Plasma p-tau231 and p-tau217 inform predominantly on tau tangle accumulation in cognitively impaired individuals

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Abstract

Introduction: Recent studies suggest that phosphorylated tau (p-tau) at threonine 231 and/or 217 reflect amyloid-β (Aβ) deposition rather than tau tangle pathology in preclinical Alzheimer's disease (AD). Here, we evaluate plasma biomarkers' contribution to identifying brain Aβ and tau pathologies in both cognitively unimpaired (CU) and impaired individuals (CI).

Methods: We assessed 138 CU and 87 CI individuals with Aβ- and tau-PET, as well as plasma biomarkers. Receiver operating characteristic analyses, linear regressions, and Akaike Information Criterion tested the performance of plasma p-tau at threonine 231, 217^{+}, and 181, Aβ42/40, glial fibrillary acidic protein (GFAP), and neurofilament light chain to identify Aβ and tau-PET deposition states beyond the information provided by demographics (age, sex and *APOE* ε4).

Results: In CU individuals, plasma p-tau231 and p-tau217^{+} were the only markers that significantly added to the demographics to detect Aβ-PET pathology, while no plasma biomarker added information to identify tau-PET signal. In CI individuals, p-tau217^{+} and GFAP contributed significantly to demographics in identifying tau and Aβ accumulation as determined by PET, while p-tau231 only provided complementary information to identify tau-PET pathology.

Discussion: Our results support previous studies suggesting that plasma p-tau231 and p-tau217^{+} are state markers of Aβ pathology in CU. Furthermore, we showed that plasma p-tau231 mainly informs on the accumulation of tau tangles in CI, whereas p-tau217 remains linked with Aβ deposition but also provides information on tau accumulation in this population. Our results support p-tau231 and p-tau217 as state markers of early Aβ deposition, but with disease progression they also inform on brain accumulation of tau tangles.

Introduction

Brain accumulation of amyloid-β (Aβ) plaques and tau neurofibrillary tangles are the hallmark pathological features of Alzheimer's disease (AD) [1]. Quantification of brain deposits of Aβ and tau proteins in living people can increase clinicians' diagnostic accuracy when assessing cognitively impaired (CI) individuals [2], and inform on the risk of progression to dementia in cognitively unimpaired (CU) individuals[3]. Reduction of Aβ42/40 and increased tau (both total [t-tau] and phosphorylated [p-tau] tau) levels are the cerebrospinal fluid (CSF) signatures of AD[4]. Similarly, positron emission tomography (PET) imaging can be used for the visual identification and quantification of Aβ[5] and tau[6] aggregates. More recently, plasma p-tau, Aβ, neurofilament light chain (NfL), and glial fibrillary acidic protein (GFAP) biomarkers have been associated with the presence of AD hallmark proteins in the living human brain[7-14].

Although early observations suggested that plasma levels of phosphorylated tau protein are highly associated with both tau and Aβ pathologies[7, 9, 10, 15], recent studies using predominantly CU populations have suggested that these markers are state markers of Aβ deposition[10, 16]. This may be explained by the fact that p-tau may become hyperphosphorylated in response to initial Aβ aggregation[10, 16-18]. In addition, plasma Aβ can be used to detect Aβ-PET accumulation[9, 19-22], while NfL, a marker of neurodegeneration, has also been associated with Aβ and tau deposition in populations with AD [14, 23-26]. Furthermore, plasma GFAP, a marker of astrocyte reactivity, has been highly associated with Aβ-PET [8, 12, 27], which may be attributed to an increased expression of GFAP in regions surrounding Aβ plaques[11, 28].

The utility of these biomarkers ultimately depends on the information they can provide in real clinical settings, where clinical context and demographic information are available. In this regard, studies have shown that the combination of demographic information (age, sex, *APOE* ε4) with plasma biomarkers is more highly associated with AD pathology? diagnosis? than plasma biomarkers alone. Here, we intend to elucidate the complementary contribution of each plasma biomarker on top of information already provided by demographics to inform on the state of brain deposition of Aβ and tau tangles for use in clinical settings.

Methods

Study participants

We assessed 225 individuals [138 CU elderly adults, 53 mild cognitive impairment (MCI), and 34 AD dementia participants] from the Translational Biomarkers of Aging and Dementia (TRIAD) cohort of McGill University, Canada[29]. The participants underwent clinical and neuropsychological assessments, including Mini-Mental State Examination (MMSE) and the Clinical Dementia Rating (CDR). CU participants had a CDR score of 0 and no objective cognitive impairment. Participants with MCI had subjective and objective cognitive impairments, a CDR score of 0.5, and preserved activities of daily living. AD dementia patients had a CDR score between 0.5 and 2 and met the National Institute on Aging and the Alzheimer's Association criteria for probable Alzheimer's disease determined by a physician[30]. This study classified individuals diagnosed with AD or MCI as cognitive impairment (CI). Details on the information gathered from participants can be found here: https://triad.tnl-mcgill.com/.

Neuroimaging

Participants included in this study had magnetic resonance imaging 3D T1-weighted MRI (3 T Siemens), tau [^{18}F]MK6240 PET, and Aβ [^{18}F]AZD4694 PET scans with a brain-dedicated Siemens High Resolution Research Tomograph at the Montreal Neurological Institute. The acquisition and processing of the images followed standard protocols[29]. Braak stages were calculated according to previously described methods[31]. We considered individuals as tau-PET positive if they were Braak stage I or above[32]. Global [^{18}F]AZD4694 standardized uptake value ratio (SUVR) was estimated from the precuneus, prefrontal, orbitofrontal, parietal, temporal, anterior, and posterior cingulate cortices[29]. We used the published [^{18}F]AZD4694 cut-off value of 1.55 global SUVR to classify the participants as Aβ negative or positive[29].

Plasma measurements

Plasma p-tau181, p-tau231, and NfL concentrations were measured using in-house Single molecule array (Simoa) methods on an HD-X instrument (Quanterix, Billerica, MA, USA) at the Clinical Neurochemistry Laboratory, University of Gothenburg, Mölndal, Sweden[7, 33, 34]. Plasma GFAP concentration was measured by Simoa using a commercial single-plex assay (No. 102336), while plasma Aβ42 and Aβ40 concentrations were quantified using a commercially available multiplexed assay (Quanterix, Billerica, MA). Plasma p-tau217 concentration was quantified by Janssen R&D using an assay specific for phosphorylation at

amino acid 217, but with enhanced sensitivity if amino acid 212 is also phosphorylated (p-tau217^{+}). Values below the lower limit of detection (0.013 pg/mL) were excluded.

Statistical analysis

Statistical analyses were performed using R statistical software version 4.0.5 (http://www.r-project.org/). Voxel-wise statistics were conducted using MATLAB software version 9.2 (http://www.mathworks.com) with the VoxelStats package[35]. Models containing demographics-only (PET ~ sex + age) and with the addition of plasma biomarker (PET ~ plasma biomarker + sex + age) were compared using Akaike Information Criterion (AIC; lower indicates better model fit). Descriptive statistics, including means and standard deviations, were calculated for CU and CI groups, and comparisons were performed using student t-tests for continuous variables and chi-square tests for the categorical ones. The association between the plasma biomarkers was assessed using Pearson correlations and linear regressions. Partial correlation analyses were conducted to determine the extent to which biomarker concentrations were associated with Aβ and tau-PET using the ppcor package. We conducted multiple comparisons correction at $P < 0.05$ using random field theory (RFT)[36] and the Bonferroni method for voxel-wise and ROI-based analyses, respectively. We assessed and compared the discriminative performances of each model using receiver operating characteristic (ROC) and compared the area under the curve (AUC) with the DeLong test. Model goodness-of-fit was evaluated using R-square analyses.

Results

Participant characteristics and biomarker profile

In the CU group, 25.8% of individuals were Aβ-positive, and 24.3% were tau-positive. In the CI group, 80.7% were Aβ-positive, and 76.3% were tau-positive. The concentration of p-tau217^{+} was 2.4-fold higher in the CI compared to the CU group ($P < 0.0001$). The concentrations of p-tau231 and GFAP were 1.5-fold higher in the CI compared to the CU group ($P < 0.0001$). Similarly, p-tau181 concentration was 1.7-fold higher ($P < 0.0001$) and NfL concentration was 1.2-fold higher in the CI group ($P = 0.010$). Plasma Aβ42/40 ratio was not significantly different between the diagnostic groups. Demographic and biomarker characteristics of the population are summarized in **Table 1** and **Supplemental Table 1**.

Correlations between plasma biomarkers

The distribution and Pearson correlation coefficients between plasma biomarkers are shown in **Supplemental Figure 1-2**. In the CU group, correlations between plasma p-tau231, p-tau217^{+}, and p-tau181 measures ($P < 0.001$), as well as GFAP with p-tau181 ($P = 0.0049$) and NfL ($P = 0.002$) were statistically significant after multiple comparison correction. Plasma Aβ42/40 was not significantly associated with any other plasma marker. In the CI group, p-tau was significantly associated with GFAP ($P < 0.001$), while Aβ42/40 and NfL were not significantly associated with each other or the other plasma biomarkers.

Prediction of Aβ-PET positivity using plasma biomarkers

In the CU group, plasma p-tau231 (AUC = 87.7%) followed by p-tau217^{+} (AUC = 85.2%) presented a significantly contribution to demographics-only (sex and age) model to predict Aβ-PET positivity (**Table 2** and **Supplemental Table 2)**. In contrast, the addition of plasma p-tau181, Aβ42/40, GFAP, and NfL to the demographics did not significantly improve the models. In the CI group, the addition of GFAP (AUC = 83.8%) followed by p-tau217^{+} (AUC = 79.6%) significantly contributed to the models with demographics-only to detect Aβ deposition (**Table 2** and **Supplemental Table 2**). On the other hand, adding plasma p-tau231, p-tau181, Aβ42/40, and/or NfL did not significantly contribute to the demographics-only model to predict Aβ-PET positivity. The inclusion of *APOE* ε4 as part of demographics already provided did not change the results (**Table 2** and **Supplemental Table 2)**.

Prediction of tau-PET positivity with plasma biomarker concentrations

In the CU group, no plasma biomarker significantly added to the demographic-only model to predict tau-PET positivity (**Table 2** and **Supplemental Table 2**). In the CI group, the addition of p-tau217^{+} (AUC = 92.6%), p-tau231 (AUC = 80.2%) and GFAP (AUC = 87.1%) significantly contributed to the demographics-only models to detect tau tangle (**Table 2** and **Supplemental Table 2**). On the other hand, adding plasma p-tau181, Aβ42/40 and NfL did not significantly contribute the demographics-only model to predict tau-PET positivity. The inclusion of *APOE* ε4 as part of demographics already provided did not change the results (**Table 2** and **Supplemental Table 2)**.

Association of global Aβ-PET SUVR with plasma biomarkers

In the CU group, we found a significant positive association between p-tau231, p-tau217^{+}, and GFAP concentrations with global Aβ-PET SUVR values (**Supplemental Table 3**). No significant association was found between global Aβ-PET SUVR values and Aβ42/40, p-

tau181, or NfL. Plasma p-tau217^{+} explained the highest variance of global Aβ-PET SUVR values (R-squared: 0.37), closely followed by p-tau231 (R-squared: 0.33). In the CI group, p-tau231, p-tau217^{+}, ptau181, and GFAP concentrations were significantly positively associated with global Aβ-PET SUVR. No significant association was found between global Aβ-PET SUVR and Aβ42/40 and NfL. Plasma p-tau217^{+} explained the highest variance of global Aβ-PET SUVR values (R-squared: 0.25), closely followed by GFAP (R-squared: 0.24) (**Supplemental Table 3**).

Association of tau-PET SUVR with plasma biomarkers

In the CU group, we found a significant positive association between p-tau231, p-tau217^{+}, and GFAP concentrations with entorhinal tau-PET SUVR values (**Supplemental Table 3**). No significant association was found between entorhinal tau-PET SUVR values and Aβ42/40, p-tau181, GFAP, or NfL. Plasma p-tau217^{+} concentration best explained variance in the entorhinal tau-PET SUVR values (R-squared: 0.18), closely followed by p-tau231 (R-squared: 0.15). In the CI group, p-tau231, p-tau217^{+}, p-tau181, NfL, and GFAP concentrations were significantly positively associated with entorhinal tau-PET SUVR values. No significant association was found between entorhinal tau-PET SUVR values and Aβ42/40 values. Plasma p-tau217^{+} explained the highest variance in the entorhinal tau-PET SUVR (R-squared: 0.35), closely followed by p-tau231 (R-squared: 0.24) and GFAP (R-squared: 0.31) (**Supplemental Table 3**).

Finally, comparison of the correlation coefficient for plasma p-tau biomarkers demonstrated that in CU individuals, p-tau231 and p-tau217 were more closely associated with Aβ-PET than with tau-PET (**Supplemental Table 4**). On the other hand, in the CI group, we revealed that plasma p-tau217^{+} was more closely associated with tau-PET than with Aβ-PET, whereas no differences were detected for plasma p-tau231.

Voxel-wise associations of Aβ-PET SUVR with plasma biomarkers

Voxel-wise linear regression analysis confirmed previous studies showing that plasma biomarkers are directly associated with Aβ-PET. In the CU group, we showed a significant positive association between Aβ-PET SUVR and plasma p-tau (p-tau231 > p-tau217^{+} > p-tau181) and GFAP (**Figure 1A**) in AD-related regions. No significant associations were found between Aβ-PET and plasma Aβ42/40 ratio or NfL concentration. In the CI group, we showed a significant positive association between Aβ-PET SUVR with plasma p-tau (**Figure 1C**) and

GFAP (**Supplemental Figure 3**) concentrations after correction for multiple comparisons. Plasma NfL showed small clusters with significant negative association with Aβ-PET SUVR after multiple comparison corrections.

Then, we evaluated the brain regions to which the addition of each plasma biomarker significantly improved the demographics-only model to predict Aβ-PET regional SUVR. In the CU group, plasma p-tau231 significantly contributed to predict Aβ signal in the lateral and medial temporal, posterior cingulate and precuneus, and medial frontal lobe, *i.e.*, in areas typically associated with AD (**Figure 2A**). P-tau217^{+} showed a significant contribution in similar regions but the associations were weaker than those observed for p-tau231. Plasma p-tau181, GFAP, Aβ42/40, and NfL did not improve the demographics-only model. In the CI group, GFAP and p-tau217^{+} increased the predictive performance of demographics-only in AD-related regions (**Figure 2C** and **Supplemental Figure 4).** Although plasma p-tau231 and p-tau181 concentrations were correlated with Aβ-PET, they did not contribute to the demographics-only model to predict Aβ-PET signal (**Figure 2C**). Plasma Aβ42/40 and NfL also did not contribute to the demographics-only model.

Voxel-wise association of tau-PET SUVR with plasma biomarkers

Voxel-wise linear regression confirmed previous studies showing that plasma biomarkers are directly associated with tau-PET (**Figure 1**). In the CU group, we observed a significant positive association between tau-PET SUVR and p-tau231 and p-tau217^{+} in small clusters in the precuneus and temporal lobe (**Figure 1B**). No significant association was found between tau-PET and plasma Aβ42/40 ratio, or GFAP or NfL concentrations. In the CI group, we observed significant positive associations between tau-PET SUVR and plasma p-tau231, p-tau217^{+}, and GFAP concentrations across the brain cortex (**Figure 1D** and **Supplemental Figure 3**). Plasma p-tau181 showed a significant positive association in the precuneus and temporal cortices. No significant association was found between tau-PET signal and plasma Aβ42/40 ratio or NfL concentration.

Next, we examined which plasma biomarkers could improve prediction of tau-PET SUVR in which brain region compared with the demographics-only model. In the CU group, plasma p-tau, GFAP, Aβ42/40, and NfL did not significantly contribute to the demographics-only model (**Figure 2B** and **Supplemental Figure 4**). In the CI group, p-tau217^{+} showed a significant contribution to the demographics-only model through the whole cortex comprising regions of

early and late Braak stages (**Figure 2D**). Plasma p-tau231 showed a significant contribution in similar regions. Plasma GFAP increased the predictive performance of the demographics-only model in AD-related regions **(Supplemental Figure 4)**. Aβ42/40 and NfL did not contribute to the demographics-only model to predict tau-PET regional concentrations.

Finally, we assessed the brain regions where the plasma biomarker contribution to the demographic-only model overlapped to detect Aβ and tau-PET signals. In CU, plasma biomarkers were only regionally associated with Aβ-PET (**Figure 3A-B** and **Supplemental Figure 5**). In CI, plasma p-tau231 provided additional information on tau tangle accumulation in AD-related regions (**Figure 3C**). On the other hand, for plasma p-tau217+, 3% of brain regions were only associated with Aβ-PET, 35% only with tau-PET, and 39% of regions overlap for both (**Figure 3D**), further supporting that this marker reflects both pathologies. Similarly, for plasma GFAP, 23% of brain regions were only associated with Aβ-PET, 13% only with tau-PET, and 31% overlap for both (**Supplemental Figure 5**).

Discussion

Our results support previous literature suggesting that, in preclinical AD, plasma p-tau231 and p-tau217^{+} are closely related to brain Aβ deposition. Our main finding was that in the CI population, plasma p-tau231 and p-tau217^{+} also inform on tau tangle deposition/load? in clinical settings where demographic information is available.

We demonstrated that in CI individuals, plasma p-tau231 may provide information regarding the presence of tau tangles, while p-tau217^{+} inform on both tau tangle and Aβ deposition, which would be relevant to the clinical setting when patients with symptoms are evaluated. Notably, our findings also support previous studies showing that p-tau231 and p-tau217 are highly associated with Aβ pathology in CU individuals,[16] which thus would be relevant if the context of use is screening. In addition, our results add to this model suggesting that p-tau231 and p-tau217 become more closely related to tau tangles in later disease stages, when Aβ levels have reached a plateau. These results align with postmortem observations showing that plasma p-tau231 levels were elevated as a function of Braak stages[37], a finding also observed *in vivo*[38]. Furthermore, our results corroborate findings showing that p-tau217 is associated with both Aβ and tau tangle aggregation in symptomatic individuals[18, 39, 40]. Taken together, these results support a model suggesting that tau phosphorylation may result from early Aβ aggregation in CU individuals, while it becomes the building blocks of, and therefore highly associated with,

tau tangles in CI individuals, when Aβ plaques have reached a plateau and tau tangles continue to develop. This highlights that the associations of plasma p-tau with brain deposits of Aβ and tau tangles are dynamic and may change with the progression of the disease and the amount of each pathology.

Our results demonstrated that plasma p-tau231 and p-tau-217^{+} provide additional data to the information already available in demographics to identify brain Aβ pathology and tau tangles. This is important to determine the clinical usefulness of these markers since age and sex are always available, whereas the *APOE* genotype is often available in clinical settings. Our results align with a growing body of evidence suggesting that plasma p-tau231 and p-tau217 highly correlate with AD pathologies, and outperform p-tau181, Aβ, and NfL[16, 40]. The results also indicate that these markers have the additional advantage of providing complementary information to the demographics to identify AD[16]. Corroborating previous literature, we showed that plasma p-tau181 and p-tau231 were directly correlated with Aβ-PET in CI [7, 10]; however, the data provided by these markers overlapped with the information already provided by the demographics. Altogether, these results support that plasma p-tau231 and p-tau217 are robust markers of AD pathophysiology that can potentially add information to evaluating patients in clinical settings.

Plasma GFAP significantly contributed to the demographic information to identify AD pathophysiology in CI but not in CU individuals. The significant association between GFAP and Aβ-PET in CU individuals corroborated recent literature in asymptomatic individuals [8, 41], but did not significantly add to the information provided by demographics. Our results in CI individuals align with recent findings showing that GFAP levels progressively increase with AD progression in late disease stages[8, 12, 42]. Interestingly, GFAP levels were also associated with tau-PET in CI individuals. Because it has already been demonstrated that the association of GFAP concentration with tau pathology is mediated by Aβ pathology [8], we speculate that the performance of GFAP to predict tau-PET may be due to the fact that tau and Aβ are related. Conversely, the fact that there are brain regions where GFAP is associated with tau tangles but not with Aβ deposition does not allow us to exclude that GFAP performance to identify tau-PET is due to its increased concentration in response to tau tangle formation[43]. These results support that plasma GFAP has the potential to be clinically used as a marker of brain AD pathophysiology in CI individuals.

Plasma Aβ42/40 provided information that overlapped with demographic data to identify Aβ-PET deposition. Specifically, we demonstrated that adding Aβ42/40 did not increase the predictive performance of the demographics-only model to identify Aβ- or tau-PET. These results add to the conflicting literature where some studies suggest that plasma Aβ42/40 strongly associates with brain Aβ-PET deposition[19, 20], whilst others report only a moderate association between blood and brain Aβ levels [44, 45 46]. Several biological and analytical factors may contribute to the divergence of the results. For example, peripheral Aβ expression account for > 50% of the global plasma Aβ [47]. In addition, the modest fold-change between CU and CI in plasma (10–20%) compared with CSF Aβ (40–60%) may lead to the high susceptibility of plasma Aβ measures to small variabilities in pre-analytical approaches and cohort characteristics [19, 22, 45]. Notably, differences in the analytical performance of different Aβ assays used across studies may explain some of the variation in the results [21]. However, most data suggest that plasma Aβ42/40 ratio as such is not a very robust biomarker for biological reasons, and that improved methods might only partially solve the problem.

The main strength of the study is the use of a well-characterized cohort of individuals that underwent state-of-the-art harmonized biomarker acquisitions and quantifications, including most of the currently available promising AD biomarkers. However, some limitations should be considered. The cohort is composed of individuals motivated to participate in a dementia study, potentially being a source of self-selection bias. It would be highly desirable to replicate these results in a clinical setting. In this study, we only had available measures of plasma Aβ using Simoa; however, it has already been shown that mass spectrometry-based has a higher performance[48]. Moreover, this study includes mostly White individuals, which limits the generalizability of our results to other populations such as Black and Latinx.

In conclusion, our results support that in preclinical AD plasma p-tau231 and p-tau217^{+} are state markers of Aβ, whereas in CI they additionally inform on brain deposition of tau neurofibrillary tangles.

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Funding

This research is supported by the Weston Brain Institute, Canadian Institutes of Health Research (#MOP-11-51-31; RFN 152985, 159815, 162303; PR-N), Canadian Consortium of Neurodegeneration and Aging (MOP-11-51-31 - team 1; PR-N), the Alzheimer's Association (#NIRG-12-92090, #NIRP-12-259245; PR-N), Brain Canada Foundation (CFI Project 34874; 33397; PR-N), the Fonds de Recherche du Québec – Santé (Chercheur Boursier, #2020-VICO-279314; PR-N). TAP and PR-N are members of the CIHR-CCNA Canadian Consortium of Neurodegeneration in Aging. TAP is supported by the NIH (#R01AG075336 and #R01AG073267) and the Alzheimer's Association (#AACSF-20-648075). PLF receives funding from Alzheimer Association (#AARFD-22-923814). JPF-S receives financial support from CAPES [88887.627297/2021-00]. CT receives funding from Faculty of Medicine McGill and IPN McGill. DTL is supported by a NARSAD Young Investigator Grant from the Brain & Behavior Research Foundation (#29486). WSB is supported by CAPES (#88887.372371/2019-00 and #88887.596742/2020-00). JT is supported by the Canadian Institutes of Health Research & McGill Healthy Brains Healthy Lives initiative. ALB is supported by the Swedish Alzheimer Foundation, Stiftelsen för Gamla Tjänarinnor, and Stohne Stiftelsen. KB is supported by the Swedish Research Council (#2017-00915), the Alzheimer Drug Discovery Foundation (#RDAPB-201809-2016615), the Swedish Alzheimer Foundation (#AF-742881), Hjärnfonden (#FO2017-0243), the Swedish state under the agreement between the Swedish government and the County Councils, the ALF-agreement (#ALFGBG-715986), the European Union Joint Program for Neurodegenerative Disorders (#JPND2019-466-236), the NIH (#1R01AG068398-01), and the Alzheimer's Association 2021 Zenith Award (#ZEN-21-848495). HZ is a Wallenberg Scholar supported by grants from the Swedish Research Council (#2022-01018), the European Union's Horizon Europe research and innovation programme under grant agreement No 101053962, Swedish State Support for Clinical Research (#ALFGBG-71320), the Alzheimer Drug Discovery Foundation (ADDF), USA (#201809-2016862), the AD Strategic Fund and the Alzheimer's Association (#ADSF-21-831376-C, #ADSF-21-831381-C, and #ADSF-21-831377-C), the Bluefield Project, the Olav Thon Foundation, the Erling-Persson Family Foundation, Stiftelsen för Gamla Tjänarinnor, Hjärnfonden, Sweden (#FO2022-0270), the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-

Curie grant agreement No 860197 (MIRIADE), the European Union Joint Programme – Neurodegenerative Disease Research (JPND2021-00694), and the UK Dementia Research Institute at UCL (UKDRI-1003). TK is funded by the Swedish Research Council's career establishment fellowship (#2021-03244), the Alzheimer's Association Research Fellowship (#850325), the BrightFocus Foundation (#A2020812F), the International Society for Neurochemistry's Career Development Grant, the Swedish Alzheimer Foundation (Alzheimerfonden; #AF-930627), the Swedish Brain Foundation (Hjärnfonden; #FO2020-0240), the Swedish Dementia Foundation (Demensförbundet), the Swedish Parkinson Foundation (Parkinsonfonden), Gamla Tjänarinnor Foundation, the Aina (Ann) Wallströms and Mary-Ann Sjöbloms Foundation, the Agneta Prytz-Folkes & Gösta Folkes Foundation (#2020-00124), the Gun and Bertil Stohnes Foundation, and the Anna Lisa and Brother Björnsson's Foundation. ERZ receives financial support from CNPq (#435642/2018-9 and #312410/2018- 2), Instituto Serrapilheira (#Serra-1912-31365), Brazilian National Institute of Science and Technology in Excitotoxicity and Neuroprotection (#465671/2014-4), FAPERGS/MS/CNPq/SESRS–PPSUS (#30786.434.24734.231120170), and ARD/FAPERGS (#54392.632.30451.05032021) and Alzheimer's Association [AARGD-21-850670].

Competing interests

K.B. has served as a consultant, at advisory boards, or at data monitoring committees for Abcam, Axon, Biogen, JOMDD/Shimadzu. Julius Clinical, Lilly, MagQu, Novartis, Prothena, Roche Diagnostics, and Siemens Healthineers, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program, all unrelated to the work presented in this paper. HZ has served at scientific advisory boards and/or as a consultant for Abbvie, Acumen, Alector, Alzinova, ALZPath, Annexon, Apellis, Artery Therapeutics, AZTherapies, CogRx, Denali, Eisai, Nervgen, Novo Nordisk, Optoceutics, Passage Bio, Pinteon Therapeutics, Prothena, Red Abbey Labs, reMYND, Roche, Samumed, Siemens Healthineers, Triplet Therapeutics, and Wave, has given lectures in symposia sponsored by Cellectricon, Fujirebio, Alzecure, Biogen, and Roche, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program (outside submitted work). All other authors declare no competing interests.

Table 1 - Demographics and key characteristics of the population

Characteristic	CU (N=138)	CI (N=87)	P-value
Demographic characteristics			
Age, y[a]	71.7 (5.93)	69.8 (7.30)	0.06
Education, y[a]	15.4 (3.69)	14.8 (3.53)	0.346
Female, No. (%)	95 (66.9)	48 (54.5)	0.756
APOEε4 carrier, number (%)	39 (27.5)	42 (47.7)	0.002
Cognition			
MMSE[a]	29.1 (1.18)	24.9 (5.41)	< 0.0001
Plasma measures, pg/mL			
p-tau231[a]	14.7 (7.35)	22.1 (10.8)	< 0.0001
p-tau217+ [b]	0.06 (0.05)	0.17 (0.124)	< 0.001
p-tau181[a]	10.8 (4.93)	18.7 (8.89)	< 0.0001
GFAP[a]	215 (112)	319 (170)	< 0.0001
Aβ40[a]	254 (62.3)	247 (56.5)	0.321
Aβ42[a]	8.99 (2.98)	9.39 (3.07)	0.213
Aβ42/Aβ40[a]	0.05 (0.20)	0.05 (0.19)	0.260
NfL [a]	25.0 (16.9)	31.0 (13.2)	0.010
Neuroimaging			
[^{18}F]AZD4694 global SUVR[a]	1.45 (0.35)	2.20 (0.59)	< 0.0001
Aβ+ (%)	24.3	80.7	
[^{18}F]MK−6240 global SUVR[a]	1.04 (0.29)	2.16 (0.99)	< 0.0001
Tau + (%)	25.8	76.34	

P-values indicate the variance analysis results to assess the difference between groups except for gender and *APOE* ε4 status, where a contingency chi-square was performed. [a]Mean (SD). [b]Assessed in a subset of 166 individuals (**Supplementary Table 1**). CU = cognitively unimpaired, CI = cognitively impaired. Aβ = amyloid-β. p-tau231 = tau phosphorylated at threonine 231, p-tau217 = tau phosphorylated at threonine 217, p-tau181 = tau phosphorylated at threonine 181, GFAP = glial fibrillary acidic protein, NfL = neurofilament light chain, MMSE = Mini Mental State Examination.

Table 2. Additive performance of plasma biomarkers to predict Aβ-PET and tau-PET in CU and CI individuals.

	Predict Aβ-PET positivity		**Predict tau-PET positivity**	
	CU	**CI**	**CU**	**CI**
Biomarker	**AUC (95% confidence interval)**	**AUC (95% confidence interval)**	**AUC (95% confidence interval)**	**AUC (95% confidence interval)**
Demographics-only (age + sex)	0.660 (0.552-0.768)	0.606 (0.473-0.739)	0.609 (0.508-0.706)	0.604 (0.465-0.743)
Demographics (age + sex) plus plasma biomarker:				
p-Tau231	0.877* (0.784-0.940)	0.752* (0.654-0.877)	0.751 (0.662-0.831)	0.802* (0.689-0.915)
p-tau217+	0.852* (0.772-0.921)	0.796* (0.685-0.912)	0.713 (0.606-0.820)	0.926* (0.865-0.988)
p-Tau181	0.755 (0.635-0.846)	0.777* (0.656-0.886)	0.701 (0.612-0.803)	0.771* (0.657-0.901)
GFAP	0.793 (0.702-0.893)	0.838* (0.731-0.925)	0.671 (0.569-0.772)	0.871* (0.795-0.940)
Aβ42/Aβ40	0.674	0.682	0.687	0.676

	(0.592-0.817)	(0.507-0.856)	(0.569-0.805)	(0.487-0.879)
NfL	0.699	0.609	0.670	0.659
	(0.594-0.805)	(0.463-0.745)	(0.564-0.777)	(0.534-0.772)
Demographics-only (age + sex + APOE ε4)	0.663	0.650	0.642	0.653
	(0.556-0.770)	(0.519-0.781)	(0.572-0.768)	(0.537-0.770)
Demographics (age + sex + APOE ε4) plus plasma biomarker:				
p-Tau231	0.880*	0.779	0.753*	0.803*
	(0.813-0.947)	(0.678-0.880)	(0.666-0.839)	(0.695-0.912)
p-tau217+	0.856*	0.803*	0.713	0.927 *
	(0.767-0.931)	(0.698-0.906)	(0.606-0.820)	(0.865-0.988))
p-Tau181	0.754	0.768	0.709	0.791*
	(0.660-0.848)	(0.657-0.879)	(0.614-0.805)	(0.716-0.919)
GFAP	0.799	0.841*	0.675	0.908*
	(0.705-0.893)	(0.749-0.932)	(0.571-0.772)	(0.842-0.974)
Aβ42/Aβ40	0.662	0.712	0.678	0.683
	(0.518-0.785)	(0.551-0.873)	(0.569-0.805)	(0.508-0.859)
NfL	0.695	0.677	0.671	0.653
	(0.589-0.799)	(0.542-0.809)	(0.564-0.777)	(0.534-0.772)

DeLong test provided significant differences between the model with demographics-only and models with the addition of each biomarker. *P < 0.05. CU = cognitively unimpaired, CI = cognitively impaired.

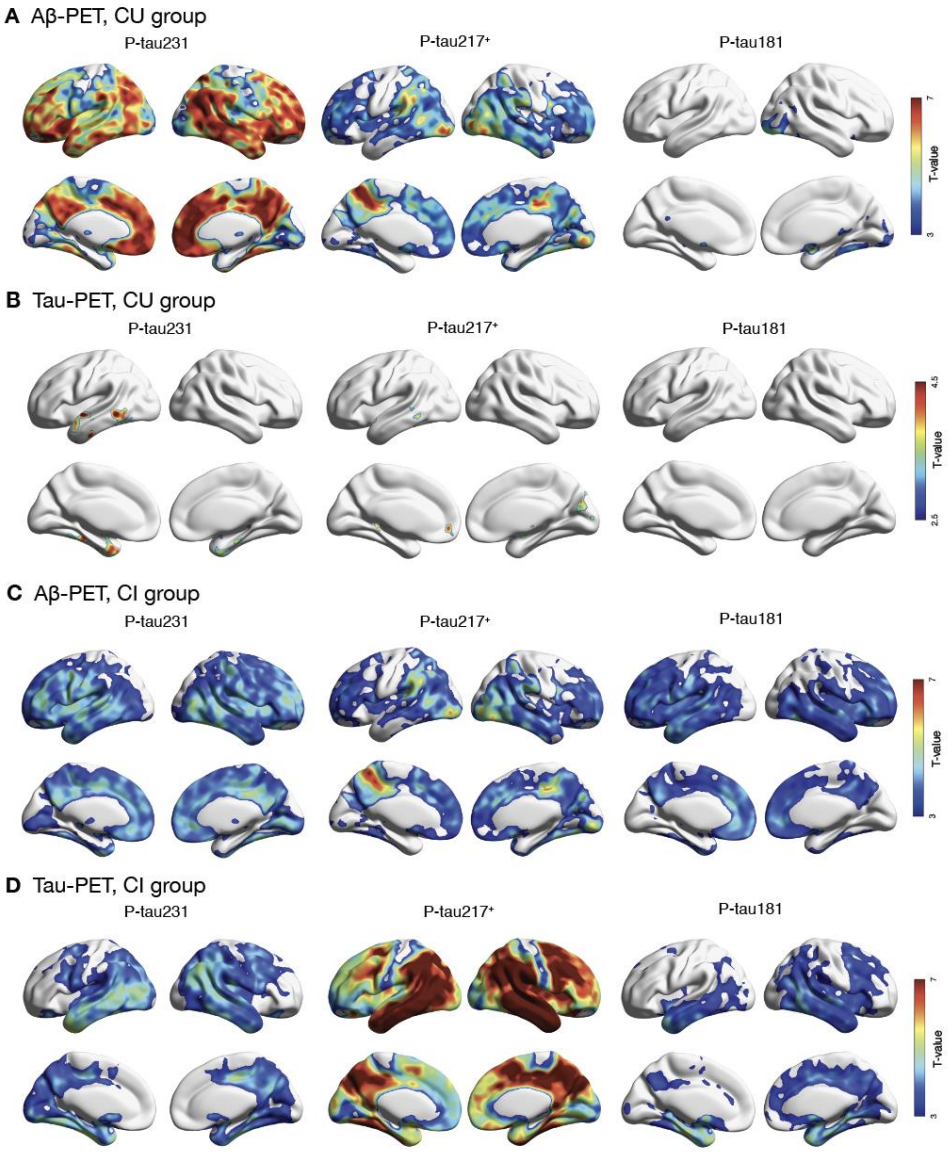

Figure 1. Regional associations between Aβ-PET and tau-PET with plasma biomarker concentrations in CU and CI individuals. Panels **A** and **D** show the regions with a significant positive association between Aβ-PET SUVR and plasma markers in CU and CI individuals. In the CI group, a significant negative association with plasma NfL and Aβ-PET SUVR were found in small clusters after multiple comparison corrections. No significant associations were found between plasma Aβ42/40 and Aβ-PET SUVR. Panels **B** and **D** show the regions indicating a significant positive association between tau-PET SUVR and plasma markers. No significant associations were found between plasma Aβ42/40 and NfL and tau-PET SUVR after multiple comparison corrections. Associations for plasma GFAP are shown in Supplementary Figure 3. CU = Cognitively unimpaired, CI = Cognitively impaired.

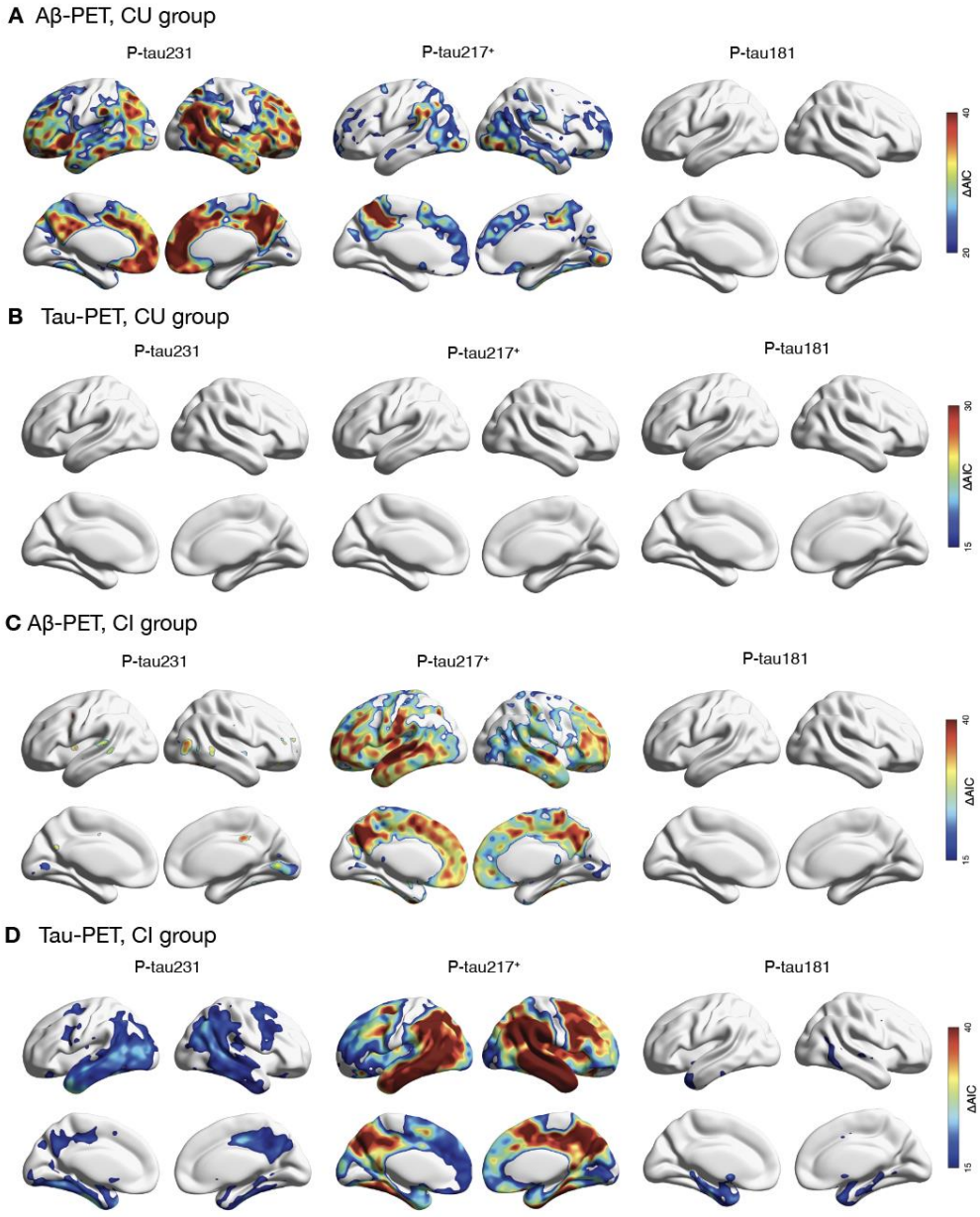

Figure 2. Brain regions that each plasma biomarker contributes to the model with demographics-only to predict Aβ-PET and tau-PET concentrations. The figure shows voxel-wise AIC maps of regressions between Aβ- and tau-PET and plasma markers [PET ~ sex + age + (plasma biomarker)] after FDR correction at $P < 0.05$. Figure **A** and **C** show the regions where the addition of plasma biomarkers contributes significantly to the demographics-only model to depict Aβ-PET concentrations in CU and CI individuals. Figure **B** shows that the addition of plasma biomarkers in CU did not contribute to the demographic-only model to depict tau-PET concentration. Figure **D** shows the regions where adding plasma biomarkers to the demographics-only model contributes to depicting tau-PET

concentrations in CI. The ΔAIC higher than 10 represents the significant additive effect of the plasma biomarker on the model in comparison with the model with only demographics. Regional associations for plasma GFAP are shown in Supplementary Figure 3. CU = Cognitively unimpaired, CI = Cognitively impaired. Aβ42/40 and NfL did not show a significant additive effect on the model. Demographics = age and sex.

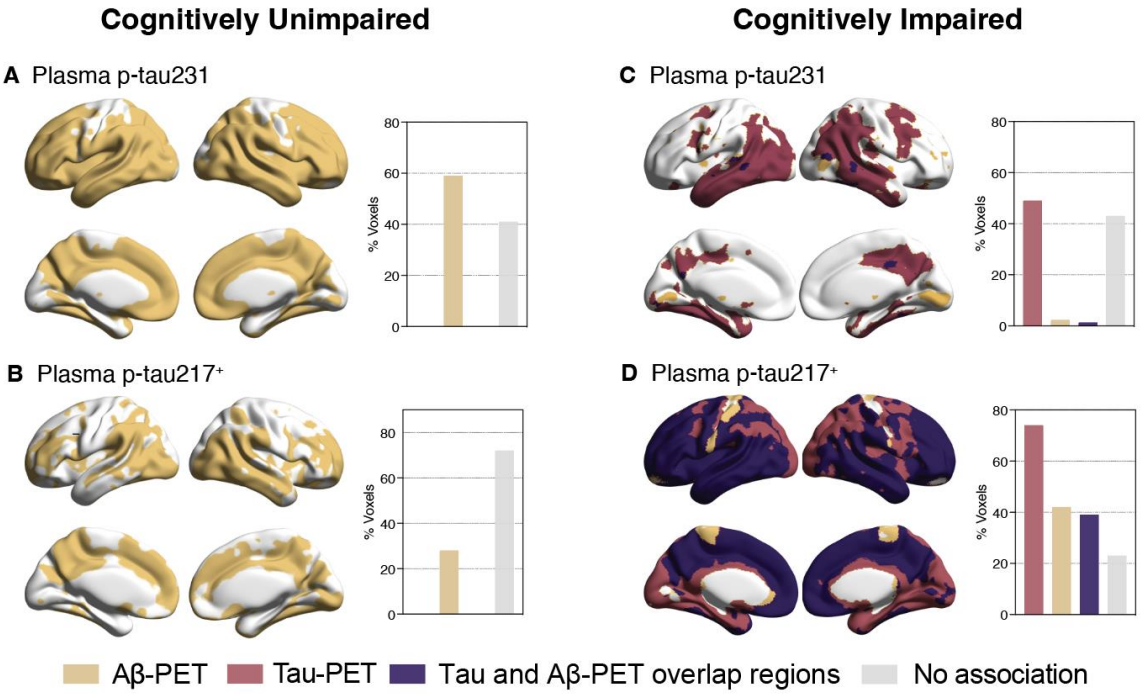

Figure 3. Topographic overlap between brain regions shows that plasma p-tau231 and p-tau217+ provided additional information to demographics to predict Aβ-PET and tau-PET concentrations. Figure **A** and **B** represent the percentage of significant voxels that plasma p-tau231 significantly contribute to the demographics-only model and the overlap regions between Aβ and tau-PET. Figure **C** and **D** represents the percentage of significant voxels that plasma p-tau217^{+} significantly added to the demographics-only model and the overlap regions between Aβ and tau-PET. Biomarkers not shown in the figure did not present significant results. Demographics = age and sex.