

α -Synuclein Seed Amplification Status Modifies the Relationship Between Plasma p-tau217, Amyloid and Fibrillar Tau

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Research Article

Keywords: plasma p-tau217, α -synuclein seed amplification assay, co-pathology, amyloid PET, tau PET, ADNI

Posted Date: October 22nd, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-11271891/v1>

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Additional Declarations: Competing interest reported. Íñigo Rodríguez-Baz has received personal fees for service on advisory boards, speaker honoraria, or educational activities from Nutricia, Almirall, and Esteve. Ignacio Illán-Gala participated in advisory boards from UCB and Nutricia, and received speaker honoraria from Almirall, Esteve Pharmaceuticals S.A, Kern Pharma, Krka Farmacéutica S.L., Lilly, Nutricia, and Zambon S.A.U. No other competing interests were reported. Judit Selma-González has received a speaker honoraria from Almirall. Valle Camacho has received personal fees for service on advisory boards, speaker honoraria or educational activities from General Electric, Life Molecular Imaging, Novartis, Lilly and Curium. Juan Fortea reported serving on the advisory boards, adjudication committees, or speaker honoraria from AC Immune, Adamed, Alzheon, Biogen, Eisai, Esteve, Fujirebio, Ionis, Laboratorios Carnot, Life Molecular Imaging, Lilly, Novo Nordisk, Perha, Roche, Zambón. JF reports holding a patent for markers of synaptopathy in neurodegenerative disease (licensed to ADx, WO2019175379). Alberto Lleó has served as a consultant or on advisory boards for Almirall, Beckman Coulter, Fujirebio-Europe, NovoNordisk, Roche, Biogen, Grifols, Novartis, Eisai, Lilly, and Nutricia, outside the submitted work. AL reports holding a patent for markers of synaptopathy in neurodegenerative disease (licensed to ADx, WO2019175379). Mireia Tondo has received personal fees for service on advisory boards, speaker honoraria, or educational activities from Grifols, Adium, Lilly and Esteve. Carla Abdelnour serves as a consultant for BioArctic, has received speaker honoraria from Hoffman-La Roche LTD, Nutricia, Schwabe Farma Ibérica SAU and Zambon; and is a member of the Board of Directors of the Lewy Body Dementia Association. No other competing interests were reported. Daniel Alcolea participated in advisory boards from Fujirebio-Europe, Roche Diagnostics, Grifols S.A., Sysmex, Schwabe and Lilly, and received speaker honoraria from Fujirebio-Europe, Roche Diagnostics, Nutricia, Sysmex, Krka Farmacéutica S.L., Zambon S.A.U., Neuraxpharm, Almirall, Alter Medica, Sysmex, Lilly and Esteve Pharmaceuticals S.A. DA declares holding a patent for markers of synaptopathy in neurodegenerative disease (licensed to ADx NeuroSciences N.V., WO2019175379 Markers of synaptopathy in neurodegenerative diseases).

Abstract

Background. Plasma p-tau217 is sufficient to establish a biological diagnosis of Alzheimer's disease (AD) and is increasingly used as a routine diagnostic tool. Many patients with AD also carry α -synuclein pathology, detectable by cerebrospinal fluid (CSF) seed amplification assay (α SynSAA). Seeding activity has been linked to more fibrillar tau than amyloid burden alone would predict, but whether this influences plasma p-tau217 levels, or compromises classification at clinical thresholds, is unknown.

Methods. Cross-sectional analysis of the Alzheimer's Disease Neuroimaging Initiative. Plasma p-tau217 (Fujirebio Lumipulse) was compared by α SynSAA status conditional on amyloid PET Centiloids, the CSF p-tau181/A β 42 ratio and tau PET, using generalised additive models adjusted for age, sex, APOE ϵ 4 status and cognitive stage (n = 193, of whom 33 α SynSAA-positive; CSF-referenced n = 286; amyloid PET-referenced n = 246). We quantified the attenuation contributed by each reference measure, modelled fibrillar tau against amyloid and against plasma p-tau217 by seeding status, and reclassified α SynSAA-positive participants after removing the estimated seeding-associated increment.

Results. α SynSAA-positive participants had higher plasma p-tau217 levels at a given amyloid PET burden (27.3%, 95% CI 4.9–54.5; p = 0.015) and CSF ratio (22.9%, 1.7–48.7; p = 0.033), but not at a given tau PET burden (12.1%, – 10.3–40.3; p = 0.313); tau PET attenuated the association by 55.4%, more than the CSF ratio (31.1%) or amyloid PET (25.4%). The amyloid–tau relationship was steeper in α SynSAA-positive participants (p = 0.047), yet a given plasma p-tau217 level corresponded to the same fibrillar tau burden in both groups (2.5% higher, – 2.7–8.0; p = 0.347). Removing the increment did not improve agreement with either reference standard and modestly worsened it (accuracy 93.9% to 92.4% against the CSF ratio; 84.6% to 82.7% against amyloid PET), with the loss falling on sensitivity.

Conclusions. Plasma p-tau217 is elevated in α SynSAA-positive individuals at equivalent AD pathology measured by amyloid PET or the CSF ratio, and this elevation is attenuated most by fibrillar tau. Neither analysis designed to detect artefactual inflation found evidence of it. A plasma p-tau217 result should not be down-weighted in patients with concomitant Lewy-related pathology.

INTRODUCTION

The revised Alzheimer's Association criteria define Alzheimer's disease (AD) biologically and make two relevant changes[1]. First, an accurate plasma phosphorylated tau at threonine 217 (p-tau217) assay is accepted as a Core 1 biomarker, sufficient on its own to establish the diagnosis, provided its accuracy against amyloid positron emission tomography (PET) or approved cerebrospinal fluid (CSF) assays reaches that of the latter in the intended-use population. Second, the classification is extended beyond amyloid, tau and neurodegeneration to include markers of common co-pathologies, among them α -synuclein, which can now be detected in life by CSF seed amplification assay (α SynSAA). These two developments intersect, as plasma p-tau217 moves into routine diagnostic pathways [2, 3], a substantial proportion of the patients tested will also carry α -synuclein pathology, and whether this alters the interpretation of a plasma result is unknown.

α SynSAA positivity is common in the Alzheimer's continuum and has been consistently associated with greater amyloid burden, but not with tau, across large independent cohorts, whether amyloid is measured in cerebrospinal fluid or by PET [4, 5]. This apparent independence from tau does not survive conditioning on amyloid. At a given amyloid burden, α SynSAA positive (α SynSAA+) participants show higher CSF phosphorylated tau at threonine 181 / amyloid-beta 1–40 (p-tau181/A β 40) ratio and higher tau PET, and accumulate fibrillar tau faster over follow-up [6], indicating that seeding displaces the relationship between amyloid and downstream tau measures.

Whether this displacement is detectable in blood is unknown. Plasma p-tau217 detects amyloid deposition accurately in biologically defined Neuronal Synuclein Disease [7, 8], and among patients with a clinical diagnosis of AD those who are α SynSAA+ have higher plasma p-tau217, but not adjusted for clinical severity or AD biomarker burden [9]. However, it is unknown whether seeding status is associated with plasma p-tau217 at an equivalent level of AD pathology, nor whether any such difference could represent an artefactual inflation and impact the classification obtained with clinical thresholds. This has practical implications, since plasma p-tau217 is increasingly used to diagnose AD in clinical and trial settings. If α SynSAA+ individuals show elevated p-tau217 independent of AD pathology burden, applying a single threshold across the population could inflate false-positive results among those with co-pathology.

We hypothesised that α SynSAA+ status is associated with higher plasma p-tau217 at equivalent AD pathology burden, and that this difference reflects fibrillar tau rather than an α -synuclein-driven artefact. We set out three objectives. First, to estimate the difference in plasma p-tau217 by seeding status at a given amyloid PET burden, CSF p-tau181/A β 42 ratio, and tau PET burden, and to determine which of these measures accounts for most of that difference. Second, to test whether α SynSAA+ participants carry more fibrillar tau at a given amyloid burden, and whether a given plasma p-tau217 concentration corresponds to the same fibrillar tau burden in both groups. Third, to test whether removing the estimated difference improves the agreement of plasma p-tau217 with the reference standards used in practice.

METHODS

Study design and participants

Data used in the preparation of this article were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (adni.loni.usc.edu). The ADNI was launched in 2003 as a public-private partnership, led by Principal Investigator Michael W. Weiner, MD. The primary goal of ADNI has been to test whether serial magnetic resonance imaging (MRI), positron emission tomography (PET), other biological markers, and clinical and neuropsychological assessment can be combined to measure the progression of mild cognitive impairment (MCI) and early Alzheimer's disease (AD). We performed a cross-sectional analysis of data from the ADNI [10]. Data were downloaded between 4 and 10 August 2026. The source population comprised every ADNI participant with a CSF α SynSAA result (1,658 records from 1,638 participants; repeated records were concordant and were collapsed). Results reported as Type 2 seeds (the multiple system atrophy profile) or as indeterminate were excluded ($n = 14$), leaving 1,624 participants. Seeding status was analysed as a binary exposure: Type 1 seeds (the Lewy body disease profile) versus not detected.

CSF collection and α -synuclein seed amplification assay

CSF was collected under standardised ADNI procedures, frozen on dry ice within one hour and shipped to the ADNI Biomarker Core (University of Pennsylvania), where pristine 0.5 mL aliquots were stored at -80°C . α SynSAA was performed by Amprion Clinical Laboratory (CLIA ID 05D2209417; CAP 8168002) under a clinically validated CLIA-compliant protocol. Each sample was assayed in triplicate in 96-well plates, with 40 μL CSF in a 100 μL reaction containing recombinant α -synuclein and thioflavin T, two silicon nitride beads per well, and positive and negative controls on every plate. Plates were incubated for 20 h at 42°C in a FLUOstar Ω reader with alternating cycles of 1 min shaking and 14 min rest, with fluorescence read after each cycle. A proprietary algorithm classified each sample from the maximum fluorescence of its triplicates as Type 1 detected, Type 2 detected, not detected or indeterminate. Analysts were blinded to demographic, clinical and AD biomarker data.

CSF AD-related biomarkers

CSF amyloid-beta 1–42 (A β 42) and p-tau181 were measured at the ADNI Biomarker Core in singlicate by electrochemiluminescence immunoassay (Elecsys, cobas e 601), using a single reagent lot per analyte. Values outside the assay measuring ranges (A β 42 200–1,700 pg/mL; p-tau181 8–120 pg/mL) were winsorised to the corresponding limit, since values above the upper limit are extrapolated from the calibration curve and values below the lower limit are not reliably quantifiable; in both cases the reported concentration conveys only that the sample lies outside the validated range. The two analytes were combined into the p-tau181/A β 42 ratio (CSF ratio), with a ratio ≥ 0.025 defining CSF amyloid positivity [11].

Plasma p-tau217

Blood was drawn into EDTA tubes and processed to plasma according to the ADNI procedures manual, with 0.5 mL aliquots stored at -80°C until analysis. Plasma p-tau217 was measured at the ADNI Biomarker Core in singlicate on the Fujirebio Lumipulse G1200 automated chemiluminescent enzyme immunoassay platform, with two quality-control plasma pools included in every analytical run; run-to-run precision for p-tau217 was 6.2–9.9% and lot-to-lot agreement across the two reagent lots used was high ($R^2 = 0.989$) [12]. No participant in any analytic sample came from a batch documented as showing a possible high bias. Values outside the measuring range were winsorised to the technical limits (lower limit of quantification 0.047 pg/mL; upper limit 10.000 pg/mL). The number and percentage of winsorised values for each CSF and plasma biomarker are presented in Supplementary Table S1. Clinical classification used the cut points published for this assay (negative < 0.128 pg/mL, positive > 0.300 pg/mL, indeterminate in between) [12].

Amyloid and tau PET

Amyloid PET (florbetapir or florbetaben) and tau PET (flortaucipir) were acquired at each site under standardised ADNI protocols and processed by the ADNI PET Core (UC Berkeley) in native space, using the structural MRI closest in time to each scan, with FreeSurfer v7.1.0 segmentation, SPM coregistration and harmonisation to a uniform 6 mm resolution [13]. Amyloid burden was summarised as a cortical summary Standardised Uptake Value Ratio (SUVR; frontal, cingulate, lateral parietal and lateral temporal regions) normalised to the whole cerebellum and converted to Centiloids, with positivity defined as ≥ 25 Centiloids [6, 12]. Tau burden was summarised as a temporal meta-ROI SUVR (entorhinal cortex, amygdala, fusiform, inferior and middle temporal gyri) normalised to inferior cerebellar grey matter [14]; only flortaucipir scans were used, since SUVRs from different tau tracers are not comparable. Scans failing quality control were excluded; this did not change the analytic samples, as no participant meeting all other inclusion criteria had a failed scan.

Demographic, genetic and clinical variables

Sex and race were self-reported; age was computed at the date of αSynSAA sampling. *apolipoprotein E (APOE)* $\epsilon 4$ carriage was defined as one or more $\epsilon 4$ alleles. Cognitive stage was derived from the global Clinical Dementia Rating [15] at the visit closest to αSynSAA sampling (0, cognitively unimpaired; 0.5, mild cognitive impairment; ≥ 1 , dementia).

Analytic samples

Each non- αSynSAA measurement was linked to the αSynSAA sampling date by taking the temporally nearest record per participant and retaining it only if the interval did not exceed one year (Supplementary Table S2). CSF for αSynSAA and for the Elecsys biomarkers came from the same lumbar puncture. Analyses were complete-case; no values were imputed.

Because the three reference measures were not available in all participants, three nested samples were used (Supplementary Figure S1). All analyses that compare reference measures with one another were confined to the main analytic sample (CSF biomarkers, plasma p-tau217, amyloid PET and tau PET all available; $n = 193$), since such comparisons are interpretable only within the same individuals. Analyses referenced to a single measure were repeated in the maximum sample available for that measure, the CSF-referenced sample ($n = 286$) and the amyloid PET-referenced sample ($n = 246$), as a robustness check. The counterfactual classification analysis was performed in the maximum sample for each reference standard, using the seeding coefficient estimated in that same sample. Sample sizes were determined by data availability in ADNI at download; all eligible participants were included. Participants in the main analytic sample were compared with the remainder of the CSF-referenced sample to assess selection bias.

Statistical analysis

Descriptive statistics and multiplicity: baseline characteristics are summarised as median [IQR] or n (%). Between-group differences were assessed with the Wilcoxon rank-sum test (continuous) or Fisher's exact test (categorical). The CSF- and PET-referenced analyses address the same hypothesis with different reference measures and are treated as a consistency check rather than as independent comparisons. No formal adjustment for multiplicity was applied and all p -values are unadjusted.

General specification: plasma p-tau217, the CSF ratio and tau PET SUVR were log-transformed (because they are right-skewed); Centiloids were left untransformed. Coefficients from log-outcome models are reported as percentage differences with 95% confidence interval (CI). Unless stated otherwise, models were generalised additive models (Gaussian, identity link, Restricted Maximum Likelihood [REML]) with the reference measure as a penalised thin-plate smooth, adjusted for age, sex, *APOE* $\epsilon 4$ carriage and cognitive stage. A penalised smooth was used throughout because the question of whether two groups' curves differ in shape cannot be addressed by a linear term; where the data do not support curvature, the penalty shrinks the smooth towards a straight line. Shape differences by seeding status were tested with a group-specific difference smooth (a shared reference smooth plus a second smooth capturing the deviation of the α SynSAA+ curve from it); the p -value of this second smooth is reported and is referred to as an interaction throughout. Where no shape difference was evident, the smooth was retained as common to both groups and a single constant proportional difference by seeding status was estimated. A site random effect was retained only for tau PET (intraclass correlation 0.032); site variance was zero for the centrally assayed plasma measure. All tests were two-sided with $\alpha = 0.05$. Fitted curves are adjusted predictions with 95% confidence bands; covariate reference levels are given in each figure legend.

Objective 1: difference in plasma p-tau217 by seeding status at equivalent AD pathology burden, and its attenuation across pathology measures. Log plasma p-tau217 was regressed on seeding status, a penalised smooth of the reference measure and the covariate set, once for each of amyloid PET Centiloids, the CSF ratio and tau PET SUVR. To compare directly how much of the seeding-status association each measure accounts for, the same four models were re-estimated in the 193 participants of the main analytic sample under a single common fixed-degrees-of-freedom specification: a reference model with no pathology term, and three models each adding one measure as a natural cubic spline (3 df, fixed knots), with HC3 standard errors. Attenuation is expressed as the percentage reduction in the seeding-status coefficient relative to the reference model, computed on the log scale on which the models were fitted. The functional form for these fixed-df models was chosen by comparing linear and spline specifications with a nested F-test rather than assuming curvature. Because fibrillar tau plausibly lies on the causal path between α -synuclein seeding and plasma p-tau217, these models are a descriptive decomposition of the association across alternative pathology measures, not a control for confounding.

Objective 2: fibrillar tau at a given amyloid burden, and calibration of plasma p-tau217 against fibrillar tau. Two models with log tau PET SUVR as the outcome examined how amyloid burden and plasma p-tau217 relate to fibrillar tau by α SynSAA status. In the first, amyloid burden was entered as a penalised smooth fitted separately by seeding status, with the covariate set and a site random effect; group differences at specified amyloid values were estimated as contrasts between the fitted curves, excluding the site term. As a sensitivity specification, this model was repeated adjusting only for age, sex and site, matching the adjustment set used previously in this cohort [6]. In the second, log tau PET SUVR was regressed on seeding status, a penalised smooth of log plasma p-tau217 (centred at the clinical positive threshold), a smooth of Centiloids, the covariate set and a site random effect; as a sensitivity analysis, the shape test was repeated after restricting both groups to the plasma range in which both are represented, truncating at the 90th percentile of the α SynSAA+ plasma distribution (n = 186).

Objective 3 (exploratory): counterfactual clinical classification. To quantify the clinical consequence of the estimated seeding-associated increment, the estimated increment was removed from each α SynSAA+ participant's plasma value and the participant reclassified using the manufacturer's cut-off points [12]. The increase removed was the seeding-status coefficient estimated in the same sample and against the same reference measure. Observed and counterfactual classifications were compared against CSF p-tau181/A β 42 ≥ 0.025 and amyloid PET ≥ 25 Centiloids. Each comparison was performed in the maximum sample available for the corresponding reference measure (CSF-referenced, n = 286; amyloid PET-referenced, n = 246). Participants classified as negative or indeterminate were grouped as non-positive, reflecting that only an unambiguous positive result (> 0.300 pg/mL) would be treated as clinically actionable without further testing. Sensitivity, specificity and accuracy were computed among α SynSAA+ participants only. The analysis was repeated at amyloid thresholds of 20 and 30 Centiloids and with the seeding-status coefficient set to each 95% CI limit. Because the number of α SynSAA+ participants is limited, this analysis is exploratory and descriptive.

Additional covariates and collinearity: two further covariates were examined because each acts on plasma rather than brain concentrations of p-tau217. Estimated glomerular filtration rate (calculated with CKD-EPI formula, 2021, within 90 days; n = 120) and body mass index (within one year; n = 191) were each added to the primary models, with a subsample-restricted refit as comparison. By a prespecified rule, these covariates were to enter the primary models only if they predicted the outcome and shifted the seeding-status estimate more than sample restriction alone did. Collinearity was assessed by variance inflation factors and, in the generalised additive models, by concurvity.

Software: analyses were performed in R 4.5.1, using mgcv for generalised additive models, splines for natural cubic splines, sandwich and lme4/lmerTest for HC3 standard errors, lme4/lmerTest for the site random-intercept checks, car for variance inflation factors.

RESULTS

Study population

The main analytic sample comprised 193 participants with concurrent plasma p-tau217, CSF biomarkers, amyloid PET and tau PET, of whom 33 (17.1%) were α SynSAA+. α SynSAA+ participants were older (73.4 [IQR 69.3, 78.4] vs 71.1 [66.4, 76.5] years; p = 0.036), more frequently had dementia (21.2% vs 5.6%; p = 0.008) and had higher values of tau PET (1.274 [1.179, 1.833] vs 1.214 [1.143, 1.315] meta-ROI SUVR; p = 0.029) and CSF ratio (0.0260 [0.0158, 0.0581] vs 0.0181 [0.0125, 0.0442]; p = 0.020), though the amyloid PET difference did not reach significance (39.0 [4.0, 118.0] vs 13.0 [3.0, 73.2] Centiloids; p = 0.134). The largest between-group difference was in the outcome itself: unadjusted plasma p-tau217 was 0.360 [0.144, 0.792] vs 0.141 [0.087, 0.347] pg/mL (p = 0.002; Table 1).

Table 1
Baseline characteristics by αSynSAA status in the main analytic sample

Variable	αSynSAA- (n = 160)	αSynSAA+ (n = 33)	p-value
Age, median [IQR]	71.1 [66.4, 76.5]	73.4 [69.3, 78.4]	0.036
Female sex, n (%)	88 (55.0%)	15 (45.5%)	0.343
White race, n (%)	150 (93.8%)	32 (97.0%)	0.693
APOE ε4 carrier, n (%)	68 (42.5%)	15 (45.5%)	0.847
Cognitively unimpaired, n (%)	79 (49.4%)	13 (39.4%)	0.341
MCI, n (%)	72 (45.0%)	13 (39.4%)	0.701
Dementia, n (%)	9 (5.6%)	7 (21.2%)	0.008
Plasma p-tau217, pg/mL, median [IQR]	0.141 [0.087, 0.347]	0.360 [0.144, 0.792]	0.002
CSF Abeta42, pg/mL, median [IQR]	1030.0 [686.9, 1700.0]	893.2 [557.1, 1440.0]	0.284
CSF p-tau181, pg/mL, median [IQR]	21.8 [15.8, 28.3]	26.4 [19.7, 35.2]	0.025
CSF p-tau181/Abeta42, median [IQR]	0.0181 [0.0125, 0.0442]	0.0260 [0.0158, 0.0581]	0.020
Amyloid PET, centiloids, median [IQR]	13.0 [3.0, 73.2]	39.0 [4.0, 118.0]	0.134
Tau-PET, meta-ROI SUVR, median [IQR]	1.214 [1.143, 1.315]	1.274 [1.179, 1.833]	0.029
Amyloid PET positive (≥ 25 CL), n (%)	67 (41.9%)	20 (60.6%)	0.056
CSF amyloid positive (ratio ≥ 0.025), n (%)	59 (36.9%)	18 (54.5%)	0.078
Plasma p-tau217 negative (< 0.128 pg/mL), n (%)	75 (46.9%)	8 (24.2%)	0.020
Plasma p-tau217 indeterminate (0.128–0.300 pg/mL), n (%)	40 (25.0%)	8 (24.2%)	1.000
Plasma p-tau217 positive (> 0.300 pg/mL), n (%)	45 (28.1%)	17 (51.5%)	0.013

Continuous variables are shown as median [IQR]; categorical variables as n (%). Between-group differences were tested with the Wilcoxon rank-sum test (continuous) or Fisher's exact test (categorical); p-values are descriptive and not adjusted for multiple comparisons. Amyloid PET positivity is defined as ≥ 25 centiloids (Cousins et al. 2025); CSF amyloid positivity as p-tau181/Abeta42 ratio ≥ 0.025 ; plasma p-tau217 categories use the manufacturer's thresholds (negative < 0.128 pg/mL, indeterminate 0.128–0.300 pg/mL, positive > 0.300 pg/mL). Abbreviations: APOE4, apolipoprotein E ε4; αSynSAA, alpha-synuclein seed amplification assay; CL, centiloids; CSF, cerebrospinal fluid; IQR, interquartile range; MCI, mild cognitive impairment; PET, positron emission tomography; SUVR, standardised uptake value ratio.

Participants retained in the main analytic sample were younger, less frequently demented and less frequently CSF amyloid-positive than the remaining 93 participants of the CSF-referenced sample who lacked tau PET, consistent with PET being offered to less advanced participants (Supplementary Table S3).

Plasma p-tau217 is higher in αSynSAA+ participants at equal amyloid, but not at equal fibrillar tau

We first asked whether plasma p-tau217 differs by seeding status once AD pathology is taken into account, and which pathology measure accounts for that difference. Before any adjustment for pathology, α SynSAA+ participants had 36.6% higher plasma p-tau217 (95% CI 1.1 to 84.4%). The difference persisted when referenced to amyloid PET (27.3%, 4.9 to 54.5%; $p = 0.015$) and to the CSF ratio (22.9%, 1.7 to 48.7%; $p = 0.033$), but not when referenced to tau PET (12.1%, - 10.3 to 40.3%; $p = 0.313$; Table 2). No shape difference by seeding status was evident for any reference measure (amyloid PET $p = 0.61$; CSF ratio $p = 0.41$; tau PET $p = 0.14$), so a single constant proportional difference was retained throughout; the fitted curves in Fig. 1 are therefore parallel.

Table 2
Adjusted association between α SynSAA status and plasma p-tau217

Model	β [95% CI]	SE	p	% Change [95% CI]
Amyloid PET	0.24 [0.05, 0.43]	0.098	0.015	27.3 [4.9, 54.5]
CSF p-tau181/A β 42 ratio	0.21 [0.02, 0.40]	0.096	0.033	23.0 [1.7, 48.7]
Tau PET	0.11 [- 0.11, 0.34]	0.113	0.313	12.1 [- 10.3, 40.3]

All models adjust for age, sex, APOE ϵ 4 carriage and cognitive stage in addition to the reference measure shown, entered as a penalised smooth term. Abbreviations: CI, confidence interval; CSF, cerebrospinal fluid; PET, positron emission tomography; SE, standard error.

Each panel plots plasma p-tau217 (pg/mL, log scale) against amyloid PET burden (centiloids, linear scale), the CSF p-tau181/A β 42 ratio (log scale), or tau-PET signal in a temporal meta-ROI SUVR (log scale). Points are the same 193 participants in every panel, coloured by α SynSAA status (blue, α SynSAA-; red, α SynSAA+). Lines are fitted values from the primary generalised additive model for each panel, with age, sex, APOE ϵ 4 carrier status and cognitive stage held at reference levels; shaded bands are 95% confidence intervals. β , 95% CI and p-value annotated in each panel refer to the α SynSAA coefficient of that panel's model (log scale). Abbreviations: A β 42, amyloid-beta 42; APOE4, apolipoprotein E ϵ 4 allele; α SynSAA, α -synuclein seed amplification assay; β , regression coefficient; CI, confidence interval; CSF, cerebrospinal fluid; pg/mL, picograms per millilitre; PET, positron emission tomography; ROI, region of interest; SUVR, standardised uptake value ratio.

The same ordering was obtained when all four contrasts were re-estimated in the identical 193 participants under a common fixed-degrees-of-freedom specification: tau PET attenuated the seeding-status coefficient by 55.4% (to 14.9%, 95% CI - 9.8 to 46.4%), the CSF ratio by 31.1% and amyloid PET by 25.4% (Fig. 2, Supplementary Table S4). The confidence intervals of the three adjusted estimates overlap, so the measures cannot be formally distinguished from one another; what separates tau PET is the magnitude of its point attenuation.

Each row shows the α SynSAA coefficient from a separate linear model, adjusted for age, sex, APOE ϵ 4 carrier status and cognitive stage, fitted to the same 193 participants. The reference model (Ref.) includes no pathology measure; in the CSF ratio, amyloid PET and tau-PET rows, that measure enters as a natural cubic spline (3 degrees of freedom), and the percentage beside each row is the attenuation in the α SynSAA coefficient relative to the reference model. Attenuation is computed on the log-scale coefficients and therefore does not equal the difference between the back-transformed percentages. Error bars are 95% confidence intervals from heteroskedasticity-consistent (HC3) standard errors. Abbreviations: APOE4, apolipoprotein E ϵ 4 allele; α SynSAA, α -synuclein seed amplification assay; CI, confidence interval; CSF, cerebrospinal fluid; HC3, heteroskedasticity-consistent (type 3); PET, positron emission tomography; Ref., reference.

These estimates were robust to sample and specification. Estimates referenced to amyloid PET and to the CSF ratio were consistent in the maximum sample available for each (Supplementary Table S5). Adding renal function or body

mass index left the seeding-status coefficient essentially unchanged, and neither covariate met the prespecified rule for entry into the primary models (Supplementary Tables S6 and S7). Neither variance inflation factors (maximum 1.32) nor concurvity in the generalised additive models (maximum worst-case 0.44) indicated collinearity among model terms; both are well below conventional thresholds.

Fibrillar tau at a given amyloid burden, and calibration of plasma p-tau217 against fibrillar tau

We next examined how amyloid burden and plasma p-tau217 each relate to fibrillar tau by seeding status. The shape of the amyloid-tau relationship differed between groups (interaction $p = 0.047$), with a non-linear fitted curve in αSynSAA^- participants (edf 2.62) and an essentially linear deviation in αSynSAA^+ participants (difference-smooth edf ≈ 1), corresponding to a modestly steeper slope (Fig. 3). The point contrast at the amyloid positivity threshold of 25 Centiloids was small and not significant (6.1% higher tau PET in αSynSAA^+ vs αSynSAA^- ; $p = 0.104$), and the curves separate most at advanced amyloid burden, where αSynSAA^+ participants are sparse. The shape difference was stronger under the sparser age-, sex- and site-only adjustment used previously in this cohort [6] ($p = 0.012$; Supplementary Figure S2).

Lines and shaded bands are model-adjusted fits from the primary GAM (age, sex, APOE $\epsilon 4$ carrier status, cognitive stage and site held at reference levels), with 95% confidence bands, fitted separately by αSynSAA status. Each group's curve is drawn only over the range of the x-axis variable observed in that group. The percentage difference, 95% CI, p-value and interaction p-value annotated in the panel refer to the formal contrast between the fitted curves at the amyloid positivity threshold and the difference-smooth test for shape, respectively. The dashed line marks the amyloid positivity threshold (25 CL). Point colour indicates αSynSAA status (blue, αSynSAA^- ; red, αSynSAA^+); point shape indicates cognitive stage (diamond, cognitively unimpaired; triangle, mild cognitive impairment; square, dementia). Tau-PET SUVR is shown on the log scale, matching the outcome scale used in the model. Abbreviations: CL, centiloids; CU, cognitively unimpaired; MCI, mild cognitive impairment; PET, positron emission tomography; SUVR, standardised uptake value ratio.

Modelled in the other direction, a given plasma p-tau217 concentration corresponded to the same fibrillar tau burden in both groups. No shape difference was evident (interaction $p = 0.39$), and the constant-difference model estimated only 2.5% higher tau PET in αSynSAA^+ participants (95% CI - 2.7 to 8.0; $p = 0.347$). Plasma p-tau217 predicted tau PET strongly (deviance explained 69.9%) and the two fitted curves were closely superimposed across the observed range (Fig. 4). The absence of a shape difference persisted after restricting both groups to the plasma range in which both are represented (≤ 1.007 pg/mL, the 90th percentile of the αSynSAA^+ distribution; 4 αSynSAA^+ and 3 αSynSAA^- participants removed, $n = 186$; interaction $p = 0.56$).

Lines and shaded bands are model-adjusted fits from the primary GAM (age, sex, APOE $\epsilon 4$ carrier status, cognitive stage, centiloids and site held at reference levels), with 95% confidence bands. β , 95% CI and p-value annotated in the panel refer to the αSynSAA coefficient of this model (log scale). The dashed lines mark the plasma p-tau217 thresholds separating negative from indeterminate (0.128 pg/mL) and indeterminate from positive (0.300 pg/mL) results. Point colour indicates αSynSAA status (blue, αSynSAA^- ; red, αSynSAA^+); point shape indicates cognitive stage (diamond, cognitively unimpaired; triangle, mild cognitive impairment; square, dementia). Both axes are shown on the log scale, matching the outcome and predictor scales used in the model. Abbreviations: CU, cognitively unimpaired; MCI, mild cognitive impairment; PET, positron emission tomography; SUVR, standardised uptake value ratio.

Removing the seeding-associated increment does not improve clinical classification

Finally, we asked what the αSynSAA+ associated plasma p-tau217 increment does to clinical classification. Removing it changed the plasma category of 1 of 66 αSynSAA+ participants when judged against the CSF ratio and 3 of 52 when judged against amyloid PET; approximately 60% of αSynSAA+ participants were already above the positive threshold before any adjustment (baseline characteristics in Supplementary Table S8).

Agreement did not improve against either reference standard, and fell modestly: accuracy 93.9% to 92.4% against the CSF ratio and 84.6% to 82.7% against amyloid PET (Table 3). The loss arose entirely from sensitivity (91.3% to 89.1% and 82.9% to 77.1%), as correctly classified participants fell below the threshold, while specificity was unchanged (100.0%) or marginally better (88.2% to 94.1%). The direction held across amyloid positivity thresholds of 20 and 30 Centiloids and across both limits of the 95% CI of the seeding coefficient, with counterfactual accuracy never exceeding the observed value (Supplementary Table S9). Given the number of αSynSAA+ participants available, these findings are exploratory.

Table 3
Observed vs counterfactual plasma p-tau217 classification in αSynSAA+ participants

Reference	Scenario	Plasma non-positive	Plasma positive	True non-positive	True positive	Sensitivity (%)	Specificity (%)	Accuracy (%)
CSF ratio	Observed	24	42	20	42	91.3	100	93.9
CSF ratio	Counterfactual	25	41	20	41	89.1	100	92.4
Amyloid PET	Observed	21	31	15	29	82.9	88.2	84.6
Amyloid PET	Counterfactual	24	28	16	27	77.1	94.1	82.7

"Counterfactual" removes the αSynSAA-associated increase in plasma p-tau217 from each αSynSAA+ participant. Reference thresholds are externally defined: CSF p-tau181/Abeta42 ≥ 0.025 (Hansson et al. 2018) and amyloid PET ≥ 25 CL (Cousins et al. 2025). Abbreviations: αSynSAA, alpha-synuclein seed amplification assay; CL, Centiloids, CSF, cerebrospinal fluid; PET, positron emission tomography.

DISCUSSION

Plasma p-tau217 was higher in αSynSAA+ than in αSynSAA- participants at equivalent levels of AD pathology, referenced either to amyloid PET burden or to a CSF ratio. No comparable difference was evident when the comparison was referenced to fibrillar tau, and adjustment for tau PET absorbed more of the seeding-associated difference than either of the other two measures did. One explanation could be that αSynSAA+ participants carry more fibrillar tau than their amyloid burden alone would predict, and that plasma p-tau217 reflects that position [16] while a Centiloid or CSF ratio values do not. This displacement of the amyloid-tau relationship has been described previously in this cohort using CSF phosphorylated tau, where αSynSAA positivity was associated with steeper amyloid-related tau increases confined to amyloid-positive individuals [6]. Our results are consistent with that report. What is new is the compartment: the same phenomenon is detectable in blood, with a clinically deployed assay and clinically defined thresholds. That matters because plasma p-tau217 is entering routine diagnostic pathways [1, 3, 17], where the confirmatory test is amyloid PET or CSF rather than tau PET.

Reversing the model, a given plasma p-tau217 concentration corresponded to the same fibrillar tau burden in both groups: the fitted curves were closely superimposed and their shapes indistinguishable, including after restriction to the range of plasma values common to both groups. We then removed the estimated seeding-associated increment

from each α SynSAA+ participant's plasma value and reclassified against both reference standards [12]. Had the increment been artefactual, agreement should have improved. It did not, accuracy fell against both standards, with the loss falling entirely on sensitivity. It should be noted that this counterfactual analysis applied the seeding-associated increment estimated from, and removed it within, the same cohort used to derive the beta estimates; an independent cohort would be needed to rule out overfitting of this correction [18].

This finding has clinical implications. An α SynSAA+ patient could have a positive plasma result but a negative amyloid confirmation and be judged a plasma false positive. Two explanations are possible. In the first, α -synuclein pathology raises plasma p-tau217 through a route unrelated to AD pathology; the increment would then be artefactual and would move patients across decision thresholds without a corresponding increase in the pathology those thresholds are meant to detect. In the second, the increment is biological: it reflects the additional fibrillar tau that α SynSAA+ patients carry at a given amyloid burden [6]. The elevation against the amyloid- and CSF-referenced measures is compatible with either explanation, but the two analyses above favour the biological one. Whichever explanation holds, the implications extend beyond individual diagnosis. The same misclassification risk applies to trial enrolment [19] and, potentially, to the response to anti-amyloid or other disease-modifying therapies, given the higher fibrillar tau burden [20]. Reporting α SynSAA status, and where feasible stratifying by it, would allow this source of heterogeneity to be examined directly.

These results may appear to conflict with previous literature, since plasma p-tau217 is generally understood to become abnormal earlier than tau PET [1, 21], which raises the question of why fibrillar tau accounts for more of the seeding-associated difference than amyloid does. Two explanations may account for this. First, plasma p-tau217 correlates with amyloid but not tau in early disease stages, and becomes more strongly associated with tau in later stages [16]; our α SynSAA+ participants, with amyloid burden well beyond the positivity threshold, occupy this later stage. Second, our models are conditional throughout, and the attenuation seen for tau PET partly reflects the larger between-group difference in tau PET itself compared with Centiloids or the CSF ratio.

For practice, the implication is that a plasma p-tau217 result does not need to be discounted in a patient with concomitant Lewy-related pathology. Where plasma and an amyloid confirmatory test disagree in such a patient, our data favour the interpretation that the amyloid measure lags the tau process [16] rather than that the plasma measure has erred. Previous work showed that plasma p-tau217 reliably identifies amyloid in neuronal synuclein disease [8], and that among patients with a clinical diagnosis of AD those who are α SynSAA+ have higher plasma p-tau217 [9]; our findings extend these by showing that the elevation persists at equivalent AD pathology burden, and that p-tau217 remains informative when AD pathology and α -synuclein seeding co-occur.

We stop short of concluding that plasma p-tau217 is unaffected by seeding: it was elevated at matched amyloid burden, and a cross-sectional design cannot establish the absence of an effect. Still, in this exploratory analysis, limited by sample size, we could not detect any compromise of diagnostic utility from this difference, consistent with the increment behaving as information about AD pathology rather than as noise. A likely explanation for why so few classifications changed is that α SynSAA+ participants already had substantially higher pathology on the very measure each classification was judged against (Supplementary Table S8), and correspondingly high plasma p-tau217; about 60% were already above the positive threshold before any adjustment. Consistent with this, reclassification affected only 1 of 66 α SynSAA+ participants against the CSF ratio and 3 of 52 against amyloid PET. The seeding-associated excess in plasma p-tau217 is therefore real, but in this cohort it rarely changed the clinical category assigned to an individual patient. Whether this holds earlier in the disease course, when plasma values sit closer to the cutpoint, remains to be tested in cohorts enriched for prodromal or indeterminate-range participants.

Our conclusions are bounded in several ways. The design is cross-sectional, so the interpretation that α SynSAA+ participants sit further along a shared amyloid-to-tau trajectory remains inferential; serial measurement of plasma p-tau217 and tau PET in the same individuals would help determine whether the plasma excess is followed by the fibrillar accumulation it appears to anticipate. Because cognitive impairment lies downstream of fibrillar tau, adjusting for cognitive stage may remove part of the effect under study, which is the most likely reason the shape difference was stronger under the sparser adjustment set used previously in this cohort [6]; our primary estimates are therefore conservative [22]. The main analytic sample was restricted to participants with tau PET, who were less advanced than the remainder of the CSF-referenced sample, leaving α SynSAA+ participants sparse at high amyloid burden. The counterfactual analysis carries an asymmetric logic: an improvement in agreement would have been strong evidence of artefact, whereas its absence is weaker evidence. In addition, ADNI is enriched for AD and includes few participants with a primary diagnosis of Lewy body disease; whether the same amyloid-tau displacement and plasma p-tau217 behavior generalize to cohorts where Lewy body pathology is the primary driver of clinical impairment remains to be established. Finally, ADNI is predominantly non-Hispanic White and highly educated. Replication in wider, more diverse cohorts, and in samples with better representation of earlier AD stages, is needed to confirm these findings.

CONCLUSION

Plasma p-tau217 is elevated in α SynSAA+ individuals at equivalent levels of AD pathology measured by amyloid PET or the CSF ratio, and that elevation is attenuated most by fibrillar tau, suggesting that α -synuclein co-pathology accompanies more tau than amyloid alone would predict, and that this displacement is detectable in blood. The concern that seeding might increase plasma p-tau217 independently of AD pathology is reasonable, but neither analysis designed to detect such inflation found any sign of it. The difference between groups is real, but it does not appear to translate into a loss of diagnostic precision, and it does not justify down-weighting a plasma p-tau217 result in a patient with concomitant Lewy-related pathology.

Declarations

CONFLICT OF INTEREST STATEMENT

Íñigo Rodríguez-Baz has received personal fees for service on advisory boards, speaker honoraria, or educational activities from Nutricia, Almirall, and Esteve. Ignacio Illán-Gala participated in advisory boards from UCB and Nutricia, and received speaker honoraria from Almirall, Esteve Pharmaceuticals S.A, Kern Pharma, Krka Farmacéutica S.L., Lilly, Nutricia, and Zambon S.A.U. No other competing interests were reported. Judit Selma-González has received a speaker honoraria from Almirall. Valle Camacho has received personal fees for service on advisory boards, speaker honoraria or educational activities from General Electric, Life Molecular Imaging, Novartis, Lilly and Curium. Juan Fortea reported serving on the advisory boards, adjudication committees, or speaker honoraria from AC Immune, Adamed, Alzheon, Biogen, Eisai, Esteve, Fujirebio, Ionis, Laboratorios Carnot, Life Molecular Imaging, Lilly, Novo Nordisk, Perha, Roche, Zambón. JF reports holding a patent for markers of synaptopathy in neurodegenerative disease (licensed to ADx, WO2019175379). Alberto Lleó has served as a consultant or on advisory boards for Almirall, Beckman Coulter, Fujirebio-Europe, NovoNordisk, Roche, Biogen, Grifols, Novartis, Eisai, Lilly, and Nutricia, outside the submitted work. AL reports holding a patent for markers of synaptopathy in neurodegenerative disease (licensed to ADx, WO2019175379). Mireia Tondo has received personal fees for service on advisory boards, speaker honoraria, or educational activities from Grifols, Adium, Lilly and Esteve. Carla Abdelnour serves as a consultant for BioArctic, has received speaker honoraria from Hoffman-La Roche LTD, Nutricia, Schwabe Farma Ibérica SAU and Zambon; and is a member of the Board of Directors of the Lewy Body Dementia Association. No other competing interests were

reported. Daniel Alcolea participated in advisory boards from Fujirebio-Europe, Roche Diagnostics, Grifols S.A., Sysmex, Schwabe and Lilly, and received speaker honoraria from Fujirebio-Europe, Roche Diagnostics, Nutricia, Sysmex, Krka Farmacéutica S.L., Zambon S.A.U., Neuraxpharm, Almirall, Alter Medica, Sysmex, Lilly and Esteve Pharmaceuticals S.A. DA declares holding a patent for markers of synaptopathy in neurodegenerative disease (licensed to ADx NeuroSciences N.V., WO2019175379 Markers of synaptopathy in neurodegenerative diseases).

Competing Interests

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Author Contribution

I.R.B. conceived the original idea. I.R.B., C.A. and D.A. designed the study and wrote the first draft of the manuscript. I.R.B. accessed and curated the data from the ADNI database and performed the statistical analyses. I.R.B., R.F., C.A., M.S.F., E.V.C. and D.A. contributed to data interpretation. C.A. and D.A. supervised the project. All authors critically reviewed and revised the manuscript and approved the final version.

Acknowledgement

Data collection and sharing for the Alzheimer's Disease Neuroimaging Initiative (ADNI) is funded by the National Institute on Aging (National Institutes of Health Grant U19 AG024904). The grantee organization is the Northern California Institute for Research and Education. In the past, ADNI has also received funding from the National Institute of Biomedical Imaging and Bioengineering, the Canadian Institutes of Health Research, and private sector contributions through the Foundation for the National Institutes of Health (FNIH) including generous contributions from the following: AbbVie, Alzheimer's Association; Alzheimer's Drug Discovery Foundation; Araclon Biotech;

BioClinica, Inc.; Biogen; Bristol-Myers Squibb Company; CereSpir, Inc.; Cogstate; Eisai Inc.; Elan Pharmaceuticals, Inc.; Eli Lilly and Company; EuroImmun; F. Hoffmann-La Roche Ltd and its affiliated company Genentech, Inc.; Fujirebio; GE Healthcare; IXICO Ltd.; Janssen Alzheimer Immunotherapy Research & Development, LLC.; Johnson & Johnson Pharmaceutical Research & Development LLC.; Lumosity; Lundbeck; Merck & Co., Inc.; Meso Scale Diagnostics, LLC.; NeuroRx Research; Neurotrack Technologies; Novartis Pharmaceuticals Corporation; Pfizer Inc.; Piramal Imaging; Servier; Takeda Pharmaceutical Company; and Transition Therapeutics. Íñigo Rodríguez-Baz acknowledges support from Instituto de Salud Carlos III and co-funded by the European Union (CM22/00052, CD25/00223), and the Alzheimer's Association (AACSF-25-1486364). Lucia Maure-Blesa was supported by Instituto de Salud Carlos III through the Río Hortega Fellowship "CM23/00291" and co-funded by the European Union. Judit Selma-González is supported by the Contratos Predoctorales de Formación en investigación en salud program (FI25/00235) associated to the project funded by Fondo de Investigaciones Sanitarias (PI24/00598 to I.I.G.) from Instituto de Salud Carlos III (ISCIII), Spain. Ignacio Illán-Gala acknowledges support from the Institute of Health Carlos III (ISCIII), Spain (PI21/00791 and PI24/00598) jointly funded by Fondo Europeo de Desarrollo Regional, Unión Europea, "Una manera de hacer Europa" and by the Department of Health Generalitat de Catalunya PERIS program (SLT042/25/000032). I. Illán-Gala is a senior Atlantic Fellow for Equity in Brain Health at the Global Brain Health Institute (GBHI) and receives funding from the Alzheimer's Association (AACSF-21-850193), and the Alzheimer Society (GBHI ALZ UK-21-72097). I. Illán-Gala was also supported by the Juan Rodés Contract (JR20/0018) from the Carlos III National Institute of Health of Spain, partly funded by the European Social Fund. Alexandre Bejanin acknowledges support from Instituto de Salud Carlos III (ISCIII) and co-funded by the European Union - ERDF- through the Miguel Servet grant (CP20/00038) and Fondo de Investigaciones Sanitarias (PI22/00307), the Alzheimer's Association (AARG-22-923680), and the Ajuntament de Barcelona, in collaboration with Fundació La Caixa (23S06157-001). Juan Fortea reports grants from the Fondo de Investigaciones Sanitarias, Carlos III Health Institute (INT21/00073, PI20/01473 and PI23/01786 to J.F.) and the Centro de Investigación Biomédica en Red sobre Enfermedades Neurodegenerativas (CIBERNED) Program 1, jointly funded by Fondo Europeo de Desarrollo Regional, Unión Europea, Una manera de hacer Europa. This work was also supported by the National Institutes of Health grants (1R01AG056850-01A1; R21AG056974, R01AG061566, 1R01AG081394-01 and 1R61AG066543-01 to J.F.), the Department de Salut de la Generalitat de Catalunya (SLT006/17/00119 to JF), Fundació Tatiana Pérez de Guzmán el Bueno (IIBSP-DOW-2020-151). It was also supported by Horizon2020– Research and Innovation Framework Programme from the European Union (H2020-SC1-BHC-2018-2020 to JF). Mireia Tondo acknowledges support from Instituto de Salud Carlos III (ISCIII) grants PI21/00140 and PI24/00513 jointly funded by Fondo Europeo de Desarrollo Regional, Unión Europea, "Una manera de hacer Europa". Carla Abdelnour acknowledges support from the Susan and Charles Berghoff Foundation, the ARISTOS program co-funded by the European Union's Horizon Europe research and innovation programme under the Marie Skłodowska-Curie grant agreement No 101081334 and the Spanish Institute of Health Carlos III (ISCIII) Juan Rodés grant JR25/00034. Daniel Alcolea acknowledges support from Institute of Health Carlos III (ISCIII), Spain (PI22/00611, PI25/00422, INT23/00048) jointly funded by Fondo Europeo de Desarrollo Regional, Unión Europea, "Una manera de hacer Europa", and by the Department of Health Generalitat de Catalunya PERIS program (SLT006/17/125, SLT042/25/000034). He also received support for Research Groups funding from the Department of Research and Universities from the Generalitat de Catalunya (2021 SGR 00979).

DATA AVAILABILITY STATEMENT

The ADNI data used in this study were obtained from the ADNI database (<https://adni.loni.usc.edu>). All ADNI data are shared without embargo through the LONI Image and Data Archive (IDA).

CONSENT STATEMENT

ADNI study was approved by each ADNI study site's respective institutional review board and informed written consents were obtained from all participants.

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Figures

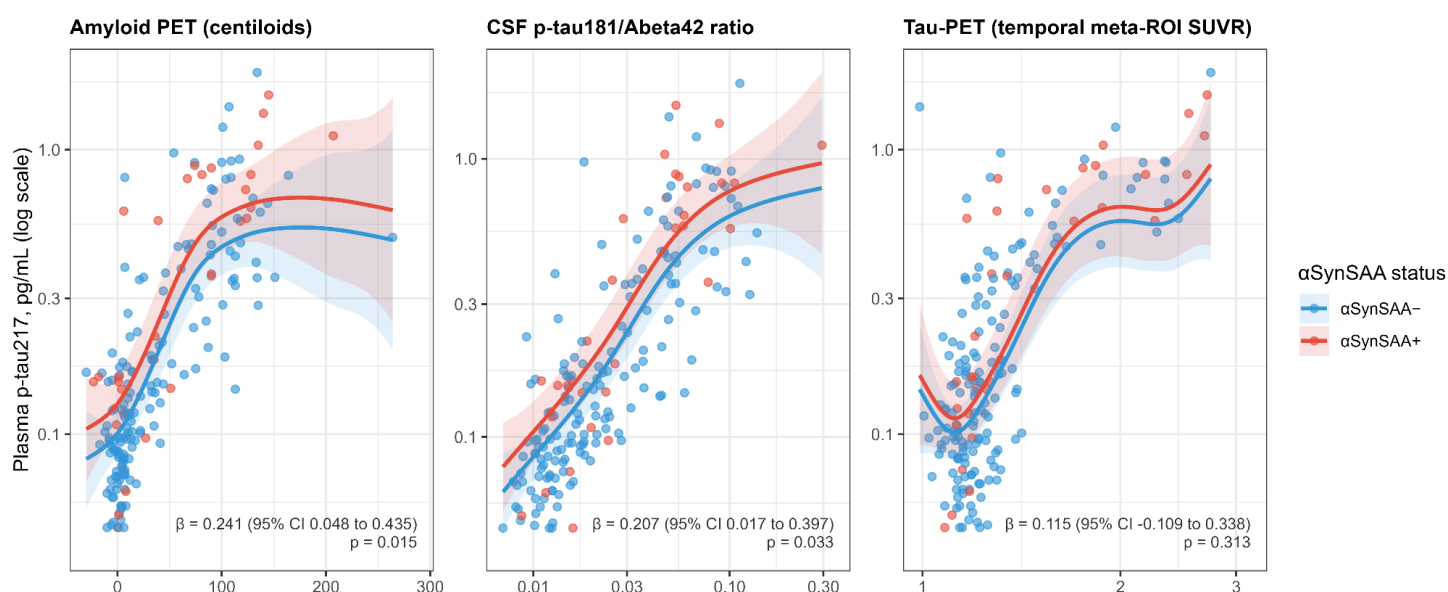


Figure 1

Plasma p-tau217 against each reference measure of pathology

Each panel plots plasma p-tau217 (pg/mL, log scale) against amyloid PET burden (centiloids, linear scale), the CSF p-tau181/Aβ42 ratio (log scale), or tau-PET signal in a temporal meta-ROI SUVR (log scale). Points are the same 193 participants in every panel, coloured by αSynSAA status (blue, αSynSAA-; red, αSynSAA+). Lines are fitted values from the primary generalised additive model for each panel, with age, sex, APOE ε4 carrier status and cognitive stage held at reference levels; shaded bands are 95% confidence intervals. β, 95% CI and p-value annotated in each panel refer to the αSynSAA coefficient of that panel's model (log scale). Abbreviations: Aβ42, amyloid-beta 42; APOE4, apolipoprotein E ε4 allele; αSynSAA, α-synuclein seed amplification assay; β, regression coefficient; CI, confidence interval; CSF, cerebrospinal fluid; pg/mL, picograms per millilitre; PET, positron emission tomography; ROI, region of interest; SUVR, standardised uptake value ratio.

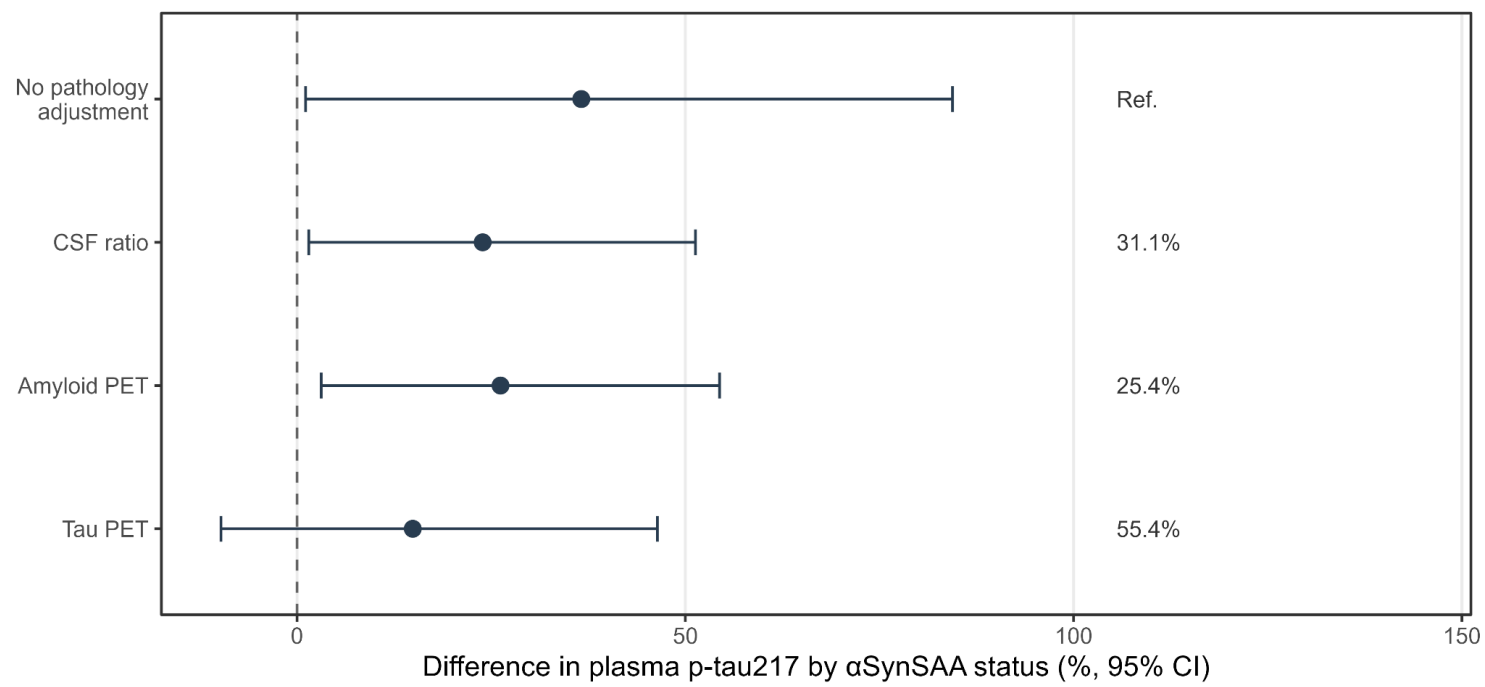


Figure 2

Attenuation of the plasma p-tau217 association by pathology measure

Each row shows the αSynSAA coefficient from a separate linear model, adjusted for age, sex, APOE ε4 carrier status and cognitive stage, fitted to the same 193 participants. The reference model (Ref.) includes no pathology measure; in the CSF ratio, amyloid PET and tau-PET rows, that measure enters as a natural cubic spline (3 degrees of freedom), and the percentage beside each row is the attenuation in the αSynSAA coefficient relative to the reference model. Attenuation is computed on the log-scale coefficients and therefore does not equal the difference between the back-transformed percentages. Error bars are 95% confidence intervals from heteroskedasticity-consistent (HC3) standard errors. Abbreviations: APOE4, apolipoprotein E ε4 allele; αSynSAA, α-synuclein seed amplification assay; CI, confidence interval; CSF, cerebrospinal fluid; HC3, heteroskedasticity-consistent (type 3); PET, positron emission tomography; Ref., reference.

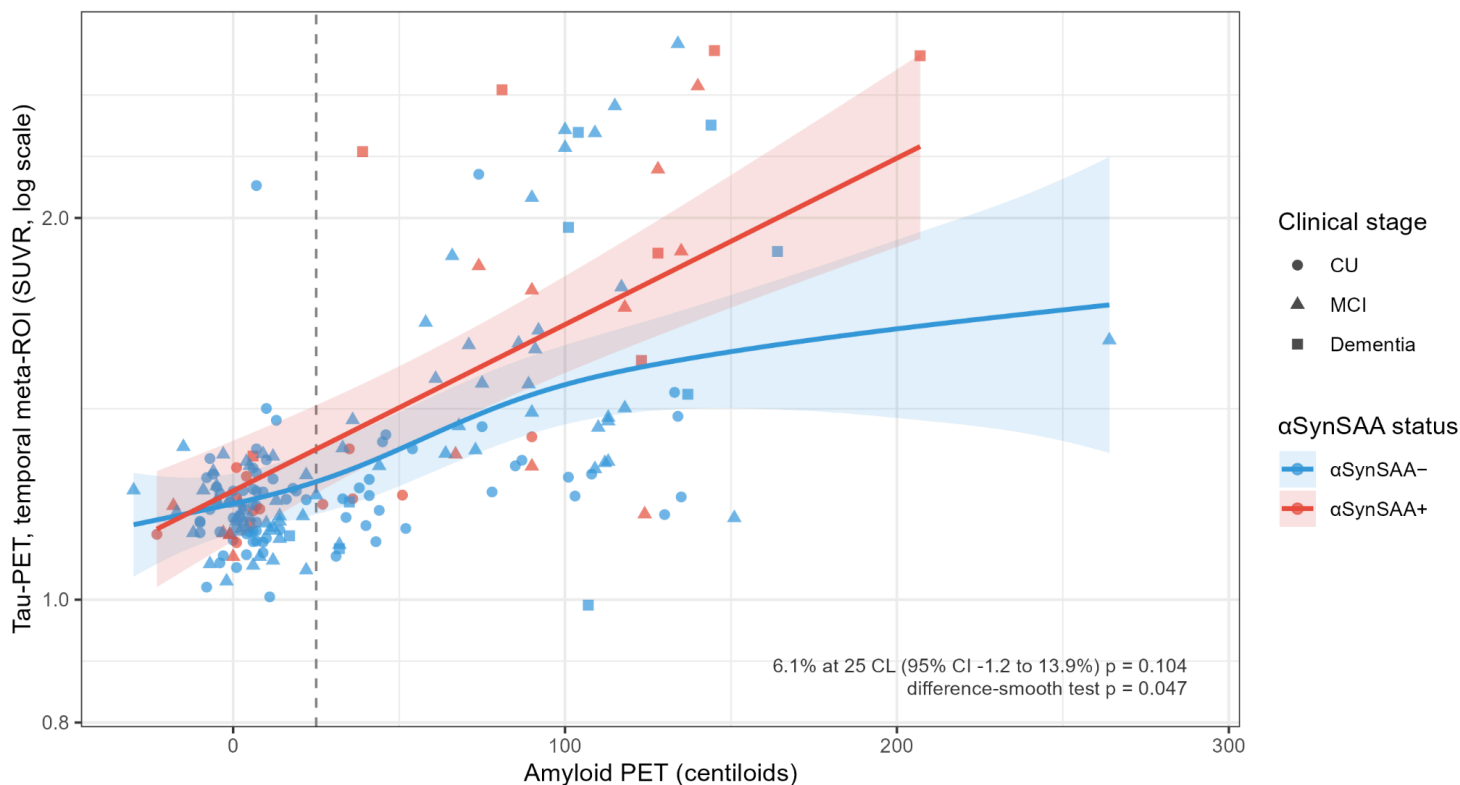


Figure 3

Amyloid burden and fibrillar tau by α SynSAA status

Lines and shaded bands are model-adjusted fits from the primary GAM (age, sex, APOE $\epsilon 4$ carrier status, cognitive stage and site held at reference levels), with 95% confidence bands, fitted separately by α SynSAA status. Each group's curve is drawn only over the range of the x-axis variable observed in that group. The percentage difference, 95% CI, p-value and interaction p-value annotated in the panel refer to the formal contrast between the fitted curves at the amyloid positivity threshold and the difference-smooth test for shape, respectively. The dashed line marks the amyloid positivity threshold (25 CL). Point colour indicates α SynSAA status (blue, α SynSAA-; red, α SynSAA+); point shape indicates cognitive stage (diamond, cognitively unimpaired; triangle, mild cognitive impairment; square, dementia). Tau-PET SUVR is shown on the log scale, matching the outcome scale used in the model. Abbreviations: CL, centiloids; CU, cognitively unimpaired; MCI, mild cognitive impairment; PET, positron emission tomography; SUVR, standardised uptake value ratio.

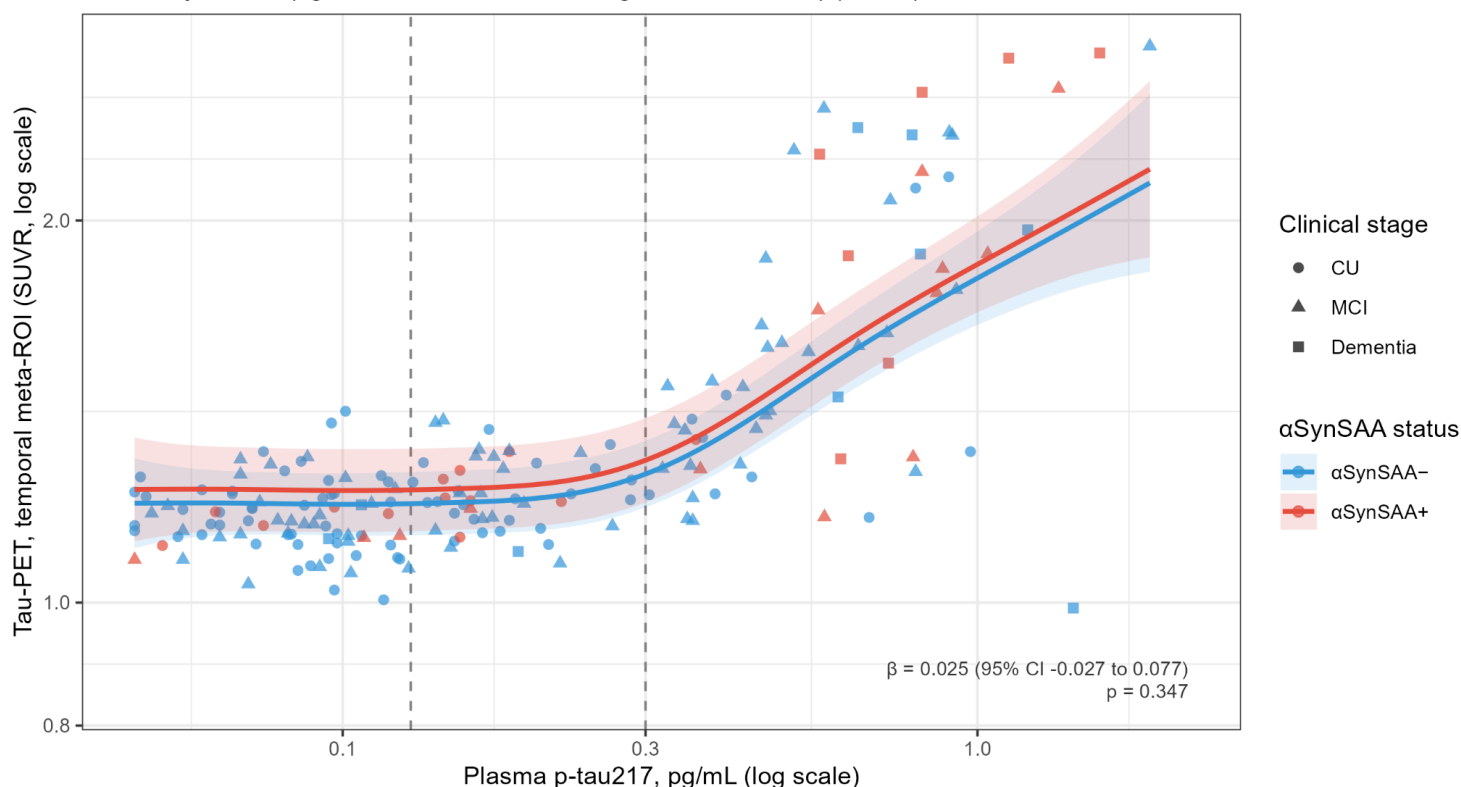


Figure 4

Plasma p-tau217 and fibrillar tau by αSynSAA status

Lines and shaded bands are model-adjusted fits from the primary GAM (age, sex, APOE ε4 carrier status, cognitive stage, centiloids and site held at reference levels), with 95% confidence bands. β , 95% CI and p -value annotated in the panel refer to the αSynSAA coefficient of this model (log scale). The dashed lines mark the plasma p-tau217 thresholds separating negative from indeterminate (0.128 pg/mL) and indeterminate from positive (0.300 pg/mL) results. Point colour indicates αSynSAA status (blue, αSynSAA-; red, αSynSAA+); point shape indicates cognitive stage (diamond, cognitively unimpaired; triangle, mild cognitive impairment; square, dementia). Both axes are shown on the log scale, matching the outcome and predictor scales used in the model. Abbreviations: CU, cognitively unimpaired; MCI, mild cognitive impairment; PET, positron emission tomography; SUVR, standardised uptake value ratio.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SynucleinSAAp-tau217suppl.docx](#)