

Plasma P-tau181 in Alzheimer's disease: relationship to other biomarkers, differential diagnosis, neuropathology and longitudinal progression to Alzheimer's dementia

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Plasma phosphorylated tau181 (P-tau181) might be increased in Alzheimer's disease (AD), but its usefulness for differential diagnosis and prognosis is unclear. We studied plasma P-tau181 in three cohorts, with a total of 589 individuals, including cognitively unimpaired participants and patients with mild cognitive impairment (MCI), AD dementia and non-AD neurodegenerative diseases. Plasma P-tau181 was increased in preclinical AD and further increased at the MCI and dementia stages. It correlated with CSF P-tau181 and predicted positive Tau positron emission tomography (PET) scans (area under the curve (AUC) = 0.87–0.91 for different brain regions). Plasma P-tau181 differentiated AD dementia from non-AD neurodegenerative diseases with an accuracy similar to that of Tau PET and CSF P-tau181 (AUC = 0.94–0.98), and detected AD neuropathology in an autopsy-confirmed cohort. High plasma P-tau181 was associated with subsequent development of AD dementia in cognitively unimpaired and MCI subjects. In conclusion, plasma P-tau181 is a noninvasive diagnostic and prognostic biomarker of AD, which may be useful in clinical practice and trials.

Accumulation of misfolded β -amyloid ($A\beta$) peptides in extracellular plaques and phosphorylated tau protein as paired helical filaments (PHFs) in intraneuronal neurofibrillary tangles (NFTs) are defining neuropathological features of Alzheimer's disease (AD). The diagnostic workup of AD has been revolutionized by the use of cerebrospinal fluid (CSF) and positron emission tomography (PET) biomarkers¹. Core CSF biomarkers include $A\beta$ 42, total tau (T-tau) and phosphorylated tau (P-tau). Reduced $A\beta$ 42 and increased P-tau are believed to reflect $A\beta$ and tau pathologies in AD, while increased T-tau is a more nonspecific marker of neuronal injury². PET imaging can directly visualize $A\beta$ and tau aggregates in the brain. However, both CSF measurements and PET imaging have notable hurdles. They are invasive, time-consuming and expensive, and they may have side effects and have limited availability, especially in primary care. There is therefore a great need for less invasive, cost-effective and easily accessible biomarkers, preferably blood tests.

Assays have been developed for detection of blood tau phosphorylated at threonine 181 (P-tau181)^{3–5}, which is higher in people with AD dementia compared to cognitively unimpaired individuals and correlates with $A\beta$ PET and tau PET uptake^{3–5}. These results are promising regarding plasma P-tau181 as an AD biomarker,

but several issues remain unclear. It is not known whether plasma P-tau181 can (1) differentiate AD from non-AD neurodegenerative diseases, (2) predict future progression to AD dementia in nondemented people, (3) identify individuals with pathological Tau PET uptake or (4) detect AD neuropathology. Furthermore, it is unclear how plasma P-tau181 correlates with CSF P-tau181 and how the diagnostic performance compares to that of other plasma biomarkers in AD (for example, T-tau, $A\beta$ 42/ $A\beta$ 40 ratio and neurofilament light (NfL))^{6–9}. We addressed these issues in two prospective cohorts, including 526 individuals. Plasma P-tau181 correlated with CSF P-tau181 and PET measures of $A\beta$ and tau pathologies, and predicted Tau PET and $A\beta$ PET positivity. Plasma P-tau181 was increased early in AD, continued to increase with disease progression and distinguished AD dementia from other neurodegenerative diseases. Plasma P-tau181 predicted longitudinal progression to AD dementia (better than other candidate plasma biomarkers) and was increased in converters to AD dementia compared to those who converted to dementia due to non-AD diseases. In a separate cohort of 63 individuals with neuropathological data, antemortem plasma P-tau181 was associated with AD neuropathology at autopsy. These results suggest that plasma P-tau181 may be used as a first line of testing to identify patients likely to be tau positive when tested by

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PET or CSF biomarkers, either to distinguish AD from other non-AD neurodegenerative diseases in cases with mild to moderate dementia or to predict future development of AD in cases with mild cognitive impairment (MCI).

Results

The two prospective cohorts had similar study designs, but cohort 1 ($n=182$) also included Tau PET imaging and cohort 2 ($n=344$) also included longitudinal follow-up to track conversion to AD dementia over a period of up to 8 yr. Cohort 1 included 64 cognitively unimpaired participants (60% A β ⁺), 28 A β ⁺ MCI, 38 A β ⁺ AD dementia and 52 patients with non-AD neurodegenerative diseases (Table 1). Cohort 2 included 219 cognitively unimpaired participants (42% A β ⁺) and 125 patients with MCI (65% A β ⁺) (Table 1). The neuropathology cohort (cohort 3) included 16 autopsy-confirmed AD dementia and 47 autopsy-confirmed non-AD individuals (Supplementary Table 1).

Plasma P-tau181 and CSF P-tau181 are correlated. If changes in plasma P-tau181 stem from changes in the central nervous system, plasma P-tau181 should correlate with CSF P-tau181 (ref. ¹⁰). We found clear associations between plasma and CSF levels of P-tau181 in cohort 1 (β -coefficient (β)=0.73, $P<0.001$, $n=172$) and cohort 2 (β =0.52, $P<0.001$, $n=343$). The association was significant in A β ⁺, including in the presymptomatic stage (A β ⁺ cognitively unimpaired), but not in A β ⁻ individuals (Fig. 1a and Extended Data Fig. 1c for cohort 1, Extended Data Fig. 1a,d for cohort 2).

These correlations suggest that, to some extent, plasma P-tau181 reflects changes in hyperphosphorylated tau in the central nervous system that occur in A β ⁺ individuals.

Plasma P-tau181 is associated with Tau PET. Tau PET can quantify insoluble PHF-tau aggregates¹¹, with potential to revolutionize clinical trials¹². We tested associations between plasma P-tau181 and Tau PET in 174 participants (cohort 1). We first tested associations in a priori defined brain regions linked to tau pathology in AD, including the Braak I–IV region of interest (ROI) (temporal ‘meta-ROI’) as well as the Braak I–II, III–IV and V–VI ROIs and inferior temporal cortex¹³. Higher P-tau181 was associated with increased Tau PET standardized uptake value ratio (SUVR) in Braak I–IV ROI in the whole cohort (β =0.71, $P<0.001$), and in A β ⁺ (β =0.69, $P<0.001$) but not in A β ⁻ individuals (β =0.11, $P=0.48$; Fig. 1b and Supplementary Table 2). The results are similar for the other four ROIs (Extended Data Fig. 2 and Supplementary Table 2). In the cognitively unimpaired group, plasma P-tau181 was positively related to Tau PET in all regions except the late Braak V–VI ROI regions, whereas in cognitively impaired participants (A β ⁺ MCI and A β ⁺ AD dementia) the associations were significant in all regions except the early Braak I–II ROI (Supplementary Table 2). In voxel-wise analyses, plasma P-tau181 was mainly associated with Tau PET in temporo-parietal regions (Fig. 1d).

Next, the cohort was divided into those without significantly elevated Tau PET measurements in any Braak ROI (‘Tau PET⁻’), and those with significantly elevated measurements in one or more of these ROIs, including (1) Braak I–II (but not III–VI), (2) Braak III–IV (but not V–VI) and (3) Braak V–VI. We observed that P-tau181 differed between the Braak I–II⁺, III–IV⁺ and V–VI⁺ groups compared to the group with normal Tau PET ($F=43.5$, $P<0.001$). Plasma P-tau181 was increased in Braak III–IV⁺ and V–VI⁺ compared with Tau PET⁻, and was also higher in Braak V–VI⁺ compared with Braak I–II⁺ and Braak III–IV⁺ (all $P<0.001$; Fig. 2a and Extended Data Fig. 3a). These results are similar to those for CSF P-tau181 (Fig. 2b and Extended Data Fig. 3b), except that there was also a difference ($P<0.001$) between Tau PET⁻ and Braak I–II⁺ ($P=0.056$ for plasma P-tau181).

Plasma P-tau181 predicted abnormal Tau PET status in both the earlier Braak I–IV ROI (area under the curve (AUC)=0.87, sensitivity=80%, specificity=78%) and the later Braak V–VI ROI (AUC=0.91, sensitivity=93%, specificity=81%). Regarding AUC, sensitivities and specificities for detection of abnormal Tau PET were slightly lower for plasma P-tau181 than for CSF P-tau181, and similar or higher than for CSF T-tau and A β 42/A β 40 (Fig. 2c and Table 2).

These results show that plasma P-tau181 correlates with increased Tau PET measurements similarly or better than CSF AD biomarkers. Since Tau PET has been validated against neuropathology with very high correlations to the quantities of PHF-tau deposits in the brain^{11,14}, this suggests that plasma P-tau181 may increase in relation to tau accumulation in the brain. We also verified that plasma P-tau181 collected antemortem was increased in neuropathologically confirmed cases with AD compared to non-AD cases ($P<0.001$) and could accurately differentiate neuropathologically confirmed AD from non-AD, with AUC=0.85 (Extended Data Fig. 4).

Plasma P-tau181 is associated with A β accumulation as quantified by A β PET. The second key hallmark of AD besides tau accumulation is A β pathology. A key question in AD research is to understand links between altered A β and tau metabolism. We performed [¹⁸F]flutemetamol PET for A β in 129 participants in cohort 1 and 324 participants in cohort 2. Increased P-tau181 was associated with increased A β PET (using a global cortical composite measure) in cohort 1 (β =0.54, $P<0.001$) and in cohort 2 (β =0.53, $P<0.001$). These associations were significant in A β ⁺ but not in A β ⁻ individuals (Fig. 1c for cohort 1, Extended Data Fig. 1b for cohort 2). Voxel-wise analyses showed weaker associations of plasma P-tau181 with A β PET than Tau PET (Fig. 1e). We compared P-tau181 to plasma Elecsys A β biomarkers that predict A β PET status¹⁵. The associations with [¹⁸F]flutemetamol were significantly higher for plasma P-tau181 than for plasma A β 42 and A β 42/A β 40 (Supplementary Table 3).

P-tau181 differentiated normal versus pathological A β PET in cohort 1 (AUC=0.80, sensitivity=89%, specificity=67%) and in cohort 2 (AUC=0.81, sensitivity=79%, specificity=75%). The performance of P-tau181 was slightly better than A β 42/A β 40 (AUC=0.77) (Supplementary Table 4). Furthermore, AUCs were higher when combining P-tau181 with A β 42/A β 40 (AUC=0.84) compared with A β 42/A β 40 alone (AUC=0.77, $P<0.001$ for AUC comparison). These results indicate that plasma P-tau181 might be combined with plasma A β 42/A β 40 to identify cerebral A β pathology with high accuracy. The performance of CSF AD biomarkers was somewhat better than plasma biomarkers (Supplementary Table 4).

Since plasma P-tau181 may start to increase at a certain level of A β pathology, we tested a nonlinear spline model with continuous A β PET SUVR as predictor of plasma P-tau181 and determined an A β PET threshold for when P-tau181 levels were elevated. In cohort 1, plasma P-tau181 was significantly increased at A β PET SUVR=0.72 (Extended Data Fig. 5a). This was slightly higher than for CSF P-tau181 (SUVR=0.66) and close to the threshold for A β PET positivity (SUVR=0.74, using the target ROI and reference region described in Methods)¹⁶. The results were replicated in cohort 2 (SUVR=0.70; Extended Data Fig. 5b). These findings show that plasma P-tau181 increases early in AD, around the timepoint of A β positivity, and support plasma P-tau181 as a possible early marker of AD.

Plasma P-tau181 differentiates between different stages of AD, and between AD and non-AD neurodegenerative diseases. Clinical diagnosis of AD is often difficult, and CSF and PET biomarkers improve the diagnostic workup¹. We tested whether plasma P-tau181 could differentiate between (1) healthy controls without

Table 1 | Demographic and clinical characteristics

	Cohort 1						Cohort 2				
	Aβ ⁻ CU, n = 26	Aβ ⁺ CU, n = 38	Aβ ⁺ MCI, n = 28	Aβ ⁺ AD, n = 38	Non-AD diseases*, n = 52	P value	Aβ ⁻ CU, n = 126	Aβ ⁺ CU, n = 93	Aβ ⁺ MCI, n = 44	Aβ ⁺ MCI, n = 81	P value
Age, yr	74 (71–78)	75 (71–79)	72 (69–78)	73 (67–78)	69 (66–73)	0.002	71 (68–75)	72 (69–75)	69 (63–74)	73 (69–76)	0.003
Sex F/M, n	10/16	23/15	9/19	17/21	20/32	0.148	78/48	48/45	13/31	35/46	0.001
Education, yr ^b	12 (9–15)	11 (9–15)	12 (9–15)	11 (9–14)	12 (9–15)	0.829	12 (9–14)	12 (10–14)	11 (8–12)	10 (8–13)	0.006
MMSE ^{b,c}	29 (28–30)	29 (29–30)	26 (24–29)	21 (18–24)	26 (23–28)	1.3 × 10 ⁻¹⁹	29 (28–30)	29 (28–30)	28 (26–28)	27 (26–28)	3.3 × 10 ⁻¹⁴
CSF Aβ ₄₂ , pg ml ⁻¹	598 (549–817)	405 (326–452)	335 (257–386)	276 (209–361)	497 (337–693)	4.9 × 10 ⁻¹⁵	771 (651–910)	401 (322–466)	774 (654–903)	345 (261–457)	3.3 × 10 ⁻⁵¹
CSF T-tau, pg ml ⁻¹	308 (238–394)	387 (307–512)	579 (466–712)	572 (424–732)	285 (227–362)	2.7 × 10 ⁻¹⁵	276 (239–336)	309 (201–457)	279 (241–333)	472 (341–611)	6.1 × 10 ⁻¹¹
CSF P-tau181, pg ml ⁻¹	93 (78–137)	170 (114–241)	339 (249–520)	390 (268–492)	88 (64–153)	1.8 × 10 ⁻²¹	94 (77–112)	122 (71–238)	86 (79–100)	266 (144–378)	2.4 × 10 ⁻¹⁶
Plasma P-tau181, pg ml ⁻¹	1.3 (0.9–2.4)	1.9 (1.4–2.8)	3.8 (2.5–5.7)	4.4 (3.3–6.4)	1.2 (0.8–1.7)	2.2 × 10 ⁻¹⁷	1.2 (0.9–1.7)	2.2 (1.4–3.1)	1.3 (0.9–1.7)	2.8 (2.0–4.8)	1.5 × 10 ⁻²⁰
Plasma Aβ ₄₂ , pg ml ^{-1b}	N/A	N/A	N/A	N/A	N/A	N/A	33 (30–35)	31 (28–34)	33 (31–37)	31 (28–35)	0.002
Plasma Aβ ₄₂ /Aβ ₄₀ ^b	N/A	N/A	N/A	N/A	N/A	N/A	0.069 (0.065–0.072)	0.065 (0.060–0.070)	0.068 (0.063–0.074)	0.063 (0.060–0.067)	5.8 × 10 ⁻¹⁰
Plasma T-tau ^b	N/A	N/A	N/A	N/A	N/A	N/A	16 (13–19)	17 (15–21)	18 (15–22)	18 (16–23)	0.0001
Plasma NFL, pg ml ^{-1b}	N/A	N/A	N/A	N/A	N/A	N/A	17 (13–22)	22 (15–26)	19 (14–27)	23 (17–31)	4.9 × 10 ⁻⁵
[¹⁸ F]flutemetamol, SUVR ^b	0.64 (0.63–0.67)	0.89 (0.74–0.95)	1.01 (0.96–1.05)	1.10 (0.97–1.19)	0.71 (0.64–0.84)	6.3 × 10 ⁻¹⁴	0.64 (0.62–0.66)	0.77 (0.67–0.95)	0.66 (0.63–0.69)	1.01 (0.83–1.10)	2.3 × 10 ⁻³⁶
[¹⁸ F]flortaucipir, SUVR ^b											
Temporal meta-ROI Braak I–IV ROI	1.17 (1.10–1.20)	1.15 (1.12–1.22)	1.56 (1.25–1.80)	1.92 (1.60–2.29)	1.13 (1.10–1.19)	3.3 × 10 ⁻²¹	N/A	N/A	N/A	N/A	N/A
Braak I–II ROI	1.06 (1.01–1.12)	1.09 (1.02–1.22)	1.43 (1.25–1.78)	1.67 (1.50–1.75)	1.05 (1.01–1.16)	5.7 × 10 ⁻¹⁹	N/A	N/A	N/A	N/A	N/A
Braak III–IV ROI	1.17 (1.10–1.20)	1.16 (1.12–1.23)	1.56 (1.24–1.82)	1.95 (1.60–2.31)	1.14 (1.10–1.19)	5.5 × 10 ⁻²¹	N/A	N/A	N/A	N/A	N/A
Braak V–VI ROI	1.05 (1.00–1.07)	1.04 (1.00–1.07)	1.22 (1.06–1.28)	1.45 (1.18–1.64)	1.03 (0.99–1.08)	9.0 × 10 ⁻¹⁷	N/A	N/A	N/A	N/A	N/A
Inferior temporal cortex	1.21 (1.14–1.24)	1.21 (1.15–1.26)	1.65 (1.30–1.90)	2.09 (1.70–2.54)	1.17 (1.13–1.24)	1.2 × 10 ⁻¹⁹	N/A	N/A	N/A	N/A	N/A

Data are median (interquartile range) unless otherwise specified. N/A indicates missing data. *P* values are derived from chi-square (sex) and Kruskal–Wallis tests. *Non-AD neurodegenerative diseases included the following patients: 11 PD, 17 PDD, 6 PSP, 6 DLB, 6 CBS, 2 SD and 4 bvFTD. ^bIn cohort 1, education information was missing for two study participants; MMSE was missing for eight study participants; CSF Aβ₄₂, Aβ₄₂/Aβ₄₀ and T-tau were missing for two study participants; Aβ status was not available for two patients with non-AD neurodegenerative diseases; [¹⁸F]flutemetamol data were missing for 53 participants; and [¹⁸F]flortaucipir data were missing for eight participants. In cohort 2, education was missing for three study participants; CSF P-tau181 and plasma NFL were missing for one study participant; plasma Aβ₄₂ and T-tau were missing for six study participants; and [¹⁸F]flutemetamol data were missing for 20 participants. ^cRange, 0–30; lower scores indicate worse global cognition. bvFTD, behavioral-variant frontotemporal dementia; CU, cognitively unimpaired; F, female; M, male; SD, semantic dementia.

signs of preclinical AD and individuals in the AD continuum (the preclinical, prodromal or dementia stages of the disease), as well as between (2) patients with AD dementia and those with non-AD neurodegenerative diseases.

Plasma P-tau181 varied between groups, in both cohort 1 ($F = 36.0$, $P < 0.001$) and cohort 2 ($F = 39.5$, $P < 0.001$) (Fig. 2d,e and Extended Data Fig. 3c,d). In cohort 1, we found higher P-tau181 in Aβ⁺ cognitively unimpaired, Aβ⁺ MCI and Aβ⁺ AD dementia than in Aβ⁻ cognitively unimpaired ($P = 0.044$, $P < 0.001$ and $P < 0.001$, respectively) and non-AD disease groups (all $P = 0.001$). P-tau181 was also increased in Aβ⁺ MCI and Aβ⁺ AD dementia compared with Aβ⁺ cognitively unimpaired groups (both $P < 0.001$). In cohort 2, the early changes in P-tau181 were replicated with higher levels in Aβ⁺ cognitively unimpaired and Aβ⁺ MCI compared with Aβ⁻ cognitively unimpaired and

Aβ⁻ MCI, and also in Aβ⁺ MCI compared with Aβ⁺ cognitively unimpaired groups (all $P < 0.001$).

Plasma P-tau181 accurately discriminated AD dementia from non-AD neurodegenerative diseases with AUC = 0.93, sensitivity = 92% and specificity = 87%. Plasma P-tau181 had similar or better performance than CSF Aβ₄₂/Aβ₄₀ and T-tau (Supplementary Table 5). The performance of plasma P-tau181 was slightly worse compared with that of CSF P-tau181 and Tau PET (Fig. 2f and Supplementary Table 5), but with small differences (AUC = 0.03–0.04, sensitivity = 6%, specificity = 7–9%).

These results show that plasma P-tau181 may be increased early in AD, potentially even in some Aβ⁺ cognitively unimpaired individuals (preclinical AD¹⁷). Plasma P-tau181 then increased further during the symptomatic (prodromal and dementia) stages of AD. In contrast, plasma P-tau181 was not increased in non-AD. These

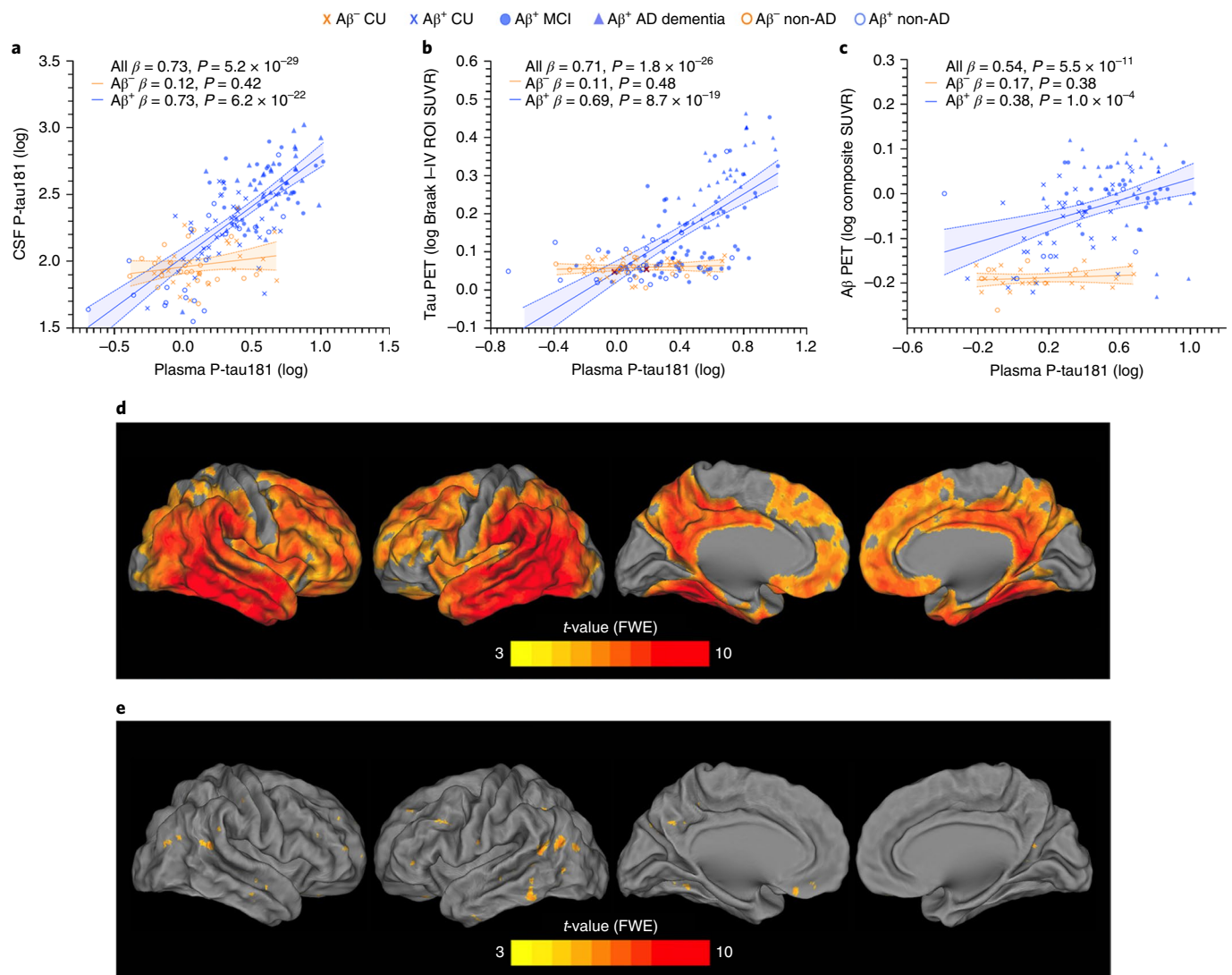


Fig. 1 | Association of plasma P-tau181 with CSF P-tau181, Tau PET and A β PET in cohort 1. **a, Association between plasma P-tau181 and CSF P-tau181 ($n=172$ ($A\beta^+ n=125$; $A\beta^- n=47$)). **b**, Association between plasma P-tau181 and Tau PET in Braak I-IV (temporal 'meta-ROI' ($n=174$ ($A\beta^+ n=124$; $A\beta^- n=48$; $A\beta$ undefined $n=2$)). **c**, Association between plasma P-tau181 and A β PET ($n=129$ ($A\beta^+ n=100$; $A\beta^- n=29$)). **a–c**, Data are shown as β (standardized coefficient) and P values derived from linear regression models adjusted for age and sex; linear regression lines with 95% confidence interval (CI) (shaded areas) are derived from unadjusted models. **d,e**, Voxel-wise regression analysis of Tau PET (**d**, $n=124$) and A β PET (**e**, $n=115$) adjusted for age in a subcohort of cognitively unimpaired patients and those with MCI and AD dementia. A β positivity was defined using either CSF A $\beta_{42} < 510$ pg ml $^{-1}$ or A β PET > 0.743 SUVR. Study participants who underwent both lumbar puncture and A β PET imaging were considered A β^+ if either CSF A β_{42} or A β PET measures were abnormal. Two study participants with undefined A β status are represented by red crosses. FWE, family-wise error.**

characteristics mark out plasma P-tau181 as a promising biomarker to track disease progression in AD and to differentiate AD from non-AD conditions, with utility for patient management in clinical practice, research and trials.

Plasma P-tau181 predicts progression to AD dementia.

The results showing that plasma P-tau181 is related to CSF P-tau181, Tau PET, different stages of AD and that it starts to increase early in the disease in terms of A β PET load suggested that it might be used to predict future progression to AD dementia in people who are cognitively unimpaired or have MCI. This is an essential question in the clinical management of patients with MCI, and in clinical trials at the preclinical and early clinical stages. We therefore performed survival analyses for progression to AD dementia in 332 participants (cohort 2) who were all nondemented at baseline, and followed with repeated clinical examinations up to 8 yr. Over

a mean follow-up of 4.9 yr (s.d.=1.3 yr), 62 (18.7%) developed AD dementia and 33 (9.9%) dementia due to other diseases (vascular dementia, $n=15$), dementia with Lewy bodies (DLB, $n=6$), Parkinson's disease with dementia (PDD, $n=4$), frontotemporal dementia (FTD, $n=2$), dementia due to normal-pressure hydrocephalus ($n=2$), cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL; $n=1$) or dementia due to unspecified cause ($n=3$). Baseline plasma P-tau181 was increased in those who subsequently developed AD dementia compared to those that did not develop any dementia (both A β^+ and A β^- individuals) or developed dementia due to other causes ($F=45.7$, $P<0.001$; post hoc tests: $P<0.001$; Fig. 3a and Extended Data Fig. 3e). Furthermore, plasma P-tau181 levels were increased in A β^+ cognitively unimpaired individuals and those with A β^+ MCI who progressed to AD dementia compared with A β^- cognitively unimpaired and A β^- MCI individuals who did not convert

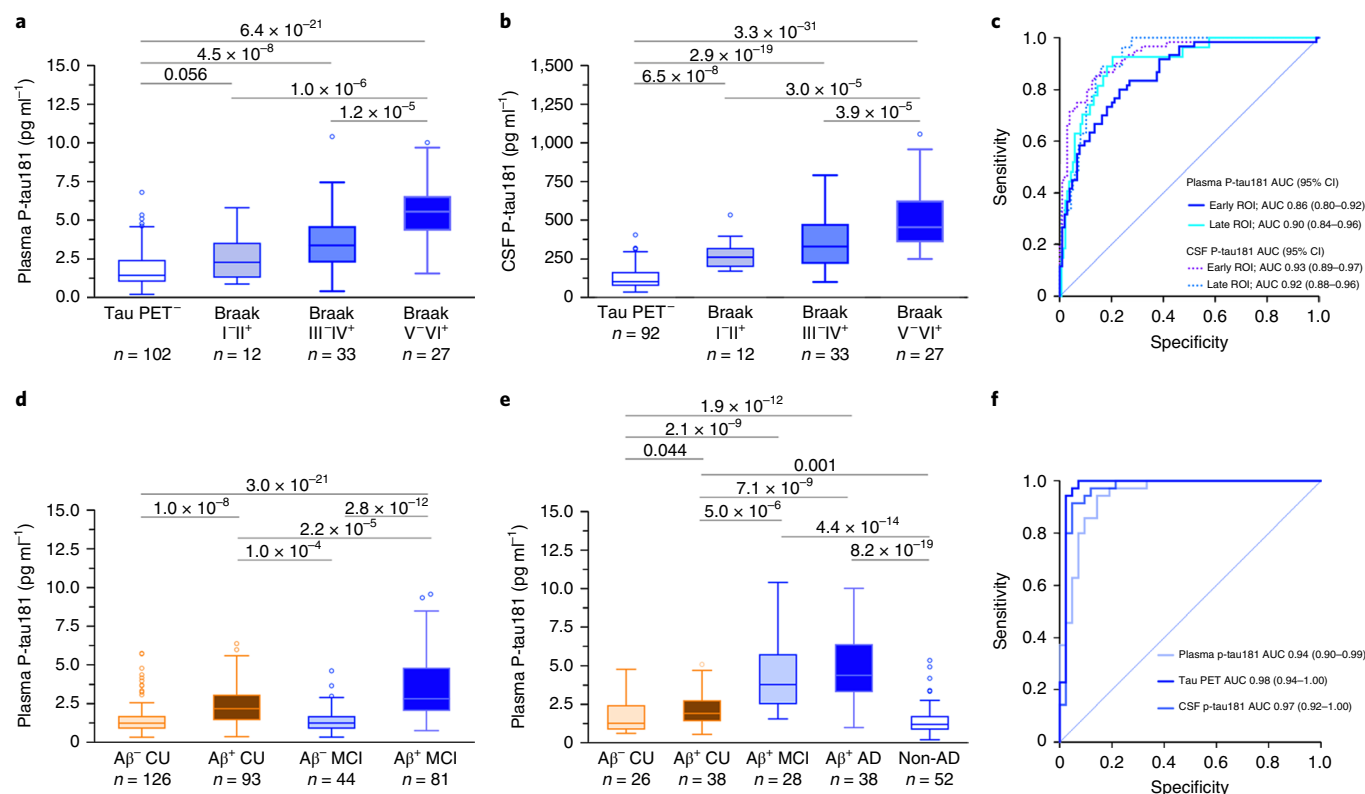


Fig. 2 | Plasma P-tau181, CSF P-tau181 and Tau PET in different diagnostic groups. **a,b**, Cohort 1; plasma (**a**) and CSF (**b**) concentrations of P-tau181 in individuals without significantly elevated Tau PET measurements in any Braak ROI (Tau PET⁻), and those with significantly elevated measurements in one or more of these ROIs, including (1) Braak I–II (but not III–VI), (2) Braak III–IV (but not V–VI) or (3) Braak V–VI. Tau data were binarized based on the SUVR cutoff of 1.3 (ref. ²⁶). **c**, Cohort 1; ROC curve analyses for prediction of abnormal Tau PET status in the earlier Braak I–IV and later Braak V–VI ROIs (n=164; see Table 2 for sensitivity and specificity measures). **d**, Cohort 2; plasma P-tau181 concentrations in Aβ⁺ and Aβ⁻ cognitively unimpaired (CU) and patients with MCI. **e**, Cohort 1; plasma P-tau181 concentrations in Aβ⁺ and Aβ⁻ CU and in the Aβ⁺ MCI, Aβ⁺ AD dementia and non-AD neurodegenerative (ND) disease groups. **f**, Cohort 1; ROC curve analyses of plasma P-tau181 and Tau PET in Braak I–IV ROI for distinguishing AD dementia from non-AD ND in a subcohort of 77 patients (35 AD dementia and 42 non-AD ND (8 PD, 13 PDD, 4 PSP, 5 DLB, 6 CBS, 2 SD and 4 bvFTD) where all these measures were available (see Supplementary Table 5 for sensitivity and specificity measures). CSF P-tau181 and Tau PET data in **f** have previously been published in an overlapping subcohort and are shown for reference²³. **a,b,d,e**, P-values derived from univariate general linear models adjusted for age and sex as described in Methods; individual measures are shown in Extended data Fig. 3a–d; boxes show interquartile range, the horizontal lines are medians and the whiskers were plotted using the Tukey method.

to AD dementia (Extended Data Fig. 6). We also observed higher plasma P-tau181 levels in Aβ⁺ MCI who converted to AD dementia than in Aβ⁺ cognitively unimpaired and Aβ⁺ MCI non-converters (Extended Data Fig. 6).

When adjusting for age, sex and education, each 1 s.d. increment in the log of baseline plasma P-tau181 was associated with greater risk of future AD dementia (hazard ratio (HR) = 3.8, $P < 0.001$; Table 3). For comparison, the HR of CSF P-tau181 was 3.9 (95% CI = 3.0–4.9, $P < 0.001$). We found similar associations between plasma P-tau181 (continuous) and increased risk after additional adjustment for plasma T-tau, Aβ42/Aβ40 and NfL (HR = 3.6, $P < 0.001$; Table 3). None of the latter biomarkers were independently related to risk of AD dementia in a multivariable analysis that included all plasma biomarkers.

Plasma P-tau181 was associated with increased risk of AD dementia in both cognitively unimpaired participants (HR = 2.5, $P = 0.01$) and MCI (HR = 3.1, $P < 0.001$), and after additional adjustment for plasma T-tau, Aβ42/Aβ40 and NfL (cognitively unimpaired, HR = 2.4, $P = 0.04$; MCI, HR = 3.0, $P < 0.001$; Table 3).

We binarized baseline plasma P-tau181 data using the Youden-based cutoff of 1.81 pg ml⁻¹ for differentiation of Aβ⁻ cognitively unimpaired who did not convert to AD dementia from those who progressed to AD dementia. Compared to normal plasma levels

of P-tau181 (≤ 1.81 pg ml⁻¹), abnormal levels (> 1.81 pg ml⁻¹) were associated with increased risk of future AD dementia (HR = 10.9, 95% CI = 5.0–24.0, $P < 0.001$; Fig. 3b).

Plasma P-tau181 may be used to predict subsequent progression to AD dementia in both cognitively unimpaired (for example, in clinical trials aimed at the preclinical stage of AD) and MCI (relevant for clinical practice and drug trials aimed at symptomatic stages). Plasma P-tau181 may be a more powerful indicator of AD risk than other candidate blood-based biomarkers, including plasma T-tau^{8,9} and plasma NfL⁷.

Discussion

Plasma P-tau181 (1) correlated to CSF P-tau181, (2) identified increased Tau PET and Amyloid PET uptake, (3) increased along the AD continuum—from preclinical to prodromal and dementia stages, (4) distinguished AD from non-AD neurodegenerative diseases, including neuropathologically verified AD from non-AD, and (5) predicted longitudinal conversion to AD dementia with greater precision than previously established plasma AD biomarkers, including Aβ42/Aβ40 ratio, NfL and T-tau^{5–9}.

The correlations found with CSF P-tau181 are in line with results from a much smaller study ($n = 11$)⁴ and suggest that, to a large degree, plasma P-tau181 is derived from the central nervous

Table 2 | ROC analysis of plasma P-tau181 and CSF AD biomarkers for identifying Tau PET⁺ status in Braak I–IV ROI and Braak V–VI ROI (cohort 1)

Biomarkers ^a	AUC (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Braak I–IV ROI (60 Tau PET ⁺ and 104 Tau PET [−])			
Plasma P-tau181	0.86 (0.80–0.92)	80 (70–90)	77 (69–85)
CSF P-tau181	0.93 (0.89–0.97)	85 (75–93)	87 (80–92)
CSF T-tau	0.87 (0.81–0.92)	80 (70–90)	78 (70–86)
CSF Aβ ₄₂ /Aβ ₄₀	0.80 (0.73–0.86)	73 (62–85)	76 (67–84)
Braak V–VI ROI (27 Tau PET ⁺ and 137 Tau PET [−])			
Plasma P-tau181	0.90 (0.84–0.96)	93 (81–100)	79 (72–86)
CSF P-tau181	0.92 (0.88–0.96)	89 (78–100)	84 (77–90)
CSF T-tau	0.86 (0.79–0.93)	89 (78–100)	69 (61–77)
CSF Aβ ₄₂ /Aβ ₄₀	0.80 (0.73–0.87)	100 ^b	55 (46–63)

AUCs (95% CIs) derived from logistic regression models for prediction of Tau PET positivity (SUVR > 1.3). Sensitivities and specificities are for cutpoints defined by the Youden index (maximizing the sum of sensitivity and specificity). ^aData derived from a subcohort of 164 individuals in which plasma P-tau181 and the other biomarker measures were all available. Data in the main text derived from all individuals in cohort 1 (*n* = 174) who underwent Tau PET imaging. ^bCI not calculated due to 100% sensitivity rate.

system in AD. One finding was that plasma P-tau181 and CSF P-tau181 correlated only in Aβ⁺ individuals, indicating that CSF and blood P-tau181 may be differently regulated depending on Aβ status. Supporting this, plasma P-tau181 had greater variability than CSF P-tau181 in the Aβ[−] group, and some individuals had increased plasma P-tau181 but not increased CSF P-tau181 (Fig. 1 and Extended Data Fig. 1). Furthermore, plasma P-tau181 and CSF P-tau181 correlated even in preclinical AD (Extended Data Fig. 1c,d) when passive secretion of P-tau181 due to neuronal cell death would be unlikely to affect P-tau181 in either blood or CSF. Other studies have shown much weaker correlations between plasma T-tau and CSF T-tau levels than we found for P-tau181^{8,9,18,19}, indicating that plasma P-tau181 is superior in the detection of pathological brain changes^{3,8}. Plasma P-tau181, but not T-tau, was independently associated with increased risk of progression to AD dementia. One possible explanation for these differences between plasma P-tau181 and plasma T-tau is that currently available assays for T-tau may measure a form of tau that is susceptible to proteolytic degradation in blood resulting in a very short apparent half-life of the molecule (hours in plasma compared with weeks in CSF)²⁰. The antibody combination used to measure plasma P-tau181 most probably measures a tau fragment that is resistant to this degradation. Another possibility is that plasma P-tau181 is more brain-specific, while T-tau can potentially also be produced outside the central nervous system²¹.

The associations between P-tau181 and Tau PET (which is associated with tau neuropathology^{11,14}), together with increased plasma P-tau181 in neuropathologically confirmed AD dementia, indicate that plasma P-tau181 is related to insoluble tau deposits in AD. Plasma P-tau181 correlated to Tau PET in all tested regions when including both cognitively unimpaired and cognitively impaired cases, and to all regions except Braak I–II (entorhinal cortex) in Aβ⁺ cognitively impaired individuals. Entorhinal cortex is one of the earliest sites engaged by tau pathology in AD and one of the first sites with increased Tau PET¹², and thus it is possible that Tau PET may be saturated there in most symptomatic individuals. When using a Tau PET staging system where individuals were grouped into Tau PET[−], Braak I–II⁺, III–IV⁺ and V–VI⁺, plasma P-tau181 increased numerically stepwise between the stages. However, while there was a strong trend for higher plasma

P-tau levels (*P* = 0.056), CSF P-tau181 was significantly increased in the Braak I–II⁺ group. Given the small sample size of this group, future studies in larger cohorts are warranted to determine whether plasma P-tau181 is a sufficiently sensitive biomarker to detect abnormal Tau PET at the early Braak I–II ROI. At the same time, plasma P-tau181 identified individuals with positive Tau PET in the Braak I–IV and V–VI composites (AUC = 0.86–0.90; Fig. 2c), supporting its usefulness as a noninvasive biomarker to predict Tau PET positivity—for example, in clinical trials aimed at tau⁺ participants. Given the correlations between plasma P-tau181 and Tau PET, the findings of stepwise increases in plasma P-tau181 from Aβ[−] cognitively unimpaired to Aβ⁺ cognitively unimpaired, and further to Aβ⁺ MCI, were expected and in line with previous data^{22,23}. Our findings suggest that plasma P-tau181 can be used as a blood-based biomarker of cerebral PHF-tau pathology. This may be used in epidemiological or interventional studies with longitudinally banked plasma samples to study the effects on tau pathology of risk factors, protective factors and different interventions. The potential mechanisms underlying increased fluid P-tau181 in AD have been elucidated by recent stable isotope-labeling kinetics experiments²⁴, which showed that neurons affected by AD or exposed to Aβ have increased synthesis and secretion of tau. These neurons may eventually degenerate and develop tangle pathology. This is a likely explanation for the positive correlation of plasma P-tau181 concentration with Tau PET signal (rather than tangle pathology directly driving the increase in P-tau181).

Differential diagnosis of AD dementia from other diseases is difficult using clinical testing²⁵. Tau PET has excellent performance in differentiating AD dementia from non-AD neurodegenerative diseases, since abnormal Tau PET is very rare in most other dementias²⁶. Plasma P-tau181 had very similar performance (AUC = 0.94) to Tau PET (AUC = 0.98) for AD versus other neurodegenerative diseases (Fig. 2f). Plasma P-tau181 may therefore be an alternative to Tau PET for differential diagnosis. If the results are replicated, plasma P-tau181 may be used to improve differential diagnosis of patients with cognitive impairment which, in turn, might improve symptomatic treatment (with acetylcholinesterase inhibitors) and patient management. One additional implication is that plasma P-tau181 may be used as a screening tool to identify patients who are in need of confirmatory tau diagnostics (PET or CSF).

Prediction of longitudinal impairment to dementia is a key question for management of individuals with mild cognitive deficits, and for the design of clinical trials in early AD. We show that, similarly to CSF AD biomarkers²⁷, plasma P-tau181 accurately predicts development of AD dementia in people without dementia (Fig. 3b). When compared directly in the same subjects, plasma P-tau181 had very similar performance to CSF P-tau181 in predicting conversion to AD dementia. This suggests that plasma P-tau181 may have a similarly high utility to that of CSF P-tau181 in predicting future AD dementia, including when risk-stratifying individuals in trials. When combining plasma P-tau181 with other plasma biomarkers, including T-tau⁹, Aβ₄₂/Aβ₄₀ (ref. 6) and NfL⁷, only P-tau181 was associated with increased risk of AD dementia. However, it is possible that different assays for plasma Aβ₄₂/Aβ₄₀ may have different precision and, in particular, mass spectrometry-based assays might be more accurate than the assays used here^{28,29}. Therefore, further studies are required to test whether a combination of plasma P-tau181 and mass spectrometry-based Aβ₄₂/Aβ₄₀ might yield an even better performance in predicting future development of AD.

The finding that plasma P-tau181 correlated to Aβ PET points to a link between metabolism of plasma P-tau181 and Aβ aggregation. The AUC to differentiate Aβ[−] and Aβ⁺ individuals was 0.81, which increased to 0.84 when combined with plasma Aβ₄₂/Aβ₄₀. Our analyses suggest that plasma P-tau181 may start to increase

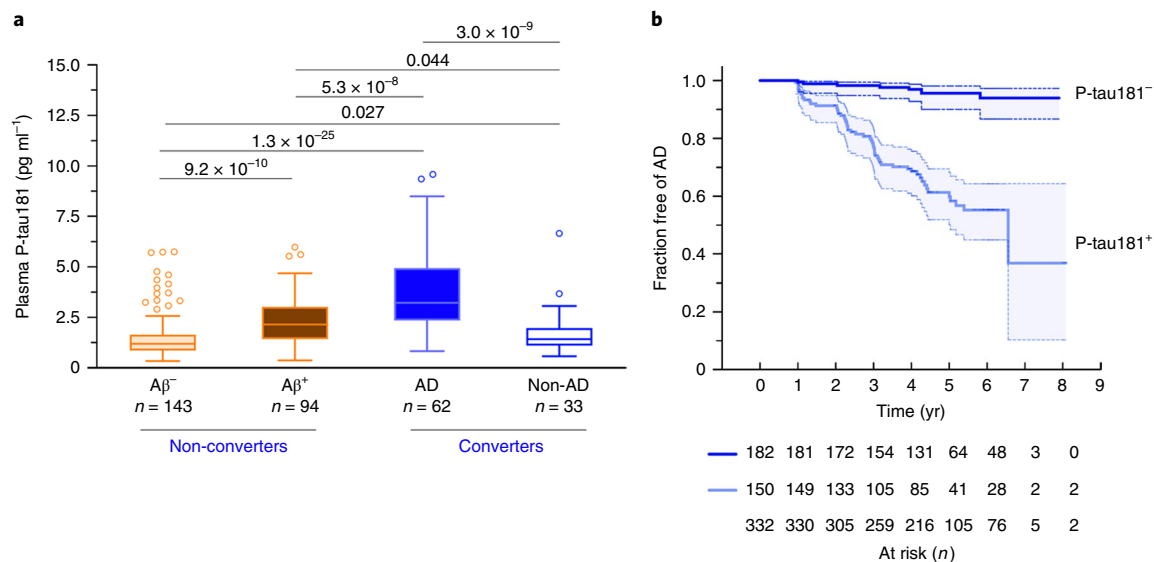


Fig. 3 | Plasma P-tau181 and progression to AD dementia. **a**, Plasma P-tau181 concentrations in Aβ⁺ and Aβ[−] individuals who did not develop dementia, developed AD dementia or developed non-AD neurodegenerative disease during clinical follow-up. *P* values derived from univariate general linear models adjusted for age, sex and years of education (as described in Methods); individual measures are shown in Extended Data Fig. 3e; boxes show interquartile range, the horizontal lines are medians and the whiskers were plotted using the Tukey method. **b**, Survival curves for progression from cognitively unimpaired or MCI to AD dementia among participants with normal versus abnormal baseline plasma P-tau181 levels (*n* = 332). Plasma P-tau181 data were binarized, using the Youden-based cutoff of 1.81 pg ml^{−1}, for differentiation of Aβ[−] cognitively unimpaired who did not convert to AD dementia from those who progressed to AD dementia.

Table 3 | Multivariate Cox proportional hazards models for progression to AD dementia (cohort 2)

Biomarkers	CU + MCI	CU	MCI
	HR (95% CI), <i>P</i> value	HR (95% CI), <i>P</i> value	HR (95% CI), <i>P</i> value
Model 1 ^a			
P-tau181	3.81 (2.77–5.23), 1.5 × 10 ^{−16}	2.48 (1.20–5.14), 0.01	3.07 (2.12–4.42), 2.5 × 10 ^{−9}
Model 2 ^b			
P-tau181	3.59 (2.55–5.04), 1.9 × 10 ^{−13}	2.37 (1.03–5.45), 0.04	3.04 (2.03–4.56), 7.1 × 10 ^{−8}
Aβ42/Aβ40	0.81 (0.64–1.02), 0.07	0.66 (0.42–1.04), 0.07	0.86 (0.60–1.23), 0.41
T-tau	0.98 (0.73–1.32), 0.90	0.72 (0.36–1.43), 0.34	0.97 (0.68–1.37), 0.86
NfL	1.07 (0.80–1.44), 0.67	1.56 (0.52–4.64), 0.42	1.00 (0.75–1.33), 0.99

^aData derived from cohort 2, including 332 individuals (213 CU and 119 MCI) who were followed with repeated clinical examinations for up to 8 yr. ^bData derived from a subcohort of 325 individuals (208 CU and 117 MCI) where plasma P-tau181 and all other biomarker measures were available. Data derived from models combining all plasma biomarkers and including age, sex and education (yr) as covariates. HRs represent fold increase in risk of AD for each 1 s.d. increase in log₁₀-transformed biomarker data.

around the time of Aβ positivity, and also continue to increase as Aβ accumulates, as shown by the correlations with Aβ PET even within the Aβ⁺ subgroup.

One study limitation was that some subgroups were relatively small, especially the non-AD neurodegenerative disease groups (cross-sectionally tested in cohort 1 and longitudinally in cohort 2) and the neuropathologically confirmed group. Future studies are needed to validate P-tau181 as a biomarker for differential diagnosis of AD (for example, distinguishing AD from FTD). Our

findings should also be replicated in a primary care setting. This is important, since the practical utility of all diagnostic tests may vary depending on the prevalence of the target condition in the tested population. Plasma P-tau181 was measured using a research-grade assay. A fully validated clinical-grade assay, together with a certified reference material³⁰, is needed for implementation in clinical practice and establishment of universal cutoffs. The results currently available suggest that one cutoff may be used to identify AD at both the MCI and dementia stages. Twenty samples (3.8%) were below the limit of detection (see Methods). This may be improved by assay development, including implementation on more sensitive platforms such as, for example, Simoa technology or mass spectrometry-based assays^{31,32}. Regarding the longitudinal analyses, at present we unfortunately cannot test whether plasma P-tau181 can predict conversion from negative to positive status on Tau PET or Amyloid PET, which will be important to study in the future.

Plasma P-tau181 is promising regarding the diagnosis and prognostication of AD, especially in facilities with limited access to CSF or PET testing. The most likely clinical applications are to distinguish AD from other non-AD neurodegenerative diseases in cases with mild to moderate dementia (to ensure optimal patient management, including access to available treatments), and to predict future development of AD in cases with MCI. Plasma P-tau181 is also promising regarding trials to enrich participants and potentially also to monitor the effects of treatments. For trials of preclinical AD, plasma P-tau181 may be used together with a measure of plasma Aβ42/Aβ40 (see Supplementary Table 4). A recent framework on AD research¹ suggests that CSF P-tau181 can be used to indicate tau status. Given the obvious benefit of a blood test, together with close correlations to CSF P-tau181, Tau PET, AD diagnosis and neuropathology, we suggest that plasma P-tau181 can also be used to indicate tau status. The capability of plasma P-tau181 to predict future development of AD dementia independently of, and superior to, other plasma AD biomarkers might be valuable in clinical practice and trials.

Online content

Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at <https://doi.org/10.1038/s41591-020-0755-1>.

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Methods

The study was approved by the Regional Ethics Committee in Lund, Sweden. All participants gave written informed consent to participate. The study was conducted in accordance with Standards for Reporting of Diagnostic Accuracy (STARD) guidelines.

Participants. This project included three separate cohorts. The first two cohorts were part of the prospective Swedish BioFINDER study, with participants recruited at Skåne University Hospital and the Hospital of Ängelholm, Sweden. The third cohort was from the Arizona Study of Aging and Neurodegenerative Disorders/Brain and Body Donation Program at Banner Sun Health Institute, USA. Cohort 1 included 182 participants ($n = 182$) enrolled between November 2014 and January 2018 who underwent Tau ($[^{18}\text{F}]\text{florbetapir}$) PET imaging, and cohort 2 included nondemented participants ($n = 344$) enrolled between January 2010 and December 2014 who were followed longitudinally for up to 8 yr. The demographics of each cohort are described in Table 1. The inclusion criteria for cognitively healthy elderly individuals were (1) absence of cognitive symptoms as assessed by a physician with special interest in cognitive disorders; (2) age ≥ 60 yr; (3) Mini Mental State Examination (MMSE) 28–30 points at screening visit; (4) did not fulfill the criteria for MCI or any dementia disorder; and (5) fluency in Swedish. The exclusion criteria were (1) significant unstable systemic illness, such as terminal cancer, or organ failure that made it difficult to participate in the study; (2) current significant alcohol or substance misuse; and (3) significant neurological or psychiatric illness. The inclusion criteria for patients with subjective cognitive decline (SCD) or MCI (defined using criteria by Petersen³³) were (1) referred to a participating memory clinic because of cognitive complaints; (2) age 60–80 yr; (3) did not fulfill the criteria for any dementia disorder; and (4) fluency in Swedish. The exclusion criteria were (1) significant unstable systemic illness or organ failure, such as terminal cancer, that made it difficult to participate in the study; (2) current significant alcohol or substance misuse; and (3) cognitive impairment that, without doubt, could be explained by other specific non-neurodegenerative disorders such as brain tumor or subdural hematoma. Following neuropsychological assessment including a test battery evaluating verbal ability, episodic memory function, visuospatial construction ability and attention and executive functions, patients were classified as SCD or MCI as previously described³⁴. In accordance with the research framework of the National Institute on Aging–Alzheimer's Association¹, study participants with SCD were analyzed together with cognitively healthy participants (and combined in the cognitively unimpaired group).

In cohort 2, the study participants (that is, participants who were cognitively unimpaired or had MCI at baseline) were followed over time with repeated cognitive, neurologic and psychiatric assessments by a physician, and also underwent repeated cognitive testing, magnetic resonance imaging (MRI) and collection of CSF and blood. The follow-up visits were performed every 2 yr in the cognitively healthy group and every year for patients with SCD and MCI (mean follow-up = 4.9 yr, s.d. = 1.3 yr). During the follow-up, 62 (18.7%; 11 A β^+ cognitively unimpaired, 50 A β^+ MCI and one A β^- MCI) developed AD dementia and 33 (9.9%; four A β^- cognitively unimpaired, three A β^+ cognitively unimpaired, 10 A β^+ MCI and 16 A β^- MCI) dementia due to other neurodegenerative diseases (that is, vascular dementia ($n = 15$), DLB ($n = 6$), PDD ($n = 4$) and FTD ($n = 2$), or dementia due to normal-pressure hydrocephalus ($n = 2$), CADASIL ($n = 1$) or unspecified cause ($n = 3$)).

In cohort 1, the study protocol was a priori designed to enroll cognitively unimpaired participants with a high prevalence ($>50\%$) of A β positivity, and only A β^+ patients with MCI, because the focus was to study tau pathology in the predementia stages of AD. In cohort 1, we also included patients with AD dementia who fulfilled the Diagnostic and Statistical Manual of Mental Disorders-5 (DSM-5) criteria for major neurocognitive disorder (dementia) due to AD and were A β^+ (ref. ¹). The non-AD neurodegenerative disease group included patients with PDD, DLB, FTD (all fulfilling the DSM-5 criteria for the respective disease), Parkinson's disease (PD; fulfilling the criteria defined by Gelb et al.³⁵), progressive supranuclear palsy (PSP; fulfilling the criteria defined by Litvan et al.³⁶) and Höglinger et al.³⁷) and corticobasal syndrome (CBS) (fulfilling the criteria defined by Armstrong et al.³⁸).

Cohort 3 included 16 AD dementia and 47 non-AD neuropathologically confirmed cases who provided plasma samples 0.02–2.9 yr before death. The demographics of cohort 3 are described in Supplementary Table 1. The AD dementia group included cases with a high likelihood that dementia was due to AD histopathology according to National Institute on Aging (NIA)–Reagan criteria³⁹. The non-AD group ($n = 47$) had none or sparse neuritic plaques according to the score of the Consortium to Establish a Registry for Alzheimer's Disease, and cases with clinical diagnoses included in the non-AD group were amyotrophic lateral sclerosis ($n = 1$), controls without a major neuropathological diagnosis ($n = 14$), corticobasal degeneration ($n = 1$), dementia with unspecified etiology ($n = 2$), FTD with TDP-43 ($n = 1$), MCI ($n = 3$), multiple sclerosis ($n = 1$), PD ($n = 15$), parkinsonism ($n = 2$), primary lateral sclerosis ($n = 1$), PSP ($n = 2$), vascular cognitive impairment ($n = 2$), vascular dementia ($n = 1$) and vascular parkinsonism ($n = 1$). All subjects or their legal representatives signed an Institutional Review Board-approved informed consent form before taking part in the study.

All eligible subjects in all cohorts (which were convenience cohorts) were included, to maximize sample sizes.

Plasma and CSF sampling and analysis. Blood and CSF samples were collected in the morning during the same visit, with participants non-fasting. For each cohort 1 and 2 study participant, blood was collected in six EDTA-plasma tubes (Vacutainer K₂EDTA tube, BD Diagnostics) and centrifuged (2,000g, $+4^\circ\text{C}$) for 10 min. Following centrifugation, plasma from all six tubes was transferred into one 50-ml polypropylene tube, mixed and 1 ml was aliquoted into 1.5-ml polypropylene tubes and stored at -80°C within 30–60 min of collection. For cohort 3, blood processing was similar except for centrifugation at 1,500 r.p.m. for 15 min followed by transfer to 1.7-ml polypropylene microcentrifuge tubes and a second centrifugation at 4°C for 5 min at 14,000 r.p.m. Lumbar puncture and CSF handling followed a structured protocol⁴⁰.

Plasma P-tau181 was analyzed in the three cohorts as previously described³. In short, the assay was performed on a streptavidin plate on the Meso Scale Discovery (MSD) platform. Biotinylated-AT270 was used as capture antibody (anti-pT181 Tau antibody; Thermo Fisher, no. MN1050) and SULFO-TAG-LRL (anti-tau monoclonal antibodies developed by Lilly Research Laboratory) as detector. Antibodies were conjugated with sulfo-NHS-biotin (Thermo Scientific, no. 21327) or MSD GOLD SULFO-TAG NHS-ester (MSD, no. R91AO) according to the manufacturer's protocol. The assay was calibrated using a recombinant tau (4R2N) protein that was phosphorylated *in vitro*. The assay uses 80 μl of plasma diluted with an equal volume of sample diluent, to allow for duplicate measurements. Data for quality control and control samples are shown in Supplementary Table 6. In cohort 1, five (2.7%) samples from non-AD neurodegenerative diseases were below the level of detection (LOD, 0.54 pg ml⁻¹). In cohort 2, 15 (4.4%) samples were $>\text{LOD}$ including nine (2.6%) A β cognitively unimpaired, four (1.2%) A β^- MCI and two A β^+ cognitively unimpaired (0.6%). All plasma samples with P-tau181 concentrations $<\text{LOD}$ were included in statistical analysis using values extrapolated from the standard curve.

In cohort 2, plasma A β 42, A β 40 and T-tau were measured using Elecsys fully-automated immunoassays on a cobas e 601 analyzer, and plasma NFL concentrations were measured using monoclonal antibodies as in the NF-light assay (UmanDiagnostics), together with an in-house calibrator (purified bovine NFL) transferred onto the Simoa platform using a homebrew kit (Quanterix), as described previously^{15,41}.

CSF from cohorts 1 and 2 was analyzed for A β 42, A β 40 and T-tau using ELISA (Euroimmun) according to the manufacturer's recommendations. CSF P-tau181 was quantified using ELISA. Anti-P-tau181 antibody was used as a capture antibody in the P-tau181 assay. Antibodies were conjugated with biotin (Thermo Scientific, no. 21329) or SULFO-TAG (MSD, no. R91AO-1). The assay was calibrated using a recombinant tau (4R2N) protein that was phosphorylated *in vitro* using a reaction with glycogen synthase kinase-3 and characterized by mass spectrometry. The samples were thawed on wet ice, briefly vortexed and diluted 1/8 in Diluent 35 (MSD, no. R50AE) with the addition of a heterophilic blocking reagent to a concentration of 200 $\mu\text{g ml}^{-1}$ (Scantibodies, no. 3KC533). To perform the assays, MSD small-spot streptavidin-coated plates (MSD, no. L45SA) were blocked for 1 h at room temperature with 200 μl of 3% bovine serum albumin in Dulbecco's phosphate buffered saline, with 650 r.p.m. shaking on a plate shaker. The plates were then washed three times with 200 μl of wash buffer (PBS + 0.05% Tween 20), and 25 μl of biotinylated capture antibody at 1 $\mu\text{g ml}^{-1}$ was added to the wells followed by incubation for 1 h at room temperature with 650 r.p.m. shaking on a plate shaker. The plates were again washed three times with 200 μl of wash buffer and 50 μl of diluted calibrator, or sample was added to each well and incubated for 2 h at room temperature with 650 r.p.m. shaking on a plate shaker. The plates were then washed three times with 200 μl of wash buffer, and 25 μl of SULFO-tagged LRL detection antibody was added at 3 $\mu\text{g ml}^{-1}$ and incubated for 1 h at room temperature with 650 r.p.m. shaking on a plate shaker. The plates were washed a final time with 200 μl of wash buffer, and 150 μl of 2 \times MSD Read Buffer T with Surfactant (MSD, no. R92TC) was added to each plate and read on the MSD SQ120 within 10 min of read buffer addition. Samples were analyzed in duplicates, and the mean of duplicates was used in statistical analysis.

All plasma and CSF analyses were performed by technicians who were blinded to the clinical and imaging data. All assessments of clinical and imaging data were done blinded to the plasma P-tau181 data.

Tau and A β PET imaging and processing. [^{18}F]florbetapir (cohort 1, $n = 174$) and [^{18}F]flutemetamol (cohort 1, $n = 129$; cohort 2, $n = 324$) were used for Tau PET and A β PET imaging, respectively. [^{18}F]florbetapir and [^{18}F]flutemetamol were synthesized at Skåne University Hospital, Lund, and PET scans were performed on a GE Discovery 690 PET scanner (Florbetapir; General Electric Medical Systems) and a Philips Gemini TF16 scanner (Flutemetamol; Philips Healthcare), respectively, as described previously^{13,42}.

[^{18}F]florbetapir PET was performed in cohort 1. The mean injected dose of [^{18}F]florbetapir was ≈ 370 megabecquerel (MBq), and participants underwent a PET scan 80–100 min after injection. Images were motion corrected with the AFNI 3dvolreg, time averaged and rigidly coregistered to the skull-stripped MRI scan. SUVR images were created using inferior cerebellar gray matter as reference

region⁴³. FreeSurfer (v.5.3) parcellation of the T1-weighted MRI scan was applied to the PET data transformed to participants' native T1 space to extract mean regional SUVR values for each participant in four predefined ROIs, including inferior temporal cortex and three regions corresponding to different image-based stages of tau as described in Cho et al.⁴⁴. ROIs Braak I–II (entorhinal cortex), III–IV (parahippocampal gyrus, fusiform gyrus, amygdala, inferior temporal and middle temporal gyri) and V–VI (posterior cingulate gyrus, caudal anterior cingulate gyrus, rostral anterior cingulate gyrus, precuneus, inferior parietal lobule, superior parietal lobule, insula, supramarginal gyrus, lingual gyrus, superior temporal gyrus, medial orbitofrontal gyrus, rostral middle frontal gyrus, lateral orbitofrontal gyrus, caudal middle frontal gyrus, superior frontal gyrus, lateral occipital gyrus, precentral gyrus, postcentral gyrus and paracentral gyrus). For voxel-wise analysis between [¹⁸F]flortaucipir and CSF and plasma P-tau181, magnetic resonance and PET images were transformed into Montreal Neurological Institute space (2-mm MNI152 MRI template), and voxel-wise correlations were made using multiple regressions adjusting for age in SPM12 (<http://www.fil.ion.ucl.ac.uk/spm>). Images were thresholded using family-wise error (FWE) correction at $P < 0.05$. The thresholded images were overlaid on a population-average, landmark- and surface-based image⁴⁵ using CARET v.5.65 (Van Essen Lab; <http://brainvis.wustl.edu>). For some analyses, [¹⁸F]flortaucipir data were binarized based on the SUVR cutoff of 1.3 (ref. ²⁶).

Aβ ([¹⁸F]flutemetamol) PET was performed in cohorts 1 and 2, the mean injected dose being ≈ 185 MBq. PET images were acquired 90–110 min after injection. The scanning and processing procedures have been described previously^{40,46}. The weighted mean SUVR from a global neocortical region of interest was calculated relative to a composite reference region (white matter, cerebellum and brainstem)^{15,46}.

Aβ positivity. Cutoffs for CSF Aβ42 (< 510 pg ml⁻¹) and Aβ PET (SUVR > 0.743) were computed using Gaussian mixture modeling. Study participants who underwent both lumbar puncture and Aβ PET imaging were considered Aβ⁺ if either CSF Aβ42 or Aβ PET measures were abnormal.

Statistical analysis. Associations between plasma P-tau181 and CSF P-tau181 or PET measures were examined using linear regression, adjusted for age and sex. Group differences were assessed in univariate general linear models, adjusted for age and sex and least significant difference tests (post hoc) for pairwise group comparisons.

Diagnostic accuracies were assessed with receiver operating characteristic (ROC) curve analysis and logistic regression models. We used a bootstrap procedure ($n = 2,000$ iterations) to compare ROC curves and compute 95% CI of sensitivities and specificities (at Youden index thresholds).

To assess associations between plasma P-tau181 (as a continuous variable) and risk of incident AD dementia, we used Cox proportional hazard regression models adjusted for age, sex and education (yr). In a secondary analysis, we covaried for other plasma biomarkers (T-tau, Aβ42/Aβ40 and NfL). All participants were censored at their last follow-up visit or diagnosis of AD dementia. Results were expressed as HR. The proportionality of hazards assumption was assessed using the Schoenfeld residuals.

Associations between log-transformed plasma P-tau181 and continuous Aβ PET uptake were tested with nonlinear polynomial spline models (using the I-spline basis), to detect Aβ PET thresholds for increased P-tau181 (the Aβ level where the spline increased by at least two standard errors from baseline).

Log-transformed biomarker and PET measures were used in regression analyses. When comparing plasma P-tau181 to other blood biomarkers in Cox proportional hazard regression models, biomarker data were standardized within the sample. Outliers with plasma P-tau181 levels > 3 s.d. above the mean ($n = 7$) were excluded from the main analysis. We performed a sensitivity analysis including the outliers, with very similar results (Supplementary Results).

Unadjusted two-sided $P < 0.05$ was considered statistically significant. The results were similar after adjustment for multiple comparisons using false discovery rate adjustment (Supplementary Results). All analyses were performed using SPSS v.24 (IBM), Rstudio and R v.3.5.3 (packages *pROC* and *splines2*). Data were visualized using either Prism 8 (Graphpad) or Rstudio.

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

Data availability

Anonymized data will be shared by request from any qualified investigator for the sole purpose of replicating procedures and results presented in the article, providing 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.

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Author contributions

S.J., N.M., S.P., R.S., T.G.B., G.E.S., X.C., N.K.P., U.E., H.Z., K.B., E.M.R., E.S., J.L.D. and O.H. collected the data and reviewed the manuscript for intellectual content. S.J., N.M. and O.H. analyzed and interpreted the data, prepared figures and co-wrote the manuscript. O.H. was the principal designer and coordinator of the study and overviewed collection, analysis and interpretation of the study data.

Competing interests

S.J., S.P., E.S. and G.E.S. report no conflicts of interest. N.M. has been a consultant for ADNI. R.S. has served as a (nonpaid) consultant for Roche. H.Z. has served at scientific advisory boards for Roche Diagnostics, Wave, Samumed and CogRx, has given lectures in symposia sponsored by Alzecure and Biogen and is a co-founder of Brain Biomarker Solutions, a GU Ventures-based platform company at the University of Gothenburg, all unrelated to the work presented in this paper. K.B. has served as a consultant or at advisory boards for Alector, Biogen, CogRx, Lilly, MagQu, Novartis and Roche Diagnostics, and is a co-founder of Brain Biomarker Solutions, all unrelated to the work presented in this paper. E.M.R. is a scientific advisor to Alzheon, Aural Analytics, Denali, Green Valley, MaQ and United Neuroscience, and to Roche and Roche Diagnostics (compensation for travel only). His NIH-supported

studies include research contracts with Avid/Eli Lilly, Genentech/Roche and Novartis/Amgen. O.H. has acquired research support (for the institution) from Roche, GE Healthcare, Biogen, AVID Radiopharmaceuticals and Euroimmun. In the past 2 years he has received consultancy/speaker fees (paid to the institution) from Biogen and Roche. T.G.B. has served on a scientific advisory board and has been a consultant for Vivid Genomics and Prothena Biosciences. The sponsors mentioned above had no role in the design and conduct of the study, collection, management, analysis and interpretation of the data, nor in preparation, review and approval of the manuscript. U.E. is an employee of the Roche Group. X.C., N.K.P. and J.L.D. are employees of Eli Lilly and Company.

Additional information

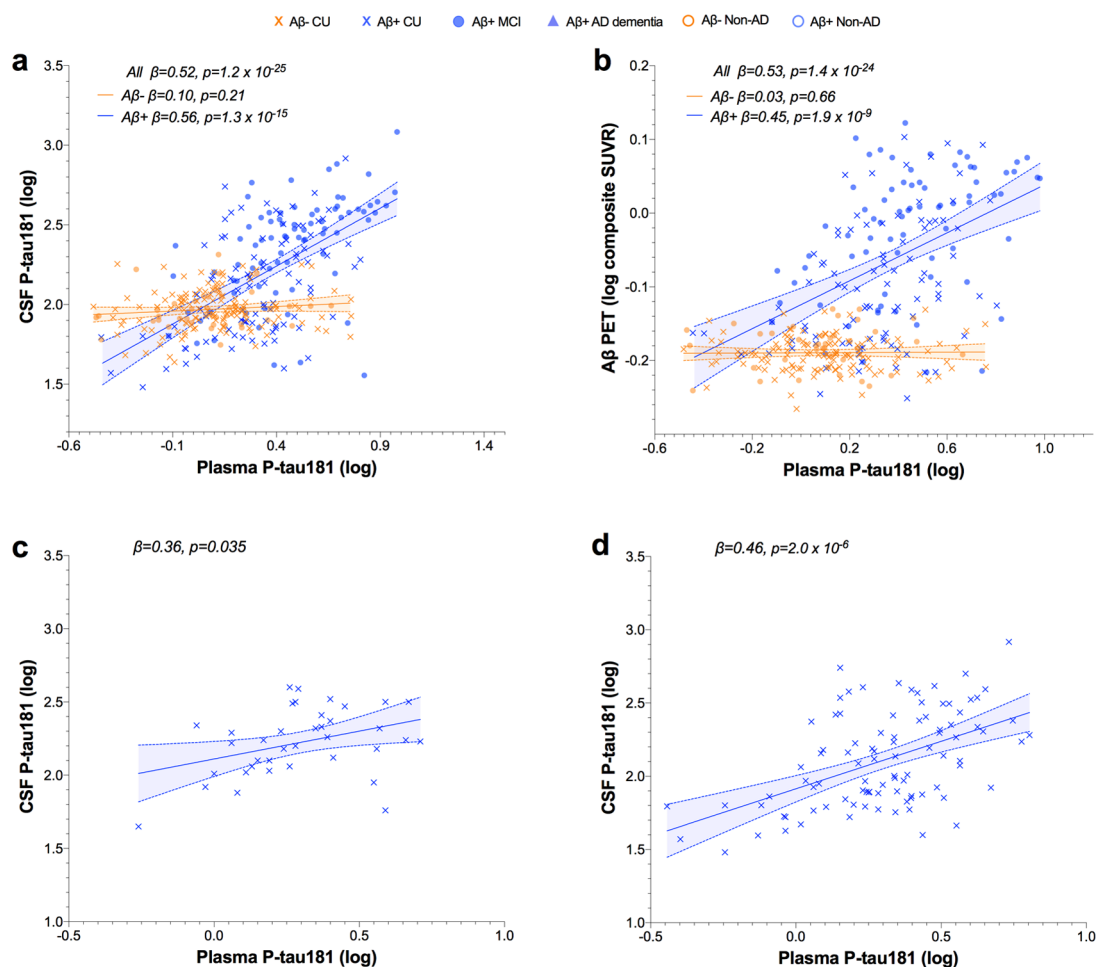
Extended data is available for this paper at <https://doi.org/10.1038/s41591-020-0755-1>.

Supplementary information is available for this paper at <https://doi.org/10.1038/s41591-020-0755-1>.

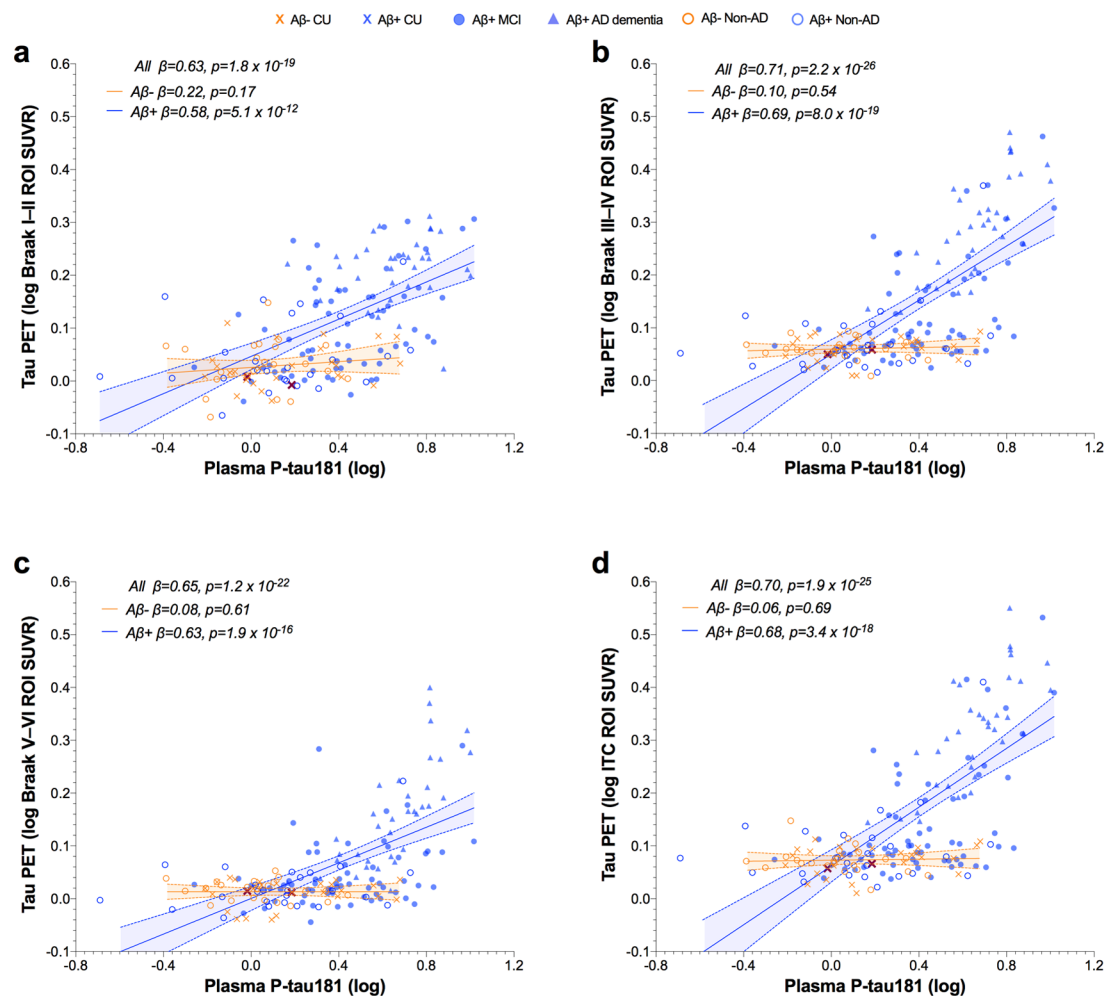
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Peer review information Brett Benedetti and Kate Gao were the primary editors on this article and managed its editorial process and peer review in collaboration with the rest of the editorial team.

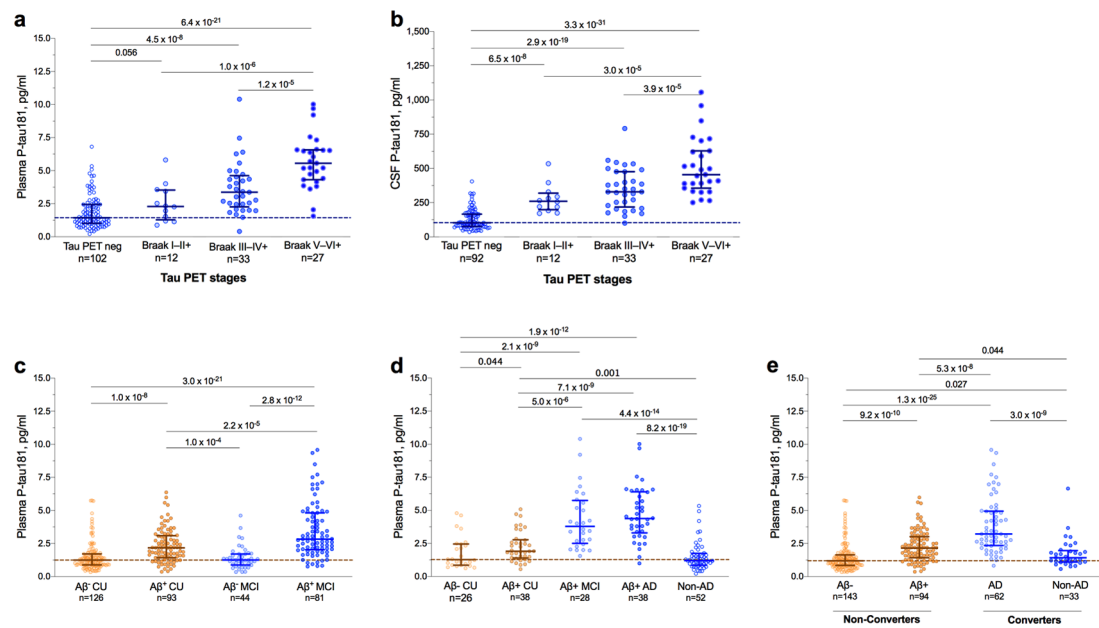
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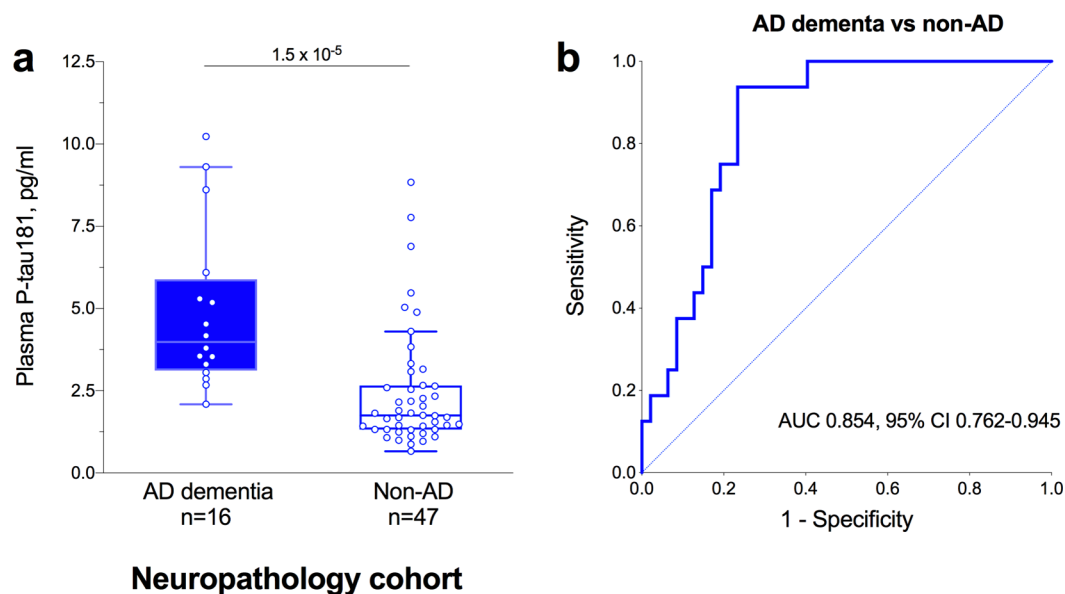
Extended Data Fig. 1 | Association of plasma P-tau181 with CSF P-tau181 and A β PET. (a) Association between plasma and CSF P-tau181 in cohort 2 ($n = 343$ [A β^+ $n = 173$; A β^- $n = 170$]). **(b)** Association between plasma P-tau181 and A β PET in cohort 2 ($n = 324$ [A β^+ $n = 164$; A β^- $n = 160$]). **(c)** Association between plasma and CSF P-tau181 in A β^+ cognitively unimpaired in cohort 1 ($n = 37$). **(d)** Association between plasma and CSF P-tau181 in A β^+ cognitively unimpaired in cohort 2 ($n = 93$). Data are shown as β (standardized coefficient) and p value from linear regression adjusted for age and sex as covariates; linear regression lines with 95% CI (shaded regions) are from unadjusted models. A β positivity was defined using CSF A β 42 cutoff value of < 510 pg/ml and A β PET SUVR cutoff value of > 0.743 . Study participants who underwent both lumbar puncture and A β PET imaging were considered A β positive if either CSF A β 42 or A β PET measures were abnormal.



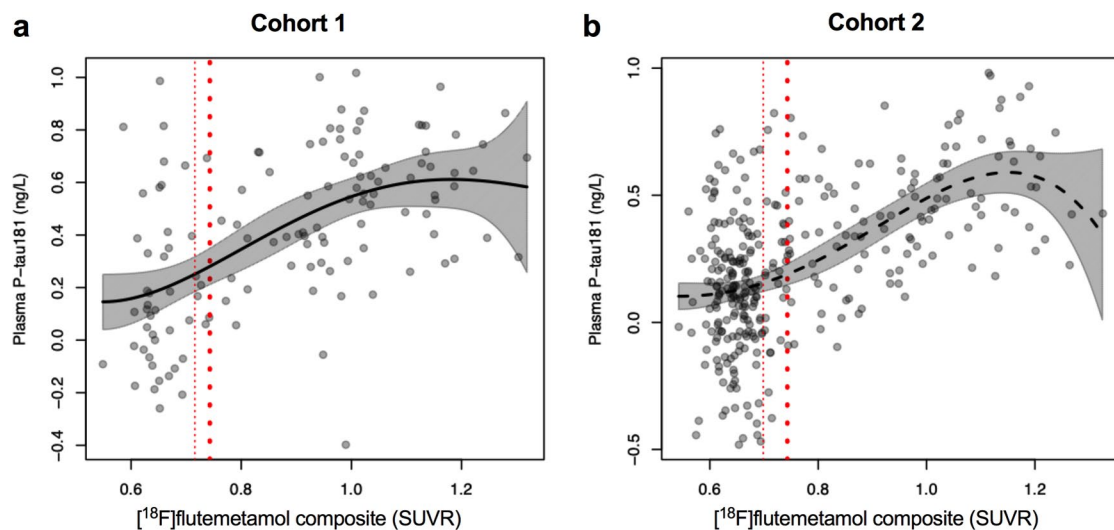
Extended Data Fig. 2 | Plasma P-tau181 and Tau PET in cohort 1. Associations between plasma P-tau181 and Tau PET ($n=174$ [A β + $n=124$; A β - $n=48$; A β undefined $n=2$] in *a priori* defined brain regions linked to tau pathology in AD, the Braak I–II ROI (**a**), III–IV ROI (**b**), V–VI ROI (**c**) and inferior temporal cortex ROI (**d**). Data are shown as β (standardized coefficient) and p value from linear regression adjusted for age and sex; linear regression lines with 95% CI (shaded regions) are from unadjusted models. A β positivity was defined using CSF A β 42 cutoff value of <510 pg/ml and A β PET SUVR cutoff value of >0.743 . Study participants who underwent both lumbar puncture and A β PET imaging were considered A β positive if either CSF A β 42 or A β PET measures were abnormal. Two study participants with undefined A β status are represented as red x points. AD = Alzheimer disease; A β + = Amyloid- β positive; A β - = Amyloid- β negative; MCI = mild cognitive impairment; PET = positron emission tomography; ITC = inferior temporal cortex; ROI = region of interest; SUVR = standardized uptake value ratio.



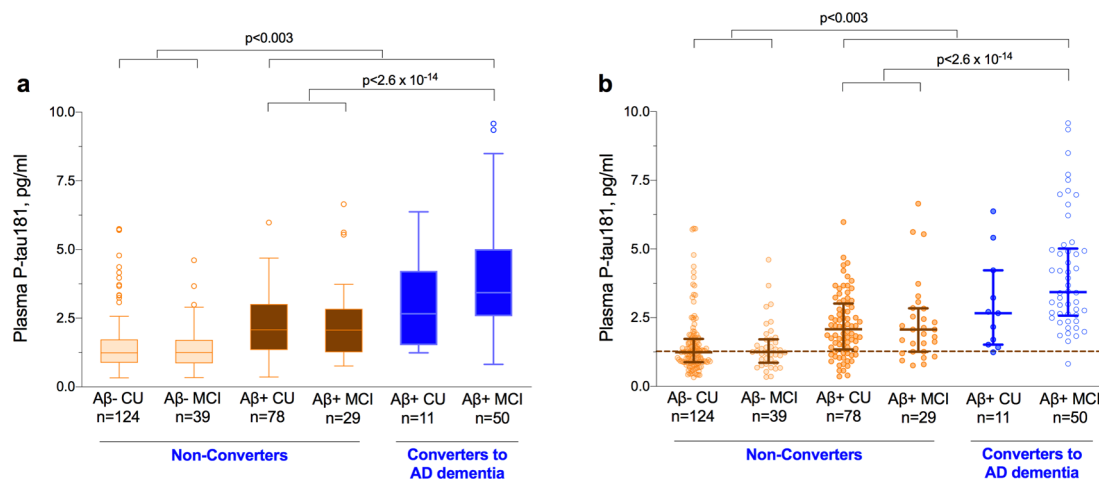
Extended Data Fig. 3 | Scatter plots of plasma P-tau181. (a) Scatter plot for Fig. 2a. (b) Scatter plot for Fig. 2b. (c) Scatter plot for Fig. 2d. (d) Scatter plot for Fig. 2e. (e) Scatter plot for Fig. 3a. P-values are from univariate general linear models adjusted for age and sex and additionally for years of education in (e) as described in the methods. Solid horizontal lines represent median and error bars correspond to interquartile range; dashed horizontal lines indicated median in the Tau PET negative group (a, b), Aβ- CU group (c, d) and Aβ- group (e).



Extended Data Fig. 4 | Plasma P-tau181 in cohort 3. (a) Plasma concentrations of P-tau181 in cases with AD dementia and high likelihood that dementia was due to AD histopathology according to NIA-Reagan criteria versus individuals with no or sparse neuritic plaques. P-values are from univariate general linear models adjusted for age and sex; boxes show interquartile range, the horizontal lines are medians and the whiskers were plotted using Tukey method. (b) ROC curve analyses for distinguishing the AD dementia group (n=16) from non-AD group (n=47). AD = Alzheimer disease; AUC = area under the curve; CI = confidence interval; NIA = NIA-R, National Institute on Aging-Reagan Institute Working Group; ROC = receiver operating characteristic.



Extended Data Fig. 5 | Plasma P-tau181 and Aβ PET. Plasma P-tau181 in relation to global cortical Aβ load in cohort 1 (**a**, $n=129$) and cohort 2 (**b**, $n=324$). The solid lines are fits from spline models of P-tau181 on $[^{18}\text{F}]$ flutemetamol. Associations between plasma P-tau181 (log-transformed) and continuous Aβ PET uptake were tested with non-linear polynomial spline models (using I-spline basis), to detect Aβ PET thresholds for increased P-tau181 (the Aβ level where the spline increased at least two standard errors from baseline). The shaded area represents 95% CI. The thick dotted line shows an a priori Aβ PET threshold for Aβ PET positivity (0.743 SUVR). The thin dotted lines indicate the $[^{18}\text{F}]$ flutemetamol level where plasma P-tau181 is significantly increased from baseline. PET = positron emission tomography; SUVR = standardized uptake value ratio.



Extended Data Fig. 6 | Plasma P-tau181 and progression to AD dementia. Boxplot (**a**) and scatterplot (**b**) for plasma P-tau181 concentrations in Aβ⁺ and Aβ⁻ cognitively unimpaired (CU) and MCI patients who did not develop AD dementia or developed AD dementia during clinical follow-up. No participants from the Aβ⁻ CU group progressed to AD dementia. Data from one Aβ⁻ MCI who converted to AD dementia was excluded from the figure. P-values are from univariate general linear models adjusted for age and sex as described in the methods. In (**a**) boxes show interquartile range, the horizontal lines are medians and the whiskers were plotted using Tukey method; in (**b**) solid horizontal lines represent median, error bars correspond to interquartile range and dashed horizontal lines indicated median in the Aβ⁻ CU group. AD = Alzheimer disease; Aβ⁺ = Amyloid-β positive; Aβ⁻ = Amyloid-β negative; MCI = Mild cognitive impairment.

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Software and code

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Data collection

Data is exported to csv-files and stored at Skåne University Hospital, Sweden.

Data analysis

SPSS version 24 (IBM, Armonk, NY, US), R studio and R version 3.5.3 (packages pROC and splines2); FreeSurfer (version 5.3), SPM12 (<http://www.fil.ion.ucl.ac.uk/spm>), CARET v5.65 (Van Essen Lab; <http://brainvis.wustl.edu>), Prism 8.

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Life sciences study design

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Sample size	The study included two prospective cohorts (from the Swedish BioFINDER study) with large sample size (n=182 and n=344) and neuropathology confirmed cohort (n=63) from the Arizona Study of Aging and Neurodegenerative Disorders/Brain and Body Donation Program. All three cohorts were convenience cohorts and all available plasma samples were analyzed in this study. The results are strongly convergent and positive. There is no indication that we were insufficiently powered for these analyses.
Data exclusions	Outliers with values more than 3 SD above the mean (n=7) were excluded from the main analysis. A sensitivity analysis including the outliers was performed with very similar results which are described in Supplementary Results. We used a commonly accepted criteria (mean+3SD) for exclusion of the outliers. The fact the results were similar when excluding these outliers confirms that the results are very robust.
Replication	The study included two different prospective cohorts with similar study designs, but cohort 1 also included Tau PET imaging and cohort 2 included longitudinal follow-up to track conversion to AD dementia. When similar types of data were available in both cohorts (e.g. correlations of plasma P-tau181 with CSF P-tau181, Tau PET or clinical diagnosis) we reproduced the results in both cohorts (all attempts at replication were successful). Furthermore, in an independent cohort with neuropathological data, antemortem plasma P-tau181 was associated with AD neuropathology at autopsy. Finally, our manuscript has been submitted together with another manuscript from Dr. Adam Boxer's group at UCSF for consideration of joint publication. These two manuscripts provide important replication and cross-validation of the results in multiple independent cohorts.
Randomization	In these 3 cohort studies (observational studies) no allocation into experimental groups were performed, therefore randomization is not relevant to this study. Statistical analyses were controlled for potential confounding effect of age and sex.
Blinding	All plasma, CSF and PET analyses were performed by individuals who were blinded to the clinical data.

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<input type="checkbox"/>	<input checked="" type="checkbox"/> Clinical data		

Antibodies

Antibodies used	Detailed information regarding the antibodies as applicable for immunoassays is provided in the manuscript. The assay was performed on a streptavidin plate on the Meso Scale Discovery (MSD) platform. Biotinylated-AT270 was used as capture antibody (anti-pT181 Tau antibody; Thermo Fisher, catalog number: MN1050) and SULFO-TAG-LRL (anti-tau monoclonal antibodies developed by Lilly Research Laboratory) as detector. Antibodies were conjugated with Sulfo-NHS-Biotin (Thermo Scientific, catalog number: 21327) or MSD GOLD SULFO-TAG NHS-Ester (MSD, catalog number: R91AO) according to the manufacturer's protocol. The assay was calibrated using a recombinant tau (4R2N) protein that was phosphorylated in vitro.
Validation	Immunoassay for detection of P-tau181 in human plasma has been previously described by Mielke et al. (Alzheimers Dement 14, 989-997, 2018). Fit for purpose assay validation has been performed by Eli Lilly according to Andreasson et al. (Frontiers in Neurology 2015) and within study validation is included within the manuscript supplement (eTable 6).

Human research participants

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Population characteristics	Detailed information is given in table 1, e-tables 1 and eMethods. In short, we present results for analyses from three different cohorts. Cohort 1 and cohort 2 had similar study designs, but cohort 1 also included Tau PET imaging and cohort 2 included longitudinal follow-up to track conversion to AD dementia over a period of up eight years. Cohort 1 included 64 cognitively unimpaired participants, 28 mild cognitive impairment (MCI) due to AD, 38 AD dementia, and 52 patients with non-AD neurodegenerative diseases. Cohort 2 included 219 cognitively unimpaired participants and 125 MCI patients. In cohort 1, out of 182 participants (median (IQR) age, 73 (67-78) years), 79 were women. In cohort 2, out of 344 participants (median (IQR) age, 72 (68-75) years), 174 were women. Cohort 3 included 16 autopsy confirmed AD dementia, and 47 autopsy confirmed non-AD individuals from the Arizona Study of Aging and Neurodegenerative Disorders/Brain and Body Donation Program.
Recruitment	This project was done as part of the prospective Swedish BioFINDER study and included two separate cohorts recruited at Skåne University Hospital and the Hospital of Ängelholm, Sweden. Cohort 1 included 182 participants enrolled between November 2014 and January 2018 and cohort 2 included 344 non-demented participants enrolled between January 2010 and December 2014 who were followed longitudinally up to eight years. Recruitment of patients with cognitive impairment or neurological diseases was done at Memory clinics and Neurology clinics. Recruitment of cognitively unimpaired controls was done through advertisements. The results for the patients may therefore be biased for a specialist setting. As we already state in the discussion our findings should be validated in a primary care setting
Ethics oversight	The study was approved by the Regional Ethics Committee in Lund, Sweden. All participants gave their informed consent to participate in the study. In cohort 3, all subjects or their legal representatives signed an Institutional Review Board-approved informed consent form before taking part in the study.

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

Clinical data

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Clinical trial registration	NCT01208675
Study protocol	Provision of the clinical protocol will be considered upon request by qualified researchers.
Data collection	Full details were provided at https://clinicaltrials.gov/ct2/show/NCT01208675?term=biofinder&rank=2 . Cohorts 1 and 2 were part of the prospective Swedish BioFINDER study with participants recruited at Skåne University Hospital and the Hospital of Ängelholm, Sweden. Cohort 1 enrolled between November 2014 and January 2018 and cohort 2 included non-demented participants enrolled between January 2010 and December 2014. Data was collected between January 2010 and May 2019. In cohort 3, fluid data was collected from February 2007 until November 2018.
Outcomes	The main outcomes of this particular study were differences in plasma P-tau181 between diagnostic groups, prediction of conversion to AD dementia, associations with other fluid and imaging biomarkers, and detection AD pathology (as explained in the introduction). These outcomes follow from our overall outcomes for the BioFINDER study (as explained at Clinicaltrials.gov , see link: https://clinicaltrials.gov/ct2/show/NCT01208675?term=biofinder&rank=2), which also involves other biomarkers.