Supplementary Online Content

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This supplementary material has been provided by the authors to give readers additional information about their work.

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1. eMethods

1.1 Further information about the participants of the Neuropathology cohort (cohort-1)

The Brain and Body Donation Program at Banner Sun Health Research Institute[1] consisted of subjects who had comprehensive annual assessments, provided blood samples near the end of their lives, and had comprehensive neuropathological assessments after they died. Neuropathological diagnosis of Alzheimer disease (AD) was based on National Institute on Aging-Reagan Institute (NIA-RI) criteria[2], which are dependent on Consortium to Establish a Registry for Alzheimer's Disease (CERAD) (neuritic Aβ plaque) scores[3] and Braak (tau tangle) stage.[4] Subjects with NIA-RI intermediate likelihood of AD (neurofibrillary tangles in limbic regions [Braak stages III-IV] and moderate or frequent neuritic Aβ plaques; n=18) or high likelihood (neurofibrillary tangles in the neocortex [Braak stages V-IV] and moderate or frequent neuritic Aβ plaques; n=16)[2] were termed "AD". The term "non-AD" was used for those with no or sparse neuritic Aβ plaques (n=47).[3] Our primary analysis included 34 donors with at least intermediate AD pathophysiologic change and 47 non-AD donors to characterize the accuracy of antemortem plasma P-tau217 vs other plasma measurements for the neuropathological diagnosis of AD; a secondary analysis compared the 16 brain donors with high (but not intermediate) AD pathophysiological change to non-AD donors (n=47). Histopathological scoring was performed blinded to clinical and neuropathological diagnosis as well as levels of the plasma biomarkers. Aβ plaque and neurofibrillary tangle density were graded at standard sites in frontal, temporal, parietal and occipital cortices as well as hippocampus and entorhinal cortex, based on the aggregate impression from 80 μm sections stained with thioflavin S, Campbell-Switzer and Gallyas methods. The total plaque score, considering all types of plaques (cored, neuritic and diffuse) together, is derived from the Campbell-Switzer stain while the thioflavin S stain was used for estimating neuritic plaque densities. All three stains show neurofibrillary changes and therefore this score is estimated after viewing slides stained with all three. Both total and neuritic plaque densities are rated as none, sparse, moderate and frequent, using the published CERAD templates.[3] Conversion of the descriptive terms to numerical values give scores of 0–3 for each area, with a maximum score of 15 for all five areas combined. Neurofibrillary tangle abundance and distribution is similarly graded in these thick sections, again using the CERAD templates for this, while the original Braak protocol[4] is used for estimating the topographical distribution of neurofibrillary tangle change.

1.2 Further information about the participants of the BioFINDER-2 cohort (cohort-2)

The BioFINDER-2 study enrolls participants in five sub-cohorts.

Cohort A and B includes neurologically and cognitively healthy controls. The inclusion criteria are: i) ages 40-65 years (cohort A) and ages 66-100 years (cohort B); ii) absence of cognitive symptoms as assessed by a physician specialized in cognitive disorders; iii) MMSE score 27-30 (A) or 26-30 (cohort B) at screening visit; iv) do not fulfill the criteria for mild or major neurocognitive disorder (MCI or dementia) according to DSM-5[5]; and v) fluent in Swedish. The recruitment process of cohorts A and B is designed to build two study populations with 50% *APOE* ε4 carriers in each.

Cohort C comprises participants with subjective cognitive decline (SCD) or MCI. Inclusion criteria are: i) ages 40-100 years; ii) referred to the memory clinics due to cognitive symptoms; iii) MMSE score of 24-30 points; iv) does not fulfill the criteria for any dementia (major neurocognitive disorder) according to DSM-5[5], v) fluent in Swedish. In accordance with the research framework by the National Institute on Aging-Alzheimer's Association[6] study participants with SCD were analyzed together with the cognitively healthy participants (and combined in the cognitively unimpaired [CU] group). Participants were classified as having MCI if they performed worse than -1.5 SD in any cognitive domain according to age and education stratified test norms. The neuropsychological battery covered the domains attention (Trail Making Test A and Symbol Digit Modalities Test), executive function (Trail Making B and A Quick Test of cognitive speed [AQT]), verbal ability (verbal fluency animals and the 15 word short version of the Boston Naming Test), memory (immediate and delayed recall from the Alzheimer's Disease Assessment Scale [ADAS]), and visuospatial function (incomplete letters and cube analysis from the Visual Object and Space Perception battery [VOSP]). Those that were not classified as MCI were considered to have SCD.

According the updated NIA-AA criteria for AD, cognitively unimpaired (CU; i.e. cognitively healthy controls and SCD participants) were classified as "preclinical AD" if they were Aβ-positive and tau-positive and "AD with MCI" if they had MCI and were Aβ-positive and tau-positive according to CSF AD biomarkers[6] (Aβ-PET was only available for a subset of participants, according to the study design). Aβ-status (positive/negative) was defined using CSF Aβ42/Aβ40 with a cutoff of <0.752 (determined using mixture modelling).[7] Tau-status was also defined using CSF to have a uniform type of biomarkers for both classifications (and also in agreement with the suggested biomarkers for Aβ and tau in the NIA-AA criteria[6]). Tau-positivity was determined using the CSF P-tau217 cutoff >101.95 pg/mL. Since CSF P-tau217 did not have a bimodal distribution, the cutoff was determined based on the mean value + 2 standard deviations (SD) in Aβ-negative CU (the same way all present cutoffs, except for the Aβ biomarkers, were established).

Cohort D consists of participants with dementia due to AD. Inclusion criteria are: i) ages 40-100 years; ii) referred to the memory clinics due to cognitive symptoms; iii) MMSE score of ≥12 points; iv) fulfill the DSM-5 criteria for dementia (major neurocognitive disorder) due to Alzheimer disease[5]; and v) fluent in Swedish. Clinical AD dementia was diagnosed according to the DSM-5 criteria for major neurocognitive disorder due to AD[5], but also with the requirement that they were Aβ-positive in agreement with the updated NIA-AA criteria for AD[6] (the latter requirement resulted in the exclusion of two AD dementia participants who were not Aβ-positive, see eFig. 1).

Cohort E covers other non-AD dementias and neurodegenerative disorders. Inclusion criteria are: i) ages 40-100 years; ii) fulfillment of criteria for dementia (major neurocognitive disorder) due to frontotemporal dementia[5], Parkinson's disease (PD) with dementia[5], or subcortical vascular dementia[5], PD[8], progressive supranuclear palsy[9], multiple system atrophy[10], corticobasal syndrome[11] or semantic variant primary progressive aphasia[12]; and iii) fluent in Swedish.

Exclusion criteria for all sub-cohorts are: i) significant unstable systemic illness that makes it difficult to participate in the study; ii) current significant alcohol or substance misuse; iii) refusing lumbar puncture, MRI or PET.

1.3 Further information about the participants of the Colombia kindred registry of autosomal-dominant AD (cohort-3)

Participants were considered cognitively unimpaired if they had an MMSE score ≥26 points, a functional assessment staging test (FAST) score ≤2, and no cognitive impairment on the Consortium to Establish a Registry for Alzheimer's disease (CERAD) battery.[13] Cognitive impairment was defined as a FAST score of ≥3 or MCI or dementia due to AD.[14,15] Individuals with significant medical, psychiatric or neurological disorders, or a history of stroke, seizures, substance abuse, or other disorders that affect motor, visuospatial or cognitive abilities were excluded. Only those who were 18 years old or above were included in the present study. For cognitively impaired participants, a partner or offspring serving as the legal representative gave the informant consent to participate.

1.4 Description of the plasma sampling

In cohort-1, blood was collected in the morning with participants non-fasting. It was collected in EDTA-plasma tubes (Vacutainer® K2EDTA tube, BD Diagnostics) and centrifuged (1,500g, +4 °C) for 15 min. Following centrifugation, plasma from all tubes were transferred

into one 50-ml polypropylene tube, mixed, and 0.5 ml was aliquoted into 1.5ml polypropylene tubes and stored at −80 °C within 30–60 min of collection.

In cohort-2, blood was collected in the morning with participants non-fasting. It was collected in EDTA-plasma tubes (Vacutainer® K2EDTA tube, BD Diagnostics) and centrifuged (2,000g, +4°C) for 10 min. Following centrifugation, plasma from all tubes were transferred into one 50 ml polypropylene tubes tube, mixed and 1ml was aliquoted into 1.5ml polypropylene tubes and stored at -80°C within 30–60 min of collection. All plasma samples underwent one freeze-thaw cycle when 200µl were further aliquoted into 0.5ml Lobind tubes (Eppendorf Nordic A/S, Denmark); the 200µl aliquots were stored at -80°C.

In cohort-3, blood was collected in the morning with participants under optional fasting. It was collected in EDTA-plasma tubes (Vacutainer® K_2EDTA tube, BD Diagnostics) and centrifuged (1200g, +4°C) for 5 min. Following centrifugation, plasma from all tubes were transferred into one 50-ml polypropylene tube, mixed, and three 1 ml aliquots were pipetted into 1.5ml polypropylene Cryovials (Corning, USA) to be stored at −80 °C within 30–60 min of collection. All plasma samples underwent one freeze-thaw cycle when 200µl were further aliquoted into 0.5ml Lobind tubes (Eppendorf Nordic A/S, Denmark); the 200µl aliquots were stored at -80°C.

1.5 Analysis of plasma P-tau217

Analysis of plasma P-tau217 was performed at Eli Lilly and Company using the MSD platform (Meso Scale Discovery). Biotinylated-IBA493 was used as a capture antibody and SULFO-TAG-4G10-E2 (anti-Tau) as the detector. 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 sample was thawed on wet ice, briefly vortexed, and centrifuged at 2,000 g for 10 min, and diluted 1:2 in sample buffer (50 mM HEPES, 60 mM NaCl, 5 mM EDTA, 5 mM EGTA, 1% Triton X-100, 1% MSD blocker A, 2% PEG) with the addition of heterophilic blocking reagent 1 to a concentration of 200 µg/ml (Scantibodies Inc). MSD small-spot streptavidin-coated plates were blocked for 1 h at room temperature with 200 µl of 3% BSA in DPBS. The plates were then washed three times with 200 µl of wash buffer (PBS + 0.05% Tween 20), and 25 µl of biotinylated-IBA493 capture antibody at 0.5 µg/ml (diluted in DPS + 0.1% BSA + 0.05% Tween 20 + 2% PEG) was added to the wells and incubated for 1 h at room temperature. 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. The plates were then washed three times with 200 µl of wash buffer, and 25 µl of SULFO-tagged

E2 detection antibody was added at 0.02 µg/ml (diluted in MSD Diluent 35 + 2% PEG) and incubated for 1 h at room temperature. The plates were washed a final time with 200 µl of wash buffer and 150 µl of 2×MSD Read Buffer T with Surfactant was added to each plate and read on the MSD SQ120 within 10 min of read buffer addition. All plate incubations were performed with 650 rpm shaking on a plate shaker.

Plasma samples from study participants were analyzed in duplicates with a mean intra-assay coefficient of variation (CV) of 13.9%. The mean inter-assay CVs of quality control samples were 3.4-5.5%. The lower limit of detection of the plasma P-tau217 assay was 0.48 pg/mL. In *cohort-1*, 13 plasma samples (11 out of 47 non-AD and 2 out of 34 AD) were below the detection limit of the assay. In *cohort-2*, 190 plasma samples were below the detection limit of the assay. The majority of these samples (n=178 [93.7%]) were in the Aβ-negative (Aβ-) groups (99 out of 224 Aβ- CU, 51 out of 86 Aβ- MCI, 28 out of 84 Aβ- non-AD) with only 12 (6.3%) in the Aβ+ groups (8 out of 77 Aβ+ CU, 4 out of 92 Aβ+ MCI and 0 out of 136 Aβ+ AD or other neurodegenerative diseases). In *cohort-3*, 26 plasma samples (20 out of 257 non-carriers, 6 out of 259 unimpaired carriers and 0 out of 106 impaired carriers) were below the detection limit of the assay. In the main analysis, plasma P-tau217 values below the lower detection limit of the assay were interpolated from the standard curve or if this was not possible due to the very low signal the values were imputed to the lowest interpolated value. Overall, the results were very similar when excluding all cases with values below limit of detection (see sensitivity analyses below).

In cohort-3, plasma samples were run in two separate batches. We included 269 identical samples in both analyses. A subtraction factor of 1.06, determined based on the difference between the P-tau217 values for these samples, was applied to harmonize the data between the two batches. The analysis in cohort-3 was also performed using raw values (i.e. non-harmonized data) with similar results (data not shown).

1.6 Analyses of CSF P-tau217, CSF P-tau181, CSF Aβ42, CSF Aβ40, plasma P-tau181, plasma Aβ42, plasma Aβ40, plasma total-tau, and plasma neurofilament light

At Eli Lilly and Company, analysis of CSF P-tau217 was performed using the MSD platform (Meso Scale Discovery) as previously described.[16] In cohort-1, plasma P-tau181 was performed using the MSD platform (Meso Scale Discovery) as previously described.[17]

At the Clinical Neurochemistry Laboratory in Gothenburg, plasma P-tau181 in cohort-2 was quantified using an in-house Simoa-based immunoassay, and these P-tau181 results have been partly included in a previous study.[18] CSF P-tau181 (this is the P-tau variant used in clinical practice and in most research studies) was quantified using Innotest® immunoassay

(Fujirebio; Gent, Belgium) and CSF Aβ42 and CSF Aβ40 using Meso Scale Discovery immunoassays (MSD; Rockville, MD, USA). Further, plasma neurofilament light (NfL) was quantified in cohort-2 and 3 using Simoa assay[19] and total-tau (T-tau) was analyzed in cohort-1 and 2 using Simoa kit (Quanterix, Lexington, MA).

At Lund University, plasma Aβ42 and Aβ40 were analyzed in cohort-1 and 2 using Euroimmun ELISAs (Euroimmun, Lubeck, Germany) and plasma NfL in cohort-1 using Simo kit (Quanterix, Lexington, MA).

1.7 MRI procedures in the BioFINDER-2 cohort (cohort-2)

Structural MRI was performed using a Siemens 3T MAGNETOM Prisma scanner (Siemens Medical Solutions), with high resolution T1-weighted anatomical magnetization-prepared rapid gradient echo (MPRAGE) images (1mm isotropic voxels) acquired for PET image co-registration and template normalization. Following spatial normalization for further use in the PET processing pipeline, T1-images underwent volumetric segmentation and parcellation using FreeSurfer (v.6.0, https://surfer.nmr.mgh.harvard.edu). Hippocampal volumes were used in the analyses as the combined volumes from the left and right hemisphere divided by the total intracranial volume. The AD-specific cortical thickness meta-ROI encompassed temporal regions with known susceptibility to atrophy in AD (mean thickness in the bilateral entorhinal, inferior temporal, middle temporal and fusiform cortices, adjusted for surface area) as previously described.[20]

1.8 Tau- and Aβ-PET procedures in the BioFINDER-2 cohort (cohort-2)

Approval for PET imaging was obtained from the Swedish Medical Products Agency. Tau-PET images were acquired on digital GE Discovery MI scanners 70-90 min post injection of ~370 MBq [^{18}F]RO948. Standardized uptake value ratio (SUVR) were created using the inferior cerebellar cortex as reference region.[21] Aβ-PET imaging was performed on the same platform as tau-PET 90-110 min after the injection of ~185 MBq [^{18}F]Flutemetamol. SUVR-values were calculated with pons as reference region. In order to capture brain regions affected by tau and Aβ pathology in AD,[4] volume weighted FreeSurfer-based composite regions of interest (ROI) were created. These included a temporal meta-ROI for tau-PET (entorhinal cortex, inferior and middle temporal cortices, fusiform gyrus, parahippocampal cortex and amygdala)[22] and a neocortical meta-ROI for Aβ-PET (prefrontal, lateral temporal, parietal, anterior cingulate, and posterior cingulate/precuneus).[23,24] Additional ROIs for tau-PET included the entorhinal cortex[25], the inferior temporal cortex[25,26] and a meta-ROI capturing late stage neocortical tau pathology (Braak stages V-VI).[25] Tau-PET (temporal

meta-ROI) was binarized using a predefined cutoff of 1.36 SUVR.[27] Aβ-PET data was binarized using a cutoff derived from mixture modeling in cohort-2 (0.53 SUVR).[28] Out of 699 participants who underwent tau-PET imaging, 167 (23.9%) had abnormally high tau-PET ligand retention in the temporal meta-ROI. A total of 488 participant underwent Aβ-PET imaging, of whom 162 (33.2%) had abnormally high SUVR.

1.9 Additional statistical analyses

Plasma P-tau217 and P-tau181 had skewed distributions and were used after log10 transformation in all analyses except for the ROC and non-parametric analyses. In cohort-1, Spearman correlation was used to examine the association between tangles and plasma P-tau217. Confidence intervals (CI) for AUCs were calculated based on 2000 bootstrap samples using the normal approximation method. In Table 1, CIs were calculated using the percentile method (due to the small sample sizes in some other neurodegenerative subgroups) based on 2000 bootstrap samples. The biomarker cutoffs in cohort-2 were determined using the mean + 2 SD in Aβ-negative controls in cohort-2, except for Aβ biomarkers where the cut-off was determined using mixture modelling statistics.[28] When establishing the cutoff for plasma P-tau181 one outlier was excluded and two outliers were excluded for plasma P-tau217. In cohort-1, the cut-offs for plasma P-tau217 and plasma P-tau181 were established at the highest Youden Index (highest combined sensitivity and specificity to discriminate between AD vs non-AD) in each ROC analysis, respectively (eTable 10), since no control sample existed in that cohort. The relationship of plasma P-tau217 with tau-PET uptake and CSF P-tau217 in cohort-2 was modelled using monotone penalized regression splines with generalized cross-validation to tune the smoothing parameter (eFig. 5).[29] For voxel-based analyses between tau- or Aβ-PET and plasma P-tau217, multiple regression models were used, adjusted for age and sex ($P<0.05$, familywise error rate corrected). To examine plasma P-tau217 and NfL levels as a function of age in cohort-3, log10 transformed P-tau217 and NfL levels were fitted to a restricted cubic spline model separately for the PSEN1 mutation carriers and noncarriers.[30] Model parameters were obtained using a Hamiltonian Markov Chain Monte Carlo (HMCMC) approach implemented in Stan (http://mc-stan.org) to determine the median P-tau217 level as a function of age as well as the 99% credible intervals.[30] The age at which the P-tau217 level separated between the carrier and noncarrier groups was also determined based on the model curves. The HMCMC is a resampling approach that allows the determination of credible intervals around the model fits which could then be used to estimate the timing of biomarker profile separation between carriers and noncarriers. This statistical approach in cohort-3 was chosen to match some of the recent

similar publications on autosomal-dominant AD using tau-PET[31] and plasma NfL.[30] In eTables 19-20, combination of biomarkers were examined using logistic regression models with the biomarkers as independent variables and diagnosis (AD dementia vs other neurodegenerative diseases) as dependent variable. The probability output from the logistic regression was then used as independent variable in ROC analysis with AD vs other neurodegenerative diseases as dependent variable.

2. eResults - sensitivity analysis excluding participants with plasma P-tau217 levels below the lower detection limit of the assay

Below is a description of the results obtained when excluding those with plasma P-tau217 levels below the lower detection limit of the assay

2.1 Study cohorts when excluding participants with plasma P-tau217 levels below the detection limit

The neuropathology cohort (cohort-1) without participants with plasma P-tau217 values below the detection limit included 68 participants, 32 (47.1%) cases with intermediate to high likelihood of AD and 36 (52.9%) non-AD cases.

The BioFINDER-2 sample (cohort-2) without participants with plasma P-tau217 values below the detection limit included 509 participants of which 194 (38.1%) were CU, 123 (24.2%) had MCI, 121 (23.8%) AD dementia and 71 (13.9%) other neurodegenerative diseases (n=25 PD/PDD, n=16 PSP, n=8 MSA, n=1 CBS, n=8 VaD, n=7 bvFTD, n=1 progressive non-fluent aphasia [PNFA], and n=5 svPPA).

The Colombian *PSEN1* mutation cohort (cohort-3) without participants with plasma P-tau217 values below the detection limit included 237 non-carriers and 359 mutation carriers (253 unimpaired carriers and 106 impaired carriers).

2.2 Summary of the difference between the main analysis and the sensitivity analysis in the Neuropathology cohort (cohort-1)

The sensitivity analysis excluding cases with plasma P-tau217 levels below the detection limit is shown in eFig. 8. Overall, the results were similar compared with the main analysis presented in Fig 1 and eFig 2 that included all cases (i.e. including those with values below the limit of detection). The difference in AUCs between the main analysis and sensitivity

analysis (ΔAUC) was 0.03 for plasma P-tau217 and 0.01 for plasma P-tau181 for differentiating AD from non-AD. The correlations between tau tangles and plasma P-tau217 were virtually unchanged in both Aβ-positive (ΔRho <0.04) and Aβ-negative (non-significant in both analyses) groups.

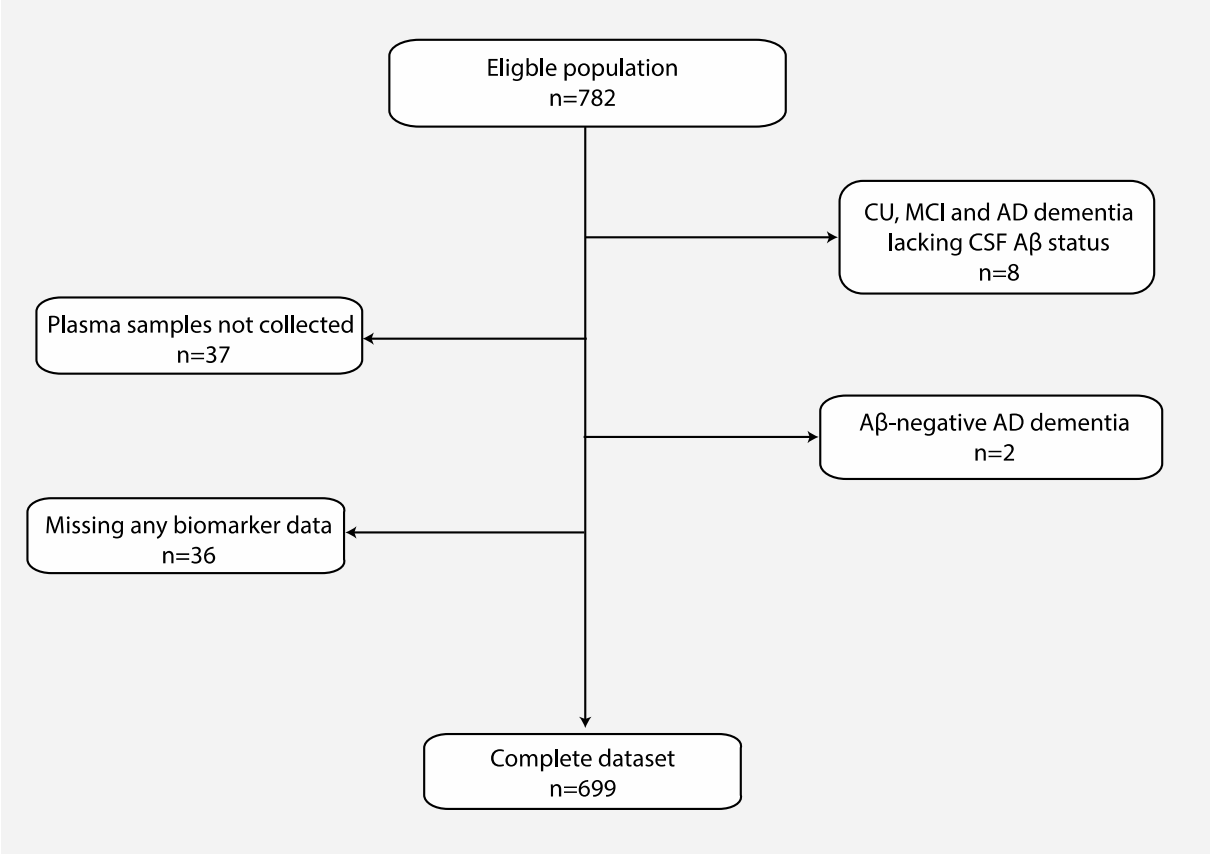
2.3 Summary of the difference between the main analysis and the sensitivity analysis in the BioFINDER-2 cohort (cohort-2)

The sensitivity analysis excluding cases with plasma P-tau217 levels below the detection limit is shown in eFig. 9-10. Overall, the results were similar to the main analysis described in Fig. 2-3. The ΔAUC of plasma P-tau217 for discriminating AD vs the other groups were ≤0.02, the Δr^2 for the association with tau-PET in Aβ-positive participants using splines models was <0.01, the ΔAUC for discriminating abnormal vs normal tau-PET was 0.01, and the ΔAUC for discriminating abnormal vs normal of Aβ-PET was <0.05.

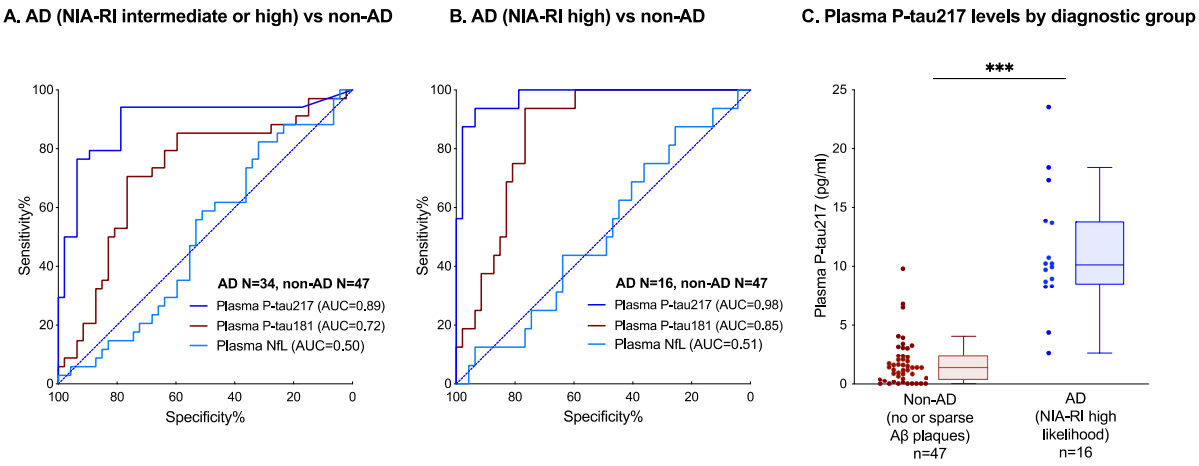
2.4 Summary of the difference between the main analysis and the sensitivity analysis in the Colombia kindred registry of autosomal-dominant AD (cohort-3)

The plasma P-tau217 trajectories in carriers and non-carriers were very similar in the sensitivity analysis compared to the main analyses (compare eFig. 11A with Fig. 4A). Plasma P-tau217 increased significantly in mutation carriers at the age of 24.5 (eFig. 11B) compared to 24.9 in the main analysis (eFig. 7).

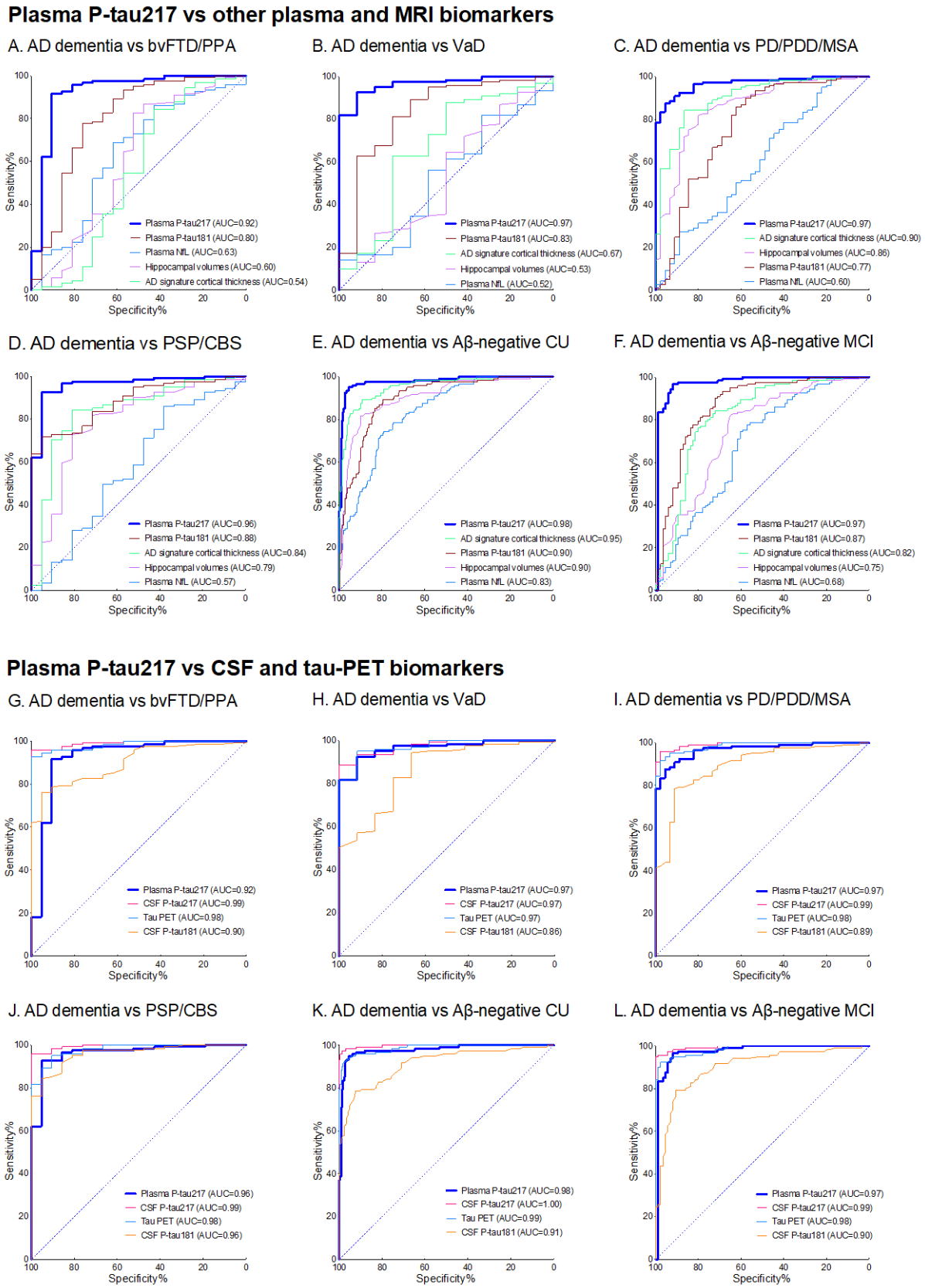
3. eFigures



eFigure 1. Enrollment flowchart for the present sample (n=699) from the BioFINDER-2 study (cohort-2).
Eligible population was defined as enrolled in the BioFINDER-2 study before Sep 6, 2019, diagnosed as CU, MCI, AD dementia or specific other neurodegenerative disorder.
Abbreviations: AD, Alzheimer disease; Aβ, β-amyloid; CSF, cerebrospinal fluid; CU, cognitively unimpaired; LP, lumbar puncture; MCI, mild cognitive impairment; MRI, magnetic resonance imaging

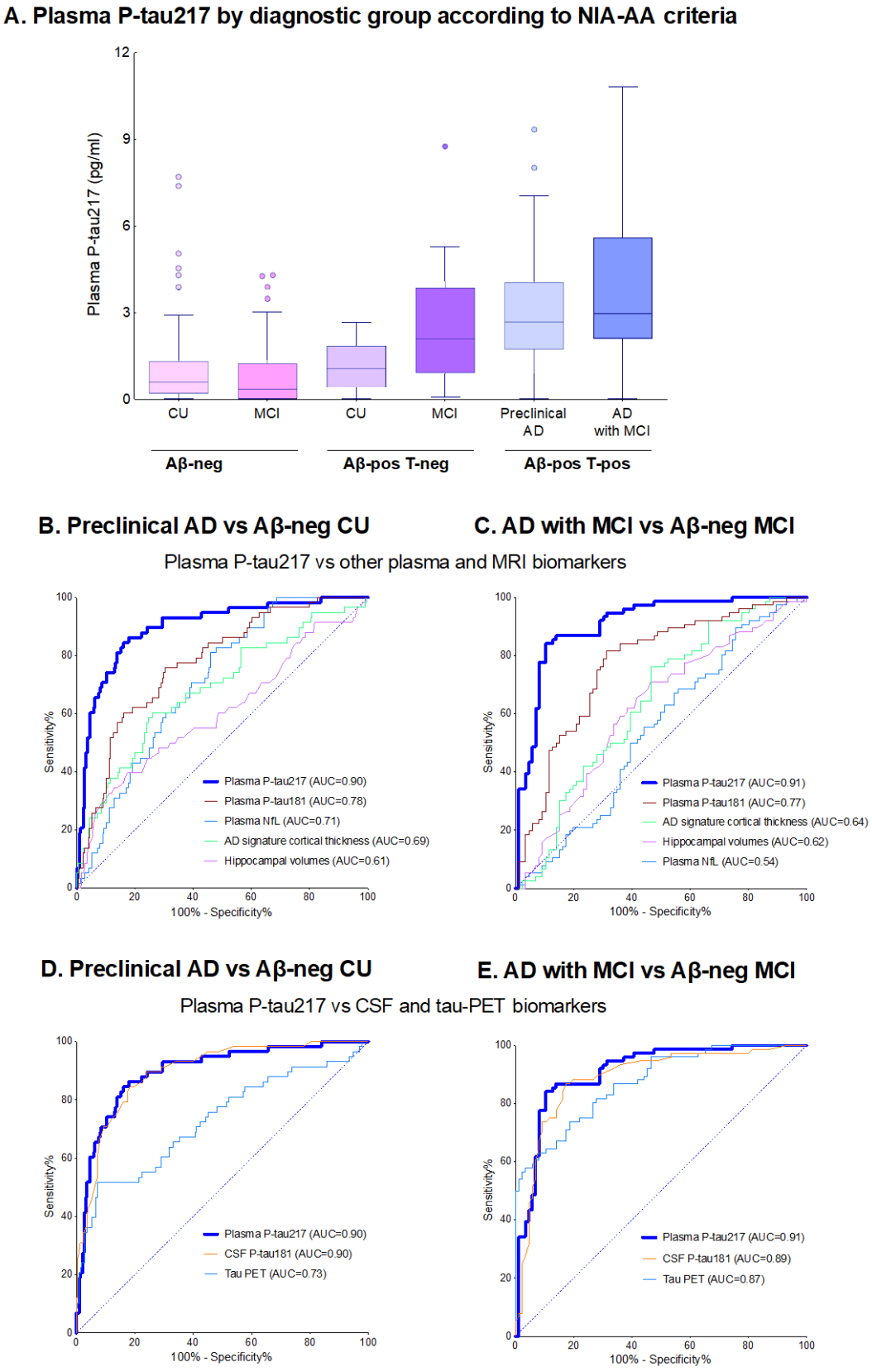


eFigure 2 Plasma P-tau217 in the neuropathology cohort (cohort-1). A, ROC curves using non-AD (n=47) vs intermediate to high likelihood of AD (n=34) as reference standard. **B**, ROC curves using non-AD (n=47) vs high likelihood of AD (n=16) as reference standard. The ROC curve for plasma P-tau181 in panel **B** is shown for comparison with P-tau217 and has also been included in another paper.[17] **C,** Antemortem plasma P-tau217 concentrations in the high likelihood of AD and non-AD groups. Boxes show interquartile range, the horizontal lines are medians and the whiskers were plotted using the Tukey method.
Abbreviations: AD, Alzheimer disease; AUC, area under the ROC curve; NIA-RI, National Institute on Aging-Reagan Institute Working Group; ROC, receiver operating characteristic.



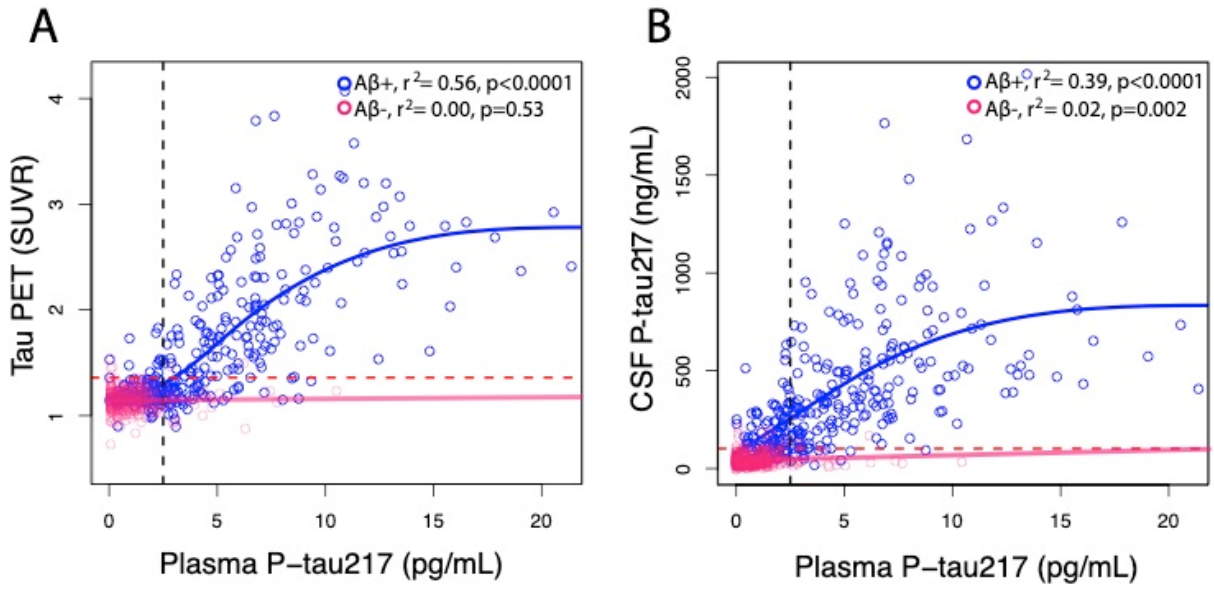
eFigure 3 Discriminative accuracy for AD vs other neurodegenerative diseases, Aβ-negative CU and MCI using plasma, CSF, tau-PET and MRI biomarkers in the BioFINDER-2 cohort (cohort-2). A-F, ROC curve analyses of plasma P-tau217, other plasma biomarkers and MRI for discriminating AD dementia (n=121) from (**A**) bvFTD/PPA (n=21), (**B**) VaD (n=12), (**C**) PD/PDD/MSA (n=45), (**D**) PSP/CBS (n=21), (**E**) Aβ-negative CU (n=224) and (**F**) Aβ-negative MCI (n=86). **G-L**, ROC curve analyses of plasma P-tau217, CSF biomarkers and tau-PET for discriminating AD dementia (n=121) from (**G**) bvFTD/PPA (n=21), (**H**) VaD (n=12), (**I**) PD/PDD/MSA (n=45), (**J**) PSP/CBS (n=21), (**K**) Aβ-negative CU (n=224) and (**L**) Aβ-negative MCI (n=86)

Abbreviations: AD, Alzheimer disease; AUC, area under the curve; Aβ, β-amyloid; bvFTD, behavioral variant of frontotemporal dementia; CSF, cerebrospinal fluid; CU, cognitively unimpaired; MCI, mild cognitive impairment; MSA, multiple system atrophy; PD, Parkinson's disease; PDD, Parkinson's disease dementia; PET, positron emission tomography; PPA, primary progressive aphasia; PSP, progressive supranuclear palsy; ROC, receiver operating characteristic; VaD, vascular dementia.

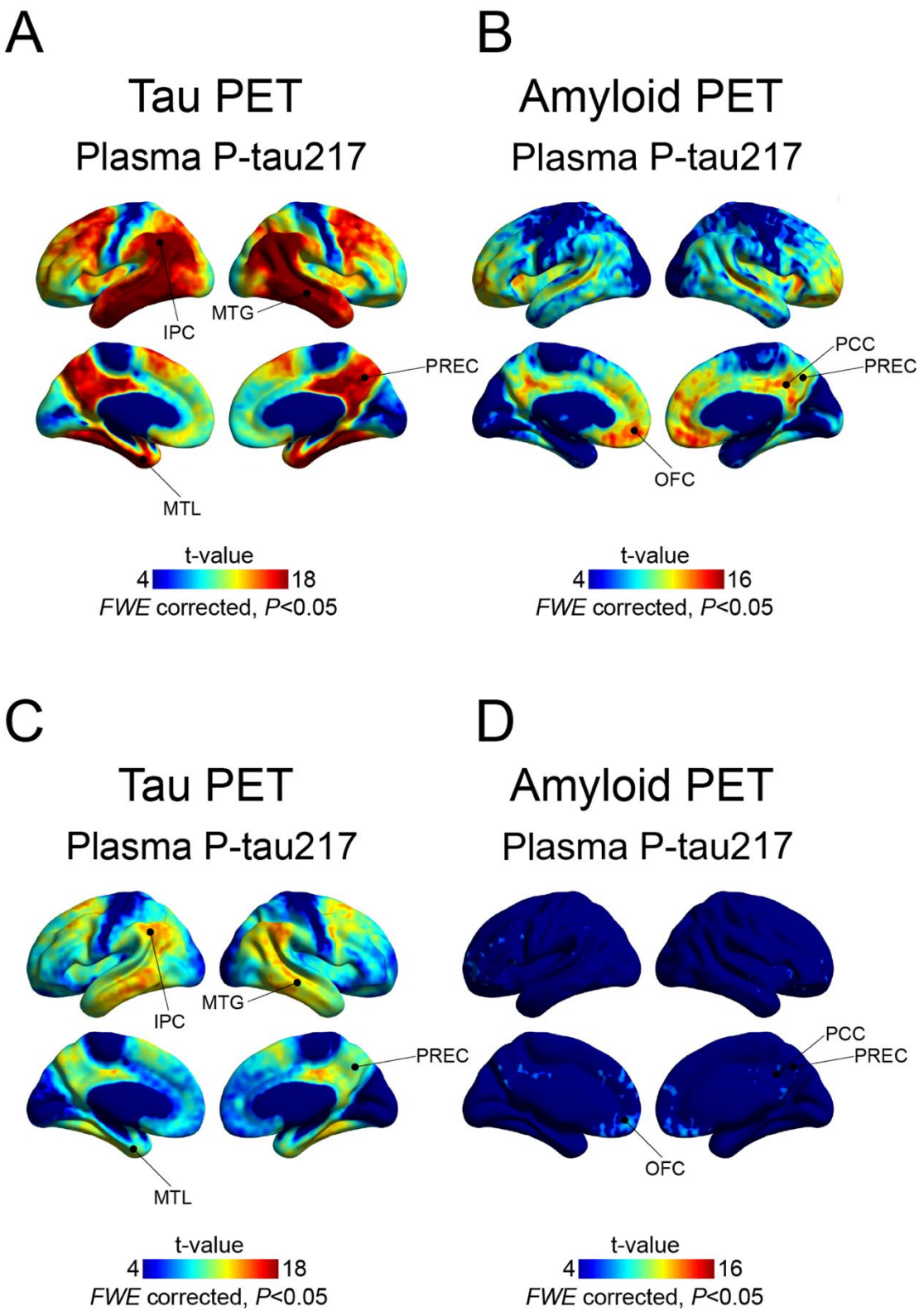


eFigure 4. Plasma P-tau217 in the BioFINDER-2 cohort (cohort-2) using the updated diagnostic framework to define pre-dementia AD. Aβ-positivity was defined as previously described in eMethods (CSF Aβ42/40 < 0.752), and tau (T)-positivity was defined as CSF P-tau217 > 101.95 pg/mL (mean + 2 SD of Aβ-negative CU participants). **A**, Plasma P-tau217 concentrations in Aβ-negative CU (n=224), Aβ-negative MCI (n=86), Aβ-pos/T-neg CU ("CU with Alzheimer pathological change"; n=19), Aβ-pos/T-neg MCI ("MCI with Alzheimer pathological change"; n=16), preclinical AD (Aβ-pos/T-pos CU, n=58), AD with MCI (Aβ-pos/T-pos MCI, n=76). Boxes show interquartile range, the horizontal lines are medians and the whiskers were plotted using Tukey method. Five outliers are not shown in A, but were included in all statistical analysis. P-values from

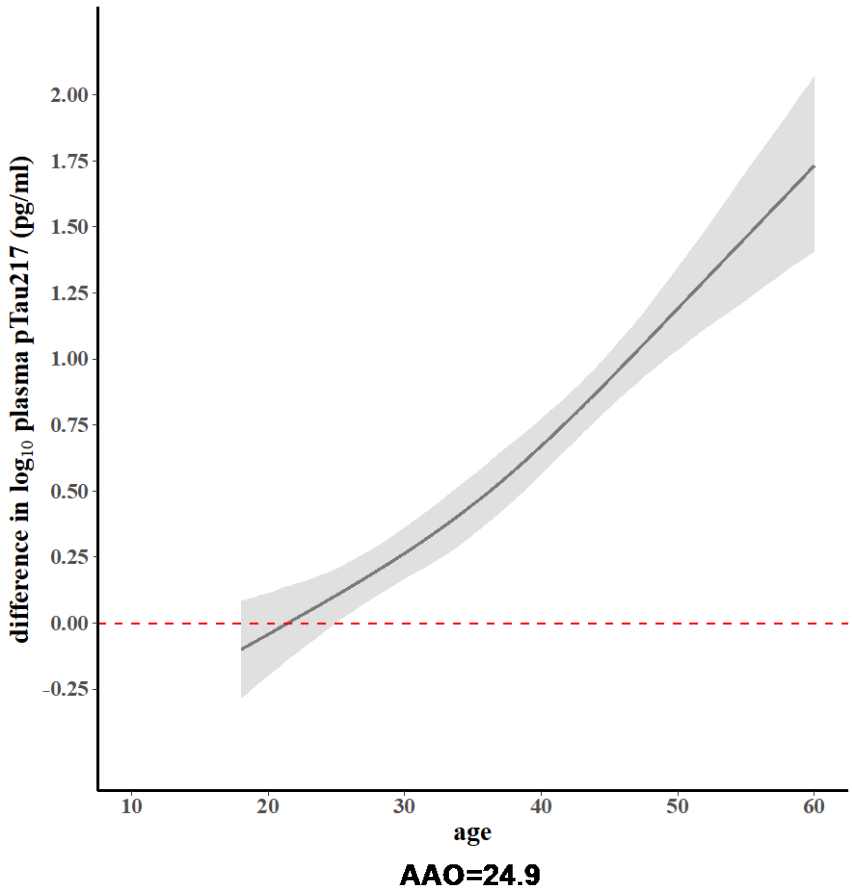
group comparisons are shown in eTable 13. **B** and **C**, ROC curve analyses of plasma P-tau217, other plasma biomarkers and MRI for discriminating preclinical AD from Aβ- CU (**B**) and AD with MCI from Aβ- MCI (**C**). **D** and **E**, Plasma P-tau217, CSF P-Tau181, and tau-PET (temporal meta-ROI SUVR) for discriminating preclinical AD from Aβ- CU (**D**) and AD with MCI from Aβ- MCI (**E**).
CSF P-tau217 and CSF Aβ42/40 were not included since they were used to define preclinical AD and AD with MCI. Corresponding sensitivities, specificities and % correctly classified cases are shown in eTables 14-15.
Abbreviations: AD, Alzheimer disease; AUC, area under the curve; Aβ-pos, β-amyloid positive; Aβ-neg, β-amyloid negative; CSF, cerebrospinal fluid; CU, cognitively unimpaired controls; MCI, mild cognitive impairment; PET, positron emission tomography; ROC, receiver operating characteristic; T-neg, tau negative; T-pos, tau positive.



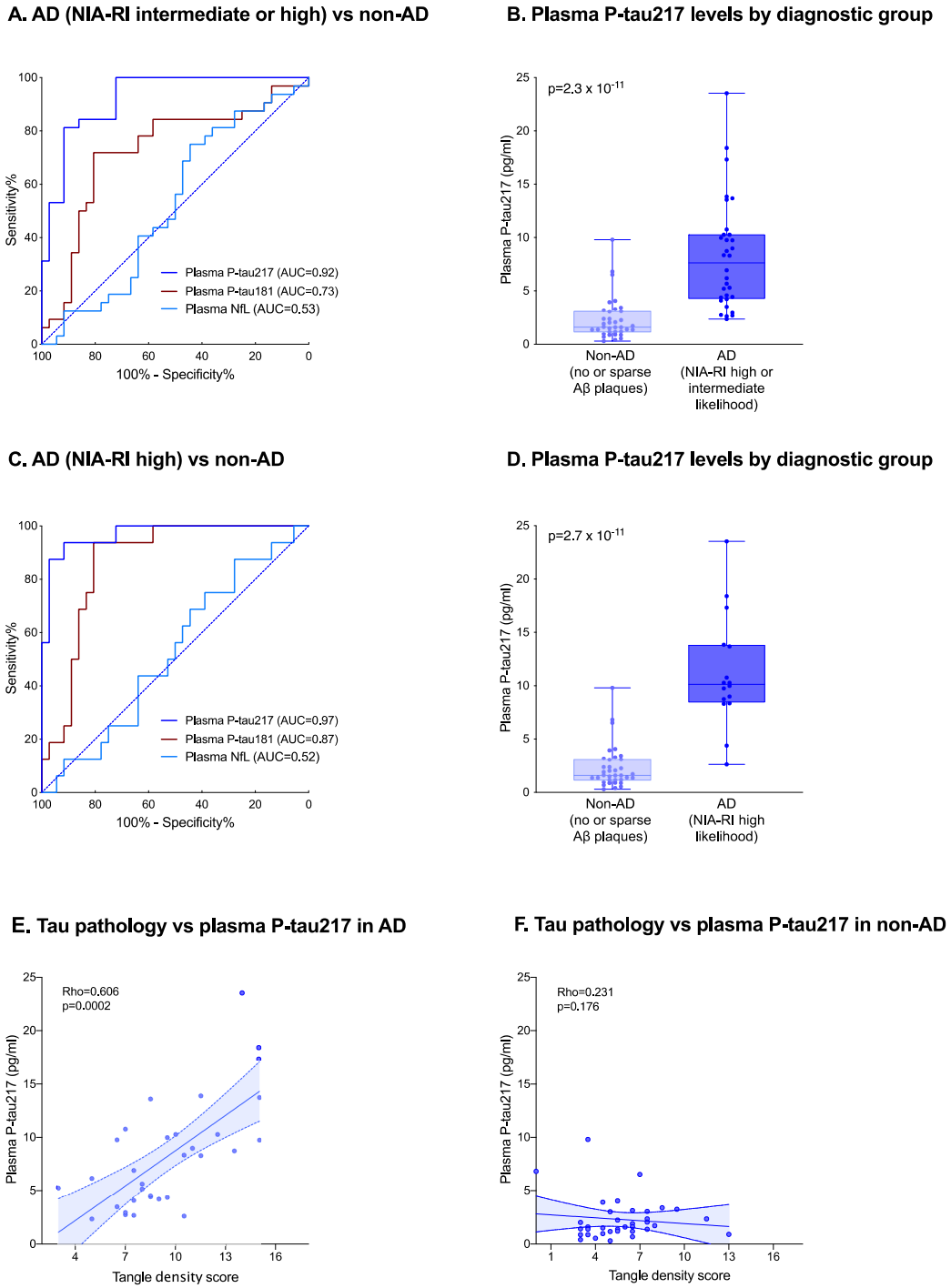
eFigure 5. Associations of plasma P-tau217 with tau-PET and CSF P-tau217 in the BioFINDER-2 cohort (cohort-2). Association of plasma P-tau217 with tau-PET in the temporal meta-ROI (A) and CSF P-tau217 (B) in Aβ-positive (n=305) and Aβ-negative (n=394) study participants. Dashed lines show the threshold for plasma P-tau217 (black), CSF P-tau217 (red) and tau-PET (red) positivity (2.5 pg/mL, 101.9 pg/mL and 1.36 SUVR, respectively). One plasma P-tau217 outlier is not shown but was included in the analysis. Blue and pink lines show spline models separately in Aβ+ and Aβ- participants with corresponding r^2 values in the upper right corners.
Abbreviations: Aβ+, β-amyloid positive; Aβ-, β-amyloid negative; CSF, cerebrospinal fluid; PET, positron emission tomography; ROI, region of interest; SUVR, standardized uptake values ratio**.**



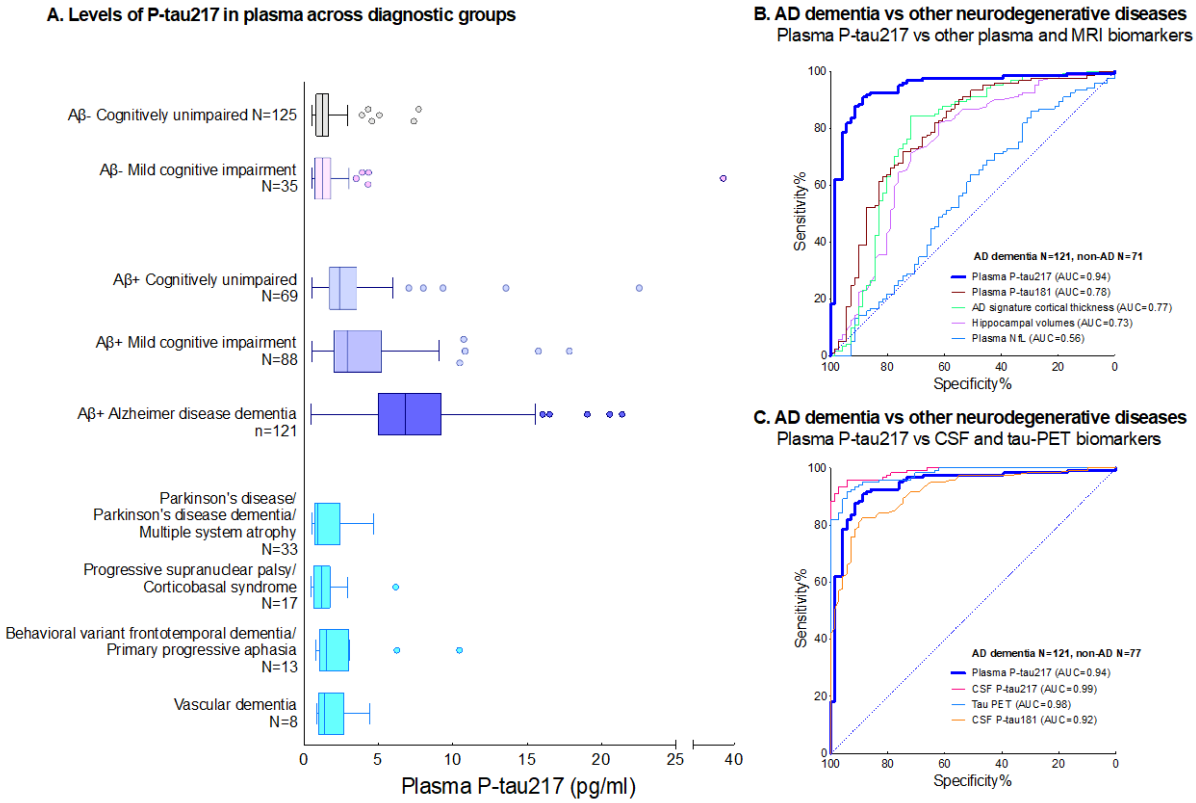
eFigure 6. Voxel-based associations of plasma P-tau217 with tau-PET and Aβ-PET in the BioFINDER-2 study (cohort-2). A and **B,** voxel-wise multiple regression analysis of tau-PET and Aβ-PET, adjusted for age and sex, in the whole BioFINDER-2 cohort (cohort-2; n=699 for tau-PET; n=488 for Aβ-PET). **C** and **D**, voxel-wise multiple regression analysis of tau-PET and Aβ-PET in Aβ+ CU (n=77), Aβ+ MCI (n=92), and Aβ+AD dementia (n=121) in cohort-2, adjusted for age and sex.
Abbreviations: AD, Alzheimer disease; β-amyloid; CU, cognitively unimpaired; FWE, familywise error; MCI, mild cognitive impairment; PET, positron emission tomography.



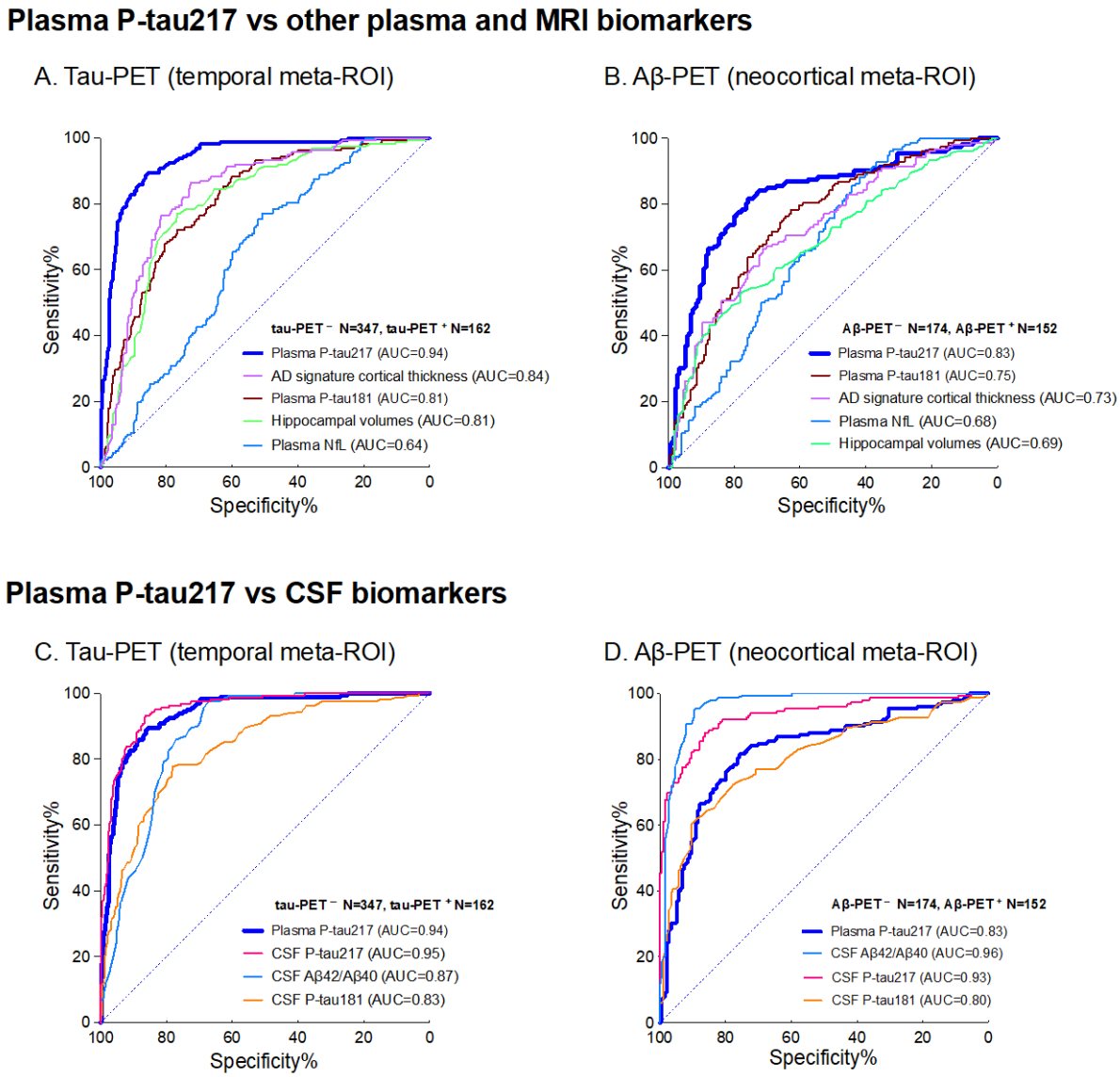
eFigure 7. Difference in plasma P-tau217 between carriers and non-carriers as a function of age in the autosomal dominant AD kindred (cohort-3). Differences in plasma P-tau217 (log-transformed) between PSEN1 E280A mutation carriers and non-carriers and as a function of age. Non-carrier levels are set at zero. The shaded areas represent the 99% credible intervals around the model estimates. The curves and credible intervals are drawn from the actual distributions of model fits derived by the Hamiltonian Markov chain Monte Carlo analyses.
Abbreviations: AAO, age at onset (time point where the biomarker differed between mutation carriers and non-carriers).



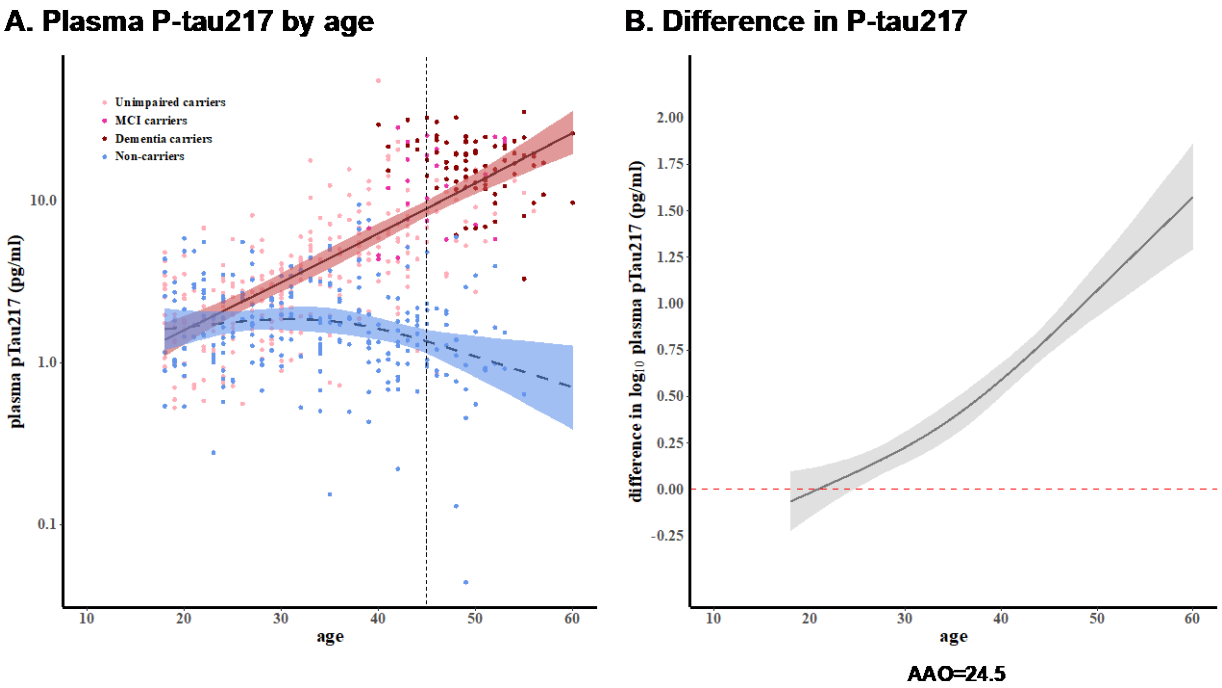
eFigure 8. Sensitivity analysis (excluding plasma P-tau217 values below the detection limit) in the neuropathology cohort (cohort-1). **A** and **C**, ROC curve analyses for distinguishing AD with high or intermediate (**A**) or high (**C**) likelihood of AD from non-AD. **B** and **D**, Antemortem plasma P-tau217 concentrations in the AD and non-AD groups. The AD group included 32 cases with intermediate or high (**A-B**) or 16 cases with high (**C-E**) likelihood of AD according to NIA-Reagan criteria.[2] The non-AD group, included 36 cases with none or sparse neuritic plaques. **E** and **F**, Associations between plasma P-tau217 and total tangle density score in the AD (n=32) and non-AD groups (n=36). The dots indicate individuals. Boxes show interquartile range, the horizontal lines are medians and the whiskers were plotted down to the minimum and up to the maximum value. In **B** and **D**, P-values are shown for group (AD or non-AD) from linear regression model using plasma P-tau217 levels as outcome and group, age, sex and time between sample collection and death as independent variables. In **E** and **F**, data are from Spearman correlation tests.
Abbreviations: AD, Alzheimer disease; AUC, area under the curve; NIA-RI, National Institute on Aging-Reagan Institute Working Group; ROC, receiver operating characteristic.



eFigure 9. Sensitivity analysis (excluding plasma P-tau217 values below the detection limit) in the BioFINDER-2 cohort (cohort-2) (group comparisons). A, plasma P-tau217 concentrations across the different diagnostic groups. Boxes show interquartile range, the vertical lines are medians and the whiskers were plotted using the Tukey method. **B-C** show ROC curve analyses with AD dementia (n=121) as reference standard vs all non-AD neurodegenerative diseases (n=71) using the biomarkers plasma P-tau217, other plasma biomarkers and MRI (**B**); and plasma P-tau217, CSF biomarkers and tau-PET (**C**).
Abbreviations: AD, Alzheimer disease; AUC, area under the curve; Aβ+, β-amyloid-positive; Aβ-, β-amyloid negative; CSF, cerebrospinal fluid; PET, positron emission tomography; ROC, receiver operating characteristic.



eFigure 10. Sensitivity analysis (excluding plasma P-tau217 values below the detection limit) in the BioFINDER-2 cohort (cohort-2) (associations with tau- and Aβ-PET). ROC curve analyses of plasma P-tau217, other plasma biomarkers and MRI (**A, B**) as well as CSF biomarkers (**C, D**) using tau-PET status in the temporal meta-ROI (**A, C**) and Aβ-PET positivity in the neocortical meta-ROI (**B, D**) as reference standard. Abbreviations: AUC, area under the curve; Aβ, β-amyloid; CSF, cerebrospinal fluid; PET, positron emission tomography; ROC, receiver operating characteristic; ROI region of interest.



eFigure 11. Sensitivity analysis (excluding plasma P-tau217 values below the detection limit) in the Colombia kindred registry of autosomal-dominant AD (cohort-3). A, plasma P-tau217 (log10 transformed) in PSEN1 E280A mutation carriers and non-carriers as a function of age. The dotted line indicates the average onset of mild cognitive impairment in mutation-carriers (at 44 years of age). **B**, differences in plasma P-tau217 (log10 transformed) between PSEN1 E280A mutation carriers and non-carriers as a function of age. Non-carrier levels are set at zero. The shaded areas represent the 99% credible intervals around the model estimates. The curves and credible intervals are drawn from the actual distributions of model fits derived by the Hamiltonian Markov chain Monte Carlo analyses.
Abbreviation: AAO, age at onset (time point where the biomarker differed between mutation carriers and non-carriers).

4. eTables

eTable 1. Biomarker assays (all cohorts)

	Assay/platform		
	Neuropathology cohort (cohort-1)	**BioFINDER-2 Cohort** (cohort-2)	**Autosomal-dominant AD cohort** (cohort-3)
Plasma P-tau217	MSD-based assay developed by Eli Lilly	MSD-based assay developed by Eli Lilly	MSD-based assay developed by Eli Lilly
Plasma P-tau181	MSD-based assay developed by Eli Lilly	Simoa-based assay developed by the Clinical Neurochemistry Laboratory in Gothenburg, Sweden	N/A
Plasma Aβ42	ELISA (Euroimmun)	ELISA (Euroimmun)	N/A
Plasma Aβ40	ELISA (Euroimmun)	ELISA (Euroimmun)	N/A
Plasma T-tau	Simoa-based assay (Quanterix)	Simoa-based assay (Quanterix)	N/A
Plasma NfL	Simoa-based assay	Simoa-based assay	Simoa-based assay
CSF P-tau217	N/A	MSD-based assay developed by Eli Lilly	N/A
CSF P-tau181	N/A	Innotest® immunoassay (Fujirebio)	N/A
CSF Aβ42	N/A	MSD-based assay (MSD)	N/A
CSF Aβ40	N/A	MSD-based assay (MSD)	N/A

Abbreviations: N/A, not available.

eTable 2. Participant characteristics across the three cohorts.

	Neuropathology cohort (cohort-1)		**BioFINDER-2 cohort** (cohort-2)				**Autosomal-dominant AD cohort** (cohort-3)	
	Non-AD pathology n=47	AD pathology n=34	CU (n=301)	MCI (n=178)	AD dementia (n=121)	Other neurodegenerative diseases (n=99)	Non-carriers (n=257)	Carriers (n=365)
Age, median (IQR), y	84.0 (77.0-90.0)	84.0 (79.0-89.3)	66.6 (55.3-76.1)	72.2 (65.3-75.9)	74.2 (70.4-78.1)	72.4 (64.0-76.5)	34.0 (25.5-42.0)	37.0 (27.0-46.5)
Male, %	59.6	64.7	45.2	55.1	47.9	51.5	39.3	45.5
MMSE score, median (IQR)	27.0 (24.0-29.0)	20.5 (17.0-25.3)	29.0 (28.0-30.0)	27.0 (25.0-29.0)	20.0 (18.0-23.0)	27.0 (24.0-29.0)	30.0 (28.0-30.0)	29.0 (25.0-30.0)
Plasma P-tau217 pg/mL, median (IQR)	1.39 (0.41-2.36)	6.56 (3.94-10.26)	0.89 (0.33-1.79)	1.47 (0.33-3.26)	6.83 (5.00-9.24)	0.89 (0.37-1.57)	1.48 (0.95-2.49)	4.88 (2.19-12.33)

Abbreviations: AD, Alzheimer disease; CU, cognitively unimpaired; IQR, interquartile range; MCI, mild cognitive impairment; MMSE, mini-mental state examination.

eTable 3. Participant characteristics in the neuropathology cohort (cohort-1)

	Non-AD pathology no or sparse amyloid plaques, n=47	**AD pathology** NIA-R intermediate or high, n=34
Age, median (IQR), y	84.0 (77.0-90.0)	84.0 (79.0-89.3)
Male, %	59.6	64.7
Post-mortem interval, median (IQR), h	3.15 (2.50-4.25)	2.48 (3.05-3.05)
MMSE score, median (IQR) [a]	27.0 (24.0-29.0)	20.5 (17.0-25.3)
Interval MMSE to death, median (IQR), m	10.0 (5.0-14.0)	10.5 (6.0-14.3)
APOE ε4 positivity, % (n/total n) [b]	14.9 (7/47)	60.6 (20/33)
Interval plasma collection to death, median (IQR), m	11.3 (6.1-21.8)	11.7 (6.3-18.8)
Plasma P-tau217 pg/mL, median (IQR)	1.39 (0.41-2.36)	6.56 (3.94-10.26)
Plasma P-tau181 pg/mL, median (IQR)	1.75 (1.32-2.66)	3.33 (2.20-4.50)
Plasma T-tau, median (IQR)	2.03 (1.51-2.83)	2.08 (1.70-2.28)
Plasma NfL pg/mL, median (IQR)	29.2 (20.5-45.4)	28.1 (23.8-38.3)
Plasma Aβ42/Aβ40, median (IQR)	0.175 (0.160-0.191)	0.154 (0.140-0.171)
Plaque total score, median (IQR) [c]	0.00 (0.00-1.00)	14.0 (12.4-14.5)
Tangle total score, median (IQR) [d]	5.50 (4.00-7.00)	8.50 (7.00-11.5)
Braak score [e] I/II/III/IV/V/VI	1/3/20/19/4/0	0/0/4/14/12/4

[a] Range: 0 to 30, lower scores indicate worse global cognition.
[b] Data are missing for 2 participants.
[c] Arithmetic sum of scores from senile amyloid plaque density scores in standard regions of the frontal, temporal and parietal lobes, hippocampal CA1 region and entorhinal/transentorhinal region.
[d] Arithmetic sum of scores from neurofibrillary tau-tangle density score in standard regions of the frontal, temporal and parietal lobes, hippocampal CA1 region and entorhinal/transentorhinal region.
[e] Braak score is the Braak neurofibrillary stage (0-VI) as defined originally by Braak and Braak.[4]
Abbreviations: IQR, interquartile range; m, months; MMSE, Mini-mental state examination; n, number of cases; y, years.

eTable 4. Participant characteristics in the BioFINDER-2 cohort (cohort-2) [a]

	Cognitively unimpaired (n=301)	Mild cognitive impairment (n=178)	Alzheimer disease dementia (n=121)	Other neurodegenerative diseases (n=99)
Age, median (IQR), y	66.6 (55.3-76.1)	72.2 (65.3-75.9)	74.2 (70.4-78.1)	72.4 (64.0-76.5)
Male, %	45.2	55.1	47.9	51.5
Duration of education, median (IQR), years [b]	12.0 (10.0-15.0)	12.0 (9.0-15.0)	11.0 (9.0-15.0)	12.0 (10.0-15.0)
MMSE score, median (IQR) [b, c]	29.0 (28.0-30.0)	27.0 (25.0-29.0)	20.0 (18.0-23.0)	27.0 (24.0-29.0)
Aβ positivity, % (No./total No.)	26% (77/301)	52% (92/178)	100% (121/121)	15% (15/99)
APOE ε4 positivity, % (n/total n) [b]	46% (137/301)	53% (95/178)	71% (85/120)	31% (31/99)
Plasma P-tau217 pg/mL, median (IQR)	0.89 (0.33-1.79)	1.47 (0.33-3.26)	6.83 (5.00-9.24)	0.89 (0.37-1.57)
Plasma P-tau181 pg/mL, median (IQR)	6.04 (3.97-8.60)	7.06 (4.50-10.51)	11.83 (9.30-16.00)	6.58 (4.90-9.71)
Plasma Aβ42/Aβ40, median (IQR)	0.16 (0.15-0.19)	0.16 (0.14-0.18)	0.15 (0.13-0.17)	0.16 (0.14-0.19)
Plasma T-tau pg/mL, median (IQR)	1.55 (1.21-1.96)	1.63 (1.24-2.13)	1.87 (1.50-2.50)	1.20 (1.58-1.58)
Plasma NfL pg/mL, median (IQR)	13.0 (9.1-17.9)	16.2 (11.6-22.5)	20.8 (16.8-31.6)	21.2 (15.1-34.4)
CSF P-tau217 pg/mL, median (IQR)	50.8 (29.7-96.6)	94.1 (47.1-285.5)	525.9 (356.5-798.5)	48.3 (34.1-76.5)
CSF P-tau181 pg/mL, median (IQR)	41.0 (34.0-57.0)	47.0 (36.0-73.0)	84.0 (64.0-109.5)	36.0 (28.0-49.0)
CSF Aβ42/Aβ40, median (IQR)	0.98 (0.74-1.12)	0.72 (0.51-1.03)	0.49 (0.37-0.56)	0.99 (0.83-1.15)
Tau-PET SUVR, median (IQR) [d]	1.15 (1.09-1.22)	1.21 (1.13-1.33)	2.04 (1.61-2.54)	1.16 (1.11-1.22)

[a] More detailed information on the study groups is given in eTable 5 in the Online Supplement.
[b] Data is missing for 1 participant.
[c] Range: 0 to 30, lower scores indicate worse global cognition.
[d] Measured in a temporal meta-ROI.
Abbreviations: Aβ, β-amyloid; CSF, cerebrospinal fluid; IQR, interquartile range; MMSE, mini-mental state examination; ROI, region of interest; SD, standard deviation; SUVR, standardized uptake value ratio.

eTable 5. Additional participant characteristics in the BioFINDER-2 cohort (cohort-2)

	Aβ- CU (n=224)	Aβ+ CU (n=77)	Aβ- MCI (n=86)	Aβ+ MCI (n=92)	Aβ+ AD (n=121)	PD/PDD/ MSA (n=45)	PSP/ CBS (n=21)	VaD (n=12)	bvFTD/ PPA (n=21)
Age, median (IQR), y	63.1 (53.2-75.0)	73.5 (64.7-79.4)	70.9 (61.6-74.7)	73.0 (67.7-76.6)	74.2 (70.4-78.1)	73.0 (62.7-77.4)	71.2 (63.5-75.7)	74.2 (69.8-80.6)	68.8 (65.5-77.0)
Male, %	45.5	44.2	61.6	48.9	47.9	55.6	52.4	58.3	38.1
Duration of education, mean (SD), years	12.8 (10.0-15.0)	12.0 (9.0-15.0)	12.0 (9.0-14.0)	12.0 (9.0-16.0)	11.0 (9.0-15.0)	14.0 (11.0-15.3)	12.5 (10.0-15.5)	10.5 (9.3-12.8)	12.0 (9.0-14.0)
MMSE score, median (IQR)	29.0 (28.0-30.0)	29.0 (28.0-30.0)	28.0 (26.0-29.0)	27.0 (25.0-29.0)	20.0 (18.0-23.0)	29.0 (27.0-30.0)	27.0 (24.0-29.0)	23.0 (21.3-27.3)	24.0 (20.5-27.0)
Aβ positivity, % (No./total No.)	0% (0/224)	100% (77/77)	0% (0/86)	100% (92/92)	100% (121/121)	18% (8/45)	5% (1/21)	17% (2/12)	19% (4/21)
APOE ε4 positivity, % (No./total No.)	38% (84/224)	69% (53/77)	29% (25/86)	76% (70/92)	71% (85/120)	40% (18/45)	19% (4/21)	25% (3/12)	27% (6/21)
Plasma P-tau217 pg/mL, median (IQR)	0.60 (0.20-1.30)	2.28 (1.44-3.25)	0.35 (0.02-1.23)	2.86 (1.84-4.95)	6.83 (5.00-9.24)	0.77 (0.36-1.55)	0.91 (0.50-1.42)	0.97 (0.31-2.13)	1.01 (0.33-2.14)
Plasma P-tau181 pg/mL, median (IQR)	5.28 (3.58-7.32)	8.81 (5.91-11.42)	5.40 (3.56-8.48)	8.43 (6.60-12.17)	11.83 (9.30-16.00)	6.78 (4.91-10.92)	6.59 (5.29-9.27)	6.40 (4.22-9.76)	6.54 (3.75-9.61)
Plasma Aβ42/Aβ40, median (IQR)	0.17 (0.15-0.19)	0.15 (0.14-0.16)	0.17 (0.15-0.19)	0.15 (0.14-0.16)	0.15 (0.13-0.17)	0.16 (0.14-0.20)	0.16 (0.14-0.17)	0.16- (0.14-0.18)	0.17 (0.15-0.18)
Plasma T-tau pg/mL, median (IQR)	1.57 (1.24-1.96)	1.48 (1.12-2.00)	1.55 (1.22-1.97)	1.71 (1.33-2.28)	1.87 (1.50-2.50)	1.36 (1.07-1.70)	1.75 (1.20-2.10)	1.97 (1.63-2.37)	1.66 (1.39-2.24)
Plasma NfL pg/mL, median (IQR)	11.8 (8.2-16.6)	15.8 (12.3-21.4)	15.5 (10.9-23.2)	16.5 (11.8-21.8)	20.8 (16.8-31.6)	19.2 (13.1-26.9)	23.1 (17.9-39.4)	23.1 (14.7-34.4)	30.0 (17.4-43.7)
CSF P-tau217 pg/mL, median (IQR))	38.8 (26.3-56.0)	163.2 (102.8-285.4)	49.1 (32.5-71.4)	282.0 (129.7-416.7)	525.9 (356.5-798.5)	48.3 (34.7-71.5)	46.2 (29.4-66.8)	61.1 (32.0-182.9)	50.1 (32.2-86.7)
CSF P-tau181 pg/mL, median (IQR)	38.0 (31.0-48.0)	61.0 (50.0-75.5)	40.0 (31.8-46.3)	69.5 (46.3-84.0)	84.0 (64.0-109.5)	39.0 (29.5-50.0)	31.0 (19.0-38.0)	39.5 (28.0-64.8)	37.0 (29.5-52.5)
CSF Aβ42/Aβ40, median (IQR)	1.05 (0.95-1.16)	0.55 (0.46-0.65)	1.03 (0.91-1.15)	0.51 (0.44-0.60)	0.49 (0.37-0.56)	1.00 (0.82-1.11)	0.95 (0.81-1.13)	0.97 (0.88-1.21)	1.00 (0.80-1.18)
Tau-PET SUVR, median (IQR) [a]	1.14 (1.08-1.20)	1.19 (1.14-1.32)	1.13 (1.07-1.22)	1.30 (1.19-1.72)	2.04 (1.61-2.54)	1.16 (1.13-1.21)	1.15 (1.08-1.21)	1.17 (1.06-1.24)	1.17 (1.10-1.23)

[a] Measured in a temporal meta-ROI.

Abbreviations: Aβ, β-amyloid; bvFTD, behavioral variant of frontotemporal dementia; CBS, corticobasal syndrome; CSF, cerebrospinal fluid; CU, cognitively unimpaired; IQR, interquartile range; MCI, mild cognitive impairment; MSA, multiple system atrophy; PD, Parkinson's disease; PDD, Parkinson's disease dementia; PPA, primary progressive aphasia; PSP, progressive supranuclear palsy; VaD, vascular dementia.

eTable 6. Characteristics of mutation carriers and non-carriers in the Colombia kindred registry of autosomal-dominant AD (cohort-3)

	Carriers (n=365)	Non-carriers (n=257)
Age, median (IQR), y	37.0 (27.0-46.5)	34.0 (25.5-42.0)
Male, %	45.5	39.3
Duration of education, median (IQR), years	7.0 (4.0-11.0)	9.0 (5.0-11.0)
MMSE score, median (IQR) [a]	29.0 (25.0-30.0)	30.0 (28.0-30.0)
CERAD world list recall, median (IQR) [b]	5.0 (2.0-7.0)	6.0 (5.0-8.0)
Plasma P-tau217 pg/mL, median (IQR)	4.88 (2.19-12.33)	1.48 (.95-2.49)
Plasma NfL pg/mL, median (IQR)	10.10 (6.09-17.69)	6.25 (4.31-8.42)

[a] Range: 0 to 30, lower scores indicate worse global cognition; data is missing for 60 participants.
[b] Range: 0 to 10, lower scores indicate worse memory; data is missing for 67 participants.
Abbreviations: CERAD, The Consortium to Establish a Registry for Alzheimer's Disease; IQR, interquartile range; MMSE, Mini-mental state examination.

eTable 7. Characteristics of impaired and unimpaired mutation carriers and non-carriers in the Colombia kindred registry of autosomal-dominant AD (cohort-3)

	Cognitively impaired carriers (n=106)	Cognitively unimpaired carriers (n=259)	Non-carriers (n=257)
Age, mean median (IQR), y	49.0 (46.0-52.0)	31.0 (24.0-39.0)	34.0 (25.5-42.0)
Male, %	45.3	45.6	39.3
Duration of education, median (IQR), years	5.0 (2.0-7.0)	9.0 (5.0-11.0)	9.0 (5.0-11.0)
MMSE score, median (IQR) [a]	19.0 (12.3-24.0)	29.0 (28.0-30.0)	30.0 (28.0-30.0)
CERAD world list recall, median (IQR) [b]	0.00 (0.00-1.00)	6.00 (5.00-7.00)	6.0 (5.0-8.0)
Plasma P-tau217 pg/mL, median (IQR)	16.74 (11.97-21.76)	3.20 (1.76-5.65)	1.48 (.95-2.49)
Plasma NfL pg/mL, median (IQR)	24.15 (17.00-34.55)	7.41 (5.13-10.90)	6.25 (4.31-8.42)

[a] Range: 0 to 30, lower scores indicate worse global cognition; data is missing for 32 participants.
[b] Range: 0 to 10, lower scores indicate worse memory; data is missing for 44 participants.
Abbreviations: CERAD, The Consortium to Establish a Registry for Alzheimer's Disease; IQR, interquartile range; MMSE, Mini-mental state examination.

eTable 8. Individual description of the non-AD participants in the neuropathology cohort (cohort-1)

N	Braak tau tangle score	CERAD neuritc plaque score	Clinical diagnosis	Primary neuropathological diagnosis	Neuropathological co-pathologies
1	I	zero	ALS	ALS	Definite PART
2	II	zero	Multiple sclerosis	Multiple sclerosis	Definite PART
3	II	sparse	PD	PD	Possible PART
4	II	zero	Parkinsonism	White matter changes, infarcts	Definite PART
5	III	zero	Control	Definite PART	White matter changes, CAA
6	III	zero	Control	Definite PART	CAA, incidental Lewy body disease
7	III	zero	Control	Definite PART	Acute infarct
8	III	sparse	Control	Possible PART	Incidental LBD
9	III	sparse	Control	Possible PART	ARTAG
10	III	sparse	Control	Possible PART	ARTAG, microscopic changes of LBD
11	III	sparse	Control	PSP (incidental)	Schwannoma
12	III	zero	Dementia NOS	Neurofibrillary tangle-predominant dementia	Definite PART, infarcts
13	III	zero	Frontotemporal Dementia	FTLD with TDP-43 proteinopathy	Definite PART, microscopic changes of LBD
14	III	sparse	MCI	Possible PART	White matter changes
15	III	sparse	MCI	White matter changes, infarcts	Possible PART
16	III	sparse	PD	PD	Possible PART, AGD, ARTAG
17	III	zero	PD	PD	Definite PART, CAA
18	III	zero	PD	PD	Definite PART, ARTAG
19	III	zero	PD with dementia	PD	Definite PART, infarcts
20	III	zero	PD with dementia	PD	Definite PART, AGD
21	III	zero	PD with dementia	PD	Definite PART, white matter changes, infarcts, ARTAG
22	III	zero	PD with MCI	Corticobasal degeneration	PD, white matter changes, ARTAG
23	III	zero	PD with MCI	PD	Definite PART, ARTAG
24	III	sparse	PD with MCI	PD	Possible PART, ARTAG, infarcts
25	IV	zero	Control	Astrocytoma dffuse (grade II)	Definite PART, AGD, white matter changes
26	IV	zero	Control	Definite PART	None
27	IV	zero	Control	Definite PART	AGD, ARTAG
28	IV	zero	Control	Definite PART	ARTAG, incidental LBD, white matter changes
29	IV	zero	Control	Definite PART	White matter changes

N	Braak tau tangle score	CERAD neuritc plaque score	Clinical diagnosis	Primary neuropathological diagnosis	Neuropathological co-pathologies
30	IV	sparse	Control	Possible PART	AGD
31	IV	sparse	Control	Possible PART	ARTAG
32	IV	sparse	Control	Possible PART	TDP-43 proteinopathy
33	IV	zero	MCI	Definite PART	White matter changes, AGD, ARTAG
34	IV	zero	MCI	Definite PART	Incidental LBD
35	IV	zero	MCI	Definite PART	AGD, ARTAG
36	IV	zero	MCI	White matter changes, infarcts	Definite PART, AGD, ARTAG
37	IV	sparse	PD	PD	Possible PART, white matter changes
38	IV	sparse	PD	PD	Possible PART, AGD, ARTAG, white matter changes
39	IV	zero	PD with dementia	PD	Definite PART, AGD, ARTAG
40	IV	sparse	PD with MCI	PD	Possible PART, AGD, ARTAG
41	IV	zero	Parkinsonism with dementia	None	Definite PART, AGD, infarcts
42	IV	zero	Vascular Dementia	Vascular dementia	PSP, AGD
43	IV	zero	Vascular parkinsonism with dementia	Vascular dementia	Definite PART
44	V	sparse	CBD	CBD	Vascular dementia, AGD, ARTAG
45	V	sparse	Dementia NOS	Neurofibrillary tangle-predominant dementia	Microscopic changes of PSP (insufficient for diagnosis), ARTAG
46	V	zero	PD with MCI	PSP	PD, AGD
47	V	zero	PSP with dementia	PSP	White matter changes

Abbreviations: AGD, agyrophilic grain disease; ALS, amyotrophic lateral sclerosis; ARTAG, aging-related tau astrogliopathy; CAA, cerebral amyloid angiopathy; CBD, corticobasal degeneration; Definite PART, primary age related tauopathy with CERAD neuritic plaque score = zero; LBD, Lewy body disease; MCI, mild cognitive impairment; N, case number; Possible PART, primary age related tauopathy with CERAD neuritic plaque score = sparse; PD, Parkinson's disease; PSP, Progressive supranuclear palsy.

eTable 9. Diagnostic description of the other (non-AD) neurodegenerative diseases in the BioFINDER-2 cohort (cohort-2)

Clinical diagnosis	N
Parkinson's disease with/without dementia	39
Progressive supranuclear palsy	22
Vascular dementia	14
Behavioral variant of frontotemporal dementia	14
Multiple systemic atrophy	10
Semantic variant of primary progressive aphasia	6
Corticobasal syndrome	2
Progressive non-fluent aphasia	2

Abbreviations: N, number of participants.

eTable 10. ROC comparison of plasma biomarkers in the neuropathology cohort (cohort-1)

Biomarker	AUC (95% CI)	AUC difference (95% CI)	P-value (compared with plasma P-tau217)	Cut-off	Correctly classified participants	Sensitivity	Specificity
Outcome: AD (intermediate to high likelihood; n=34) vs non-AD (n=47)							
Plasma P-tau217	0.89 (0.81-0.97)	NA	NA	2.36 pg/mL	85%	94%	79%
Plasma P-tau217/Aβ42	0.90 (0.81-0.98)	-0.01 (-0.05-0.4)	1.00	0.083	89%	88%	89%
Plasma P-tau217/T-tau	0.88 (0.79-0.96)	0.01 (-0.03-0.06)	1.00	1.48	84%	82%	85%
Plasma P-tau181	0.72 (0.60-0.84)	0.17 (0.04-0.30)	**0.04**	2.66	74%	71%	77%
Plasma NfL	0.50 (0.37-0.63) [a]	0.39 (0.26-0.52)	**<0.001**	41.9	53%	82%	32%
Outcome: AD (high likelihood; n=16) vs non-AD (n=47)							
Plasma P-tau217	0.98 (0.94-1.00)	NA	NA	4.21 pg/mL	94%	94%	94%
Plasma P-tau217/Aβ42	0.95 (0.88-1.00)	0.03 (-0.05-0.10)	1.00	0.085	90%	94%	89%
Plasma P-tau217/T-tau	0.94 (0.86-1.00)	0.04 (-0.05-0.12)	1.0	2.40	94%	88%	96%
Plasma P-tau181	0.85 (0.76-0.95)	0.12 (0.04-0.20)	**0.003**	2.66 pg/mL	81%	94%	77%
Plasma NfL	0.51 (0.35-0.67) [a]	0.46 (0.31-0.61)	**<0.001**	46.1 pg/mL	40%	88%	23%

Correctly classified participants, sensitivity, and specificity were calculated using the cut-off that produced the highest Youden index (sensitivity + specificity – 1) for AD vs non-AD. P-values are Bonferroni corrected for multiple comparisons (i.e. uncorrected P-value x 4 is shown). Intermediate and high likelihood of AD was defined according to the NIA-Reagan neuropathology criteria.[2] Non-AD was used for those with no or sparse amyloid plaques in postmortem brain tissue according to the CERAD criteria.[3] P-values are from the comparison of AUCs (DeLong statistics).

[a] Not significant.

Abbreviations: AUC, area under the receiver operating characteristic curve; CERAD, the Consortium to Establish a Registry for Alzheimer's Disease CI, confidence interval; N/A, not applicable.

eTable 11. P-values from the comparisons in Figure 2A (AD dementia, Aβ-positive MCI and Aβ-positive CU vs all other groups) in the BioFINDER-2 cohort (cohort-2)

	Compared to AD	Compared to Aβ-positive MCI	Compared to Aβ-positive CU
AD	1	**<0.001**	**<0.001**
CU Aβ-negative	**<0.001**	**<0.001**	**<0.001**
CU Aβ-positive	**<0.001**	0.87	1
FTD/PPA	**<0.001**	**0.03**	0.94
MCI Aβ-negative	**<0.001**	**<0.001**	**0.03**
MCI Aβ-positive	**<0.001**	1	0.88
PD/PDD/MSA	**<0.001**	**<0.001**	**0.009**
PSP/CBD	**<0.001**	**0.002**	0.25
VaD	**<0.001**	0.16	0.86

All comparisons (P-values) were adjusted for age and sex and Bonferroni corrected (P-value x 9) to account for multiple comparisons. All AD participants were Aβ-positive. Bold P-values are significant.
Abbreviations: AD, Alzheimer disease; CBD, corticobasal degeneration; CU, cognitively unimpaired (controls and SCD); SCD, subjective cognitive decline; FTD, frontotemporal dementia; MCI, mild cognitive impairment; MSA, multiple system atrophy; PD, Parkinson's disease; PDD, PD dementia; PPA, primary progressive aphasia; PSP, progressive supranuclear palsy; VaD, vascular dementia.

eTable 12. ROC comparison of the discriminative accuracy for AD with dementia (n=121) vs other neurodegenerative diseases (n=99) in the BioFINDER-2 cohort (cohort-2)

Biomarker	AUC (95% CI)	AUC Difference (95% CI)	P-value (compared with plasma P-tau217)	Cut-off	Correctly classified participants	Sensitivity	Specificity
Plasma P-tau217	0.96 (0.93-0.98)	NA	NA	2.50	89%	93%	88%
Plasma P-tau217/Aβ42	0.94 (0.91-0.97)	0.02 (0.00-0.03)	0.50	0.095	87%	82%	92%
Plasma P-tau217/T-tau	0.93 (0.90-0.96)	0.03 (0.01-0.05)	0.16	0.11	88%	91%	85%
Plasma P-tau181	0.81 (0.74-0.87)	0.15 (0.10-0.21)	**<0.001**	11.9	67%	50%	89%
Plasma NfL	0.50 (0.42-0.58)[a]	0.46 (0.38-0.50)	**<0.001**	26.5	54%	67%	38%
MRI AD cortex	0.78 (0.72-0.85)	0.17 (0.10-0.25)	**<0.001**	2.49	74%	74%	74%
MRI hippocampus / ICV	0.74 (0.67-0.81)	0.22 (0.14-0.30)	**<0.001**	0.0019	59%	42%	80%
CSF P-tau217	0.99 (0.98-1.00)	-0.03 (-0.06-[-0.01])	0.15	101.9	92%	98%	84%
CSF P-tau181	0.90 (0.86-0.95)	0.05 (0.01-0.10)	0.21	67.1	79%	69%	92%
Tau-PET	0.98 (0.97-0.99)	-0.02 (-0.05-0.00)	0.72	1.32	94%	93%	95%

ROC analysis using AD dementia vs non-AD neurodegenerative diseases as reference standard. P-values are from the comparison of AUCs (DeLong statistics). P-values are Bonferroni corrected for multiple comparisons (i.e. uncorrected P-value x 10 is shown; including the plasma P-tau217/CSF Aβ42 ratio comparison described below). P-values in bold indicate a statistically significant difference in AUC compared with plasma P-tau217 ($P<0.05$). Cutoffs were established using the mean + 2 SD in Aβ-negative controls (i.e. established in an independent sample to avoid overfitting). Cut-offs are given in pg/mL for fluid biomarkers and in SUVR for tau-PET. Ratios of biomarkers have no unit.

Note that there are many different plasma Aβ42 assays with varying performance. Therefore, we also analyzed the plasma P-tau217/Aβ42 ratio using CSF Aβ42 as the denominator since this is the *in vivo* gold standard for Aβ42. Plasma P-tau217/CSF Aβ42 produced an AUC of 0.97 (95% CI 0.94-0.99), which was not significantly different from using just plasma P-tau217 for discriminating AD vs other neurodegenerative diseases (p=0.74).

[a] Not significant.

Abbreviations: AUC, area under the receiver operating characteristic curve; CI, confidence interval; ICV, intracranial volume; MCI, mild cognitive impairment; MRI AD cortex, cortical thickness in a region prone to atrophy in AD[20]; ROC, receiver operating characteristic; SD, standard deviation; SUVR, standardized uptake value ratio.

eTable 13. P-values from the comparisons in eFigure 4A (preclinical AD and AD with MCI vs non-AD groups) in the BioFINDER-2 cohort (cohort-2)

	Compared to preclinical AD (Aβ and tau positive CU), n=58	Compared to AD with MCI (Aβ and tau positive MCI), n=76
Non-AD CU (Aβ-negative), n=224	**<0.001**	**<0.001**
Non-AD MCI (Aβ-negative), n=86	**0.001**	**<0.001**
CU with Alzheimer pathological change (Aβ-positive), n=19	**0.047**	**0.001**
MCI with Alzheimer pathological change (Aβ-positive), n=16	1.00	0.42
Preclinical AD (Aβ- and tau-positive CU), n=58	1.00	1.00
AD with MCI (Aβ- and tau-positive MCI), n=76	1.00	1.00

Diagnostic classification according to the most recent NIA-AA criteria for AD.[6] All comparisons (P-values) were adjusted for age and sex and Bonferroni corrected for multiple comparisons (i.e. uncorrected P-value x 6 is shown).

Abbreviations: Aβ+, CSF Aβ42/40 < 0.752; AD, Alzheimer disease; CU, cognitively unimpaired; MCI, mild cognitive impairment; tau+, CSF P-tau217 > 101.95 pg/mL. Cutoffs were established using the mean + 2SD in Aβ-negative controls.

eTable 14. ROC comparison of the discriminative accuracy for preclinical AD (n=58) vs Aβ-negative CU (n=224) in the BioFINDER-2 cohort (cohort-2)

Biomarker	AUC (95% CI)	AUC Difference (95% CI)	P-value (compared with plasma P-tau217)	Correctly classified participants	Sensitivity	Specificity
Plasma P-tau217	0.90 (0.85-0.94)	NA	NA	88%	57%	96%
Plasma P-tau181	0.78 (0.72-0.85)	0.12 (0.04-0.20)	**0.02**	80%	24%	95%
Plasma NfL	0.71 (0.64-0.77)	0.19 (0.12-0.26)	**<0.001**	77%	7%	95%
MRI AD cortex	0.69 (0.60-0.77)	0.21 (0.11-0.31)	**<0.001**	79%	16%	96%
MRI hippocampus / ICV	0.61 (0.52-0.69)	0.29 (0.19-0.48)	**<0.001**	79%	9%	97%
CSF P-tau181	0.90 (0.85-0.94)	0.00 (-0.06-0.06)	1.00	85%	43%	96%
Tau-PET	0.73 (0.65-0.81)	0.17 (0.07-0.26)	**0.002**	83%	29%	97%

ROC analysis using preclinical AD vs Aβ-negative CU as reference standard. P-values are from the comparison of AUCs (DeLong statistics). P-values are Bonferroni corrected for multiple comparisons (i.e. uncorrected P-value x 6 is shown). P-values in bold indicate a statistically significant difference in AUC compared with plasma P-tau217 ($P<0.05$). Preclinical AD was defined as cognitively unimpaired Aβ-positive (CSF Aβ42/40 < 0.752) and tau-positive (CSF P-tau217 > 101.95 pg/mL) subjects. Aβ-negative CU was defined as CU subjects with CSF Aβ42/40 > 0.752. Cutoffs were established using the mean + 2 SD in Aβ-negative controls (see eTable 12 for cut-offs). CSF P-tau217 and Aβ42/40 were not included since they were used to defined preclinical AD. Abbreviations: AUC, area under the receiver operating characteristic curve; CI, confidence interval; CU, cognitively unimpaired; ICV, intracranial volume; MRI AD cortex, cortical thickness in a region prone to atrophy in AD[20]; ROC, receiver operating characteristic; SD, standard deviation; SUVR, standardized uptake value ratio.

eTable 15. ROC comparison of the discriminative accuracy for AD with MCI (n=76) vs Aβ-negative MCI (n=86) in the BioFINDER-2 cohort (cohort-2)

Biomarker	AUC (95% CI)	AUC Difference (95% CI)	P-value (Compared with plasma P-tau217)	Correctly classified participants	Sensitivity	Specificity
Plasma P-tau217	0.91 (0.86-0.95)	NA	NA	78%	62%	93%
Plasma P-tau181	0.77 (0.70-0.84)	0.14 (0.06-0.22)	**0.005**	63%	33%	90%
Plasma NfL	0.54 (0.45-0.63)[a]	0.36 (0.27-0.46)	**<0.001**	51%	16%	83%
MRI AD cortex	0.64 (0.55-0.73)	0.27 (0.16-0.37)	**<0.001**	59%	34%	81%
MRI hippocampus / ICV	0.62 (0.53-0.70)	0.29 (0.19-0.39)	**<0.001**	56%	26%	81%
CSF P-tau181	0.89 (0.83-0.94)	0.02 (-0.06-0.09)	1.00	78%	61%	93%
tau-PET	0.87 (0.82-0.92)	0.03 (-0.04-0.10)	1.00	78%	57%	97%

ROC analysis using AD with MCI vs Aβ-negative MCI as reference standard. P-values are from the comparison of AUCs (DeLong statistics). P-values are Bonferroni corrected for multiple comparisons (i.e. uncorrected P-value x 6 is shown). P-values in bold indicate a statistically significant difference in AUC compared with plasma P-tau217 ($P<0.05$). AD with MCI was defined as Aβ-positive (CSF Aβ42/40 < 0.752) and tau-positive (CSF P-tau217 > 101.95 pg/mL) patients with MCI. Aβ-negative MCI was defined as MCI patients with CSF Aβ42/40 > 0.752. Cutoffs for all included biomarkers were established using the mean + 2 SD in Aβ-negative controls (i.e. established in an independent sample to avoid overfitting; see eTable 12 for cut-offs). CSF P-tau217 and Aβ42/40 were not included since they were used to defined AD with MCI.

[a] Not significant.

Abbreviations: AUC, area under the receiver operating characteristic curve; CI, confidence interval; ICV, intracranial volume; MCI, mild cognitive impairment; MRI AD cortex, cortical thickness in a region prone to atrophy in AD[20]; ROC, receiver operating characteristic; SD, standard deviation; SUVR, standardized uptake value ratio.

eTable 16. Correlations of plasma, CSF, and MRI biomarkers with different tau-PET ROIs and plasma P-tau217 in the BioFINDER-2 cohort (cohort-2)

	Plasma P-tau217	tau-PET ERC	tau-PET ITC	tau-PET temporal meta-ROI	tau-PET Braak stages V-VI
Plasma P-tau217	1.00	0.59	0.54	0.57	0.44
Plasma P-tau181	0.54	0.52	0.45	0.47	0.35
Plasma Aβ42/Aβ40	-0.23	-0.26	-0.19	-0.20	NS
Plasma T-tau	0.21	0.14	0.15	0.14	0.18
Plasma NfL	0.35	0.35	0.32	0.33	0.18
CSF P-tau217	0.69	0.75	0.63	0.67	0.47
CSF P-tau181	0.50	0.59	0.50	0.54	0.36
CSF Aβ42/Aβ40	−0.65	−0.61	−0.54	−0.57	−0.38
MRI AD cortex	–0.42	−0.56	−0.54	–0.55	–0.36
MRI hippocampus/ICV	–0.35	−0.51	−0.46	–0.49	–0.30

All noted correlations coefficients (Spearman) were statistically significant at P<0.001 (Bonferroni corrected, i.e. P-value x 49), except for plasma T-tau ~ tau-PET ERC (p=0.02), plasma T-tau ~ tau-PET ITC (p=0.004), and plasma T-tau ~ tau-PET temporal meta-ROI (p=0.01).
Abbreviations: ERC, entorhinal cortex (corresponding to Braak tau stages I-II); ICV, intracranial volume; ITC, inferior temporal cortex, NfL, neurofilament light, NS, non-significant (p=0.08); ROI, region of interest; temporal meta-ROI, ROI corresponding to Braak tau stages I-IV.

eTable 17. Discriminative accuracy of plasma, CSF and MRI biomarkers for tau-PET status in the BioFINDER-2 cohort (cohort-2)

Biomarker	**AUC (95% CI)**	**AUC Difference (95% CI)**	**P-value (compared with plasma P-tau217)**	**Correctly classified participants**	**Sensitivity**	Specificity
Plasma P-tau217	0.93 (0.91-0.96)	NA	NA	86%	90%	85%
Plasma P-tau181	0.83 (0.80-0.87)	0.10 (0.06-0.13)	**<0.001**	80%	49%	90%
Plasma NfL	0.67 (0.63-0.72)	0.26 (0.21-0.30)	**<0.001**	71%	26%	85%
MRI AD cortex	0.84 (0.81-0.88)	0.09 (0.04-0.13)	**<0.001**	81%	64%	86%
MRI hippocampus / ICV	0.80 (0.77-0.84)	0.13 (0.08-0.17)	**<0.001**	77%	38%	89%
CSF P-tau217	0.96 (0.94-0.97)	-0.02 (-0.05-0.00)	0.22	81%	96%	76%
CSF P-tau181	0.85 (0.81-0.88)	0.09 (0.05-0.12)	**<0.001**	82%	65%	87%
CSF Aβ42/Aβ40	0.90 (0.87-0.92)	0.04 (-0.01-0.08)	**0.04**	79%	96%	73%

Outcome was temporal meta-ROI tau-PET status; normal (n=532) or abnormal (n=167). P-values are from the comparison of AUCs (DeLong statistics). P-values are Bonferroni corrected for multiple comparisons (i.e. uncorrected P value x 7 is shown). P-values in bold indicate a statistically significant difference in AUC compared with plasma P-tau217 (P<0.05). All cutoffs, except for CSF Aβ42/Aβ40, were established using the mean + 2 SD in Aβ-negative controls (i.e. established in an independent sample to avoid overfitting). For CSF Aβ42/Aβ40 the cut-off had to be established using mixture modeling (see eMethods 1.2). See eTable 12 for cut-offs.

Abbreviations: AUC, area under the receiver operating characteristic curve; CI, confidence interval; N/A, not applicable.

eTable 18. Discriminative accuracy of plasma, CSF and MRI biomarkers for Aβ-PET status in the BioFINDER-2 cohort (cohort-2)

Biomarker	AUC (95% CI)	AUC Difference (95% CI)	P-value (compared with plasma P-tau217)	Correctly classified participants	Sensitivity	Specificity
Plasma P-tau217	0.87 (0.83-0.90)	NA	NA	82%	57%	94%
Plasma P-tau181	0.76 (0.72-0.81)	0.10 (0.05-0.15)	**<0.001**	71%	27%	93%
Plasma NfL	0.69 (0.64-0.74)	0.18 (0.12-0.23)	**<0.001**	66%	15%	92%
MRI AD cortex	0.71 (0.67-0.76)	0.15 (0.09-0.22)	**<0.001**	71%	30%	92%
MRI hippocampus / ICV	0.66 (0.61-0.71)	0.21 (0.15-0.28)	**<0.001**	68%	19%	93%
CSF P-tau217	0.93 (0.91-0.96)	-0.06 (-0.10-[-0.03])	**0.003**	88%	81%	91%
CSF P-tau181	0.80 (0.75-0.84)	0.07 (0.03-0.12)	**0.02**	77%	45%	94%
CSF Aβ42/Aβ40	0.97 (0.95-0.98)	-0.10 (-0.14-[-0.05])	**<0.001**	92%	93%	92%

The analysis was performed on the subsample where Aβ-PET was available (n=488). Outcome was neocortical Aβ-PET status; normal (n=326) or abnormal (n=162). P-values are from the comparison of AUCs (DeLong statistics). P-values are Bonferroni corrected for multiple comparisons (i.e. uncorrected P-value x 7 is shown). P-values in bold indicate a statistically significant difference in AUC compared with plasma P-tau217 ($P<0.05$). All cutoffs, except for CSF Aβ42/Aβ40, were established using the mean + 2 SD in Aβ-negative controls (i.e. established in an independent sample to avoid overfitting). For CSF Aβ42/Aβ40 the cut-off had to be established using mixture modeling (see eMethods 1.2). See eTable 12 for cut-offs.
Abbreviations: AUC, area under the receiver operating characteristic curve; CI, confidence interval; N/A, not applicable.

eTable 19. Logistic regression models comparing the added value of combining plasma P-tau217 with other biomarkers for intermediate to high likelihood of AD (n=34) vs non-AD (n=47) in the neuropathology cohort (cohort-1)

Biomarkers	AUC (95% CI)	AUC Difference (95% CI)	P-value (compared with plasma P-tau217)
Plasma P-tau217	0.89 (0.81-0.97)	NA	NA
Plasma Aβ42/40	0.72 (0.60-0.83)	0.17 (0.04-0-30)	**0.04**
Plasma T-tau	0.51 (0.38-0.64)	0.38 (0.24-0.51)	**<0.001**
Plasma P-tau217 Plasma P-tau181 [a]	0.89 (0.82-0.97)	0.00 (-0.02-0.01)	1.0
Plasma P-tau217 Plasma Aβ42/40 [a]	0.89 (0.82-0.97)	0.00 (-0.02-0.01)	1.0

Biomarkers were examined using logistic regression models with the biomarkers as independent variables and diagnosis (AD dementia vs other neurodegenerative diseases) as dependent variable. Only biomarkers that were significant in univariable ROC analysis were entered in the multivariable analyses (i.e. plasma NfL was not included [see eTable 10]). See eTable 10 for a univariate comparison with plasma P-tau181. The probability output from the logistic regression was then used as independent variable in ROC analysis with AD vs other neurodegenerative diseases as dependent variable. P-values are from the comparison of AUCs (DeLong statistics). P-values are Bonferroni corrected for multiple comparisons (i.e. uncorrected P-value x 4 is shown). P-values in bold indicate a statistically significant difference in AUC compared with plasma P-tau217 (P<0.05).
[a] Not significant in multivariable model.
Abbreviations: AUC, area under the receiver operating characteristic curve; CI, confidence interval; ROC, receiver operating characteristic.

eTable 20. Logistic regression models comparing the added value of combining plasma P-tau217 with other biomarkers for AD with dementia (n=121) vs other neurodegenerative diseases (n=99) in the BioFINDER-2 cohort (cohort-2)

Biomarkers	AUC (95% CI)	AUC Difference (95% CI)	P-value (compared with plasma P-tau217)
Plasma P-tau217	0.96 (0.93-0.98)	NA	NA
Plasma Aβ42/40	0.62 (0.54-0.69)	0.34 (0.26-0.42)	**<0.001**
Plasma T-tau	0.66 (0.54-0.72)	0.30 (0.23-0.37)	**<0.001**
Plasma P-tau217 Plasma P-tau181[a]	0.96 (0.93-0.98)	0.00 (0.00-0.00)	1.0
Plasma P-tau217 Plasma Aβ42/40 [a]	0.96 (0.93-0.98)	0.00 (0.00-0.00)	1.0
Plasma P-tau217 Plasma T-tau [a]	0.96 (0.93-0.98)	0.00 (0.00-0.00)	1.0

Biomarkers were examined using logistic regression models with the biomarkers as independent variables and diagnosis (AD dementia vs other neurodegenerative diseases) as dependent variable. Only biomarkers that were significant in univariable ROC analysis were entered in the multivariable analyses (i.e. plasma NfL was not included [see eTable 12]). See eTable 12 for a univariate comparison with plasma P-tau181. The probability output from the logistic regression was then used as independent variable in ROC analysis with AD vs other neurodegenerative diseases as dependent variable. P-values are from the comparison of AUCs (DeLong statistics). P-values are Bonferroni corrected for multiple comparisons (i.e. uncorrected P value x 5 is shown). P-values in bold indicate a statistically significant difference in AUC compared with plasma P-tau217 (P<0.05).
[a] Not significant in multivariable model.
Abbreviations: AUC, area under the receiver operating characteristic curve; CI, confidence interval; ROC, receiver operating characteristic.

5. eReferences

1. Beach TG, Adler CH, Sue LI, et al. Arizona Study of Aging and Neurodegenerative Disorders and Brain and Body Donation Program. *Neuropathology.* 2015;35(4):354-389.
2. Consensus recommendations for the postmortem diagnosis of Alzheimer's disease. The National Institute on Aging, and Reagan Institute Working Group on Diagnostic Criteria for the Neuropathological Assessment of Alzheimer's Disease. *Neurobiol Aging.* 1997;18(4 Suppl):S1-2.
3. Mirra SS, Heyman A, McKeel D, et al. The Consortium to Establish a Registry for Alzheimer's Disease (CERAD). Part II. Standardization of the neuropathologic assessment of Alzheimer's disease. *Neurology.* 1991;41(4):479-486.
4. Braak H, Braak E. Neuropathological stageing of Alzheimer-related changes. *Acta Neuropathol.* 1991;82(4):239-259.
5. American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders, 5ed.* Vol fifth edition. Arlington, VA2013.
6. Jack CR, Jr., Bennett DA, Blennow K, et al. NIA-AA Research Framework: Toward a biological definition of Alzheimer's disease. *Alzheimers Dement.* 2018;14(4):535-562.
7. Bertens D, Tijms BM, Scheltens P, Teunissen CE, Visser PJ. Unbiased estimates of cerebrospinal fluid beta-amyloid 1-42 cutoffs in a large memory clinic population. *Alzheimers Res Ther.* 2017;9(1):8.
8. Gelb DJ, Oliver E, Gilman S. Diagnostic criteria for Parkinson disease. *Arch Neurol.* 1999;56(1):33-39.
9. Hoglinger GU, Respondek G, Stamelou M, et al. Clinical diagnosis of progressive supranuclear palsy: The movement disorder society criteria. *Mov Disord.* 2017;32(6):853-864.
10. Gilman S, Wenning GK, Low PA, et al. Second consensus statement on the diagnosis of multiple system atrophy. *Neurology.* 2008;71(9):670-676.
11. Armstrong MJ, Litvan I, Lang AE, et al. Criteria for the diagnosis of corticobasal degeneration. *Neurology.* 2013;80(5):496-503.
12. Gorno-Tempini ML, Hillis AE, Weintraub S, et al. Classification of primary progressive aphasia and its variants. *Neurology.* 2011;76(11):1006-1014.
13. Aguirre-Acevedo DC, Gomez RD, Moreno S, et al. [Validity and reliability of the CERAD-Col neuropsychological battery]. *Rev Neurol.* 2007;45(11):655-660.
14. Albert MS, DeKosky ST, Dickson D, et al. The diagnosis of mild cognitive impairment due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement.* 2011;7(3):270-279.
15. McKhann GM, Knopman DS, Chertkow H, et al. The diagnosis of dementia due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement.* 2011;7(3):263-269.
16. Janelidze S, Stomrud E, Smith R, et al. Cerebrospinal fluid p-tau217 performs better than p-tau181 as a biomarker of Alzheimer's disease. *Nature communications.* 2020;11(1):1683.
17. Janelidze S, Mattsson N, Palmqvist S, et al. Plasma P-tau181 in Alzheimer's disease: relationship to other biomarkers, differential diagnosis, neuropathology and longitudinal progression to Alzheimer's dementia. *Nature medicine.* 2020;26(3):379-386.
18. Karikari TK, Pascoal TA, Ashton NJ, et al. Blood phosphorylated tau 181 as a biomarker for Alzheimer's disease: a diagnostic performance and prediction modelling study using data from four prospective cohorts. *Lancet Neurol.* 2020;19(5):422-433.

19. Gisslen M, Price RW, Andreasson U, et al. Plasma Concentration of the Neurofilament Light Protein (NFL) is a Biomarker of CNS Injury in HIV Infection: A Cross-Sectional Study. *EBioMedicine.* 2016;3:135-140.
20. Jack CR, Jr., Wiste HJ, Weigand SD, et al. Different definitions of neurodegeneration produce similar amyloid/neurodegeneration biomarker group findings. *Brain.* 2015;138(Pt 12):3747-3759.
21. Baker SL, Maass A, Jagust WJ. Considerations and code for partial volume correcting [(18)F]-AV-1451 tau PET data. *Data Brief.* 2017;15:648-657.
22. Ossenkoppele R, Rabinovici GD, Smith R, et al. Discriminative Accuracy of [18F]flortaucipir Positron Emission Tomography for Alzheimer Disease vs Other Neurodegenerative Disorders. *JAMA.* 2018;320(11):1151-1162.
23. Lundqvist R, Lilja J, Thomas BA, et al. Implementation and validation of an adaptive template registration method for 18F-flutemetamol imaging data. *J Nucl Med.* 2013;54(8):1472-1478.
24. Palmqvist S, Zetterberg H, Blennow K, et al. Accuracy of brain amyloid detection in clinical practice using cerebrospinal fluid beta-amyloid 42: a cross-validation study against amyloid positron emission tomography. *JAMA Neurol.* 2014;71(10):1282-1289.
25. Cho H, Choi JY, Hwang MS, et al. In vivo cortical spreading pattern of tau and amyloid in the Alzheimer disease spectrum. *Ann Neurol.* 2016;80(2):247-258.
26. Jack CR, Jr., Wiste HJ, Weigand SD, et al. Age-specific and sex-specific prevalence of cerebral beta-amyloidosis, tauopathy, and neurodegeneration in cognitively unimpaired individuals aged 50-95 years: a cross-sectional study. *Lancet Neurol.* 2017;16(6):435-444.
27. Leuzy A, Smith R, Ossenkoppele R, et al. Diagnostic Performance of RO948 F18 Tau Positron Emission Tomography in the Differentiation of Alzheimer Disease From Other Neurodegenerative Disorders. *JAMA Neurol.* 2020.
28. Benaglia T, Chauveau D, Hunter DR, Young D. mixtools: An R Package for Analyzing Finite Mixture Models. *Journal of Statistical Software.* 2009;32(6):1-29.
29. Wood S. Monotonic Smoothing Splines Fitted by Cross Validation. *SIAM Journal on Scientific Computing.* 1994;15(5):1126-1133.
30. Preische O, Schultz SA, Apel A, et al. Serum neurofilament dynamics predicts neurodegeneration and clinical progression in presymptomatic Alzheimer's disease. *Nature medicine.* 2019;25(2):277-283.
31. Gordon BA, Blazey TM, Christensen J, et al. Tau PET in autosomal dominant Alzheimer's disease: relationship with cognition, dementia and other biomarkers. *Brain.* 2019;142(4):1063-1076.