognitively unimpaired or impaired) and dementia severity.

Results

Characteristics of cohorts

The primary cohorts for the study were from the Knight Alzheimer Disease Research Center (Knight ADRC) at Washington University in St. Louis, MO, USA. The Knight ADRC amyloid PET cohort included 750 individuals with a median age of 71.2 years (interquartile range (IQR) 65.3–76.1 years); 55% were female, 90% self-identified as white, 39% carried at least one apolipoprotein E (*APOE*) ε4 allele and 16% were cognitively impaired as defined by a Clinical Dementia Rating (CDR) of ≥0.5, which includes mild cognitive impairment (MCI) and AD dementia (Extended Data Table 1). The overlapping Knight ADRC tau PET cohort included 371 individuals (Extended Data Table 2). Individuals in the tau PET cohort who were cognitively impaired (CDR ≥ 0.5) were included in Knight ADRC tau PET symptomatic AD subcohort ($n = 55$) (Supplementary Table 1). The validation cohort included 90 individuals enrolled in the BioFINDER-2 cohort at Skåne University Hospital in Sweden with a median age of 72 years (IQR 67–76 years); 47% were female, 71% carried at least one *APOE* ε4 allele, 83 were diagnosed with MCI and 7 were diagnosed with AD dementia (Extended Data Table 3).

CSF measures and amyloid PET

MS was used to evaluate 24 CSF tau measures and automated Lumipulse assays were used to evaluate 5 measures including Aβ42/Aβ40. The coefficient of variation for each CSF tau measure was estimated by running three pooled CSF samples with each of the 26 batches. For the intermediately abnormal CSF pool, pT231/T231 had higher variance (17.5%) compared with pT217/T217 (3.0%), pT181/T181 (5.8%) and pT205/T205 (9.1%) (see Appendix 1 for methodology, quality control and performance details of the MS assay). Of note, the p-tau species served as the numerator and the corresponding nonphosphorylated tau species served as the denominator for the occupancy measures. Key findings were replicated in subcohorts with no missing CSF biomarker measures.

The relationships of the CSF measures with amyloid PET status (positive or negative according to established cut-offs) were evaluated in the large ($n = 750$) Knight ADRC amyloid PET cohort (Extended Data Fig. 1a). Concentrations of all phosphorylated and nonphosphorylated CSF tau species were higher in amyloid PET-positive compared with amyloid PET-negative individuals ($P < 0.0001$ for all) and the fold difference was largest for CSF p-tau231 concentration (7.79-fold) (Extended Data Table 4 and Supplementary Table 2). The phosphorylation occupancy at six sites (T217, T111, T231, T153, S208 and T181) and the concentrations of four p-tau species (p-tau217, p-tau231, p-tau208 and p-tau153) distinguished amyloid PET status with a receiver operating characteristic (ROC) area under the curve (AUC) > 0.90 (Supplementary Table 3). CSF pT217/T217 had the highest correspondence with amyloid PET status of all the measures, with an ROC AUC of 0.98 (95% confidence interval (CI) 0.97–0.99), which was slightly superior to Lumipulse Aβ42/Aβ40 (AUC = 0.97 (0.95–0.98), $P = 0.02$) and p-tau217 concentration (AUC = 0.95 (0.93–0.96), $P < 0.0001$; Extended Data Fig. 2a).

The correlations of CSF measures with continuous amyloid PET Centiloid values were examined (Figs. 1a and 2). CSF pT217/T217 had the highest correlation with amyloid PET Centiloid of all the measures (Spearman's $\rho = 0.76$ (95% CI 0.73–0.79), which was almost significantly higher than Lumipulse Aβ42/Aβ40 ($\rho = -0.74$ (−0.77 to −0.71), $P = 0.08$) and was significantly higher than p-tau217 concentration ($\rho = 0.71$ (0.67–0.74), $P < 0.0001$) (Supplementary Tables 4 and 5). Importantly, in amyloid PET-positive individuals, CSF pT217/T217 was strongly correlated with amyloid PET Centiloid ($\rho = 0.69$ (0.62–0.75)), whereas the correlation between Lumipulse Aβ42/Aβ40 and amyloid PET Centiloid was only modest ($\rho = -0.42$ (−0.52 to −0.32), $P < 0.0001$; Supplementary Table 6).

CSF measures and tau PET

The overlapping Knight ADRC tau PET cohort was used to evaluate the relationships between CSF measures and tau PET status (Extended Data Fig. 1b). Concentrations of almost all CSF phosphorylated and nonphosphorylated tau species were higher in tau PET-positive compared with PET-negative individuals, with the highest fold difference again being in CSF p-tau231 concentration (7.16-fold) (Supplementary Tables 7 and 8).

The phosphorylation occupancy at six sites (T217, T111, T181, T153, S208 and T231) and the concentrations of four p-tau species (p-tau217, p-tau208, p-tau231and p-tau153) that were strongly associated with amyloid PET status also distinguished tau PET status with an AUC ≥ 0.89 (Supplementary Table 9). It is interesting that two measures that were not strongly associated with amyloid PET status, pT205/T205 and p-tau205 concentration, were among the best predictors of tau PET status (AUC = 0.94 and 0.96, respectively). The CSF measures with the highest correspondence with tau PET status included pT217/T217 (AUC = 0.96 (0.94–0.98), p-tau205 concentration (AUC = 0.96 (0.94–0.98)), p-tau217 concentration (AUC = 0.95 (0.93–0.97)) and T205/T205 (AUC = 0.94 (0.91–0.97) (Extended Data Fig. 2b).

In amyloid PET-positive individuals, only CSF measures related to T205 and T217 distinguished tau PET status with an AUC > 0.75: pT205/T205 (AUC = 0.88 (0.82–0.94), p-tau205 concentration (AUC = 0.87 (0.81–0.93)), pT217/T217 (AUC = 0.83 (0.76–0.90)) and p-tau217 concentration (AUC = 0.80 (0.72–0.88)) (Extended Data Fig. 2c and Supplementary Table 10). Individuals who were both amyloid PET positive and tau PET positive could be distinguished from all other individuals by single measures, including CSF pT217/T217 (AUC = 0.96 (0.94–0.98)) (Supplementary Table 11).

Correlations of CSF measures with a continuous tau PET summary measure were evaluated (Figs. 1b and 3). In the Knight ADRC tau PET cohort, CSF concentrations of p-tau208 ($\rho = 0.52$ (0.44–0.60)), p-tau205 ($\rho = 0.51$ (0.43–0.58), pT217/T217 ($\rho = 0.47$ (0.39–0.55)) and pT205/T205 ($\rho = 0.47$ (0.39–0.55)) had among the highest correlations with the tau PET summary measure (Supplementary Tables 12 and 13). In amyloid PET-positive individuals ($n = 125$), CSF pT205/T205 had the highest correlation with the tau PET summary measure ($\rho = 0.72$ (0.63–0.80)), which was not superior statistically to p-tau205 concentration ($\rho = 0.65$ (0.54–0.74)), but was superior to pT217/T217 ($\rho = 0.55$ (0.42–0.67), $P = 0.02$) and p-tau217 concentration ($\rho = 0.48$ (0.33–0.60), $P = 0.0002$) (Supplementary Table 14).

https://doi.org/10.1038/s43587-023-00380-7

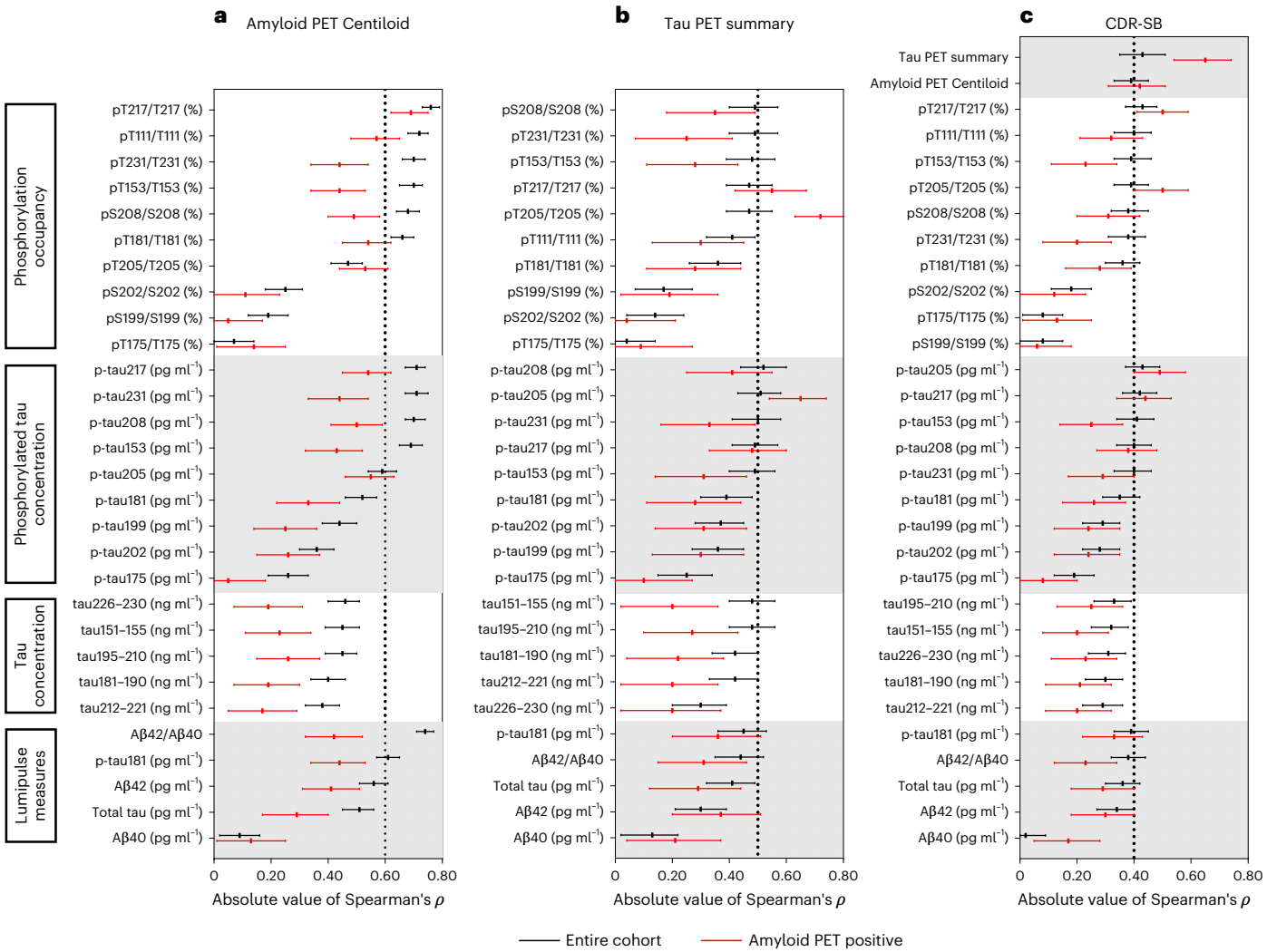


Fig. 1 | Correlations of CSF measures with amyloid PET Centiloid, the tau PET summary measure or dementia severity. a, Correlations of the CSF measures with amyloid PET Centiloid evaluated in the amyloid PET cohort ($n = 750$ individuals). **b**, Correlations with the tau PET summary measure evaluated in the tau PET cohort ($n = 371$ individuals). **c**, Correlations with dementia severity, as measured by the CDR-SB, evaluated in the larger amyloid PET cohort ($n = 750$ individuals). The lines represent Spearman's correlation (middle point) with 95% CIs. The black lines represent correlations in the entire cohort and the red lines represent correlations in amyloid PET-positive individuals.

CSF measures and regional tau PET and brain volumes

The correlation of select CSF measures with regional tau PET in amyloid PET-positive individuals in the Knight ADRC tau PET cohort is shown in Fig. 4a. CSF pT205/T205 had the highest correlation with tau PET standardized uptake value ratio (SUVR) in the amygdala and temporal regions (for example, inferior temporal gyrus, fusiform gyrus, parahippocampal gyrus, middle temporal gyrus and entorhinal), with partial Spearman's ρ values ranging from 0.68 to 0.61 for these regions (Supplementary Table 15). CSF pT217/T217 was also most highly correlated with regional tau PET in the amygdala and temporal regions, but the correlations were weaker, with partial Spearman's ρ values ranging from 0.55 to 0.49 for the top six most correlated regions of interest (ROIs) (Supplementary Table 16). The pattern of regional correlations with tau PET were similar for CSF pT181/T181 and pT231/T231, but the maximum partial Spearman's ρ value was 0.31 for both measures (Supplementary Tables 17 and 18).

The correlation of select CSF measures with regional brain volumes in amyloid PET-positive individuals in the Knight ADRC amyloid PET cohort is shown in Fig. 4b and is similar to the patterns seen with tau PET, but the correlations were more modest. CSF pT205/T205 had the highest correlation with regional brain volumes in the hippocampus, amygdala and temporal regions (for example, temporal pole, middle temporal gyrus, fusiform gyrus and entorhinal), with partial Spearman's ρ values ranging from −0.50 to −0.37 for these regions (Supplementary Table 19). CSF pT217/T217 had the highest correlation with regional brain volumes in similar areas, with partial Spearman's ρ values ranging from −0.43 to −0.28 for the top six most correlated ROIs (Supplementary Table 20). After correction for multiple comparisons, CSF pT181/T181 and pT231/T231 were not significantly correlated with regional brain volumes (Supplementary Tables 21 and 22).

Biomarker measures and dementia

The relationship of CSF measures, amyloid PET and tau PET with clinical status were evaluated in the large Knight ADRC amyloid PET cohort. Clinical status (cognitively unimpaired (CDR = 0) or cognitively impaired (CDR > 0, includes both MCI and AD dementia)) was predicted by biomarkers with and without the covariates of age, sex and years of education (Extended Data Fig. 1c and Supplementary Table 23). The tau PET summary measure best distinguished cognitively impaired from cognitively unimpaired individuals (AUC = 0.85

https://doi.org/10.1038/s43587-023-00380-7

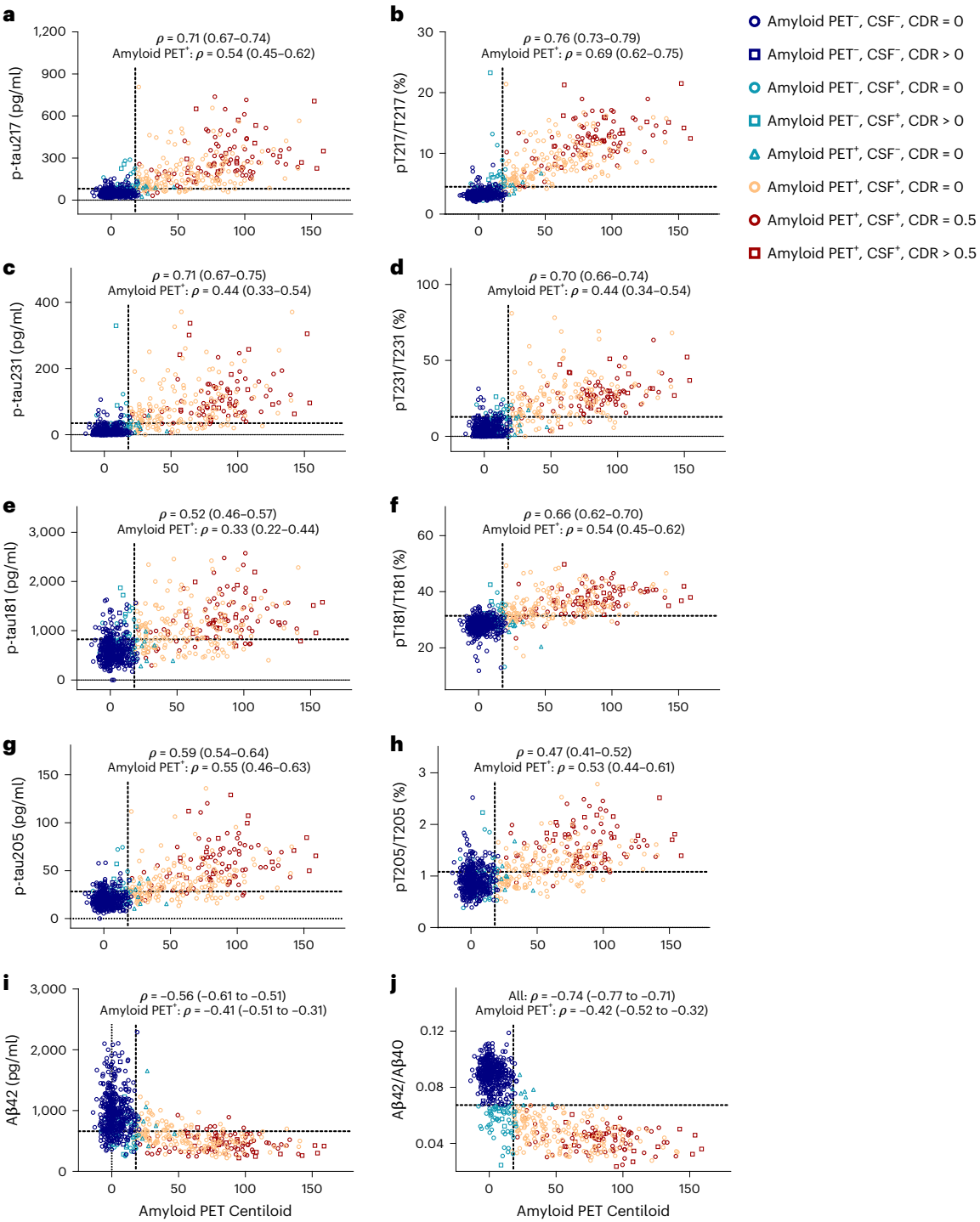


Fig. 2 | Correlations of selected CSF p-tau concentrations and tau phosphorylation occupancies with amyloid PET Centiloid. a–**j**, CSF concentrations of p-tau217 (**a**), p-tau231 (**c**), p-tau181 (**e**), p-tau205 (**g**) and Aβ42 (**i**), and tau phosphorylation occupancies at T217 (**b**), T231 (**d**), T181 (**f**) and T205 (**h**), as well as Aβ42/Aβ40 (**j**), plotted as a function of amyloid PET Centiloid. Spearman's correlations with 95% CIs are shown for the entire amyloid PET cohort ($n = 750$ individuals) and amyloid PET-positive individuals in the amyloid PET cohort ($n = 263$). The horizontal dashed lines denote the cut-offs that best distinguish amyloid PET status, based on combined sensitivity and specificity (Supplementary Table 3). The vertical dashed lines represent the cut-off for amyloid PET positivity. Each symbol represents one individual: blue circle: amyloid PET negative, CSF negative, CDR = 0; blue square: amyloid PET negative, CSF negative, CDR > 0; green circle: amyloid PET negative, CSF positive, CDR = 0; green square: amyloid PET negative, CSF positive, CDR > 0; green triangle: amyloid PET positive, CSF negative, CDR = 0; orange circle: amyloid PET positive, CSF positive, CDR = 0; red circle: amyloid PET positive, CSF positive, CDR = 0.5; and red square: amyloid PET positive, CSF positive, CDR > 0.5.

(0.78–0.91)), but this was not superior statistically to several CSF measures including pT217/T217 (AUC = 0.84 (0.80–0.89), p-tau205 concentration (AUC = 0.84 (0.80–0.88)), p-tau217 concentration (AUC = 0.83 (0.79–0.88)) or pT205/T205 (AUC = 0.81 (0.77–0.86)). In amyloid PET-positive individuals, the tau PET summary measure also had the highest correspondence with clinical status (AUC = 0.86

https://doi.org/10.1038/s43587-023-00380-7

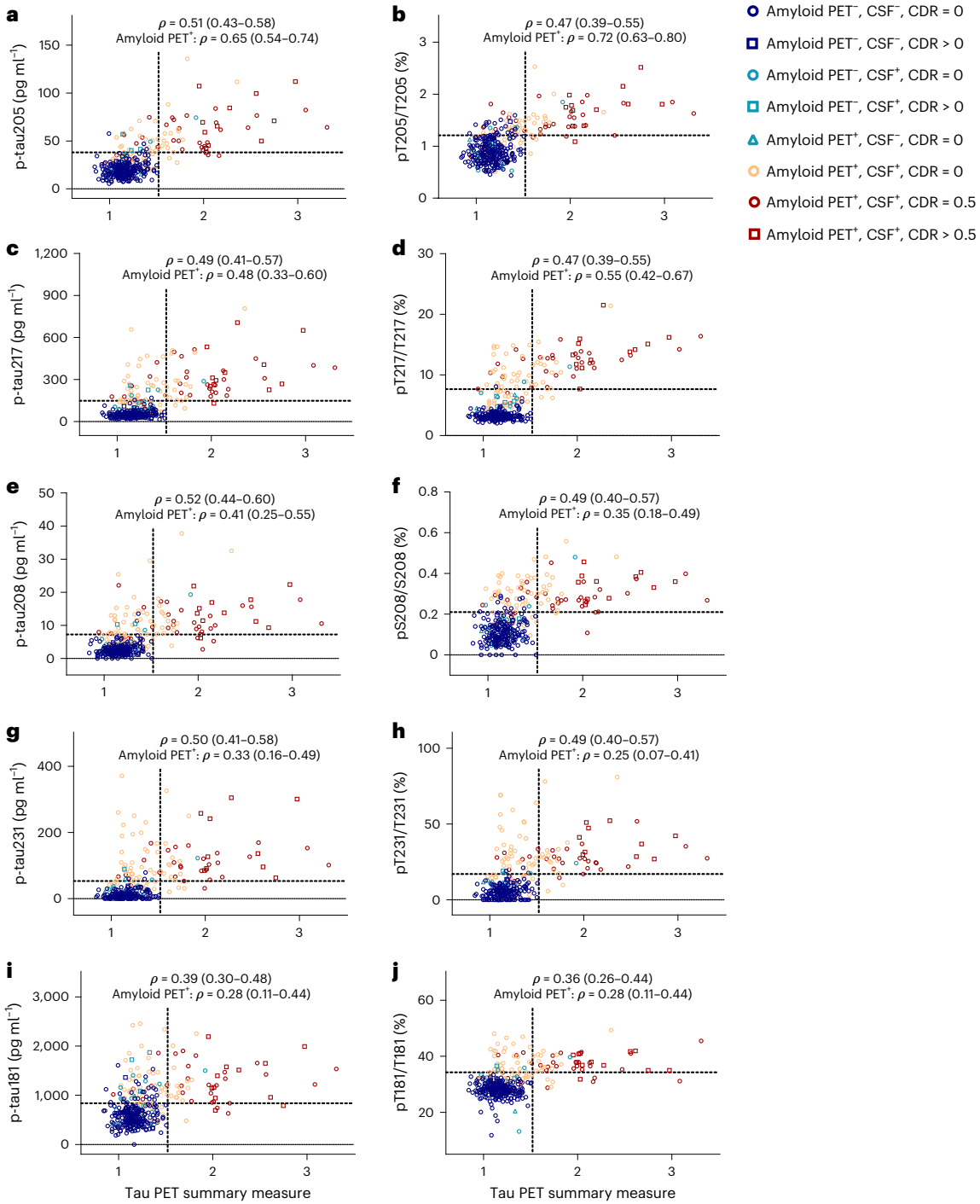


Fig. 3 | Correlations of selected CSF p-tau concentrations and tau phosphorylation occupancies with the tau PET summary measure. a–j, CSF concentrations of p-tau205 (**a**), p-tau217 (**c**), p-tau208 (**e**), p-tau231 (**g**) and p-tau181 (**i**), and tau phosphorylation occupancies at T205 (**b**), T217 (**d**), S208 (**f**), T231 (**h**), and T181 (**j**), plotted as a function of the tau PET summary measure. Spearman's correlations with 95% CIs are shown for the entire tau PET cohort ($n = 371$) and amyloid PET-positive individuals in the tau PET cohort ($n = 125$). The horizontal dashed lines denote the cut-offs that best distinguish tau PET status, based on combined sensitivity and specificity (Supplementary Table 9). The vertical dashed lines represent the cut-off for tau PET positivity. Each symbol represents one individual: blue circle: amyloid PET negative, CSF negative, CDR = 0; blue square: amyloid PET negative, CSF negative, CDR > 0; green circle: amyloid PET negative, CSF positive, CDR = 0; green square: amyloid PET negative, CSF positive, CDR > 0; green triangle: amyloid PET positive, CSF negative, CDR = 0; orange circle: amyloid PET positive, CSF positive, CDR = 0; red circle: amyloid PET positive, CSF positive, CDR = 0.5; and red square: amyloid PET positive, CSF positive, CDR > 0.5.

(0.78–0.94)), but again this was not statistically superior to pT217/T217 (AUC = 0.80 (0.74–0.85)) or pT205/T205 (AUC = 0.79 (0.73–0.84)) (Supplementary Table 24). Notably, CSF Lumipulse measures (for example, Aβ42/Aβ40, p-tau181 concentration) had relatively low performance in predicting clinical status (AUC = 0.60–0.69) in amyloid PET-positive individuals.

Correlations of amyloid PET, tau PET and CSF biomarkers with dementia severity as measured by the CDR sum of boxes (CDR-SB) were

https://doi.org/10.1038/s43587-023-00380-7

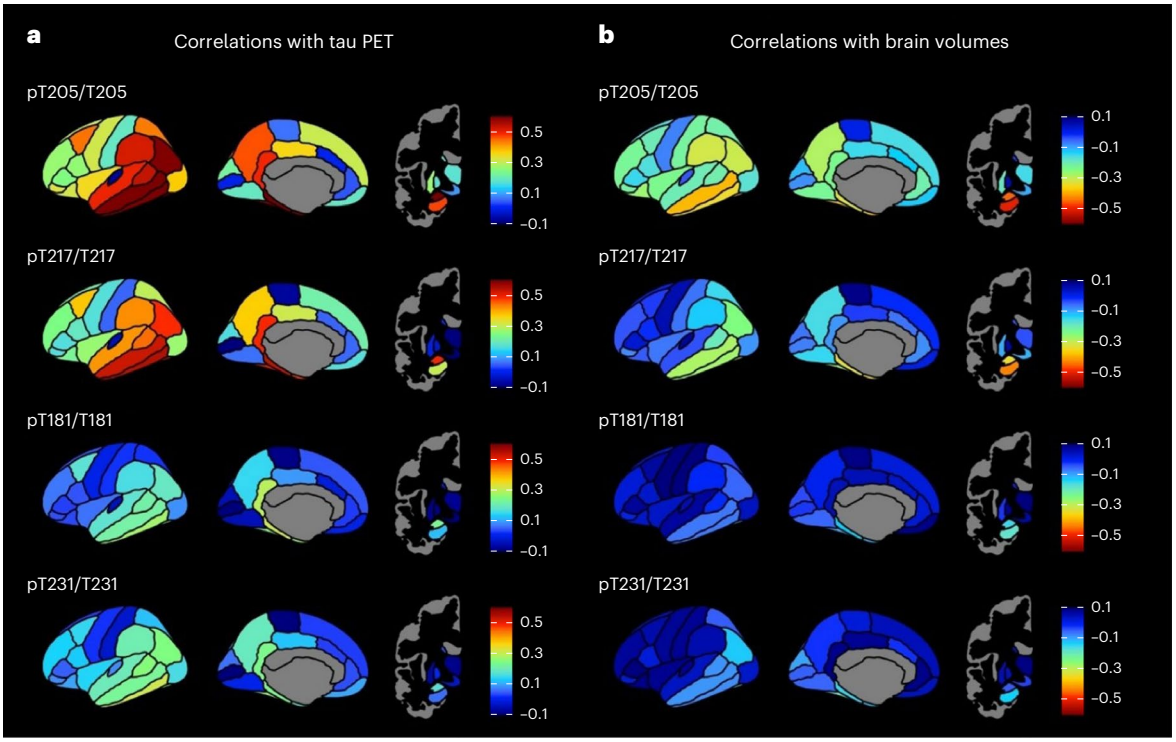


Fig. 4 | Correlations of selected CSF tau phosphorylation occupancies with regional tau PET and regional brain volumes for amyloid PET-positive individuals. a, Partial Spearman's correlations of selected CSF measures with regional tau PET adjusted for age and sex shown for amyloid PET-positive individuals in the tau PET cohort ($n = 125$). **b**, Partial Spearman's correlations of CSF measures with regional brain volumes adjusted for age and sex shown for amyloid PET-positive individuals in the larger amyloid PET cohort ($n = 263$).

also examined in the large Knight ADRC amyloid PET cohort (Fig. 1c). The tau PET summary measure ($\rho = 0.43$ (0.35–0.51), CSF pT217/T217 ($\rho = 0.43$ (0.37–0.48)) and pT205/T205 ($\rho = 0.39$ (0.33–0.45)) had some of the highest correlations with CDR-SB (Supplementary Table 25). In amyloid PET-positive individuals, the CDR-SB had the highest correlations with the tau PET summary measure ($\rho = 0.65$ (0.54–0.74)), CSF pT217/T217 ($\rho = 0.50$ (0.41–0.59)) and pT205/T205 ($\rho = 0.50$ (0.40–0.59)), whereas correlations with Lumipulse Aβ42/Aβ40 were lower ($\rho = -0.23$ (−0.34 to −0.12); Supplementary Table 26).

Biomarker measures in a symptomatic AD cohort

To evaluate the performance of these measures in a more clinically relevant cohort, CSF tau measures were examined in cognitively impaired (CDR ≥ 0.5) individuals from the Knight ADRC tau PET cohort ($n = 55$; Supplementary Table 27). There was a trend toward a higher correlation of amyloid PET Centiloid with CSF pT217/T217 ($\rho = 0.77$ (0.63–0.86)) compared with Aβ42/Aβ40 ($\rho = -0.58$ (−0.73 to −0.37), $P = 0.06$; Supplementary Table 28). CSF pT217/T217 ($\rho = 0.71$ (0.55–0.82) and/or pT205/T205 ($\rho = 0.67$ (0.50–0.80)) had higher correlations with a tau PET summary measure compared with pT181/T181 ($\rho = 0.42$ (0.18–0.62), $P = 0.006$ and $P = 0.04$) or Lumipulse p-tau181 concentration ($\rho = 0.44$ (0.20–0.63), $P = 0.003$ and $P = 0.07$; Supplementary Table 29).

Biomarker measures in a confirmatory symptomatic AD cohort

The CSF tau measures were further examined in participants with symptomatic AD (MCI or AD dementia) from the BioFINDER-2 cohort ($n = 90$; Extended Data Table 5). CSF pT217/T217 ($\rho = 0.55$ (0.38–0.68)) was better correlated with amyloid PET Centiloid than CSF Aβ42/Aβ40 ($\rho = -0.19$ (−0.38 to 0.02), $P = 0.0007$; Extended Data Fig. 3 and Supplementary Table 30). CSF pT217/T217 ($\rho = 0.76$ (0.65–0.83)) and/or pT205/T205 ($\rho = 0.72$ (0.60–0.81)) had higher correlations with a tau PET measure for the Braak I–IV region compared with pT181/T181 ($\rho = 0.59$ (0.43–0.71), $P = 0.002$ and $P = 0.14$) and CSF p-tau181 concentration by immunoassay ($\rho = 0.42$ (0.23–0.57), $P = 0.0004$ and $P = 0.02$, respectively; Extended Data Fig. 4 and Supplementary Table 31). CSF pT217/T217 ($\rho = 0.59$ (0.43–0.71)) and pT205/T205 ($\rho = 0.66$ (0.52–0.76)) were better correlated with a tau PET measure for the Braak V–VI region compared with pT181/T181 ($\rho = 0.44$ (0.25–0.59), $P = 0.004$ and 0.02) and p-tau181 concentration by immunoassay ($\rho = 0.23$ (0.02–0.42), $P = 0.0003$ and $P = 0.0007$; Extended Data Fig. 5 and Supplementary Table 32).

Discussion

In the present study, we examined the relationships of 24 CSF tau MS measures and 5 CSF Lumipulse automated immunoassay measures with amyloid PET, tau PET, regional brain volumes, clinical status and dementia severity. The CSF measure that best distinguished amyloid PET status was not Aβ42, Aβ42/Aβ40 or even p-tau217 concentration, but pT217/T217. Moreover, CSF pT217/T217 was strongly correlated with amyloid PET Centiloid in amyloid PET-positive individuals in whom the correlation between Aβ42/Aβ40 and amyloid PET Centiloid was only modest. Overall, although six of the ten sites investigated (T217, T111, T231, T153, S208 and T181) were strongly associated with amyloid PET status, only CSF pT217/T217 was also strongly associated with tau PET status. In contrast, CSF pT205/T205 was not strongly associated with amyloid status, but, in amyloid PET-positive individuals, pT205/T205 had the highest correlations with the tau PET summary measure, regional tau PET measures and regional brain volumes. In amyloid PET-positive individuals, the tau PET summary measure best distinguished cognitively impaired from cognitively unimpaired individuals, but it was not statistically superior to CSF pT217/T217 or pT205/T205. Overall, these data demonstrate that CSF pT217/T217 is a superior biomarker of amyloid plaque burden and pT205/T205 is a promising

biomarker of neurofibrillary tangle burden in individuals with brain amyloidosis.

The amyloid–tau–neurodegeneration (ATN) framework has posited that CSF Aβ42 or Aβ42/Aβ40 is a measure of amyloid plaques (A) and that CSF p-tau (especially p-tau181) is a measure of neurofibrillary tangles (T)[27]. However, our study found that CSF pT217/T217 was superior to Aβ42/Aβ40 in reflecting amyloid plaque burden and was also strongly associated with tau PET status, demonstrating that a single measure may reflect the presence of both amyloid and tau (A and T). Notably, CSF pT217/T217 was not well correlated with tau PET in amyloid PET-positive individuals, suggesting that the association of pT217/T217 with tau PET status could be partially driven by the high amyloid levels in individuals who are tau PET positive. Concentrations and/or phosphorylation occupancies associated with sites T111, T231, T153, S208 and T181 were also highly associated with amyloid PET, but unlike T217 they were not strongly associated with tau PET, suggesting that these biomarkers may more specifically reflect amyloid (A). Our findings agree with the conclusions of other studies that p-tau217, p-tau181 and p-tau231 change early in AD in response to amyloid[16,28–31]. In contrast, CSF pT205/T205 was only modestly associated with amyloid PET, but was strongly associated with tau PET in amyloid PET-positive individuals, indicating that pT205/T205 is a relatively specific biomarker of tau (T). CSF pT205/T205 had the highest correlations with brain volumes, suggesting that pT205/T205 may also be a biomarker of neurodegeneration (N). The relationships between CSF tau measures and other biomarkers probably reflect the timing of biomarker changes in AD: previous work suggests that T217 is phosphorylated early in AD around the time of amyloid plaque deposition, whereas T205 is phosphorylated later and closer to neurofibrillary tangle accumulation and symptom onset[26]. The present study demonstrates the complexity of the relationship between tau phosphorylation and AD pathology, which may defy simple categorization.

An advantage of MS over immunoassays is that different protein species can be specifically and simultaneously identified[32]. MS enables assessment of measures such as phosphorylation occupancy, which may be difficult to evaluate via immunoassay. In the present study, CSF pT217/T217 had slightly but significantly higher performance compared with p-tau217 in prediction of amyloid PET status, which could be meaningful when accuracy is highly valued (for example, clinical diagnosis). It is possible that phosphorylation occupancies attenuate interindividual variability unrelated to AD pathology which affects the concentrations of both phosphorylated and nonphosphorylated tau species[33,34]. Notably, the CSF biomarker assays that are currently available for clinical AD diagnosis do not include p-tau217, p-tau205, pT217/T217 or pT205/T205. Although immunoassays are more widely available than specialized MS assays, MS may potentially provide unique and better performing measures than immunoassays.

The study cohorts consisted of participants enrolled in research studies and, therefore, the findings are most relevant to research studies and clinical trials and less generalizable to clinical populations. The cohorts were relatively young (early to mid-70s). Older cohorts, especially clinical cohorts, would be expected to have a higher prevalence of neurological comorbidities, which could complicate biomarker relationships. The cohorts included relatively few individuals with symptomatic AD and/or moderate or high levels of tau pathology. In addition, minoritized populations were not well represented in the study cohorts and studies have found racial differences in CSF t-tau and p-tau181 concentrations[35,36]. It is unknown whether other CSF tau measures such as pT217/T217 or pT205/T205 perform similarly across racial and ethnic groups, and further studies of these measures in diverse and clinically relevant cohorts are needed.

Technical limitations include that tau was immunopurified for analysis, which may result in some species of tau not being recovered well. Data for some CSF tau measures (particularly those related to p-tau231) did not meet quality control criteria for a subset of the samples and were thus excluded from analyses. The variance for p-tau231 measures was higher than for p-tau217 measures, which could have resulted in underestimating the performance of p-tau231 measures. Measures associated with p-tau231 had the largest fold-change between amyloid PET-negative and amyloid PET-positive individuals, and between tau PET-negative and tau PET-positive individuals, but the higher variance led to reduced associations of p-tau231 measures with outcomes. A limitation of the comparisons between CSF Aβ42/Aβ40 and tau measures is that CSF Aβ peptides were measured with immunoassays rather than MS, and it is possible that MS measurements of CSF Aβ42/Aβ40 could perform better. Overall, it is important to consider that the observed relationship between a biomarker and AD depends not only on the biological relationship, but also on the technical characteristics of the assay.

Future directions for this work include evaluating longitudinal trajectories of CSF tau measures to define how these measures change over the time course of sporadic AD, and whether they predict progression from cognitive normality to symptomatic AD. It will also be important to evaluate these CSF tau measures in non-AD dementias, including primary tauopathies[37,38]. Comprehensive evaluation of corresponding tau species in blood may yield improved AD biomarkers that are more accessible to researchers and patients. In particular, plasma pT217/T217 is highly correlated with CSF pT217/T217 (ref. [14]) and a recent head-to-head study of ten plasma tau assays (including key p-tau181, p-tau217 and p-tau231 assays) found that plasma pT217/T217, as measured by MS, had the best performance in predicting amyloid PET status and progression to AD dementia[17]. Studies of the correspondence of plasma pT205/T205, tau PET and clinical AD symptoms are needed. Overall, the present study demonstrates that different CSF tau species represent different aspects of AD, and that pT217/T217 and pT205/T205 may be superior to the CSF biomarkers of AD currently in widespread use.

Methods

Participants

Participants in the Knight Alzheimer Disease Research Center (ADRC) at Washington University in St. Louis were community-dwelling volunteers enrolled in studies of memory and aging. Most participants were recruited from memory clinics or self-referred due to interest in dementia. Individuals were excluded from enrollment if they were diagnosed with a non-AD dementia at their initial assessment (for example, Parkinson's disease), had conditions that might interfere with study procedures (for example, a pacemaker that would make the participant ineligible for magnetic resonance imaging (MRI)) or had medical issues that might affect long-term participation (for example, metastatic cancer). All procedures were approved by the Washington University Human Research Protection Office (HRPO) and written informed consent was obtained from each participant or their legally authorized representative when appropriate (protocol no. 201109100). Participants received compensation for time spent undergoing procedures, as approved by the HRPO. All participants underwent a comprehensive clinical assessment that included a detailed interview of a collateral source, a neurological examination of the participant, the CDR[39], the CDR-SB and the Mini-Mental State Examination. Individuals with a CDR ≥ 0.5 were considered to have a dementia syndrome, and the probable etiology of the dementia syndrome was formulated by clinicians based on clinical features in accordance with standard criteria and methods[40]. *APOE* genotype was determined as previously described[41]. Sex and race were self-identified. Participants included in the present study underwent a clinical assessment and lumbar puncture (LP) within 2 years of an amyloid PET scan (amyloid PET cohort). An overlapping cohort was selected in which participants underwent both an amyloid PET and a tau PET scan within 2 years of an LP (tau PET cohort). Previous work has demonstrated that the relationship between CSF biomarkers and amyloid PET is stable if CSF is collected up to 6 years before or 2 years after amyloid PET[42]. If more than one CSF

sample from a participant met the criteria, the most recently obtained sample was included. No data points were excluded from analyses and outliers were not removed.

Participants in the Swedish BioFINDER-2 confirmatory cohort (NCT03174938) included individuals diagnosed with MCI due to AD or AD dementia. Details on recruitment, exclusion and inclusion criteria have been described previously[12]. All participants included underwent an LP and cognitive testing within 2 years of both an amyloid PET scan and a tau PET scan. All participants gave written informed consent and ethics approval was granted by the Regional Ethical Committee in Lund, Sweden.

CSF collection and immunoassays

For studies in the Knight ADRC cohorts, 20–30 ml of CSF was collected via LP at approximately 8am after overnight fasting. CSF was collected in a 50-ml poly(propylene) tube via a gravity drip using an atraumatic Sprotte 22-gauge spinal needle. The entire sample was gently inverted to disrupt potential gradient effects and centrifuged at low speed to pellet any cellular debris. CSF was then aliquoted into poly(propylene) tubes and stored at −80 °C. Aβ42, Aβ40, t-tau and p-tau181 concentrations were measured with a single lot of reagents for all samples with an automated immunoassay platform (LUMIPULSE G1200, Fujirebio) according to the manufacturer's specifications.

For the BioFINDER-2 confirmatory cohort, CSF was collected as previously described[12]. Aβ42, Aβ40, t-tau and p-tau181 concentrations were measured using fully automated Elecsys immunoassays[43].

Measurement of CSF tau peptides

All samples from an individual were run in the same batch. CSF tau was immunopurified and digested, then phosphorylated and nonphosphorylated peptides were quantified using high-resolution MS (HRMS) as previously described and detailed in Appendix 1 (ref. [25]). Briefly, tau was immunopurified by incubating 450 µl of CSF with tau1 and HJ8.5 antibodies covalently attached to Sepharose beads at room temperature for 4 h. Immunopurified tau was digested for 16 h at 37 °C with 400 ng of trypsin (Promega). AQUA peptides (Life Technologies) were added to a final sample concentration of 5 fmol per labeled phosphorylated peptide and 50 fmol per labeled unmodified peptide. Samples were subjected to liquid chromatography–tandem HRMS (LC-MS/HRMS) analysis on a nanoAcquity ultra-performance LC system (Waters) coupled to an Orbitrap Tribrid Eclipse mass spectrometer (Thermo Fisher Scientific) operating in parallel reaction monitoring mode. MS/HRMS transitions were extracted using Skyline v.22.2.2.278 (MacCoss lab, University of Washington). Data were aggregated using Tableau v.2022.2.2 (Tableau Software) to calculate concentrations and phosphorylation occupancies. All assays and data extraction steps were performed by operators blinded to any clinical or biomarker information.

Amyloid and tau PET imaging

Participants at the Knight ADRC underwent amyloid PET using either [^{18}F]AV45 (florbetapir) or [^{11}C]Pittsburgh Compound B (PiB). An overlapping cohort additionally underwent tau PET with [^{18}F]AV1451 (flortaucipir). Both amyloid PET and tau PET scans were performed in coordination with a 3-T structural MRI scan. T1-weighted MRIs were processed using Freesurfer 5.3 to generate ROIs used for the processing of PET data. Estimates of regional volumes were adjusted for intracranial volume using a regression approach. Data from the 30- to 60-min post-injection window for PiB, the 50- to 70-min window for florbetapir or the 80- to 100-min window for flortaucipir were converted to SUVRs using the cerebellar gray as a reference and partial volume corrected using a geometric transfer matrix[44]. Values from the following regions were averaged together to represent mean cortical SUVR for florbetapir or PiB: bilateral orbitofrontal, medial orbitofrontal, rostral middle frontal, superior frontal, superior temporal, middle temporal and precuneus. Amyloid PET positivity was previously defined as a mean cortical SUVR > 1.42 for PiB and >1.19 for florbetapir[45]. Mean cortical SUVRs were converted to Centiloid units to combine data from the two tracers[45,46]. Values from the bilateral entorhinal cortex, amygdala, lateral occipital cortex and inferior temporal cortex regions were averaged together as a summary measure of tau PET[47]. Tau PET positivity was defined as a tau PET summary measure >1.52, based on Gaussian mixture modeling (Supplementary Fig. 1).

Participants in BioFINDER-2 underwent amyloid PET using [^{18}F] flutemetamol as previously described[12]. Participants underwent tau PET using [^{18}F]RO948 as previously described[12]. For tau PET, SUVRs for the brain regions with early change (Braak I–IV region) and later change (Braak V–VI region) were calculated. The cut-off for positivity in the Braak I–IV region was SUVR > 1.32 (ref. [48]).

Statistics and reproducibility

No statistical methods were used to predetermine sample sizes but our sample sizes are similar to or larger than those used for similar studies[16,26,31]. Measured values for many biomarkers were not normally distributed and therefore nonparametric analyses were performed. The significance of differences by biomarker status (amyloid PET or tau PET status) were evaluated with Wilcoxon's rank-sum tests for continuous variables and χ^2 or Fisher's exact test for categorical variables. ROC analyses were used to evaluate the correspondence of CSF biomarker measures with amyloid PET status, tau PET status or clinical status (cognitively unimpaired (CDR = 0) or cognitively impaired (CDR > 0)). Cut-offs that best distinguished amyloid PET or tau PET status were found based on the highest combined sensitivity and specificity (Youden's index). Differences between ROC AUCs were evaluated using DeLong tests[49]. Spearman's correlations were used to evaluate the continuous relationships of CSF biomarker measures with amyloid PET Centiloid, the tau PET summary measure or the CDR-SB. For partial Spearman's correlations with amyloid PET, tau PET and brain volumes, analyses included covariates of age and sex; for correlations with CDR-SB, analyses included covariates of age, sex and years of education. Comparisons between Spearman's correlations were performed by bootstrapping. When multiple measures were compared, the significance was adjusted using the Benjamini–Hochberg procedure[50]. Analyses were replicated in the subcohort of individuals with no missing data. Statistical analyses were implemented using SAS 9.4. Plots were created with GraphPad Prism v.9.2.0. All P values were from two-sided tests and results were deemed statistically significant at $P < 0.05$.

For visualization of the associations between CSF measures and regional tau PET or brain volumes, partial Spearman's correlations, including age and sex, were calculated with partial.r from the R psych toolbox v.2.1.9. The ggseg package v.1.6.5 was used to visualize correlations and results from the left hemisphere are shown. The significance of correlations was adjusted for multiple comparisons using the Benjamini–Hochberg procedure[50].

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

Knight ADRC data are available to qualified investigators who have a proposal approved by an institutional committee (https://knightadrc.wustl.edu/Research/ResourceRequest.htm) that meets monthly. The study must be approved by an institutional review board to ensure ethical research practices and investigators must agree to the terms and conditions of the data use agreement, which includes not distributing the data without permission. For BioFINDER-2 data, anonymized data will be shared by request from a qualified academic investigator for the sole purpose of replicating procedures and results presented in the article and as long as data transfer is in agreement with EU legislation on the general data protection regulation and decisions by the Ethical

Review Board of Sweden and Region Skåne, which should be regulated in a material transfer agreement.

References

1. Villemagne, V. L., Dore, V., Burnham, S. C., Masters, C. L. & Rowe, C. C. Imaging tau and amyloid-beta proteinopathies in Alzheimer disease and other conditions. *Nat. Rev. Neurol.* **14**, 225–236 (2018).
2. Ossenkoppele, R. et al. Tau PET correlates with different Alzheimer's disease-related features compared to CSF and plasma p-tau biomarkers. *EMBO Mol. Med.* **13**, e14398 (2021).
3. Patterson, B. W. et al. Age and amyloid effects on human central nervous system amyloid-beta kinetics. *Ann. Neurol.* **78**, 439–453 (2015).
4. Mattsson-Carlgren, N. et al. Cerebrospinal fluid biomarkers in autopsy-confirmed Alzheimer disease and frontotemporal lobar degeneration. *Neurology* **98**, e1137–e1150 (2022).
5. Long, J. M. et al. Preclinical Alzheimer's disease biomarkers accurately predict cognitive and neuropathological outcomes. *Brain* **145**, 4506–4518 (2022).
6. Gordon, B. A. et al. The relationship between cerebrospinal fluid markers of Alzheimer pathology and positron emission tomography tau imaging. *Brain* **139**, 2249–2260 (2016).
7. Mattsson-Carlgren, N. et al. Abeta deposition is associated with increases in soluble and phosphorylated tau that precede a positive Tau PET in Alzheimer's disease. *Sci. Adv.* **6**, eaaz2387 (2020).
8. Barthelemy, N. R. et al. Tau protein quantification in human cerebrospinal fluid by targeted mass spectrometry at high sequence coverage provides insights into its primary structure heterogeneity. *J. Proteome Res.* **15**, 667–676 (2016).
9. Cicognola, C. et al. Novel tau fragments in cerebrospinal fluid: relation to tangle pathology and cognitive decline in Alzheimer's disease. *Acta Neuropathol.* **137**, 279–296 (2019).
10. Meredith, J. E. Jr. et al. Characterization of novel CSF Tau and ptau biomarkers for Alzheimer's disease. *PLoS ONE* **8**, e76523 (2013).
11. Sato, C. et al. Tau kinetics in neurons and the human central nervous system. *Neuron* **98**, 861–864 (2018).
12. Palmqvist, S. et al. Discriminative accuracy of plasma phospho-tau217 for Alzheimer disease vs other neurodegenerative disorders. *JAMA* **324**, 772–781 (2020).
13. Ashton, N. J. et al. Plasma p-tau231: a new biomarker for incipient Alzheimer's disease pathology. *Acta Neuropathol.* **141**, 709–724 (2021).
14. Barthelemy, N. R., Horie, K., Sato, C. & Bateman, R. J. Blood plasma phosphorylated-tau isoforms track CNS change in Alzheimer's disease. *J. Exp. Med.* **217**, e20200861 (2020).
15. Mielke, M. M. et al. Comparison of plasma phosphorylated tau species with amyloid and tau positron emission tomography, neurodegeneration, vascular pathology, and cognitive outcomes. *JAMA Neurol.* **78**, 1108–1117 (2021).
16. Mila-Aloma, M. et al. Plasma p-tau231 and p-tau217 as state markers of amyloid-beta pathology in preclinical Alzheimer's disease. *Nat. Med.* **28**, 1797–1801 (2022).
17. Janelidze, S. et al. Head-to-head comparison of 10 plasma phospho-tau assays in prodromal Alzheimer's disease. *Brain* https://doi.org/10.1093/brain/awac333 (2022).
18. Thijssen, E. H. 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.* **20**, 739–752 (2021).
19. Janelidze, S. et al. Cerebrospinal fluid p-tau217 performs better than p-tau181 as a biomarker of Alzheimer's disease. *Nat. Commun.* **11**, 1683 (2020).
20. Barthelemy, N. R. et al. Cerebrospinal fluid phospho-tau T217 outperforms T181 as a biomarker for the differential diagnosis of Alzheimer's disease and PET amyloid-positive patient identification. *Alzheimers Res. Ther.* **12**, 26 (2020).
21. Leuzy, A. et al. Comparing the clinical utility and diagnostic performance of CSF P-Tau181, P-Tau217, and P-Tau231 assays. *Neurology* **97**, e1681–e1694 (2021).
22. Karikari, T. K. et al. Head-to-head comparison of clinical performance of CSF phospho-tau T181 and T217 biomarkers for Alzheimer's disease diagnosis. *Alzheimer's Dement.* **17**, 755–767 (2021).
23. Barthelemy, N. R., Mallipeddi, N., Moiseyev, P., Sato, C. & Bateman, R. J. Tau phosphorylation rates measured by mass spectrometry differ in the intracellular brain vs. extracellular cerebrospinal fluid compartments and are differentially affected by Alzheimer's disease. *Front. Aging Neurosci.* **11**, 121 (2019).
24. Horie, K., Barthelemy, N. R., Sato, C. & Bateman, R. J. CSF tau microtubule binding region identifies tau tangle and clinical stages of Alzheimer's disease. *Brain* **144**, 515–527 (2021).
25. Barthelemy, N. R. et al. Site-specific cerebrospinal fluid tau hyperphosphorylation in response to Alzheimer's disease brain pathology: not all tau phospho-sites are hyperphosphorylated. *Alzheimer's Dement.* **85**, 415–429 (2022).
26. Barthelemy, N. R. et al. A soluble phosphorylated tau signature links tau, amyloid and the evolution of stages of dominantly inherited Alzheimer's disease. *Nat. Med.* **26**, 398–407 (2020).
27. Jack, C. R. Jr. et al. NIA-AA Research Framework: toward a biological definition of Alzheimer's disease. *Alzheimer's Dement.* **14**, 535–562 (2018).
28. Ashton, N. J. et al. Cerebrospinal fluid p-tau231 as an early indicator of emerging pathology in Alzheimer's disease. *EBioMedicine* **76**, 103836 (2022).
29. Suarez-Calvet, M. et al. Novel tau biomarkers phosphorylated at T181, T217 or T231 rise in the initial stages of the preclinical Alzheimer's continuum when only subtle changes in Abeta pathology are detected. *EMBO Mol. Med.* **12**, e12921 (2020).
30. Ashton, N. J. et al. Differential roles of Abeta42/40, p-tau231 and p-tau217 for Alzheimer's trial selection and disease monitoring. *Nat. Med.* **28**, 2555–2562 (2022).
31. Therriault, J. et al. Association of phosphorylated tau biomarkers with amyloid positron emission tomography vs tau positron emission tomography. *JAMA Neurol.* **80**, 188–199 (2022).
32. Gobom, J. et al. Antibody-free measurement of cerebrospinal fluid tau phosphorylation across the Alzheimer's disease continuum. *Mol. Neurodegen.* **17**, 81 (2022).
33. Schindler, S. E. & Karikari, T. K. Comorbidities confound Alzheimer's blood tests. *Nat. Med.* **28**, 1349–1351 (2022).
34. Pichet Binette, A. et al. Confounding factors of Alzheimer's disease plasma biomarkers and their impact on clinical performance. *Alzheimer's Dement.* https://doi.org/10.1002/alz.12787 (2022).
35. Morris, J. C. et al. Assessment of racial disparities in biomarkers for Alzheimer disease. *JAMA Neurol.* **76**, 264–273 (2019).
36. Garrett, S. L. et al. Racial disparity in cerebrospinal fluid amyloid and tau biomarkers and associated cutoffs for mild cognitive impairment. *JAMA Netw. Open* **2**, e1917363 (2019).
37. Sato, C. et al. MAPT R406W increases tau T217 phosphorylation in absence of amyloid pathology. *Ann. Clin. Transl. Neurol.* **8**, 1817–1830 (2021).
38. Horie, K. et al. CSF tau microtubule-binding region identifies pathological changes in primary tauopathies. *Nat. Med.* **28**, 2547–2554 (2022).
39. Morris, J. C. The Clinical Dementia Rating (CDR): current version and scoring rules. *Neurology* **43**, 2412–2414 (1993).

40. Morris, J. C. et al. The Uniform Data Set (UDS): clinical and cognitive variables and descriptive data from Alzheimer disease centers. *Alzheimer Dis. Assoc. Disord.* **20**, 210–216 (2006).
41. Pastor, P. et al. Apolipoprotein Eepsilon4 modifies Alzheimer's disease onset in an E280A PS1 kindred. *Ann. Neurol.* **54**, 163–169 (2003).
42. Boerwinkle, A. H. et al. Temporal correlation of CSF and neuroimaging in the amyloid-tau-neurodegeneration model of Alzheimer disease. *Neurology* **97**, e76–e87 (2021).
43. Hansson, O. et al. CSF biomarkers of Alzheimer's disease concord with amyloid-beta PET and predict clinical progression: a study of fully automated immunoassays in BioFINDER and ADNI cohorts. *Alzheimer's Dement.* **14**, 1470–1481 (2018).
44. Su, Y. et al. Partial volume correction in quantitative amyloid imaging. *NeuroImage* **107**, 55–64 (2015).
45. Su, Y. et al. Comparison of Pittsburgh compound B and florbetapir in cross-sectional and longitudinal studies. *Alzheimer's Dement.* **11**, 180–190 (2019).
46. Su, Y. et al. Utilizing the Centiloid scale in cross-sectional and longitudinal PiB PET studies. *Neuroimage Clin.* **19**, 406–416 (2018).
47. Mishra, S. et al. AV-1451 PET imaging of tau pathology in preclinical Alzheimer disease: defining a summary measure. *NeuroImage* **161**, 171–178 (2017).
48. Leuzy, A. et al. A multicenter comparison of [^{18}F]flortaucipir, [^{18}F]RO948, and [^{18}F]MK6240 tau PET tracers to detect a common target ROI for differential diagnosis. *Eur. J. Nucl. Med. Mol. Imaging* **48**, 2295–2305 (2021).
49. DeLong, E. R., DeLong, D. M. & Clarke-Pearson, D. L. Comparing the areas under two or more correlated receiver operating characteristic curves: a nonparametric approach. *Biometrics* **44**, 837–845 (1988).
50. Benjamini, Y. & Hochberg, Y. Controlling the false discovery rate—a practical and powerful approach to multiple testing. *J. R. Stat. Soc. B* **57**, 289–300 (1995).

Acknowledgements

We thank the research volunteers who participated in the studies, from whom these data were obtained, and their supportive families. We thank the Clinical, Fluid Biomarker and Imaging Cores at the Knight ADRC for sample and data collection. The present study was supported by National Institutes of Health (NIH; grant no. R01AG070941 to S.E.S.), Knight ADRC Developmental Projects (N.R.B.), Eisai industry grant (to R.J.B. and K.H.), RF1AG061900 and R56AG061900 (to R.J.B.) and P30AG066444, P01AG003991, P01AG026276, U19AG032438 and U19AG024904 (to J.C.M.). Avid Radiopharmaceuticals provided doses of [^{18}F]AV45 (florbetapir) and precursor and technology transfer for [^{18}F]AV1451 (flortaucipir) to the Washington University, but was not involved in the data analysis or interpretation. The BioFINDER-2 study was supported by the Swedish Research Council (no. 2016-00906), and all the following to O.H.: the Knut and Alice Wallenberg Foundation (no. 2017-0383), the Marianne and Marcus Wallenberg foundation (no. 2015-0125), the Strategic Research Area MultiPark (Multidisciplinary Research in Parkinson's disease) at Lund University, the Swedish Alzheimer Foundation (no. AF-939932), the Swedish Brain Foundation (no. FO2021-0293), the Parkinson foundation of Sweden (no. 1280/20), the Cure Alzheimer's fund, the Konung Gustaf V:s och Drottning Victorias Frimurarestiftelse, the Skåne University Hospital Foundation (no. 2020-O000028), Regionalt Forskningsstöd (no. 2020-0314) and the Swedish federal government under the ALF agreement (2018-Projekt0279). The precursor of [^{18}F]flutemetamol was sponsored by GE Healthcare and the precursor of [^{18}F]RO948 was provided by Roche. G.S. received funding from the European Union's (EU's) Horizon 2020 research and innovation program under the Marie Sklodowska-Curie action grant (no. 101061836), from Greta och Johan Kocks research grants and travel grants from the Strategic Research Area MultiPark (Multidisciplinary Research in Parkinson's disease) at Lund University. The funders had no role in study design, data collection and analysis, decision to publish or preparation of the manuscript.

Author contributions

N.R.B., S.E.S. and R.J.B. conceived the study. N.R.B. developed the MS assay with support from C.S. and K.H. N.R.B., Y.H., C.S. and K.H. supervised processing and LC–MS analysis of CSF samples. N.R.B., Y.H. and V.O. performed LC-MS data processing and quality control. B.A.G., T.L.S.B. and O.H. designed and implemented the MRI and PET acquisition protocols, and performed image processing and quality control. J.C.M., R.J.B., S.E.S., E.S. and O.H. collected clinical data. R.L.H., S.E.S., A.M.F., S.J. and O.H. supervised processing and analysis of CSF samples using immunoassays. N.R.B., S.E.S., B.S., Y.L. and B.A.G. performed the statistical analyses. N.R.B., S.E.S., R.J.B., B.A.G., K.H., C.S., B.S., Y.L., C.X., S.J., G.S. and O.H. helped in interpreting the results. N.R.B. and S.E.S. drafted the manuscript. All authors contributed to revision and editing of the manuscript.

Competing interests

N.R.B., K.H., C.S., V.O. and R.J.B. are coinventors on the following US patent applications: 'Methods to detect novel tau species in CSF and use thereof to track tau neuropathology in Alzheimer's disease and other tauopathies' (PCT/US2020/046224, N.R.B., K.H., C.S. and R.J.B.); 'CSF phosphorylated tau and amyloid beta profiles as biomarkers of tauopathies' (PCT/US2022/022906, N.R.B., C.S. and R.J.B.); 'Plasma based methods for detecting CNS amyloid deposition' (PCT/UC2018/030518, V.O. and R.J.B.); and 'Methods of diagnosing and treating based on site-specific tau phosphorylation' (PCT/US2019/030725, N.R.B. and R.J.B.). N.R.B., K.H., C.S., V.O. and R.J.B. may receive a royalty income based on technology licensed by Washington University to C2N Diagnostics. K.H. is an Eisai-sponsored voluntary research associate professor at Washington University and has received a salary from Eisai. A.M.F. has received research funding from Biogen, Centene, Fujirebio and Roche Diagnostics. She is a member of the scientific advisory boards for Roche Diagnostics, Genentech and Diadem. She consults for DiamiR and Seimens Healthcare Diagnostics Inc. T.L.S.B. has investigator-initiated research funding from the NIH, the Alzheimer's Association, the Barnes-Jewish Hospital Foundation and Siemens. She participates as a site investigator in clinical trials sponsored by Avid Radiopharmaceuticals, Eli Lilly, Biogen, Eisai, Janssen and Roche, and serves as a consultant to Biogen, Eli Lilly, Eisai and Siemens. C.X. consulted for DIADEM and has used funding from the NIH to hire C2N Diagnostics as a vendor in another independent NIH-funded project. He received no funding from C2N Diagnostics. Neither J.C.M. nor his family owns stock or has equity interest (outside of mutual funds or other externally directed accounts) in any pharmaceutical or biotechnology company. O.H. has acquired research support (for the institution) from ADx, AVID Radiopharmaceuticals, Biogen, Eli Lilly, Eisai, Fujirebio, GE Healthcare, Pfizer and Roche. In the past 2 years, he has received consultancy/speaker fees from AC Immune, Amylyx, Alzpath, BioArctic, Biogen, Cerveau, Eisai, Fujirebio, Genentech, Novartis, Novo Nordisk, Roche and Siemens. R.J.B. cofounded C2N Diagnostics. Washington University and R.J.B. have equity ownership interest in C2N Diagnostics and receive a royalty income based on technology (stable isotope labeling kinetics, blood plasma assay and methods of diagnosing AD with phosphorylation changes) licensed by Washington University to C2N Diagnostics. R.J.B. receives an income from C2N Diagnostics for serving on the scientific advisory board, and has received research funding from Avid Radiopharmaceuticals, Janssen, Roche/Genentech, Eli Lilly, Eisai, Biogen, AbbVie, Bristol Myers Squibb and Novartis. S.E.S. has analyzed data provided by C2N Diagnostics to Washington University, but she has not received any research funding or

personal compensation from C2N Diagnostics or any other for-profit organizations. The remaining authors declare no competing interests.

Additional information

Extended data is available for this paper at https://doi.org/10.1038/s43587-023-00380-7.

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s43587-023-00380-7.

Correspondence and requests for materials should be addressed to Nicolas R. Barthélemy or Suzanne E. Schindler.

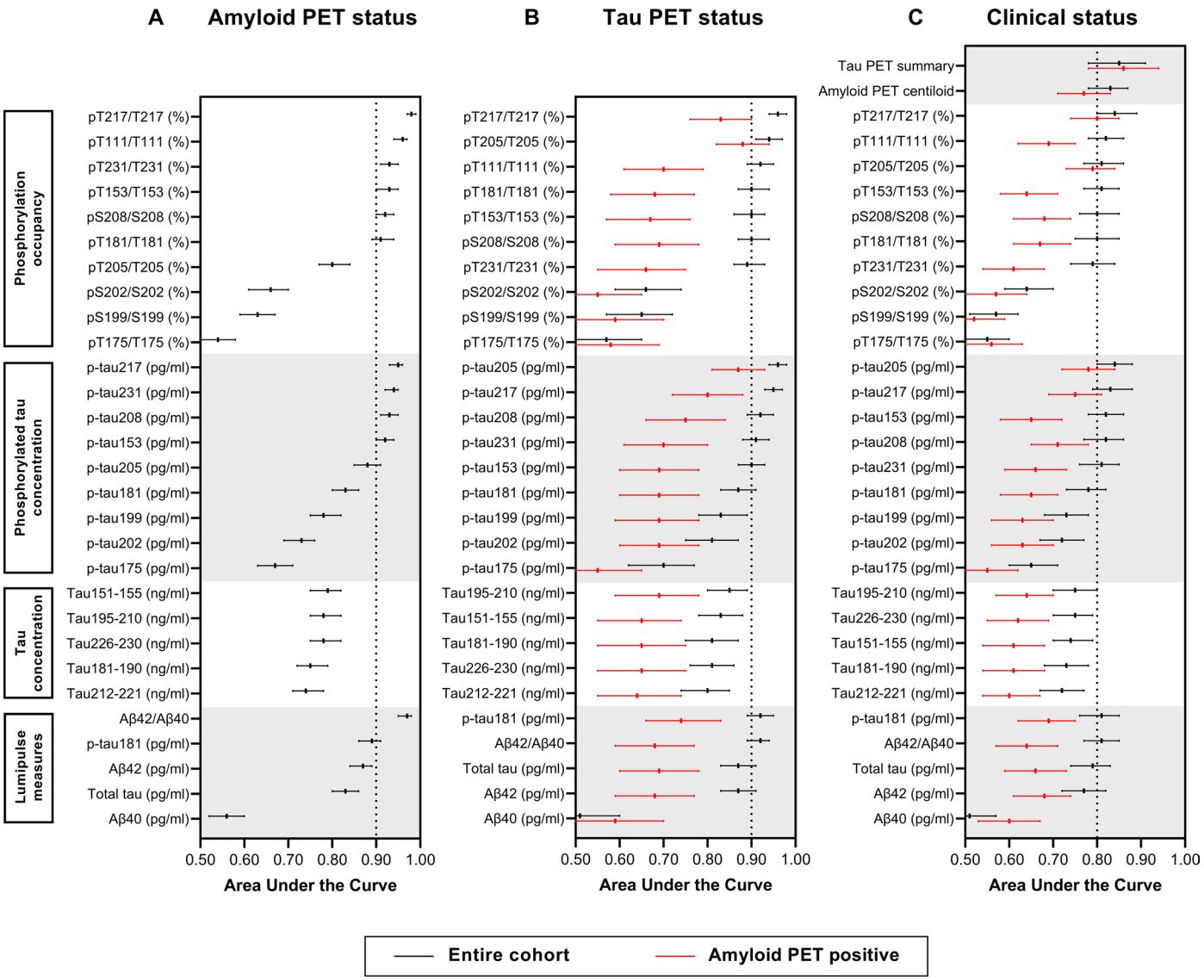
Peer review information *Nature Aging* thanks Michelle Mielke and the other, anonymous, reviewer(s) for their contribution to the peer review of this work.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

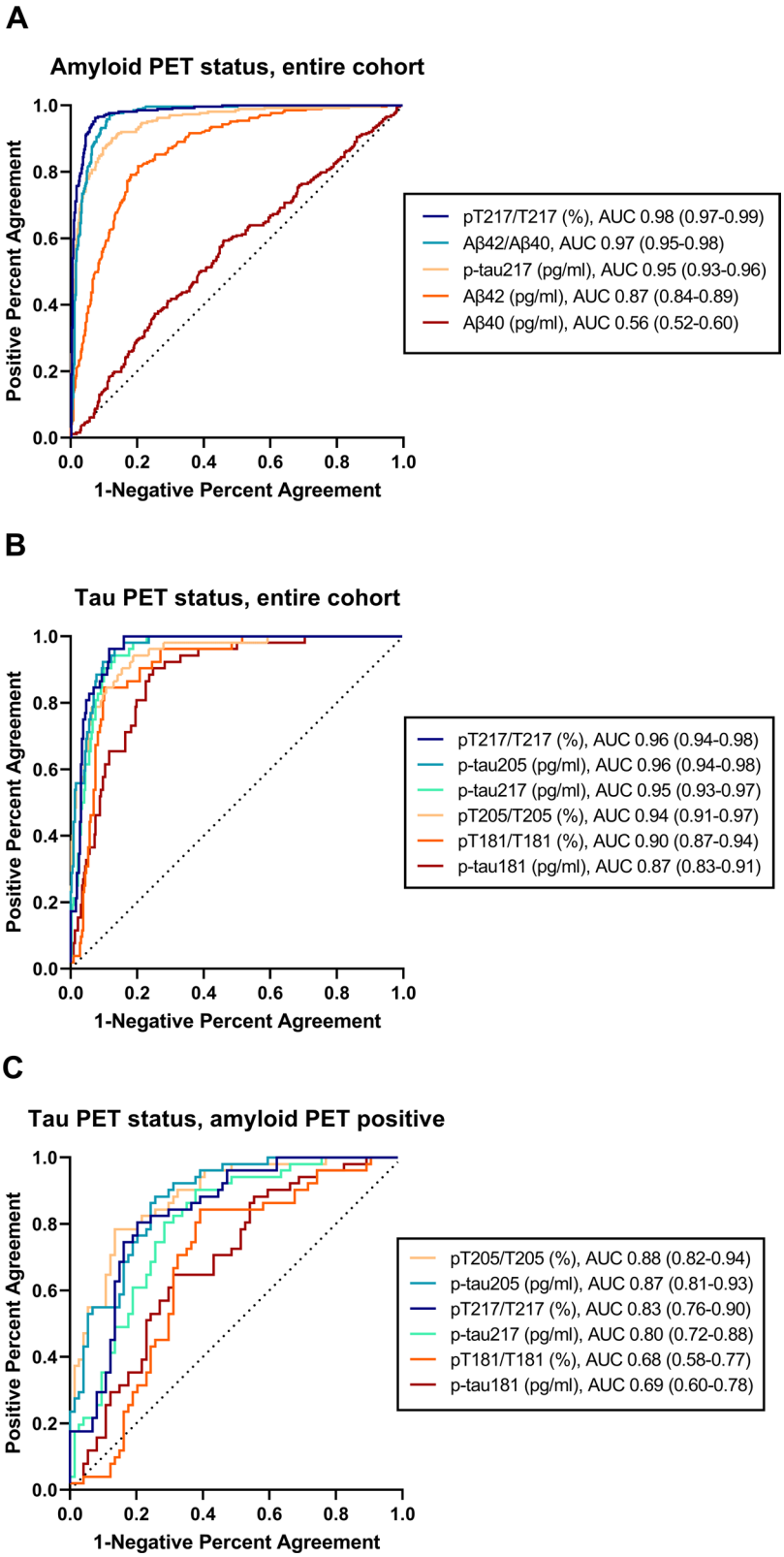
[1]Department of Neurology, Washington University School of Medicine, St. Louis, MO, USA. [2]Tracy Family SILQ Center for Neurodegenerative Biology, St. Louis, MO, USA. [3]Department of Radiology, Washington University School of Medicine, St. Louis, MO, USA. [4]Clinical Memory Research Unit, Department of Clinical Sciences Malmö, Lund University, Lund, Sweden. [5]Memory Clinic, Skåne University Hospital, Malmö, Sweden. [6]Knight Alzheimer Disease Research Center, Washington University School of Medicine, St. Louis, MO, USA. [7]Division of Biostatistics, Washington University School of Medicine, St. Louis, MO, USA. [8]These authors jointly supervised this work: Nicolas R. Barthélemy, Suzanne E. Schindler. ✉e-mail: barthelemy.nicolas@wustl.edu; schindler.s.e@wustl.edu

https://doi.org/10.1038/s43587-023-00380-7



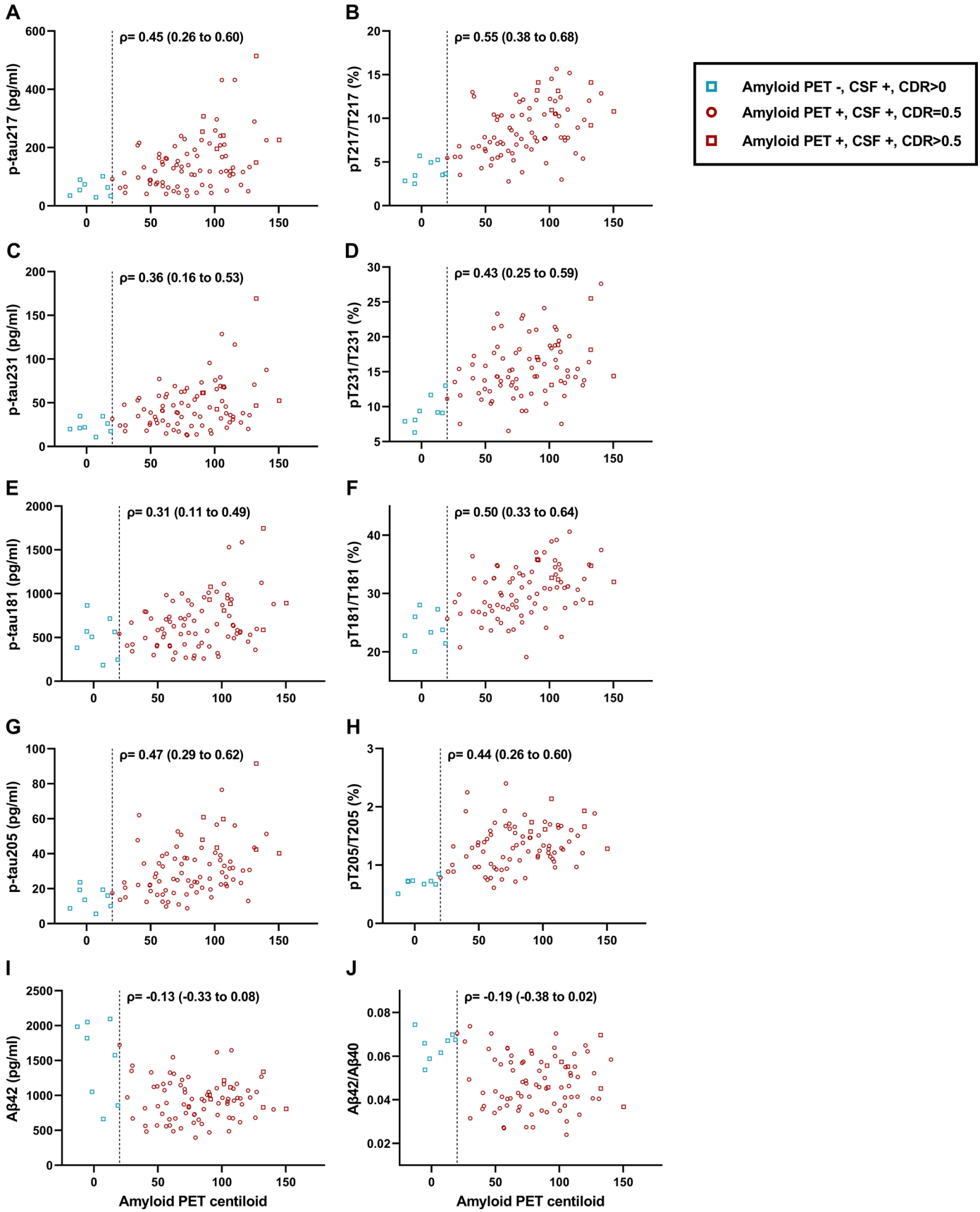
Extended Data Fig. 1 | Correspondence of CSF measures with amyloid PET status, tau PET status, or clinical status. Correspondence of the CSF measures with amyloid PET status was evaluated in the amyloid PET cohort (n = 750 individuals) (**A**), and correspondence with tau PET status was evaluated in tau PET cohort (n = 371 individuals) (**B**). Correspondence of the CSF measures with clinical status (cognitively normal [CDR = 0] or cognitively impaired [CDR > 0]) was evaluated in the larger amyloid PET cohort (n = 750 individuals) (**C**). Lines represent the receiver operating characteristic area under the curve point estimate (middle point) and 95% confidence intervals. Black lines represent correspondence in the entire cohort and the red lines represent correspondence in amyloid PET positive individuals.

https://doi.org/10.1038/s43587-023-00380-7



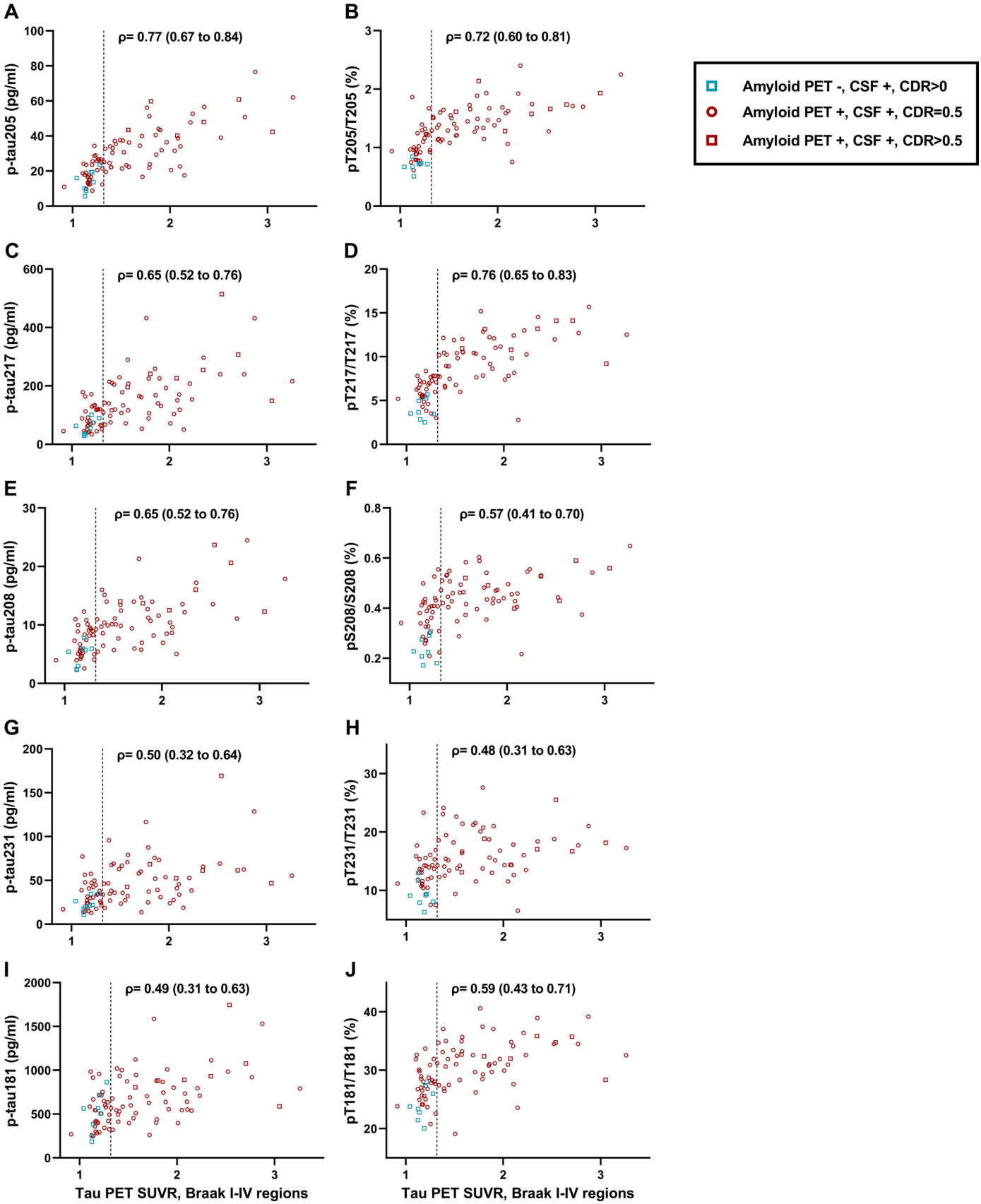
Extended Data Fig. 2 | Receiver operating characteristic curves for selected CSF measures. Correspondence of selected CSF measures with amyloid PET status was evaluated in the amyloid PET cohort; n = 750 for all measures shown (**A**). Correspondence of selected CSF measures with tau PET status was evaluated in the tau PET cohort; n = 371 for all measures shown except for pT181/T181 and p-tau181 (n = 370) (**B**). Correspondence of selected CSF measures with tau PET status was evaluated in the amyloid PET positive individuals in the tau PET cohort; n = 125 for all measures shown (**C**). For each measure, the receiver operating characteristic area under the curve with 95% confidence intervals is shown.

https://doi.org/10.1038/s43587-023-00380-7



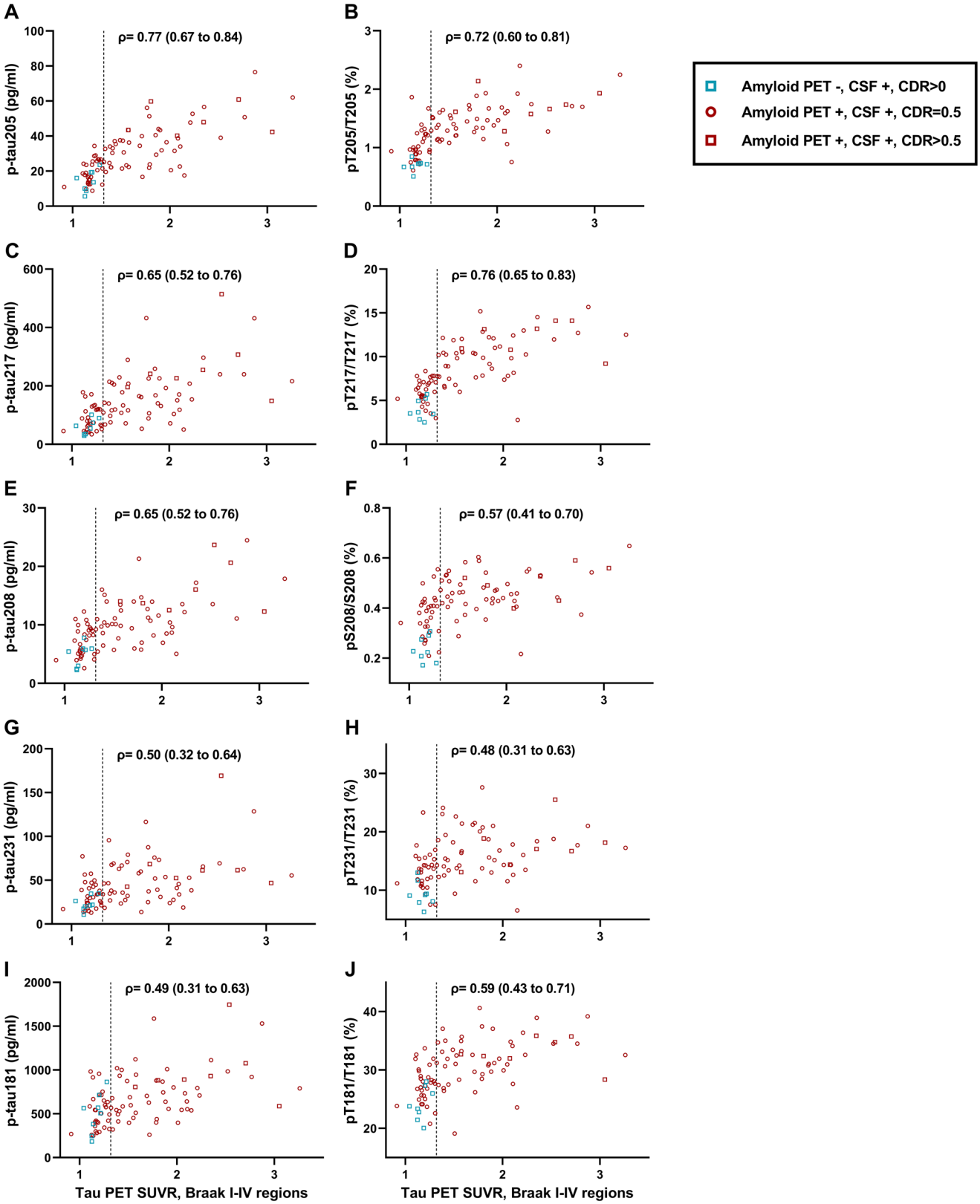
Extended Data Fig. 3 | Correlations of selected CSF p-tau concentrations and tau phosphorylation occupancies with amyloid PET Centiloid in the BioFINDER-2 cohort. CSF concentrations of p-tau217 (**A**), p-tau231 (**C**), p-tau181 by MS (**E**), p-tau205 (**G**), and Aβ42 (**I**) and tau phosphorylation occupancies at T217 (**B**), T231 (**D**), T181 (**F**), T205 (**H**), as well as Aβ42/Aβ40 (**J**), are plotted as a function of amyloid PET Centiloid. Spearman correlations with 95% confidence intervals are shown. Vertical dashed lines represent the cut-off for amyloid PET positivity. Each symbol represents one individual: green square: amyloid PET negative, CSF positive, CDR > 0; red circle: amyloid PET positive, CSF positive, CDR = 0.5; red square: amyloid PET positive, CSF positive, CDR > 0.5.

https://doi.org/10.1038/s43587-023-00380-7



Extended Data Fig. 4 | Correlations of selected CSF p-tau concentrations and tau phosphorylation occupancies with tau PET SUVR for Braak I-IV regions in the BioFINDER-2 cohort. CSF concentrations of p-tau205 (**A**), p-tau217 (**C**), p-tau208 (**E**), p-tau231 (**G**), and p-tau181 by MS (**I**) and tau phosphorylation occupancies at T205 (**B**), T217 (**D**), S208 (**F**), T231 (**H**), as well as T181 (**J**), are plotted as a function of the tau PET SUVR for Braak I-IV regions. Spearman correlations with 95% confidence intervals are shown. Vertical dashed lines represent the cut-off for tau PET positivity (SUVR > 1.32 for Braak I-IV regions). Each symbol represents one individual: green square: amyloid PET negative, CSF positive, CDR > 0; red circle: amyloid PET positive, CSF positive, CDR = 0.5; red square: amyloid PET positive, CSF positive, CDR > 0.5.

https://doi.org/10.1038/s43587-023-00380-7



Extended Data Fig. 5 | Correlations of selected CSF p-tau concentrations and tau phosphorylation occupancies with tau PET SUVR for Braak V-VI regions in the BioFINDER-2 cohort. CSF concentrations of p-tau205 (**A**), p-tau217 (**C**), p-tau208 (**E**), p-tau231 (**G**), and p-tau181 by MS (**I**) and tau phosphorylation occupancies at T205 (**B**), T217 (**D**), S208 (**F**), T231 (**H**), as well as T181 (**J**), are plotted as a function of the tau PET SUVR for Braak V-VI regions. Spearman correlations with 95% confidence intervals are shown. Each symbol represents one individual: green square: amyloid PET negative, CSF positive, CDR > 0; red circle: amyloid PET positive, CSF positive, CDR = 0.5; red square: amyloid PET positive, CSF positive, CDR > 0.5.

https://doi.org/10.1038/s43587-023-00380-7

Extended Data Table 1 | Participant characteristics for the Knight ADRC amyloid PET cohort

Characteristic	Entire cohort (n=750)		Amyloid PET negative (n=487)		Amyloid PET positive (n=263)		p=
Demographics							
Age at CSF collection (years)	750	71.2 (65.3-76.1)	487	69.6 (62.9-74.2)	263	74.5 (69.5-78.7)	<0.0001
Gender (n, % female)	750	412, 55%	487	280, 57%	263	132, 50%	0.06
APOE ε4 carrier status (n, % ε4 carrier)	740	285, 39%	481	130, 27%	259	155, 60%	<0.0001
CDR 0/0.5/1+ (% >0)	750	628/100/22 (16%)	487	462/22/3 (5%)	263	166/78/19 (37%)	<0.0001
CDR-SB	750	0.0 (0.0-0.0)	487	0.0 (0.0-0.0)	263	0.0 (0.0-1.5)	<0.0001
Years of education	750	16 (14-18)	487	16 (14-18)	263	16 (14-18)	0.66
Race (Black/White/Other)	750	69/672/9	487	61/420/6	263	8/252/3	<0.0001
CSF Lumipulse measures							
Aβ42 (pg/ml)	750	734 (526-993)	487	889 (690-1120)	263	511 (407-618)	<0.0001
Aβ40 (pg/ml)	750	10800 (8700-13200)	487	10600 (8510-12800)	263	11500 (9100-13900)	0.006
Aβ42/Aβ40	750	0.0792 (0.0498-0.0916)	487	0.0889 (0.0805-0.0943)	263	0.0459 (0.0380-0.0534)	<0.0001
Total tau (pg/ml)	750	291 (211-462)	487	244 (182-324)	263	483 (338-631)	<0.0001
p-tau181 (pg/ml)	750	37.7 (28.2-59.6)	487	31.8 (24.6-40.0)	263	67.4 (47.0-88.9)	<0.0001
Amyloid and tau PET measures							
Amyloid PET Centiloid	750	7.8 (-0.1-40.8)	487	1.4 (-2.1-7.5)	263	64.6 (36.5-87.9)	<0.0001
PIB SUVR	390	1.10 (1.01-1.82)	265	1.04 (0.99-1.11)	125	2.60 (1.94-3.15)	<0.0001
Florbetapir SUVR	360	1.05 (0.88-1.64)	222	0.92 (0.82-1.02)	138	1.91 (1.45-2.38)	<0.0001
Interval between CSF collection and amyloid PET (years)	750	0.12 (0.04-0.30)	487	0.11 (0.04-0.29)	263	0.13 (0.04-0.31)	0.35
Tau PET summary measure	361	1.20 (1.10-1.37)	237	1.15 (1.08-1.25)	124	1.41 (1.21-1.78)	<0.0001
Tau Summary PET Measure Positivity (n, % positive)	361	52, 14%	237	1, 0.4%	124	51, 41%	<0.0001
Interval between CSF collection and tau PET (years)	361	0.12 (0.04-0.25)	237	0.11 (0.04-0.26)	124	0.14 (0.04-0.25)	0.99

Continuous values are presented as the median with the IQR. The significance of differences by amyloid PET status were evaluated with Wilcoxon's rank-sum tests for continuous variables and χ^2 or Fisher's exact test for categorical variables. All tests were two sided and not adjusted for multiple comparisons.

https://doi.org/10.1038/s43587-023-00380-7

Extended Data Table 2 | Participant characteristics for the Knight ADRC tau PET cohort

Characteristic	Entire cohort (n=371)		Tau PET negative (n=319)		Tau PET positive (n=52)		p=
Demographics							
Age at CSF collection (years)	371	70.5 (64.8-75.6)	319	69.9 (63.6-74.7)	52	75.8 (71.7-81.4)	<0.0001
Gender (n, % female)	371	196, 53%	319	168, 53%	52	28, 54%	0.87
APOE ε4 carrier status (n, % ε4 carrier)	365	140, 38%	314	105, 33%	51	35, 69%	<0.0001
CDR 0/0.5/1+ (% >0)	371	316/41/14 (15%)	319	299/19/1 (6%)	52	17/22/13 (67%)	<0.0001
CDR-SB	371	0.0 (0.0-0.0)	319	0.0 (0.0-0.0)	52	1.5 (0-4)	<0.0001
Years of education	371	16 (15-18)	319	16 (16-18)	52	16 (14-18)	0.09
Race (Black/White/Other)	371	27/339/5	319	26/289/4	52	1/50/1	0.26
CSF Lumipulse measures							
Aβ42 (pg/ml)	371	773 (563-1030)	319	858 (622-1080)	52	482 (362-593)	<0.0001
Aβ40 (pg/ml)	371	11400 (9060-13500)	319	11300 (9010-13400)	52	11400 (9100-13400)	0.79
Aβ42/Aβ40	371	0.0819 (0.0511-0.0924)	319	0.0862 (0.0631-0.0931)	52	0.0432 (0.0345-0.0472)	<0.0001
Total tau (pg/ml)	371	292 (207-483)	319	257 (189-383)	52	571 (447-728)	<0.0001
p-tau181 (pg/ml)	371	36.6 (27.8-60.3)	319	34.0 (26.2-46.4)	52	81.1 (66.8-114)	<0.0001
Amyloid and tau PET measures							
Amyloid PET status (n, % positive)	371	125, 34%	319	74, 23%	52	51, 98%	<0.0001
Amyloid PET Centiloid	371	9.8 (1.40-40.6)	319	6.8 (0.7-17.8)	52	84.0 (69.7-107)	<0.0001
PIB SUVR	121	1.16 (1.06-2.12)	105	1.12 (1.04-1.50)	16	3.21 (2.76-3.40)	<0.0001
Florbetapir SUVR	250	1.01 (0.85-1.49)	214	0.957 (0.842-1.12)	36	2.28 (1.96-2.63)	<0.0001
Interval between CSF collection and amyloid PET (years)	371	0.12 (0.04-0.25)	319	0.12 (0.04-0.25)	52	0.19 (0.05-0.34)	0.10
Tau PET summary measure	371	1.20 (1.10-1.36)	319	1.17 (1.09-1.26)	52	1.97 (1.68-2.15)	<0.0001
Interval between CSF collection and tau PET (years)	371	0.12 (0.04-0.26)	319	0.11 (0.04-0.26)	52	0.19 (0.06-0.26)	0.21

Continuous values are presented as the median with the IQR. The significance of differences by tau PET status were evaluated with Wilcoxon's rank-sum tests for continuous variables and χ^2 and Fisher's exact test for categorical variables. All tests were two sided and not adjusted for multiple comparisons.

https://doi.org/10.1038/s43587-023-00380-7

Extended Data Table 3 | Participant characteristics for the BioFINDER-2 cohort

Characteristic	All (n=90)	Tau PET negative, Braak I-IV regions (n=38)	Tau PET positive, Braak I-IV regions (n=52)	p=
Demographics				
Age at CSF collection (years)	72 (67-76)	72 (70-76)	71 (67-76)	0.54
Gender (n, % female)	42, 47%	17, 45%	25, 48%	0.80
APOE ε4 carrier status (n, % ε4 carrier)	64, 71%	23, 61%	41, 79%	0.04
Diagnosis (n MCI/n AD dementia, % AD dementia)	83/7 (8%)	38/0 (0%)	45/7 (13%)	0.02
Mini-Mental State Exam Score	27 (25-28)	27 (26-28)	26 (25-28)	0.37
Years of education	11 (9-15)	11 (9-14)	12 (9-15)	0.59
CSF NeuroTool Kit measures				
Aβ42 (pg/ml)	946 (719-1160)	1010 (803-1330)	911 (657-1070)	0.03
Aβ40 (pg/ml)	20200 (14900-23600)	20400 (14600-26000)	20100 (15100-22600)	0.52
Aβ42/Aβ40	0.0488 (0.0382-0.06)	0.0569 (0.0413-0.0638)	0.0456 (0.0368-0.0551)	0.003
Total tau (pg/ml)	315 (237-407)	282 (187-358)	325 (260-429)	0.03
p-tau181 (pg/ml)	28.8 (21.1-41.8)	25.3 (17-31.3)	32 (25.6-43.8)	0.002
Amyloid and tau PET measures				
Amyloid PET status (n, % positive)	82, 91%	30, 79%	52, 100%	0.0005
Amyloid PET Centiloid	76.5 (53.4-104)	55.1 (26.1-71.9)	91.3 (71.2-109)	<0.0001
Interval between CSF collection and amyloid PET (years)	0.301 (0.126-0.413)	0.26 (0.0766-0.366)	0.306 (0.142-0.429)	0.35
Tau PET SUVR, Braak I-IV regions	1.4 (1.2-1.86)	1.18 (1.14-1.22)	1.79 (1.51-2.1)	<0.0001
Tau PET SUVR, Braak V-VI regions	1.13 (1.03-1.25)	1.03 (0.995-1.06)	1.2 (1.14-1.46)	<0.0001
Interval between CSF collection and tau PET (years)	0.25 (0.08-0.38)	0.25 (0.06-0.38)	0.254 (0.10-0.38)	0.81

Continuous values are presented as the median with the IQR. The significance of differences by tau PET status were evaluated with Wilcoxon's ranked sum tests for continuous variables and χ^2 or Fisher's exact test for categorical variables. The cut-off for tau PET positivity was set at SUVR>1.32 for the Braak I–IV region. All tests were two sided and not adjusted for multiple comparisons.

https://doi.org/10.1038/s43587-023-00380-7

Extended Data Table 4 | CSF tau measures for the Knight ADRC amyloid PET cohort

Characteristic	Entire cohort (n=750)		Amyloid PET negative (n=487)		Amyloid PET positive (n=263)		Fold difference	p=
Phosphorylation occupancies by mass spectrometry								
pT111/T111 (%)	746	3.31 (2.35-6.56)	483	2.56 (2.03-3.29)	263	7.96 (5.90-10.27)	3.11	<0.0001
pT153/T153 (%)	660	0.0599 (0.0312-0.122)	399	0.0369 (0.0211-0.0581)	261	0.133 (0.0943-0.195)	3.60	<0.0001
pT175/T175 (%)	734	0.465 (0.378-0.544)	477	0.467 (0.381-0.554)	257	0.455 (0.374-0.536)	0.97	0.11
pT181/T181 (%)	747	29.5 (27.5-33.3)	485	28.1 (26.9-29.8)	262	35.4 (31.9-39.4)	1.26	<0.0001
pS199/S199 (%)	743	0.671 (0.525-0.869)	480	0.626 (0.49-0.823)	263	0.739 (0.616-0.932)	1.18	<0.0001
pS202/S202 (%)	750	5.58 (4.65-6.55)	487	5.82 (4.95-6.84)	263	5.06 (4.22-6.02)	0.87	<0.0001
pT205/T205 (%)	749	0.982 (0.803-1.24)	486	0.894 (0.75-1.06)	263	1.29 (1.01-1.57)	1.44	<0.0001
pS208/S208 (%)	685	0.142 (0.0863-0.245)	425	0.102 (0.0704-0.143)	260	0.273 (0.202-0.347)	2.68	<0.0001
pT217/T217 (%)	750	3.58 (3.04-7.31)	487	3.16 (2.86-3.56)	263	9.20 (6.60-12.47)	2.91	<0.0001
pT231/T231 (%)	620	8.74 (3.5-21)	380	4.95 (2.02-8.68)	240	24.74 (15.8-31.2)	4.99	<0.0001
Phosphorylated tau concentrations by mass spectrometry								
p-tau153 (pg/ml)	660	1.14 (0.543-3.05)	399	0.693 (0.358-1.08)	261	3.66 (2.11-6.43)	5.28	<0.0001
p-tau175 (pg/ml)	734	10.8 (7.25-15)	477	9.66 (6.4-12.7)	257	13.4 (9.6-17.3)	1.39	<0.0001
p-tau181 (pg/ml)	747	671 (490-998)	485	590 (443-738)	262	1040 (752-1390)	1.76	<0.0001
p-tau199 (pg/ml)	743	17.1 (11.6-24.6)	480	14.6 (9.94-19.7)	263	25.0 (17.4-32.3)	1.71	<0.0001
p-tau202 (pg/ml)	750	136 (112-169)	487	126 (107-152)	263	161 (131-194)	1.28	<0.0001
p-tau205 (pg/ml)	749	23.3 (17.7-34.9)	486	19.9 (15.5-24.2)	263	40.5 (28.3-57.8)	2.04	<0.0001
p-tau208 (pg/ml)	685	3.49 (1.9-7.05)	425	2.34 (1.45-3.49)	260	8.82 (5.35-13.1)	3.77	<0.0001
p-tau217 (pg/ml)	750	62.5 (43.6-142)	487	48.6 (35.7-63.3)	263	192 (121-288)	3.95	<0.0001
p-tau231 (pg/ml)	619	19.6 (7.35-60.4)	380	9.59 (3.29-18.5)	239	74.7 (42.8-127)	7.79	<0.0001
Non-phosphorylated tau concentrations by mass spectrometry								
Tau151-155 (ng/ml)	747	2.01 (1.52-2.76)	486	1.77 (1.33-2.2)	261	2.72 (2.09-3.47)	1.54	<0.0001
Tau181-190 (ng/ml)	750	2.29 (1.76-3.07)	487	2.09 (1.61-2.54)	263	2.94 (2.29-3.66)	1.41	<0.0001
Tau195-210 (ng/ml)	750	2.46 (1.9-3.31)	487	2.22 (1.71-2.69)	263	3.29 (2.54-4.1)	1.48	<0.0001
Tau212-221 (ng/ml)	750	1.69 (1.29-2.19)	487	1.52 (1.17-1.9)	263	2.11 (1.66-2.67)	1.39	<0.0001
Tau226-230 (ng/ml)	719	0.224 (0.158-0.327)	486	0.894 (0.75-1.06)	263	1.29 (1.01-1.57)	1.44	<0.0001

Continuous values are presented as the median with the IQR. The significance of differences by amyloid PET status was evaluated with Wilcoxon's rank-sum tests for continuous variables and χ^2 or Fisher's exact test for categorical variables. The fold difference is the median biomarker value in the amyloid PET-positive group divided by the median value in the amyloid PET-negative group. All tests were two sided and not adjusted for multiple comparisons.

https://doi.org/10.1038/s43587-023-00380-7

Extended Data Table 5 | CSF tau measures for the BioFINDER-2 cohort

Characteristic	All (n=90)	Tau PET negative, Braak I-IV regions (n=38)	Tau PET positive, Braak I-IV regions (n=52)	Fold difference	p=
Phosphorylation occupancies by mass spectrometry					
pT111/T111 (%)	3.81 (3.10-5.34)	3.16 (2.36-4.03)	4.67 (3.57-5.72)	1.48	<0.0001
pT153/T153 (%)	0.168 (0.127-0.202)	0.126 (0.115-0.156)	0.178 (0.165-0.212)	1.41	<0.0001
pT181/T181 (%)	29.7 (26.8-32.7)	26.8 (24.1-28.3)	32.4 (29.7-34.8)	1.21	<0.0001
pS199/S199 (%)	0.943 (0.831-1.04)	0.904 (0.768-1.01)	0.976 (0.856-1.05)	1.08	0.08
pS202/S202 (%)	3.17 (2.59-3.57)	3.17 (2.80-3.57)	3.16 (2.55-3.51)	1.00	0.28
pT205/T205 (%)	1.28 (0.959-1.59)	0.927 (0.748-1.100)	1.49 (1.28-1.67)	1.61	<0.0001
pS208/S208 (%)	0.425 (0.340-0.494)	0.339 (0.273-0.408)	0.46 (0.42-0.53)	1.36	<0.0001
pT217/T217 (%)	7.79 (5.72-10.50)	5.58 (4.61-6.81)	10.40 (8.15-12.1)	1.86	<0.0001
pT231/T231 (%)	14.3 (11.9-17.7)	11.9 (10.4-14.2)	16.7 (13.8-18.9)	1.40	<0.0001
Phosphorylated tau concentrations by mass spectrometry					
p-tau153 (pg/ml)	2.62 (1.72-3.72)	1.87 (1.21-2.47)	3.29 (2.52-4.70)	1.76	<0.0001
p-tau181 (pg/ml)	588 (421-864)	504 (343-634)	704 (542-921)	1.40	<0.0001
p-tau199 (pg/ml)	21 (15.9-24.9)	18.2 (13.3-21.3)	23.2 (19.2-26.0)	1.27	<0.0001
p-tau202 (pg/ml)	72.2 (54.6-82.3)	63.2 (50.3-73.8)	76 (62.1-84.5)	1.20	0.004
p-tau205 (pg/ml)	25.5 (18.6-37.6)	17.9 (12.9-23.6)	36.3 (24.6-43.5)	2.03	<0.0001
p-tau208 (pg/ml)	9.23 (5.77-12.30)	5.93 (4.71-8.37)	11.1 (8.7-14.0)	1.87	<0.0001
p-tau217 (pg/ml)	119 (67.9-193)	67.9 (45.4-113)	165 (116-226)	2.43	<0.0001
p-tau231 (pg/ml)	38.0 (24.5-57.4)	26.3 (19.9-38.0)	46.7 (33.9-66.8)	1.78	<0.0001
Non-phosphorylated tau concentrations by mass spectrometry					
Tau151-155 (ng/ml)	1.65 (1.27-2.19)	1.49 (0.97-1.85)	1.80 (1.48-2.26)	1.21	0.007
Tau181-190 (ng/ml)	2.11 (1.59-2.63)	1.83 (1.24-2.24)	2.18 (1.83-2.67)	1.19	0.01
Tau195-210 (ng/ml)	2.2 (1.66-2.77)	1.92 (1.33-2.34)	2.38 (1.92-2.99)	1.24	0.002
Tau212-221 (ng/ml)	1.58 (1.17-1.93)	1.42 (0.961-1.74)	1.61 (1.35-1.93)	1.13	0.03
Tau226-230 (ng/ml)	0.278 (0.204-0.349)	0.237 (0.165-0.292)	0.288 (0.247-0.357)	1.22	0.01

Continuous values are presented as the median with the IQR. The significance of differences by tau PET status for the Braak I–IV region was evaluated with Wilcoxon's rank-sum tests for continuous variables and χ^2 or Fisher's exact test for categorical variables. The cut-off for tau PET positivity was set at SUVR>1.32 for the Braak I–IV region. The fold difference is the median biomarker value in the tau PET-positive group divided by the median value in the tau PET-negative group. All tests were two sided and not adjusted for multiple comparisons.

nature portfolio

Corresponding author(s): Suzanne E. Schindler

Last updated by author(s): Jan 28, 2023

Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our Editorial Policies and the Editorial Policy Checklist.

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed
☐	☒ The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒ The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☐	☒ A description of all covariates tested
☐	☒ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒ A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒ For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☐	☒ Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	MS/HRMS transitions were extracted using Skyline version 22.2.2.278 (MacCoss lab, University of Washington). Data aggregated using Tableau version 2022.2.2 (Tableau Software, Seattle, Washington) to calculate concentrations and phosphorylation occupancies.
Data analysis	Statistical analyses were implemented using SAS 9.4 (SAS Institute Inc., Cary, NC, USA). Plots were created with GraphPad Prism version 9.2.0 (GraphPad Software, La Jolla, CA, USA). For visualization of the associations between CSF measures and regional tau PET or brain volumes, partial Spearman correlations including age and sex were calculated with partial.r from the R psych toolbox version 2.1.9. The ggseg package version 1.6.5 was used to visualize correlations and results from the left hemisphere are shown. The significance of correlations was adjusted for multiple comparisons using the Benjamini-Hochberg procedure 50.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

Knight ADRC data are available to qualified investigators who have a proposal approved by an institutional committee (https://knightadrc.wustl.edu/Research/ResourceRequest.htm) that meets monthly. The study must be approved by an institutional review board to ensure ethical research practices and investigators must agree to the terms and conditions of the data use agreement, which includes not distributing the data without permission. For BioFINDER-2 data, anonymized data will be shared by request from a qualified academic investigator for the sole purpose of replicating procedures and results presented in the article and as long as data transfer is in agreement with EU legislation on the general data protection regulation and decisions by the Ethical Review Board of Sweden and Region Skåne, which should be regulated in a material transfer agreement.

Human research participants

Policy information about studies involving human research participants and Sex and Gender in Research.

Reporting on sex and gender	Sex and race were self-identified.
Population characteristics	The primary cohorts for the study were from the Knight Alzheimer Disease Research Center (Knight ADRC) at Washington University in St. Louis, MO, USA. The Knight ADRC amyloid PET cohort included 750 individuals with a median age of 71.2 years (interquartile range [IQR] 65.3 to 76.1 years); 55% were female, 90% self-identified as White, 39% carried at least one APOE ε4 allele (Extended Data Table 1), and 16% were cognitively impaired as defined by a Clinical Dementia Rating (CDR) of 0.5 or greater, which includes mild cognitive (MCI) impairment and AD dementia. The overlapping Knight ADRC tau PET cohort included 371 individuals (Extended Data Table 2). Individuals in the tau PET cohort who were cognitively impaired (CDR of 0.5 or greater) were included in Knight ADRC tau PET symptomatic AD sub-cohort (n=55), (Supplemental Table 1). The validation cohort included 90 individuals enrolled in the BioFINDER-2 cohort at Skåne University Hospital in Sweden with a median age of 72 years (IQR 67 to 76 years); 47% were female, 71% carried at least one APOE ε4 allele, 83 were diagnosed with MCI and 7 were diagnosed with AD dementia (Extended Data Table 3).
Recruitment	Participants in the Knight Alzheimer Disease Research Center (ADRC) at Washington University in St. Louis were community-dwelling volunteers enrolled in studies of memory and aging. Most participants were recruited from memory clinics or self-referred due to interest in dementia. Individuals were excluded from enrollment if they were diagnosed with a non-AD dementia at their initial assessment (e.g., Parkinson disease), had conditions that might interfere with study procedures (e.g., a pacemaker that would make the participant ineligible for MRI), or had medical issues that might significantly affect long-term participation (e.g., metastatic cancer). Participants in the Swedish BioFINDER-2 confirmatory cohort (NCT NCT03174938) included individuals diagnosed with MCI due to AD or AD dementia. Details on recruitment, exclusion and inclusion criteria have previously been described 12. Note that the limitations section of the discussion says the following: "The study cohorts consisted of participants enrolled in research studies, and therefore the findings are most relevant to research studies and clinical trials and less generalizable to clinical populations. The cohorts were relatively young (early to mid-70s). Older cohorts, especially clinical cohorts, would be expected to have a higher prevalence of neurological comorbidities, which could complicate biomarker relationships. The cohorts included relatively few individuals with symptomatic AD and/or moderate or high levels of tau pathology. Additionally, minoritized populations were not well represented in the study cohorts, and studies have found racial differences in CSF t-tau and p-tau181 concentrations 35,36. It is unknown whether other CSF tau measures such as pT217/T217 or pT205/T205 perform similarly across racial and ethnic groups, and further studies of these measures in diverse and clinically relevant cohorts are needed."
Ethics oversight	Washington University Human Research Protection Office (HRPO) and written informed consent was obtained from each participant or their legally authorized representative when appropriate (protocol #201109100). Participants in the Swedish BioFINDER-2 confirmatory cohort (NCT NCT03174938) included individuals diagnosed with MCI due to AD or AD dementia.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	No statistical methods were used to pre-determine sample sizes but our sample sizes are similar or larger to those used for similar studies 16,26,31.
Data exclusions	If more than one CSF sample from a participant met criteria, the most recently obtained sample was included. No data points were excluded from analyses; outliers were not removed.
Replication	Biomarker measures in a confirmatory symptomatic AD cohort The CSF tau measures were further examined in participants with symptomatic AD (MCI or AD dementia) from the BioFINDER-2 cohort (n=90, Extended Data Table 5).
Randomization	Individuals were included in groups based on the procedures they underwent (i.e., CSF collection and amyloid PET and/or tau PET). All samples from an individual were run in the same batch of assays.
Blinding	All assays and data extraction steps were performed by operators blinded to any clinical or biomarker information.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☐	☐ Eukaryotic cell lines
☐	☐ Palaeontology and archaeology
☐	☐ Animals and other organisms
☐	☒ Clinical data
☐	☐ Dual use research of concern

Methods

n/a	Involved in the study
☐	☐ ChIP-seq
☐	☐ Flow cytometry
☐	☒ MRI-based neuroimaging

Antibodies

Antibodies used	Tau was immunopurified by incubating CSF with Tau1 (provided by Drs. Nicholas Kanaan and Lester Binder) and HJ8.5 antibodies (provided by Dr. David Holtzman) at room temperature for 4 hours (3 mg antibody per g of beads) 2 (see Appendix 1 with more detailed assay methods and quality control metrics).
Validation	1. Barthelemy NR, Toth B, Manser PT, et al. Site-Specific Cerebrospinal Fluid Tau Hyperphosphorylation in Response to Alzheimer's Disease Brain Pathology: Not All Tau Phospho-Sites are Hyperphosphorylated. Journal of Alzheimer's disease : JAD 2022; 85(1): 415-29. 2. Sato C, Barthelemy NR, Mawuenyega KG, et al. Tau Kinetics in Neurons and the Human Central Nervous System. Neuron 2018; 98(4): 861-4.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	*State the source of each cell line used and the sex of all primary cell lines and cells derived from human participants or vertebrate models.*
Authentication	*Describe the authentication procedures for each cell line used OR declare that none of the cell lines used were authenticated.*
Mycoplasma contamination	*Confirm that all cell lines tested negative for mycoplasma contamination OR describe the results of the testing for mycoplasma contamination OR declare that the cell lines were not tested for mycoplasma contamination.*
Commonly misidentified lines (See ICLAC register)	*Name any commonly misidentified cell lines used in the study and provide a rationale for their use.*

Palaeontology and Archaeology

Specimen provenance	*Provide provenance information for specimens and describe permits that were obtained for the work (including the name of the issuing authority, the date of issue, and any identifying information). Permits should encompass collection and, where applicable, export.*
Specimen deposition	*Indicate where the specimens have been deposited to permit free access by other researchers.*
Dating methods	*If new dates are provided, describe how they were obtained (e.g. collection, storage, sample pretreatment and measurement), where they were obtained (i.e. lab name), the calibration program and the protocol for quality assurance OR state that no new dates are provided.*

☐ Tick this box to confirm that the raw and calibrated dates are available in the paper or in Supplementary Information.

Ethics oversight	*Identify the organization(s) that approved or provided guidance on the study protocol, OR state that no ethical approval or guidance was required and explain why not.*

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Animals and other research organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research, and Sex and Gender in Research

Laboratory animals	*For laboratory animals, report species, strain and age OR state that the study did not involve laboratory animals.*
Wild animals	*Provide details on animals observed in or captured in the field; report species and age where possible. Describe how animals were caught and transported and what happened to captive animals after the study (if killed, explain why and describe method; if released, say where and when) OR state that the study did not involve wild animals.*
Reporting on sex	*Indicate if findings apply to only one sex; describe whether sex was considered in study design, methods used for assigning sex. Provide data disaggregated for sex where this information has been collected in the source data as appropriate; provide overall numbers in this Reporting Summary. Please state if this information has not been collected. Report sex-based analyses where performed, justify reasons for lack of sex-based analysis.*
Field-collected samples	*For laboratory work with field-collected samples, describe all relevant parameters such as housing, maintenance, temperature, photoperiod and end-of-experiment protocol OR state that the study did not involve samples collected from the field.*
Ethics oversight	*Identify the organization(s) that approved or provided guidance on the study protocol, OR state that no ethical approval or guidance was required and explain why not.*

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Clinical data

Policy information about clinical studies

All manuscripts should comply with the ICMJE guidelines for publication of clinical research and a completed CONSORT checklist must be included with all submissions.

Clinical trial registration	N/A--the study is NOT a clinical trial
Study protocol	N/A--the study is NOT a clinical trial
Data collection	Participants in the Knight Alzheimer Disease Research Center (ADRC) at Washington University in St. Louis were community-dwelling volunteers enrolled in studies of memory and aging. Participants in the Swedish BioFINDER-2 confirmatory cohort (NCT NCT03174938) included individuals diagnosed with MCI due to AD or AD dementia.
Outcomes	Amyloid PET positivity was previously defined as a mean cortical SUVR > 1.42 for PiB and > 1.19 for Florbetapir 45. Tau PET positivity was defined as a tau PET summary measure > 1.52 based on Gaussian mixture modeling (Supplemental Figure 1). Participants in BioFINDER-2 underwent amyloid PET using 18F-flutemetamol as previously described 12. Participants underwent tau PET using 18F-RO948 as previously described 12. For tau PET, SUVRs for the brain regions with early change (Braak I-IV) and later change (Braak V-VI) were calculated. The cut-off for positivity in the Braak I-IV region was SUVR>1.32 48.

Dual use research of concern

Policy information about dual use research of concern

Hazards

Could the accidental, deliberate or reckless misuse of agents or technologies generated in the work, or the application of information presented in the manuscript, pose a threat to:

No	Yes	
☒	☐	Public health
☒	☐	National security
☒	☐	Crops and/or livestock
☒	☐	Ecosystems
☒	☐	Any other significant area

Experiments of concern

Does the work involve any of these experiments of concern:

No	Yes	
☒	☐	Demonstrate how to render a vaccine ineffective
☒	☐	Confer resistance to therapeutically useful antibiotics or antiviral agents
☒	☐	Enhance the virulence of a pathogen or render a nonpathogen virulent
☒	☐	Increase transmissibility of a pathogen
☒	☐	Alter the host range of a pathogen
☒	☐	Enable evasion of diagnostic/detection modalities
☒	☐	Enable the weaponization of a biological agent or toxin
☒	☐	Any other potentially harmful combination of experiments and agents

ChIP-seq

Data deposition

☐ Confirm that both raw and final processed data have been deposited in a public database such as GEO.

☐ Confirm that you have deposited or provided access to graph files (e.g. BED files) for the called peaks.

Data access links *May remain private before publication.*	*For "Initial submission" or "Revised version" documents, provide reviewer access links. For your "Final submission" document, provide a link to the deposited data.*
Files in database submission	*Provide a list of all files available in the database submission.*
Genome browser session (e.g. UCSC)	*Provide a link to an anonymized genome browser session for "Initial submission" and "Revised version" documents only, to enable peer review. Write "no longer applicable" for "Final submission" documents.*

Methodology

Replicates	*Describe the experimental replicates, specifying number, type and replicate agreement.*
Sequencing depth	*Describe the sequencing depth for each experiment, providing the total number of reads, uniquely mapped reads, length of reads and whether they were paired- or single-end.*
Antibodies	*Describe the antibodies used for the ChIP-seq experiments; as applicable, provide supplier name, catalog number, clone name, and lot number.*
Peak calling parameters	*Specify the command line program and parameters used for read mapping and peak calling, including the ChIP, control and index files used.*
Data quality	*Describe the methods used to ensure data quality in full detail, including how many peaks are at FDR 5% and above 5-fold enrichment.*
Software	*Describe the software used to collect and analyze the ChIP-seq data. For custom code that has been deposited into a community repository, provide accession details.*

Flow Cytometry

Plots

Confirm that:

☐ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☐ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).

☐ All plots are contour plots with outliers or pseudocolor plots.

☐ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation	*Describe the sample preparation, detailing the biological source of the cells and any tissue processing steps used.*
Instrument	*Identify the instrument used for data collection, specifying make and model number.*
Software	*Describe the software used to collect and analyze the flow cytometry data. For custom code that has been deposited into a community repository, provide accession details.*
Cell population abundance	*Describe the abundance of the relevant cell populations within post-sort fractions, providing details on the purity of the samples and how it was determined.*
Gating strategy	*Describe the gating strategy used for all relevant experiments, specifying the preliminary FSC/SSC gates of the starting cell population, indicating where boundaries between "positive" and "negative" staining cell populations are defined.*

☐ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.

Magnetic resonance imaging

Experimental design

Design type	Structural MRI
Design specifications	N/A
Behavioral performance measures	N/A

Acquisition

Imaging type(s)	Structural MRI
Field strength	3T
Sequence & imaging parameters	Both amyloid PET and tau PET scans were performed in coordination with a 3 Tesla structural MRI scan. T1-weighted MRIs were processed using Freesurfer 5.3 to generate regions of interest used for the processing of PET data. Estimates of regional volumes were adjusted for intracranial volume using a regression approach. Data from the 30-60 minute post-injection window for PiB, the 50-70 minute window for Florbetapir, or the 80-100 minute window for Flortaucipir were converted to standardized uptake value ratios (SUVRs) using the cerebellar grey as a reference and partial volume corrected using a geometric transfer matrix 39.
Area of acquisition	Values from the following regions were averaged together to represent mean cortical SUVR for Florbetapir or PiB: bilateral orbitofrontal, medial orbitofrontal, rostral middle frontal, superior frontal, superior temporal, middle temporal, and precuneus. Amyloid PET positivity was previously defined as a mean cortical SUVR > 1.42 for PiB and > 1.19 for Florbetapir 40. Mean cortical SUVRs were converted to Centiloid units to combine data from the two tracers 40,41. Values from the bilateral entorhinal cortex, amygdala, lateral occipital cortex, and inferior temporal cortex regions were averaged together as a summary measure of tau PET42.

Diffusion MRI ☐ Used ☒ Not used

Preprocessing

Preprocessing software	T1-weighted MRIs were processed using Freesurfer 5.3 to generate regions of interest used for the processing of PET data.
Normalization	Estimates of regional volumes were adjusted for intracranial volume using a regression approach. Data from the 30-60 minute post-injection window for PiB, the 50-70 minute window for Florbetapir, or the 80-100 minute window for Flortaucipir were converted to standardized uptake value ratios (SUVRs) using the cerebellar grey as a reference and partial volume corrected using a geometric transfer matrix 39.

Normalization template

Estimates of regional volumes were adjusted for intracranial volume using a regression approach. Data from the 30-60 minute post-injection window for PiB, the 50-70 minute window for Florbetapir, or the 80-100 minute window for Flortaucipir were converted to standardized uptake value ratios (SUVRs) using the cerebellar grey as a reference and partial volume corrected using a geometric transfer matrix 39.

Noise and artifact removal

N/A

Volume censoring

partial volume corrected using a geometric transfer matrix

Statistical modeling & inference

Model type and settings

The significance of differences by biomarker status (amyloid PET or tau PET status) were evaluated with Wilcoxon ranked sum tests for continuous variables and Chi-Square or Fisher exact tests for categorical variables. Receiver Operating Characteristic (ROC) analyses were used to evaluate the correspondence of CSF biomarker measures with amyloid PET status, tau PET status, or clinical status (cognitively unimpaired [CDR=0] or cognitively impaired [CDR>0]). Cut-offs that best distinguished amyloid PET or tau PET status were found based on the highest combined sensitivity and specificity (Youden Index). Differences between ROC areas under the curves (AUCs) were evaluated using DeLong tests 49. Spearman correlations were used to evaluate the continuous relationships of CSF biomarker measures with amyloid PET Centiloid, the tau PET summary measure, or the CDR-SB. For partial Spearman correlations with amyloid PET, tau PET, and brain volumes, analyses included covariates of age and sex; for correlations with CDR-SB, analyses included covariates of age, sex, and years of education. Comparisons between Spearman correlations were performed by bootstrapping. When multiple measures were compared, the significance was adjusted using the Benjamini-Hochberg procedure 50. Analyses were replicated in the sub-cohort of individuals with no missing data. Statistical analyses were implemented using SAS 9.4 (SAS Institute Inc., Cary, NC, USA). Plots were created with GraphPad Prism version 9.2.0 (GraphPad Software, La Jolla, CA, USA). All p values were from two-sided tests, and results were deemed statistically significant at $p < 0.05$.

For visualization of the associations between CSF measures and regional tau PET or brain volumes, partial Spearman correlations including age and sex were calculated with partial.r from the R psych toolbox. The ggseg package was used to visualize correlations and results from the left hemisphere are shown. The significance of correlations was adjusted for multiple comparisons using the Benjamini-Hochberg procedure 50.

Effect(s) tested

Differences between ROC areas under the curves (AUCs) were evaluated using DeLong tests 49. Comparisons between Spearman correlations were performed by bootstrapping.

Specify type of analysis: ☐ Whole brain ☒ ROI-based ☐ Both

Anatomical location(s)

Values from the following regions were averaged together to represent mean cortical SUVR for Florbetapir or PiB: bilateral orbitofrontal, medial orbitofrontal, rostral middle frontal, superior frontal, superior temporal, middle temporal, and precuneus. Amyloid PET positivity was previously defined as a mean cortical SUVR > 1.42 for PiB and > 1.19 for Florbetapir 40. Mean cortical SUVRs were converted to Centiloid units to combine data from the two tracers 40,41. Values from the bilateral entorhinal cortex, amygdala, lateral occipital cortex, and inferior temporal cortex regions were averaged together as a summary measure of tau PET42.

Statistic type for inference (See Eklund et al. 2016)

N/A

Correction

When multiple measures were compared, the significance was adjusted using the Benjamini-Hochberg procedure.

Models & analysis

n/a	Involved in the study
☐	☐ Functional and/or effective connectivity
☐	☐ Graph analysis
☐	☒ Multivariate modeling or predictive analysis

Functional and/or effective connectivity

Spearman correlations were used to evaluate the continuous relationships of CSF biomarker measures with amyloid PET Centiloid, the tau PET summary measure, or the CDR-SB. For partial Spearman correlations with amyloid PET, tau PET, and brain volumes, analyses included covariates of age and sex; for correlations with CDR-SB, analyses included covariates of age, sex, and years of education. Comparisons between Spearman correlations were performed by bootstrapping.

Graph analysis

N/A

Multivariate modeling and predictive analysis

Receiver Operating Characteristic (ROC) analyses were used to evaluate the correspondence of CSF biomarker measures with amyloid PET status, tau PET status, or clinical status (cognitively unimpaired [CDR=0] or cognitively impaired [CDR>0]). Cut-offs that best distinguished amyloid PET or tau PET status were found based on the highest combined sensitivity and specificity (Youden Index).