Diagnostic value of plasma phosphorylated tau181 in Alzheimer’s disease and frontotemporal lobar degeneration

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With the potential development of new disease-modifying Alzheimer’s disease (AD) therapies, simple, widely available screening tests are needed to identify which individuals, who are experiencing symptoms of cognitive or behavioral decline, should be further evaluated for initiation of treatment. A blood-based test for AD would be a less invasive and less expensive screening tool than the currently approved cerebrospinal fluid or amyloid β positron emission tomography (PET) diagnostic tests. We examined whether plasma tau phosphorylated at residue 181 (pTau181) could differentiate between clinically diagnosed or autopsy-confirmed AD and frontotemporal lobar degeneration. Plasma pTau181 concentrations were increased by 3.5-fold in AD compared to controls and differentiated AD from both clinically diagnosed (receiver operating characteristic area under the curve of 0.894) and autopsy-confirmed frontotemporal lobar degeneration (area under the curve of 0.878). Plasma pTau181 identified individuals who were amyloid β-PET-positive regardless of clinical diagnosis and correlated with cortical tau protein deposition measured by 18F-flortaucipir PET. Plasma pTau181 may be useful to screen for tau pathology associated with AD.

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at residue 181 (pTau181) in FTLD, CSF tau and pTau181 can be either elevated or decreased. Insoluble tau deposition can be visualized in the brains of living individuals with AD using florataucipir (FTP)-PET, a tracer that binds with high affinity to mixed 3 and 4 microtubule binding domain repeat (3R/4R) tau that is found in AD ν-path, neurofilibrillary tangles and can distinguish clinical AD (ADClin) from other diseases. However, FTP has low affinity for the predominantly 3R or 4R tau deposits found in most FTLD, limiting its usefulness. In contrast, levels of neurofilament light chain (NFL) a marker of axonal damage measurable in CSF, plasma and serum are increased in FTLD and correlate with survival, clinical severity and brain volume. CSF and NFL concentrations are also elevated in ADClin but less so than in FTLD. As in FTLD, serum NFL is predictive of cortical thinning and rate of disease progression in ADClin.

Recent studies have shown that the Aβ42/Aβ40 ratio measured in plasma can differentiate between healthy controls and patients with AD using immunoprecipitation mass spectrometry (IP–MS), but this technology is not accessible to most clinical laboratories. New ultrasensitive single molecule array (Simoa) antibody-based approaches measuring Aβ in blood are easier to implement but do not yet have sufficient diagnostic precision to be useful clinically. Elevated levels of total tau measured with Simoa technology in plasma are associated with cognitive decline, although there is substantial overlap between concentrations measured in normal aging and AD limiting the diagnostic usefulness of such assays.

Recently, a new plasma pTau181 assay was found to differentiate ADClin from healthy controls. We tested the diagnostic differential ability of plasma pTau181 measurements to differentiate MCI and ADClin relative to a variety of clinical FTLD phenotypes. A subset of diagnoses was verified using neuropathological examination at autopsy or by the presence of autosomal dominant mutations that lead to specific types of FTLD pathology, including mutations in the tau gene (MAPT) that lead to FTLD pure 4R tau or AD-like mixed 3R/4R tau deposition in the brain. We also compared plasma pTau181 to current clinical standards for dementia differential diagnosis, Aβ-PET and CSF pTau181, as well as to the research biomarkers plasma NFL, plasma Aβ42 and Aβ40, FTP-PET and brain atrophy measured with magnetic resonance imaging (MRI), to better evaluate the biological basis for elevated plasma pTau181.

**Results**

**Participant characteristics.** Baseline demographics, clinical assessments, imaging measures and fluid biomarker levels are shown in Table 1. The control group (HC) and the MCI group were younger than the PSP and nfPPA groups. Plasma pTau181 and NFL concentrations were similar in men and women. Plasma NFL concentrations correlated with age (ρ = 0.19, P = 0.006) and with time between blood draw and death in autopsy cases (ρ = −0.27, P = 0.009); pTau181 concentrations were not correlated with either value. Plasma pTau181 concentrations were associated with the clinical dementia rating scale sum of boxes score (CDRsb) (β = 0.184, P = 0.004, Supplementary Table 1), as were NFL concentrations (β = 0.456, P < 0.0001, Supplementary Table 2). FTP-PET binding was highest in ADClin cases compared to MCI, corticobasal syndrome (CBS), PSP, bvFTD and nfPPA. Pittsburgh Compound B (PiB) Aβ-PET binding was highest in ADClin. Overall, 27% of controls were Aβ-PET positive (visual read). CSF pTau181 was higher in ADClin compared to every other diagnosis, except for MCI and semantic variant primary progressive aphasia (svPPA).

**Plasma pTau181 and NFL comparisons by clinical diagnostic group.** Plasma pTau181 concentrations were elevated in ADClin compared to all other groups (Fig. 1a and Table 1). Plasma NFL concentrations were elevated in CBS, PSP and bvFTD compared to ADClin and MCI as well as controls (Fig. 1b). NFL concentrations were also elevated in nfPPA and svPPA as compared to controls and MCI. NFL was increased in AD compared to HC. The ratio of pTau181/NFL was decreased in all FTLD diagnoses compared to controls, ADClin and patients with MCI (extended data Fig. 1). The individuals with AD-associated logopenic variant primary progressive aphasia (lvPPA) had increased pTau181 levels compared to the those with FTLD-associated nfPPA, svPPA and controls (Fig. 1c). An age-adjusted plasma pTau181 cutoff of 8.7 pg ml⁻¹ differentiated ADClin from clinical FTLD with a receiver operating characteristic (ROC) area under the curve (AUC) of 0.894 (P < 0.0001, Fig. 1d and Table 2). The plasma Aβ42/Aβ40 ratio did not differ between the clinical diagnostic groups (Extended Data Fig. 2a), but was able to differentiate between Aβ-PET-positive and negative cases (AUC of 0.768, P < 0.0001, Extended Data Fig. 2b and Table 2) and FTP-PET-positive and negative cases (AUC of 0.782, P < 0.0001, Extended Data Fig. 2c and Table 2).

**Plasma pTau181 and NFL in pathology-verified cases and FTLD mutation carriers.** Neuropathological diagnosis was available in 82 cases. Owing to potential effects of disease severity, analyses were adjusted for age and CDRsb at the time of blood draw. Median plasma pTau181 concentrations were higher in ADClin (n = 15, 7.5 ± 8 pg ml⁻¹) compared to FTLD-tau (n = 52, 2.3 ± 3 pg ml⁻¹, P < 0.0001) and FTLD-TAR DNA-binding protein (FTLD-TDP) (n = 15, 2.1 ± 2 pg ml⁻¹, P < 0.0001, Fig. 2a). Plasma pTau181 differentiated ADClin from the combined FTLD-TDP and FTLD-tau group (AUC of 0.878, P < 0.0001, Fig. 2b), from FTLD-TDP alone (AUC of 0.947, P < 0.0001) and from FTLD-tau alone (AUC of 0.858, P < 0.0001, Table 2). Plasma NFL was a poor discriminator of ADClin from neuropathologically diagnosed FTLD (Table 2). Presence of pTau181 was associated with autopsy-defined Braak stage (β = 0.569, P < 0.0001) and was higher in Braak stage 5–6 (n = 16, 4.9 ± 4 pg ml⁻¹) compared to Braak stage 0 (n = 10, 2.1 ± 2 pg ml⁻¹, P = 0.003), Braak stage 1–2 (n = 42, 2.2 ± 2 pg ml⁻¹, P < 0.0001) and Braak stage 3–4 (n = 13, 2.3 ± 3 pg ml⁻¹, P = 0.009, Fig. 2c). NFL did not differ by Braak stage (Extended Data Fig. 3).

Seventy-six individuals were FTLD-causing mutation carriers (61 MAPT, 5 GRN and 10 C9orf72). There was no difference in pTau181 concentrations between the mutation carriers (grouped by mutated gene) or the mutation carrier groups and normal controls (Extended Data Fig. 4). Plasma pTau181 levels were increased in MAPT mutation carriers with AD-like mixed 3R/4R tau pathology (n = 17, 4.4 ± 4 pg ml⁻¹, Fig. 2d), compared to those with pure 4R tau pathology (n = 44, 2.2 ± 2 pg ml⁻¹, P = 0.024) and controls (n = 44, 2.0 ± 2 pg ml⁻¹, P = 0.011). Plasma pTau181 differentiated ADClin from neuropathologically diagnosed FTLD and mutation carriers combined (AUC of 0.854, P < 0.0001, Table 2).

**Association between plasma pTau181 and other fluid biomarkers.** Plasma pTau181 and plasma NFL concentrations were associated in combined ADClin/MCI cases (β = 0.66, P < 0.0001, Fig. 3a), but not in the whole patient sample. CSF pTau181 was associated with plasma pTau181 in the whole sample (β = 0.51, P < 0.0001; n = 74, Extended Data Fig. 5) and both within the AD/MCI group (β = 0.41, P = 0.042; n = 25) and the FTLD group (β = 0.49, P < 0.0001; n = 29), but not in controls. CSF pTau181 concentrations were higher in ADClin (45.8 ± 31 pg ml⁻¹) compared to FTLD (22.1 ± 8 pg ml⁻¹, P < 0.0001) and differentiated the two clinical diagnoses (AUC of 0.931, P < 0.0001, Tables 1 and 2).

**Plasma pTau181 and NFL associations with tau (FTP)-PET and Aβ-PET.** There were strong linear relationships between plasma pTau181 concentrations and PiB standardized uptake value ratio (SUVR) (β = 0.75, P < 0.0001, Fig. 3b) as well as global cortical FTP SUVR (β = 0.73, P < 0.0001, Fig. 3c). Plasma NFL concentration was not related to either PET measure. An age-corrected
### Table 1 | Participant characteristics, primary cohort

| Parameter | HC (N = 69) | MCI (N = 47) | AD (N = 56) | CBS (N = 39) | PSP (N = 48) | bvFTD (N = 50) | nfvPPA (N = 27) | svPPA (N = 26) | All (N = 362) |
|-----------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|
| Sex, M/F | 37/32 | 26/21 | 23/33 | 16/23 | 21/27 | 28/22 | 15/12 | 10/16 | 176/186 |
| Age, y | 60.6 (22) | 60.8 (14) | 65.0 (9) | 68.0 (8) | 69.4 (7) | 58.3 (9) | 70.5 (7) | 69.3 (7) | 1086 (28) |
| APOE E4 positivity | | | | | | | | | |
| N (%) | 18/65 (28%) | 6/34 (18%) | 20/29 (69%) | 9/36 (25%) | 9/47 (19%) | 12/48 (25%) | 5/20 (25%) | – | 82/288 (28%) |
| Average disease duration | – | 5.7 (3) | 6.0 (3) | 6.0 (4) | 6.7 (3) | 8.5 (8) | 5.9 (2) | 8.0 (4) | 6.6 (5) |
| N | – | 40 | 37 | 36 | 47 | 45 | 9 | 4 | 219 |
| Disease severity | | | | | | | | | |
| CDRb | 0 (0) | 2.0 (1) | 4.8 (3) | 3.3 (3) | 4.7 (3) | 7.8 (3) | 3.4 (3) | 6.0 (3) | 3.6 (3) |
| N | 65 | 47 | 56 | 39 | 46 | 31 | 27 | 26 | 337 |
| SEADL, % | 100 (2) | 89 (18) | 75 (16) | 47 (22) | 43 (26) | 45 (19) | 67 (25) | 58 (19) | 62.8 (30) |
| N | 34 | 14 | 6 | 29 | 45 | 21 | 22 | 17 | 188 |
| FAQ | 0.1 (0) | 4.6 (5) | 14.1 (7) | 15.0 (6) | 20.5 (6) | 8.0 (8) | 16.0 (8) | 10.3 (9) | |
| N | 63 | 45 | 39 | 45 | 51 | 31 | 22 | 26 | 326 |
| GDS | 2.0 (2) | 6.3 (6) | 6.9 (5) | 10.6 (6) | 13.3 (6) | 6.8 (6) | 5.2 (6) | 7.0 (4) | 6.8 (6) |
| N | 68 | 42 | 46 | 29 | 41 | 26 | 17 | 16 | 287 |
| Neuropsychology | | | | | | | | | |
| MMSE | 29.0 (1) | 26.8 (3) | 20.3 (6) | 23.4 (6) | 24.7 (4) | 20.7 (9) | 22.7 (6) | 19.2 (8) | 23.7 (6) |
| N | 44 | 39 | 52 | 37 | 45 | 27 | 14 | 26 | 282 |
| Modified trails test, seconds | 26.6 (17) | 40.6 (21) | 89.7 (37) | 79.4 (40) | 90.4 (36) | 76.8 (40) | 91.3 (35) | 61.3 (9) | 64.9 (40) |
| N | 43 | 34 | 28 | 39 | 45 | 31 | 22 | 26 | 324 |
| Stroop color naming, seconds | 82.3 (16) | 71.0 (18) | 51.8 (22) | 44.3 (21) | 42.4 (20) | 52.0 (16) | 34.3 (16) | 30.5 (6) | 58.4 (24) |
| N | 38 | 38 | 34 | 36 | 36 | 36 | 36 | 20 | 191 |
| Semantic fluency, words per minute | 16.2 (5) | 13.3 (4) | 9.5 (6) | 7.3 (4) | 6.6 (6) | 5.0 (3) | 3.7 (2) | 5.3 (2) | 9.8 (6) |
| N | 41 | 37 | 36 | 32 | 46 | 22 | 6 | 3 | 225 |
| BNT, number of words | 14.7 (1) | 13.6 (2) | 11.9 (3) | 12.8 (3) | 13.1 (2) | 10.9 (5) | 11.9 (4) | 1.7 (2) | 12.8 (3) |
| N | 43 | 36 | 36 | 31 | 46 | 24 | 7 | 3 | 228 |
| D-word fluency, words per minute | 10.7 (3) | 9.0 (3) | 5.2 (3) | 4.5 (3) | 5.2 (3) | 4.7 (4) | 4.8 (3) | 2.3 (1) | 6.9 (4) |
| N | 38 | 38 | 34 | 18 | 3 | 22 | 6 | 3 | 187 |
| Modified Rey copy, points | 15.5 (1) | 15.2 (1) | 12.8 (4) | 10.9 (5) | 11.5 (3) | 15 (2) | 13.2 (3) | 15.8 (1) | 13.5 (3) |
| N | 37 | 39 | 36 | 27 | 39 | 21 | 5 | 4 | 210 |
| Modified Rey recall, points | 12.6 (2) | 8.7 (4) | 3.2 (4) | 7.4 (5) | 8.9 (3) | 8.6 (5) | 10.2 (5) | 2.8 (4) | 8.2 (5) |
| N | 37 | 39 | 37 | 27 | 38 | 23 | 5 | 4 | 212 |
| Imaging | | | | | | | | | |
| Whole brain volume, l | 1.1 (0.1) | 1.1 (0.1) | 1.0 (0.1) | 1.0 (0.1) | 1.0 (0.1) | 0.9 (0.1) | 1.0 (0.2) | 1.0 (0.1) | 1.0 (0.1) |
| N | 39 | 36 | 36 | 34 | 42 | 22 | 9 | 3 | 220 |
| Bilateral hippocampal volume, mm³ | 5,332.8 (526) | 5,197.7 (757) | 4,790.5 (704) | 5,148.6 (660) | 5,188.9 (491) | 4,616.5 (612) | 5,320.2 (871.2) | 3,958.9 (622) | 5,063.3 (681) |
| N | 39 | 35 | 36 | 34 | 42 | 22 | 9 | 3 | 220 |

Continued
plasma pTau181 cutoff value for Aβ-PET positivity of 8.0 pg ml⁻¹ discriminated between all individuals who were Aβ-PET-positive and negative with 0.889 sensitivity, 0.853 specificity and AUC of 0.914 (P < 0.0001, Fig. 3d and Table 2). Plasma pTau181 also differentiated between Aβ-PET-positive and negative cases within the healthy controls and MCI groups individually. In controls, the AUC was 0.859 (P < 0.0001, 11 Aβ-PET-positive and 29 Aβ-PET-negative). Within the MCI group, the AUC was 0.944 (P < 0.0001, 18 Aβ-PET positive and 21 Aβ-PET-negative; Table 2 and Extended Data Fig. 6).

When a cortical FTP-SUVr diagnostic threshold of 1.22 was applied to designate all cases as FTP-PET-positive and negative, plasma pTau181 was also a good discriminator of FTP-PET status (AUC of 0.919, P < 0.0001, Fig. 3e). In the MCI cases alone, the AUC for FTP-PET status was 0.977 (P < 0.0001, 11 FTP-PET-positive and 20 FTP-PET-negative; Table 2). Similar relationships between plasma pTau181 and FTP-PET values were obtained with the independent cohort from an Eli Lilly ADclin/MCI clinical research study (n = 42; Supplementary Results and Supplementary Table 3). Plasma NfL did not differentiate between Aβ-PET-positive and negative cases (AUC of 0.559, P = 0.276) or between FTP-PET-positive and negative cases (AUC of 0.606, P = 0.159, Table 2). Levels of pTau181 were associated with FTP-PET-estimated Braak stage 3-5 (β = 0.610, P < 0.0001) and were higher in FTP-PET Braak stage 5–6 (n = 54, 9.2 ± 4 pg ml⁻¹) and Braak stage 3–4 (n = 8, 6.4 ± 3 pg ml⁻¹) compared to Braak stage 0 (n = 26, 2.4 ± 2 pg ml⁻¹, both P < 0.0001). NfL did not differ by FTP-estimated Braak stage (Extended Data Fig. 7).

Voxelwise analyses of FTP-PET and gray matter volume in relation to plasma pTau181 and NfL. Concentrations of pTau181 were strongly associated with FTP-PET SUVR values (Spearman’s ρ values exceeding 0.70 in peak regions) in the frontal, temporoparietal and posterior cingulate cortices and precuneus regions (Fig. 4a). Associations remained significant in the patients with only ADclin/MCI, although with slightly lower ρ values. There were insufficient data to perform the analyses in the FTLD group separately (n = 18). There was no association between NfL concentrations and FTP-PET uptake in the whole group. In the patients with only ADclin/MCI there were weak correlations in the right hemisphere that did not survive multiple-comparisons corrections, predominantly in the frontal and insular cortex and in the right temporal horn (reaching ρ ~ 0.6 in the insula; Fig. 4a).

### Table 1 | Participant characteristics, primary cohort

|                  | HC (N = 69) | MCI (N = 47) | ADclin* (N = 56) | CBS (N = 39) | PSP (N = 48) | bvFTD (N = 50) | nvPPA (N = 27) | svPPA (N = 26) | All (N = 362) |
|------------------|-------------|--------------|------------------|-------------|-------------|----------------|----------------|---------------|---------------|
| FTP-PET SUVR     | ~             | 1.2 (0)     | 1.8 (0)  | 1.1 (0)  | 1.0 (0)  | 1.0 (0)  | 1.4 (0)  | 1.5 (0)  |               |
| N                | ~             | 31           | 48            | 4          | 4          | 5              | 2              | 3              | 97            |
| PiB-PET SUVR     | 1.2 (0)      | 1.5 (0)     | 2.1 (0)  | 1.3 (0)  | 1.2 (0)  | 1.2 (0)  | 1.1 (0)  | 1.6 (1)  |               |
| N                | 10           | 37           | 36            | 13         | 6          | 12             | 6              | 4              | 124           |
| Amyloid-PET read | 29/11        | 21/18        | 0/51          | 16/3       | 6/0        | 10/3           | 5/2            | 8/2            | 95/90         |

Fluid biomarkers

|                  | Plasma pTau181 (pg ml⁻¹) | Plasma NfL (pg ml⁻¹) | Plasma pTau181/NfL ratio | Plasma Aβ42 (pg ml⁻¹) | Plasma Aβ40 (pg ml⁻¹) | Plasma Aβ42/Aβ40 ratio |
|------------------|-------------------------|----------------------|-------------------------|-----------------------|-----------------------|------------------------|
| N                | 28                      | 28                   | 26                      | 32                    | 45                    | 40                     | 9                      | 4              | 214           |
| CSF pTau181 (pg ml⁻¹) | 24.4 (12)               | 37.9 (24)            | 45.8 (31)               | 22.2 (11)             | 18.1 (5)              | 20.7 (12)              | 14.7 (15)              | 27.0 (24)       | 32.8 (20)      |
| N                | 20                      | 9                    | 16                      | 11                    | 4                     | 9                      | 3                      | 2              | 74            |
| Plasma Aβ42 (pg ml⁻¹) | 21.5 (6)                | 22.0 (8)             | 19.7 (7)                | 23.5 (9)              | 23.5 (8)              | 23.3 (6)               | 17.0 (2)               | 31.5 (-)        | 21.6 (8)       |
| N                | 38                      | 38                   | 35                      | 25                    | 27                    | 12                     | 2                      | 1              | 178           |
| Plasma Aβ40 (pg ml⁻¹) | 248.4 (52)              | 236.6 (49)           | 245.9 (43)             | 262.8 (59)            | 252.6 (50)            | 231.3 (40)             | 218.9 (9)              | 311.5 (-)       | 249.4 (55)     |
| N                | 38                      | 38                   | 35                      | 25                    | 27                    | 12                     | 2                      | 1              | 178           |
| Plasma Aβ42/Aβ40 ratio | 0.09 (0)               | 0.09 (0)             | 0.08 (0)                | 0.09 (0)              | 0.09 (0)              | 0.08 (0)               | 0.10 (-)               | 0.09 (0)        |               |
| N                | 38                      | 38                   | 35                      | 25                    | 27                    | 12                     | 2                      | 1              | 178           |

Values are shown as mean (s.d.); fluid biomarker values are shown as median (interquartile range). APOE, apolipoprotein E; GDS, geriatric depression scale; HC, healthy control; modified Rey copy, modified Rey-Benson Figure copy; SEADL, Schwab and England activities of daily living. Amyloid status was based on visual read of 18F-AV-45 and PiB-PET imaging. “Indicates a statistically significant difference between groups (P < 0.05) with HCs in post hoc pairwise comparisons. *P < 0.05 versus MCI. **P < 0.05 versus AD.”
High plasma pTau181 concentrations correlated with lower gray matter volume in the bilateral medial temporal lobe, the posterior cingulate cortex and precuneus ($r = -0.35$, $P < 0.001$, Fig. 4b). This association was driven by the patients with AD$_{clin}$/MCI, who showed the highest correlation coefficients in these regions ($r = -0.55$, $P < 0.001$). There was no association between plasma pTau181 and gray matter volume in patients with FTLD. In the combined group there were strong negative correlations between NfL and gray matter volume in the right putamen and insula ($r = -0.5$, $P < 0.001$) and to a lesser extent with gray matter volume in the medial prefrontal cortices ($r = -0.45$, $P < 0.001$). In the FTLD group, the association was maximal in the right putamen and insula ($r = -0.4$, $P < 0.001$), with lower correlations present in the frontal and lateral temporal regions and right precuneus (Fig. 4b).

**Plasma pTau181 and NfL associations with clinical disease severity and cognitive function.** Levels of pTau181 showed strong associations with baseline CDRSb scores ($\beta = 0.486$, $P < 0.0001$), functional activities questionnaire (FAQ) ($\beta = 0.541$, $P < 0.0001$) and modified Rey figure recall ($\beta = 0.585$, $P < 0.0001$) only in the AD$_{clin}$/MCI group and not in the control or FTLD groups. In contrast, NfL showed associations with CDRSb and neuropsychological performance in both the AD$_{clin}$/MCI and FTLD groups ($\beta = 0.472$, $P < 0.0001$ for CDRSb in AD$_{clin}$/MCI; $\beta = 0.244$, $P = 0.010$ in FTLD; Supplementary Tables 1 and 2). In longitudinal analyses, a higher baseline pTau181 was associated with faster rates of decline in patients with AD$_{clin}$/MCI in CDRSb, mini-mental state exam (MMSE), Rey recall, Boston naming test (BNT) and FAQ (Supplementary Table 4), whereas higher baseline NfL predicted faster decline over time in patients with FTLD in MMSE, phonemic fluency and the trail-making test (Supplementary Table 5).

**Discussion**

The main findings of this study are that plasma pTau181 concentrations differentiated patients with clinically diagnosed AD from those with FTLD and elderly controls, and that plasma pTau181 concentrations were strongly associated with currently approved AD-biomarker measurements, including Aβ-PET and CSF pTau181, regardless of clinical diagnosis. Plasma pTau181 also differentiated autopsy-diagnosed AD from FTLD with slightly lower accuracy than clinically diagnosed or PET-defined cases. Plasma pTau181 accurately identified healthy elderly controls and individuals with MCI with a positive Aβ-PET scan, suggesting underlying AD$_{path}$ changes and also differentiated between individuals with elevated cortical tau deposition, measured by FTP-PET. Elevated pTau181 concentrations correlated with higher FTP-PET uptake and more severe gray matter atrophy in AD-related brain regions. Plasma pTau181 reflected severity of cortical AD tau pathology as reflected by Braak stage measured at autopsy$^{31,36}$. Plasma pTau181 also predicted the rate of decline on clinical measures of disease severity and neuropsychological status over 2 years of follow-up in AD$_{clin}$/MCI. These findings were specifically related to plasma pTau181, as plasma concentrations of NfL, a nonspecific biomarker of neurodegeneration, were not related to AD diagnosis, Aβ or FTP-PET signal. As expected, NfL concentrations were associated with measures of disease severity, cognitive function and gray matter atrophy most strongly in patients with FTLD$^{31,32}$. Together, these data suggest that plasma pTau181 may be a useful screening tool for identifying the AD pathobiological process in individuals at risk of cognitive decline or with cognitive impairment.

Aβ-PET has established clinical utility for differential diagnosis of AD$_{clin}$ from other dementias, is associated with more severe clinical and cognitive decline$^{37}$ and has been validated as a measure...
of AD neuropathology. Plasma pTau181 accurately differentiated between AD and FTLD, similarly to the previously reported diagnostic accuracy of Aβ-PET. This suggests that the diagnostic value of plasma pTau181 could be comparable to Aβ-PET in patients who are symptomatic with MCI or dementia. We found that increased plasma pTau181 concentrations were associated with Aβ-PET positivity even in cognitively healthy controls, however plasma pTau181 is unlikely to be a direct measure of Aβ pathology. Others have found that there is often tau accumulation in healthy elderly controls who are Aβ-PET positive, suggesting that amyloid positivity is a hallmark for Alzheimer pathology and may reflect not only amyloid, but also presymptomatic tau accumulation. As plasma pTau181 was related to regional tau deposition measured by Braak stage at autopsy or estimated by FTP-PET uptake during life, this might explain the ability of pTau181 to differentiate between Aβ-PET-positive and negative controls. A limitation of our study was that we had few data from healthy controls with FTP-PET data and so we could not directly test the relationship of pTau181 to FTP-PET status in these individuals.

Whereas CSF total tau has little diagnostic value differentiating FTLD from AD, CSF pTau181 is able to differentiate clinically diagnosed AD from FTLD with a sensitivity and specificity of approximately 70–80%, which is similar to the accuracy found in this study using plasma pTau181. Using autopsy data, we determined a specific association of elevated plasma pTau181 with underlying mixed 3R/4R tau pathology that is characteristic of, but not specific to AD. We found elevated pTau181 concentrations in AD patients, which is known to have neurofibrillary tangles consisting of 3R/4R mixed tau pathology and low pTau181 concentrations in sporadic FTLD-tau, which is associated with insoluble deposits of either 3R (such as

Table 2 | Diagnostic accuracy of plasma pTau181, NFL, Aβ42/Aβ40 ratio and CSF pTau181

| Differentiated groups | Test | n per group | AUC | 95% CI | P value* | Sensitivity | Specificity | Cutoff point (pg ml⁻¹) |
|-----------------------|------|-------------|-----|--------|----------|------------|------------|----------------------|
| FTP-PET-positive versus negative, only MCI | pTau181, plasma | 11 versus 20 | 0.977 | 0.929–1.000 | <0.0001 | 0.909 | 0.950 | 8.1 |
| Autopsy confirmed: AD versus FTLD-TDP | pTau181, plasma | 15 versus 15 | 0.947 | 0.873–1.000 | <0.0001 | 1.000 | 0.800 | 9.4 |
| Aβ-PET-positive versus negative, only MCI | pTau181, plasma | 18 versus 21 | 0.944 | 0.873–1.000 | <0.0001 | 0.944 | 0.857 | 8.4 |
| Clinical AD versus FTLD | pTau181, CSF | 16 versus 29 | 0.931 | 0.854–1.000 | <0.0001 | 0.875 | 0.897 | 67.0 |
| FTP-PET-positive versus negative (all) | pTau181, plasma | 60 versus 37 | 0.919 | 0.863–0.976 | <0.0001 | 0.917 | 0.838 | 8.1 |
| Aβ-PET-positive versus negative (all) | pTau181, plasma | 90 versus 95 | 0.914 | 0.869–0.958 | <0.0001 | 0.889 | 0.853 | 8.0 |
| Clinical AD versus FTLD | pTau181, plasma | 56 versus 190 | 0.894 | 0.855–0.933 | <0.0001 | 0.982 | 0.711 | 8.7 |
| Autopsy confirmed: AD versus combined FTLD-TDP + FTLD-tau | pTau181, plasma | 15 versus 67 | 0.878 | 0.798–0.957 | <0.0001 | 1.000 | 0.672 | 9.5 |
| Aβ-PET-positive versus negative, healthy controls only | pTau181, plasma | 11 versus 29 | 0.859 | 0.732–0.986 | 0.001 | 0.818 | 0.828 | 7.6 |
| Autopsy confirmed: AD versus FTLD-tau | pTau181, plasma | 15 versus 52 | 0.858 | 0.765–0.950 | <0.0001 | 1.000 | 0.635 | 9.6 |
| Autopsy confirmed: AD versus FTLD + mutation carriers | pTau181, plasma | 15 versus 115 | 0.854 | 0.772–0.937 | <0.0001 | 1.000 | 0.626 | 8.9 |
| FTP-PET positive versus negative | Aβ42/Aβ40 ratio, plasma | 42 versus 34 | 0.782 | 0.674–0.890 | <0.0001 | 0.647 | 0.857 | 0.16 |
| Aβ-PET positive versus negative | Aβ42/Aβ40 ratio, plasma | 68 versus 67 | 0.768 | 0.686–0.849 | <0.0001 | 0.567 | 0.926 | 0.15 |
| Autopsy confirmed: AD versus FTLD-TDP | Plasma NFL | 7 versus 14 | 0.765 | 0.557–0.973 | 0.052 | 0.643 | 1.000 | 53.7 |
| Autopsy confirmed: FTLD-TDP versus FTLD-tau | pTau181, plasma | 15 versus 52 | 0.664 | 0.499–0.829 | 0.054 | 0.981 | 0.333 | 9.6 |
| Autopsy confirmed: AD versus combined FTLD-TDP + FTLD-tau | NFL, plasma | 6 versus 63 | 0.656 | 0.369–0.774 | 0.209 | 0.429 | 1.000 | 48.7 |
| Autopsy confirmed: FTLD-TDP versus FTLD-tau | NFL, plasma | 13 versus 50 | 0.655 | 0.494–0.817 | 0.086 | 0.615 | 0.700 | 55.0 |
| Autopsy confirmed: AD versus FTLD + mutation carriers | NFL, plasma | 6 versus 70 | 0.633 | 0.439–0.828 | 0.281 | 0.414 | 1.000 | 48.0 |
| FTP-PET positive versus negative | NFL, plasma | 34 versus 27 | 0.606 | 0.446–0.765 | 0.159 | 0.824 | 0.556 | 64.5 |
| Aβ-PET positive versus negative | NFL, plasma | 51 versus 67 | 0.559 | 0.453–0.664 | 0.276 | 0.433 | 0.882 | 42.7 |

Cutoff value is adjusted for age and autopsy-confirmed cutoff is adjusted for age and CDRsb. *P value corrected for false discovery rate.
The association between plasma pTau181 and estimated Braak stage by FT-PET was stronger than with neuropathological Braak stage.

Pick's disease or 4R tau (such as corticobasal degeneration or PSP) pathology. To test the hypothesis that plasma pTau181 concentrations specifically reflect mixed 3R/4R tau pathology, we measured samples from individuals with rare MAPT mutations (R406W and V337M) that lead to FTLD pathology with accumulation of neurofibrillary tangles consisting of 3R/4R tau that are similar to those seen in ADpath that often cause a clinical syndrome similar to Pick's disease.

Supportive of this hypothesis, we found an association of pTau181 with FT-PET that was stronger than with neuropathological Braak staging. Even though plasma pTau181 could differentiate late-stage tau pathology (Braak 5–6) from other stages, it could not differentiate early and moderate stages (Braak 1–2 and 3–4) from the group without pathology (Braak 0). This could indicate a limitation in the sensitivity of plasma pTau181 for AD pathology, but could also reflect differences in sample size, the more comprehensive anatomical coverage with PET and additional variability introduced by the delay from blood draw to autopsy in the pathological Braak stage analysis. The increased pTau181 concentrations in ADpath and their strong association with patterns of brain atrophy in AD suggest that plasma pTau181 is also associated with AD-related neuronal loss.

Plasma Aβ measured on an automated platform has recently been demonstrated as a promising and cost-effective tool as compared to Aβ-PET, to identify brain amyloidosis in individuals with or at risk for AD. We found that the fold change in mean plasma pTau181 concentration between individuals who were Aβ-PET positive and negative in our study exceeded the fold change found by others using plasma Aβ42/Aβ40 ratio and the overlap between groups seemed much smaller. Although we did not have access to the same automated Aβ measurement platform or to IP–MS, we measured plasma Aβ42/Aβ40 by Simoa and found a much larger fold difference in pTau181 between groups as compared to Aβ42/Aβ40. Aβ42/Aβ40 concentrations were less accurate in differentiating between individuals who were Aβ-PET positive and negative.
than pTau181. Future comparisons with more accurate plasma amyloid tests will be necessary to determine the relative value of plasma amyloid compared to pTau181 measurements.

This study has a number of important limitations. There were several outlier high plasma pTau181 values in the clinical diagnostic groups who were not expected to have elevated pTau181: two controls, one in CBS, PSP, bvFTD, nfvPPA and svPPA. These findings may reflect previously undetected brain 3R/4R tau deposition. In support of this interpretation, one of those controls was Aβ-PET positive, the individual with CBS had unknown amyloid status and AD path compared to pTau181. The sample sizes were balanced by clinical diagnosis, but more were in the FTLD spectrum. A larger number of controls and patients with MCI and AD would have offset this, although accuracy of pTau181 in these groups has been demonstrated in a previous study21. Finally, neither plasma pTau181 nor NfL was able to determine whether pTau181 associates primarily with FTP-PET or Aβ-PET. The sample sizes were balanced by clinical diagnosis, but more were in the FTLD spectrum. A larger number of controls and patients with MCI and AD would have offset this, although accuracy of pTau181 in these groups has been demonstrated in a previous study21. Finally, neither plasma pTau181 nor NfL was able to differentiate between individuals with autopsy-confirmed FTLD-tau and FTLD-TDP. More work will be necessary to identify effective biomarkers for this context of use.

This study provides strong evidence that plasma pTau181 concentration could be a useful screening blood test to identify underlying mixed 3R/4R tau pathology, consistent with AD in individuals who have symptoms of cognitive or behavioral decline in clinical settings where diagnostic status may be uncertain. Since Aβ-PET scans are expensive and require specialized imaging centers, plasma pTau181 may be a more readily accessible tool to identify individuals who should undergo more detailed diagnostic testing with this approved technology. Alternatively, given the strong relationship between plasma pTau181 and FTP-PET uptake, plasma pTau181 could be useful as a screening tool in clinical trials employing FTP-PET to measure treatment effects of new AD therapies.
**Fig. 4 | Voxelwise correlations of plasma pTau181 and plasma NfL with FTP-PET and gray matter atrophy.** **a,** Regions of correlation between plasma pTau181 concentration and FTP-PET uptake were strongest in AD-specific brain regions: frontal and temporoparietal cortex, posterior cingulate and precuneus regions ($\rho \sim 0.75$). There was no correlation of FTP-PET with plasma NfL in the whole cohort. In the ADclin/MCI group, correlations existed in the frontal and insular cortex ($\rho \sim 0.6$). **b,** Negative correlations between plasma pTau181 and gray matter volume were highest in the bilateral temporal lobe and remained in the ADclin/MCI group, but no correlation was found in the FTLD group. The correlation between plasma NfL and gray matter volume was highest in the right putamen and insular region ($\rho \sim -0.5$). The association remained in the FTLD group but was not found in the ADclin/MCI group. All correlations were thresholded on the basis of an uncorrected $P < 0.001$ at the voxel level and family-wise error-corrected $P < 0.05$ at the cluster level.

**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/s41591-020-0762-2.

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Advancing Research and Treatment for Frontotemporal Lobar Degeneration (ARTFL) investigators

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Participants. This retrospective study included 404 participants from three independent cohorts (Table 1 and Supplementary Table 3), a primary cohort of 362 individuals; 301 from the University of California San Francisco (UCSF) Memory and Aging Center and 61 from the Advancing Research and Treatment for Frontotemporal Lobar Degeneration (ARTFL) consortium and a secondary cohort of baseline data from 42 participants in an Eli Lilly sponsored research study (www.clinicaltrials.gov: NCT02624778). Participants were only included in the study when their plasma pTau181 measurement was successful. Aβ-PET was available in 226 participants, 138 had FTP-PET (79 AD/mCI and 59 FTLD in the primary cohort), 41 AD/mCI (in the secondary cohort), 220 participants had MRI (71 AD/mCI, 110 FTLD and 39 HC) and 74 individuals had previous PET, MRI, autopsy or genetic biomarker verification. Overall, 82 cases had MRI (71 AD/mCI and 110 FTLD) and 74 individuals had previous +44 with mutations that produce 4R tau (22 P301L, 11 N279K, 4 IVS9-10G T, 3 S305S, 1 S305I and 2 S305N)32. The GRN C9orf72 61 MAPT, 5 MAPT and 10 NfL to differentiate between diagnostic groups. Y ouden cutoff values natural log-transformed data or nonparametric statistics were used. Differences in biomarker values and in clinical and neuroimaging variables were assessed in statistical analyses. A group template was generated from the segmented gray-matter volume, the INNO-BIA AlzBio3 (Fujirebio) platform by a centralized laboratory. The researchers who performed the fluid biomarker analyses were blinded to the clinical information and reference standard results of the participants during sample measurement. Imaging methods. MRI acquisition. Structural MRIs were available for 221 participants and acquired at UCSF on a 3T Siemens Tim Trio or a 3T Siemens Prisma Fit scanner at an average of 20 d (±58) from the plasma sample. T-weighted magnetization prepared rapid gradient echo MRI sequences were acquired at UCSF, either on a 3T Siemens Tim Trio or a 3T Siemens Prisma Fit scanner. Both scanners had similar acquisition parameters on each scanner (sagittal slice orientation; slice thickness of 1 mm; 160 slices per slab; in-plane resolution of 1.0×1.0 mm; matrix of 240×256; repetition time of 2,300 ms; inversion time of 900 ms; and flip angle of 9°), although echo time slightly differed (2.98: Prisma: 2.9 ms). MRI preprocessing. Before preprocessing, all scans were visually inspected for quality control. Images with excessive motion or image artifact were excluded. T1-weighted images underwent bias field correction using an N3 algorithm and segmentation was performed using statistical parametric mapping (SPM12, Wellcome Trust Center for Neuroimaging, www.fion.uc.usp.uk/spm) unified segmentation. The total intracranial volume was derived from SPM12 to be used in statistical analyses. A group template was generated from the segmented gray- and white-matter tissues and CSF by nonlinear registration template generation using the large deformation diffeomorphic metric mapping framework. Native subject gray gray were normalized, modulated and smoothed in group template space with a 10-mm full width half maximum Gaussian kernel. Every step of the transformation from the native space to the group template was carefully inspected. FTP-PET acquisition. FTP-PET was acquired on a Siemens Biograph PET/CT scanner at the Lawrence Berkeley National Laboratory (LBNL) for 75 participants (65 AD/mCI and 10 FTLD) at an average of 70d (±122) from the plasma sample. FTP was synthesized and radiolabeled at LBNL Biomedical Isotope Facility. We analyzed PET data that were acquired 80–100 min after the injection of ~10 mCi of FTP (four 5-min frames). A low-dose computed tomography scan was performed for attenuation correction before PET acquisition and data were reconstructed using an ordered subset expectation maximization algorithm with weigthed attenuation and smoothed with a 4-mm isotropic Gaussian kernel to be used for voxelwise analyses. FTP-PET preprocessing. PET frames were realigned, averaged and co-registered onto their corresponding T1-MRI. S U V R images were created using the inferior cerebellum gray matter as a reference region (the region was defined using the T1-MRI was segmented using Freesurfer 5.3 (http://surfer.nmr.mgh.harvard and SPM12). Native-space FTP-SUVR images were warped to template space using the deformation parameters derived from the MRI procedure. Warped SUVR images were masked to limit contamination from nonrelevant areas (such as off- target binding from meninges, eyes or skull) and smoothed with a 4-mm isotropic Gaussian kernel to be used for voxelwise analyses. FTP-PET analyses. Using Freesurfer segmentation, the average cortical SUVR value was extracted from each patient in native space to obtain a measure of global tau burden. Patients were categorized as tau-positive or tau-negative on the basis of was excluded from all analyses. The average percentage c.v. of the samples was 7.3%. The percentage c.v. of the low quality control was 5.6% and 4.6% for the high quality control. Plasma NfL measurements. Plasma NfL concentrations were measured at three sites: Novartis Institutes for Biomedical Research, Quanterix Corp and UCSF using a commercially available NfL kit on the Simoa HD-1 platform. Samples were 4x diluted, automated by the HD-1 analyzer and measured in duplicate. The average interassay variation was 4.9% and all samples were measured well above the kit LLOQ of 0.174 pg/ml−1. One sample had an NfL concentration of 713 pg/ml−1 almost 20-times as high as the average NfL value. This value was excluded from all analyses. In a previous study, an overlapping set of samples from 186 participants was analyzed separately at Novartis and at Quanterix, showing that plasma NfL concentrations were highly correlated across sites, r = 0.958. Plasma NfL concentrations were measured at two sites also had comparable means and s.d. (21.8 ± 35.3 pg/ml−1, Quanterix and 20.2 ± 34 pg/ml−1, Novartis). Plasma Aβ42 and Aβ40 measurements. Plasma Aβ42 and 40 was measured at UCSF using the Neurology 3plex A kit from Quanterix, which measures Aβ42, Aβ40 and tau. Samples were 4x diluted, automated by the HD-1 analyzer and measured in duplicate. The average interassay variation was 6.4% for Aβ42 and 2.9% for Aβ40 and all samples were measured well above the kit LLOQ of 0.142 pg/ml−1 for Aβ42 and 0.675 pg/ml−1 for Aβ40. CSF pTau181 measurements. CSF pTau181 was measured in duplicate with the INNO-BIA AlzBio3 (Fujirebio) platform by a centralized laboratory. The researchers who performed the fluid biomarker analyses were blinded to the clinical information and reference standard results of the participants during sample measurement. Fluid biomarker methods. Plasma pTau181 measurements. Blood samples were obtained by venipuncture in EDTA tubes, following the ADNI protocol. Within 60 min, the samples were centrifuged at 3,000 rpm at room temperature, aliquoted and stored at −80°C. Plasma pTau181 levels were measured in duplicate by electrochemiluminescence using a proprietary pTau181 assay (Lilly Research Laboratory) as previously described. Briefly, samples were diluted 1:2 and 50 μl of diluted sample was used for the assay. The assay was performed on a streptavidin small spot plate using the Meso Scale Discovery platform. Biotinylated AT270 was used as a capture antibody (anti-pTau181 Tau antibody, mouse IgG1) and sulfato-TAG-Ru-IRL (anti-tau monoclonal antibodies developed by Lilly Research Laboratory) for the detector. The assay was calibrated using a recombinant tau (4R2N) protein that was phosphorylated in vitro using a reaction cocktail of glycogen synthase kinase-3 and characterized by MS. Overall, 41 of the included samples were measured below the lower limit of quantitation (LLOQ) of 1.4 pg/ml−1, none of which in the AD phenotype. One sample from an Aβ-PET negative normal control had a pTau181 concentration of 49.1 pg/ml−1, almost 12-times as high as the average pTau181 value. This individual was excluded from all analyses. The average percentage c.v. of the samples was 7.3%. The percentage c.v. of the low quality control was 5.6% and 4.6% for the high quality control.
of a previously published cortical FTP-SUV threshold of 1.22 (see Table 3 from Maas et al.16). Complementary analyses were conducted using inferior temporal lobe SUVr values to classify patients (using a 1.30 threshold, see Table 3 from Maas et al.) but results were unchanged.

Patients were assigned to a Braak stage (0–I, II, III–IV or V–VI) using the approach developed by Maas et al.16 For each patient, we extracted the average SUVr from three bilateral composite regions of interest (ROIs) in native space based on Freesurfer 5.3’s aparc+ase segmentation file, as follows:

Braak I–II ROI: entorhinal, hippocampus, Braak III–IV ROI: parahippocampal, fusiform, lingual, amygdala, middle temporal, caudal anterior cingulate, rostral anterior cingulate, posterior cingulate, isthmus cingulate, insula, inferior temporal and temporal pole.

Braak V–VI ROI: superior frontal, lateral orbitofrontal, medial orbitofrontal, frontal pole, caudal middle frontal, rostral middle frontal, pars opercularis, pars orbitalis, pars triangularis, lateral occipital, supramarginal, inferior parietal, superior temporal, superior parietal, precuneus, banks of the superior temporal sulcus, transverse temporal, pericalcarine, postcentral, cuneus, precentral and paracentral.

The Braak stage classification scheme (including thresholds) was determined by Maas et al.16 and works as follows:

Step 1. If average SUVr in Braak V–VI ROI > 1.25, participant is assigned to Braak stage V–VI; if not:

Step 2. If average SUVr in Braak III–IV ROI > 1.28, participant is assigned to Braak stage III–IV; if not:

Step 3. If average SUVr in Braak I–II ROI > 1.35, participant is assigned to Braak stage I–II; if not, participant is assigned to Braak stage 0.

FTP-PET imaging in secondary cohort (Eli Lilly). The tau PET acquisitions were performed from 75 to 105 min (6 × 15-min frames) after injection of approximately 240 MBq of FTP. Frames were aligned and averaged with an acquisition time-offset correction. An average 75–105 min image was spatially registered to the corresponding individual's MRI space and then to the MNI template in Montreal Neurological Institute stereotaxic space. Reference signal was parametrically derived in the white-matter-based region to isolate nonspecific signal using the parametric estimate of reference signal intensity method. The used weighted SUVr was designed by multiblock barycentric discriminant analysis, which has been shown to maximize the separation of diagnostic groups and amyloid status.

Aβ-PET. Aβ status was available for 166 participants (41 HC, 77 AD/MCI and 48 FTLD) and derived from PET acquired with 11C-PiB (injected dose, ~15 mCi; n = 124 participants) or 18F-florbetapir (injected dose, ~10 mCi; n = 42) at an average of 273 d (± 433) from the plasma sample. Aβ-PET data were acquired at LNBl on a Siemens ECAT EXACT HR PET scanner (n = 32) or a Siemens Biograph PET-CT scanner (n = 104) or at UCSF China Basin on a GE Discovery STE/VCT PET-CT scanner (n = 32). We created a distribution value ratio (for PiB when patients underwent a 90-min acquisition) or 50–70 min SUVr images (for florbetapir or PiB when patients only underwent a 20-min PET acquisition) as previously described, using tracer-specific reference regions: cerebellar gray matter for PiB and whole cerebellum for florbetapir. Aβ-PET positivity was on the basis of visual read, as previously validated against neuropathological standards.

Voxelwise analyses and result rendering. Voxelwise analyses were run in SPM12 to test the association between plasma markers and gray matter volume or FTP SUVr in the primary cohort (UCSF + ARTFL). Separate models were used for each pair of variable (pTau181, Volumetric, PiB, PiB181: FTP and NFT-FTL) and models were run on (1) all participants with available data; (2) patients with a clinical diagnosis of MCI/AD only; and (3) patients with a clinical diagnosis of FTLD only. Specific sample size for each analysis is indicated in the Results. Age was entered as a covariate in all models and total intracranial volume was entered in MRI models to control for inter-individual variability in head size. Resulting T-maps were thresholded (based on uncorrected P < 0.001 at the voxel level with family-wise error-corrected P < 0.05 at the cluster level) and converted to R-maps using the CAT12 toolbox (www.nitrc.org/projects/ bnv/) and default interpolation and perceptually uniform color scales (magma for MNI and viridis for tau PET; https://matto phil.org/).

An overview of the methods is provided in the Nature Research Reporting Summary linked to this article.

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

Data availability

All requests for raw and analyzed data and materials will be promptly reviewed by the corresponding author and the University of California, San Francisco to verify whether the request is subject to any intellectual property or confidentiality obligations. Some participant data not included in the paper were generated as part of clinical trials and may be subject to patient confidentiality limitations. Data and materials from participants with FTLD enrolled in ARTFL are accessible via forms that can be found on the ARTFL website (https://www.rarediseasenetwork.org/cms/artfl/Healthcare-Professionals/Collaborating). Other data and materials that can be shared will be released via a material transfer agreement.

Code availability

All requests for code used for data analyses and data visualization will be promptly reviewed by the corresponding author and the UCSD to verify whether the request is subject to any intellectual property, confidentiality or other licensing obligations. If there are no limitations, the corresponding author will communicate with the requester to share the code.

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Additional information
Extended data is available for this paper at https://doi.org/10.1038/s41591-020-0762-2.
Supplementary information is available for this paper at https://doi.org/10.1038/s41591-020-0762-2.
Correspondence and requests for materials should be addressed to A.L.B.

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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Peer review information
Brett Benedetti and Kate Gao were the primary editors on this article.
Extended Data Fig. 1 | Plasma pTau/NFL ratio per clinical diagnosis. The ratio of pTau181/NFL was decreased in all FTLD diagnoses compared to controls, ADclin, and MCI patients (n=212). **p<0.001 *p<0.05.
Extended Data Fig. 2 | Plasma Aβ 42/40 ratio per clinical diagnosis and Amyloid PET and FTP-PET status.  

a. There was no difference in plasma Aβ 42/40 ratio between the different phenotypes (n=178).  
b. The Aβ 42/40 ratio was decreased in Amyloid PET positive cases (n=135).  
c. The Aβ 42/40 ratio was decreased in FTP-PET positive cases (n=76).
Extended Data Fig. 3 | Plasma NfL concentrations per autopsy determined Braak stage. There was no difference in plasma NfL concentrations between the different Braak stages (n=69).
Extended Data Fig. 4 | Plasma pTau181 and plasma NfL concentrations in mutation carriers. a. Plasma pTau181 concentrations did not differ between mutation carriers (n=120). b. Plasma NfL concentrations were elevated in GRN and C9orf72 mutation carriers compared to the control group (p<0.0001) and MAPT mutation carriers (p<0.01) (n=59). **p<0.01.
Extended Data Fig. 5 | Association between plasma pTau181 and CSF pTau181. CSF pTau181 is associated with plasma pTau181 ($\beta=0.51$, $p<0.0001$; $n=74$), and is also associated within the AD/MCI ($\beta=0.41$, $p=0.042$; $n=25$), and the FTLD group ($\beta=0.49$, $p<0.0001$; $n=29$), but not in controls.
Extended Data Fig. 6 | Receiver Operating Characteristic analyses of plasma pTau181 for Aβ-PET status in MCI patients and in controls. 

a. Plasma pTau181 concentrations are increased in Aβ-PET positive MCI cases. pTau181 could differentiate between Aβ-PET positive and negative cases (visual read). AUC=0.944 (95% CI: 0.873-1.000, \( p < 0.0001 \), \( n=18 \) Aβ-PET positive, 21 negative), with a cut-off of 8.4 pg/mL (0.944 sensitivity and 0.857 specificity).

b. Plasma pTau181 concentrations are increased in Aβ-PET positive NC cases. pTau181 could differentiate between Aβ-PET positive and negative cases (visual read). AUC=0.859 (95% CI: 0.732-0.986, \( p = 0.001 \), \( n=11 \) Aβ-PET positive, 29 negative), with a cut-off of 7.1 pg/mL (0.818 sensitivity and 0.828 specificity). Notch displays the confidence interval around the median. ***\( p < 0.0001 \) **\( p < 0.01 \).
Extended Data Fig. 7 | Plasma pTau181 and plasma NfL concentrations per FTP-PET estimated Braak stage. 

a. Plasma pTau181 was increased in Braak stage 5-6, and Braak stage 3-4 compared to Braak stage 0 (n=97).
b. There was no difference in plasma NfL concentrations between the different Braak stages (n=61). ***p<0.0001.
Reporting Summary

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Statistics

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

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- The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
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- A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
- For null hypothesis testing, the test statistic (e.g. F, t, r) with confidence intervals, effect sizes, degrees of freedom and P value noted
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- For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
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- Estimates of effect sizes (e.g. Cohen’s d, Pearson’s r), indicating how they were calculated

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

Software and code

Policy information about availability of computer code

Data collection We worked with the commercial software integrated in Simoa HD-1, INNO-BIA AlzBio3 ELISA, MRI imaging, and Amyloid and Tau-PET technologies

Data analysis Statistical analyses were performed using SPSS (version 25; SPSS/IBM, Chicago, IL), Stata (Stata 14.0, StataCorp LLC) and R (version 3.5.1). For the analyses of the imaging data, SPM version 12, Freesurfer version 5.3. Voxelwise images were generated using the CAT12 toolbox (www.neuro.uni-jena.de/cat/) and BrainNet Viewer76 (www.nitrc.org/projects/bnv/) and default interpolation and perceptually uniform color scales (magma for MRI, viridis for tau-PET; https://matplotlib.org/).

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Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:
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The datasets generated and/or analysed during the current study are available from the corresponding author on reasonable request.
Field-specific reporting

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Life sciences study design

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

**Sample size**

There were two considerations for the samples size: first, we were limited by the number of samples that Eli Lilly was able to test. Second, we used samples that were readily available in our biobank. We had a limited number of samples available from certain patient groups due to rarity of the disease. With these restrictions in mind we balanced the patient groups with minimum 15 cases, based on experience with previous experience with analyses.

**Data exclusions**

We had no predetermined data exclusions. One sample from an Aβ-PET negative normal control had a pTau181 concentration of 49.1 pg/mL, almost 12 times as high as the average pTau181 value. One sample had an NfL concentration of 713 pg/mL, almost 20 times as high as the average NfL value. After discussing with the relevant authors we decided that these results were likely not right, and excluded these values from the dataset.

**Replication**

The plasma pTau181 measurements included both internal as external control samples that replicated successfully. Besides, a secondary cohort of baseline data from 42 participants in an Eli Lilly sponsored research study were included. The results were very similar to the results obtained in a subset of our primary cohort. Besides, this paper is submitted to be a joint publication together with Oskar Hansson group’s paper, showing very similar results in two independent cohorts.

**Randomization**

Allocation in groups was based on clinical diagnosis, following the appropriate diagnostic criteria per diagnostic group and PET biomarker status. The grouping was therefore not random, but we controlled where appropriate for age and CDRsb since it is known to affect NfL concentrations.

**Blinding**

The researchers conducting measurements of pTau and NFL concentrations were blinded to clinical data and group assignment.

Reporting for specific materials, systems and methods

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

### Materials & experimental systems

- n/a Involved in the study
  - ☒ Antibodies
  - ☒ Eukaryotic cell lines
  - ☒ Palaeontology
  - ☒ Animals and other organisms
  - ❌ Human research participants
  - ☒ Clinical data

### Methods

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

### Antibodies

**Antibodies used**

Plasma pTau measurements: Biotinylated-AT270 was used as a capture antibody (anti-pTau181 Tau antibody, mouse IgG1) and SULFO-TAG-Ru-LRL (anti-tau monoclonal antibodies developed by Lilly Research Laboratory) for the detector.

Plasma NFL measurements: The commercial NFL kit (Ref 103186) by Quanterix Corp (Boston, MA) was used.

Plasma Abeta 42 and 40 measurements: the Neurology 3-plex A kit from Quanterix was used.

**Validation**

Plasma pTau assay: Fit for purpose assay validation has been performed by Eli Lilly according to Andreasson et al (2015) Frontiers in Neurology and within study validation is included within the manuscript supplement (%CV<10%). The NFL kit was successfully validated by Quanterix Corp (within run, between lot, between instrument, between run and between day %CVs < 10%), detailed results can be found here: https://portal.quanterix.com/files/assays/HD-1/Simoa_NF-light_Data_Sheet_HD-1.pdf

The Neurotriplex kit was successfully validated by Quanterix Corp (within run, between lot, between instrument, between run and between day %CVs < 10%), detailed results can be found here: https://www.quanterix.com/sites/default/files/assays/Simoa_N3PA_Data_Sheet_HD-1_HD-X_Rev04%20%281%29.pdf
Human research participants

The primary cohort consisted of 362 cases; 70 normal controls, 103 cases in the AD spectrum: 56 AD clin per NIA-AA criteria including 14 logopenic variant PPA (lvPPA) and 47 MCI,59 and 10 patients meeting clinical criteria for a syndrome in the FTLD spectrum: 39 corticobasal syndrome (CBS), 48 PSP, 50 bvFTD, 27 nonfluent variant PPA (nfvPPA) and 26 semantic variant PPA (svPPA). These included 76 carriers of FTLD-causing mutations: 61 microtubule associated protein (MAPT), five progranulin (GRN) and ten chromosome 9 open reading frame 72 (C9orf72). The MAPT mutation carriers group included 17 individuals with mutations that produce 3R/4R tau (10 V337M and 7 R406W), and 44 with mutations that produce 4R tau (22 P901L, 11 N279K, 4 IVS9-10G>T, 3 IVS10+16C>T, 3 S305S, 1 S305I, and 2 S305N).

Recruitment

This was a retrospective cross-sectional cohort including observational cohorts at the University of California, San Francisco (UCSF), the Advancing Research & Treatment for Frontotemporal Lobar Degeneration (ARTFL) clinical research consortium and one independent clinical research study. Participants provided written informed consent at the time of recruitment. Patients were recruited from academic research centers that are specialized in these diseases. This may not reflect true population.

Ethics oversight

The study was approved by the institutional review board of each research center from which the individual was recruited.

Clinical data

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

Clinical trial registration

Secondary cohort: www.clinicaltrials.gov: NCT02624778. ARTFL: NCT02365922.

Study protocol

www.clinicaltrials.gov: NCT02624778, NCT02365922.

Data collection

Only baseline data were used

Outcomes

We did not use the primary or secondary outcomes of the trial, we only utilized the baseline data.

Magnetic resonance imaging

Experimental design

Cross-sectional Voxel-Based Morphometry Analysis (structural MRI only, no functional)

Design specifications

One T1 weighted structural MRI per subject was analyzed for voxelwise relationships with pTau and NfL.

Behavioral performance measures

n/a

Acquisition

Imaging type(s)

Structural

Field strength

3T

Sequence & imaging parameters

T1-weighted magnetization prepared rapid gradient echo (MPRAGE) MRI sequences were acquired at UCSF, either on a 3T Siemens Tim Trio or a 3T Siemens Prisma Fit scanner. Similar acquisition parameters were used on each scanner (sagittal slice orientation; slice thickness = 1.0 mm; slices per slab = 160; in-plane resolution = 1.0x1.0 mm; matrix = 240x256; repetition time = 2,300 ms; inversion time = 900 ms; flip angle = 9°), although echo time slightly differed (Trio: 2.98 ms; Prisma: 2.9ms).

Area of acquisition

Whole brain

Diffusion MRI

Not used

Preprocessing

Preprocessing software

T1-weighted images underwent bias field correction using an N3 algorithm and segmentation was performed using Statistical Parametric Mapping (SPM12; Wellcome Trust Center for Neuroimaging, London, UK, http://www.fil.ion.ucl.ac.uk/spm) unified segmentation. The Total Intracranial Volume (TIV) was derived from SPM12 to be used in statistical analyses.

Normalization

A group template was generated from the segmented gray and white matter tissues and cerebrospinal fluid by non-linear registration template generation using the Large Deformation Diffeomorphic Metric Mapping framework. Native subject space gray were normalized, modulated and smoothed in group template space with a 10mm full width half
maximum Gaussian kernel. Every step of the transformation from the native space to the group template was carefully inspected.

**Normalization template**
A study-specific group template was generated from segmented gray and white matter tissues and cerebrospinal fluid by non-linear registration template generation using the Large Deformation Diffeomorphic Metric Mapping framework.

**Noise and artifact removal**
Bias field correction was applied using an N3 algorithm.

**Volume censoring**
Not applicable to structural MRI

### Statistical modeling & inference

**Model type and settings**
Voxelwise analyses were run in SPM12 to test the association between plasma markers and gray matter volume or FTP SUVR in the primary cohort (UCSF+ARTFL). Separate models were used for each pair of variable (pTau-volume, NfL-volume, pTau-FTP, NfL-FTP) and models were run on i) all participants with available data, ii) patients with a clinical diagnosis of MCI or AD only, iii) patients with a clinical diagnosis of FTLD only. Specific sample size for each analysis is indicated in the result section and corresponding figures. Age was entered as a covariate in all models and total intracranial volume was entered in MRI models to control for inter-individual variability in head size. Resulting T-maps were thresholded (based on uncorrected p<0.001 at the voxel level with family wise error-corrected p<0.05 at the cluster level) and converted to R-maps using the CAT12 toolbox (www.neuro.uni-jena.de/cat/). Maps were rendered on a 3D brain surface using BrainNet Viewer15 (www.nitrc.org/projects/bnv/) and default interpolation and perceptually uniform color scales (magma for MRI, viridis for tau-PET; https://matplotlib.org/).

**Effect(s) tested**
We tested for associations between imaging measures (FTP, grey matter volume) and each plasma marker (pTau, NfL).

**Specify type of analysis:**
- [x] Whole brain
- [ ] ROI-based
- [ ] Both

**Statistic type for inference**
T-maps were thresholded using SPM12 default parameters, based on uncorrected p<0.001 at the voxel level with family wise error-corrected p<0.05 at the cluster level.

**Correction**
A family-wise error (FWE) correction for multiple comparison approach was used at the cluster level.

### Models & analysis

**n/a Involved in the study**
- [x] Functional and/or effective connectivity
- [ ] Graph analysis
- [x] Multivariate modeling or predictive analysis