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RESEARCH ARTICLE

Higher plasma β-synuclein indicates early synaptic degeneration in Alzheimer's disease

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Abstract

INTRODUCTION: β-Synuclein is an emerging synaptic blood biomarker for Alzheimer's disease (AD) but differences in β-synuclein levels in preclinical AD and its association with amyloid and tau pathology have not yet been studied.

METHODS: We measured plasma β-synuclein levels in cognitively unimpaired individuals with positive Aβ-PET (i.e., preclinical AD, $N = 48$) or negative Aβ-PET ($N = 61$), Aβ-positive patients with mild cognitive impairment (MCI, $N = 36$), and Aβ-positive AD dementia ($N = 85$). Amyloid (A) and tau (T) pathology were assessed by [^{18}F]flutemetamol and [^{18}F]RO948 PET.

RESULTS: Plasma β-synuclein levels were higher in preclinical AD and even higher in MCI and AD dementia. Stratification according to amyloid/tau pathology revealed higher β-synuclein in A^+T^- and A^+T^+ subjects compared with A^-T^-. Plasma β-synuclein levels were related to tau and Aβ pathology and associated with temporal cortical thinning and cognitive impairment.

DISCUSSION: Our data indicate that plasma β-synuclein might track synaptic dysfunction, even during the preclinical stages of AD.

KEYWORDS
amyloid-β PET, β-Synuclein, blood biomarker, preclinical Alzheimer's disease, synaptic degeneration, tau-PET

HIGHLIGHTS

- Plasma β-synuclein is already higher in preclinical AD.
- Plasma β-synuclein is higher in MCI and AD dementia than in preclinical AD.

Patrick Oeckl and Shorena Janelidze contributed equally to this work.

- Aβ- and tau-PET SUVRs are associated with plasma β-synuclein levels.
- Plasma β-synuclein is already higher in tau-PET negative subjects.
- Plasma β-synuclein is related to temporal cortical atrophy and cognitive impairment.

1 | BACKGROUND

Synapses, the connections between neurons, play a central role in memory formation, and synaptic dysfunction or loss is linked to various neurodegenerative diseases. Synaptic degeneration is a major hallmark of Alzheimer's disease (AD),[1] the most common form of dementia, and neuropathological studies indicate that synaptic loss is the best correlate of cognitive impairment in AD.[1] Because synaptic degeneration links pathology to clinical symptoms in AD, its assessment in patients is of high clinical relevance for diagnosis and prognosis, but also to monitor effects on synaptic function of novel therapeutic interventions. The role of synaptic loss in the preclinical phase of AD and its dynamics is less well understood because it is usually not tangible for neuropathological studies. Fluid biomarkers in cerebrospinal fluid (CSF) and blood offer the possibility to study pathological alterations in patients during lifetime and are an important tool to study synaptic degeneration in AD. Several synaptic biomarker candidates in CSF have been described such as neurogranin, GAP-43 or SNAP-25 originating from different synaptic compartments. CSF levels of them are higher in AD patients compared with healthy controls which is ascribed to the release of this proteins into the extracellular space upon synaptic disruption.[2] One emerging synaptic biomarker candidate in CSF is the presynaptic protein β-synuclein. Several studies consistently showed higher CSF levels of β-synuclein in AD patients reflecting the ongoing synaptic degeneration.[3–7]

A major achievement in biomarker research in the past years was the development of highly sensitive assays enabling the measurement of key biomarkers also in blood.[8] Blood is more readily and more convenient to collect than CSF and increases the accessibility of biomarker measurements. In this context, we could recently develop a sensitive assay to measure also the synaptic marker β-synuclein in blood samples.[3] We found that blood levels of β-synuclein are increased in sporadic AD patients and elevated levels could already be detected in a smaller sub-cohort in the early clinical phase of AD characterized by mild cognitive impairment (MCI).[3,9] β-Synuclein blood levels correlate with cognitive impairment and are mainly associated with temporal brain atrophy in AD, a region that is affected early in AD pathogenesis.[9] These data show that measurement of β-synuclein is a promising and easily accessible blood test to study synaptic degeneration in AD. However, blood levels of β-synuclein have not been investigated so far in the preclinical stage of AD, which would help to improve our knowledge about synaptic degeneration in this very early phase of the disease. It is also unclear how β-synuclein levels in blood are related to amyloid and tau pathology helping to temporally integrate synaptic degeneration in the AD continuum.

The aim of our study was to investigate plasma β-synuclein levels in different stages of AD including the preclinical phase and its relation to amyloid and tau pathology. β-Synuclein levels were measured in samples from the Swedish BioFINDER-2 study including cognitively unimpaired individuals (CU, $N = 109$) with positive Aβ-PET (CU $A\beta^+$, i.e., preclinical AD) or negative Aβ-PET (CU $A\beta^-$), $A\beta^+$ patients with MCI ($N = 36$) and AD dementia ($N = 85$). Amyloid and tau pathology was assessed by quantitative [^{18}F]flutemetamol and [^{18}F]RO948 PET, respectively and cortical thickness by MRI. Cognitive performance was analyzed using the Mini-Mental State Examination (MMSE) and modified Preclinical Alzheimer's Cognitive Composite (mPACC). We used non-parametric tests and linear regression models for group comparisons and correlation analyses between β-synuclein levels and other parameters.

2 | METHODS

2.1 | Participants

The study included participants from the Swedish BioFINDER-2 study (clinical trial no. NCT03174938) who were recruited between 2017 and 2021 in southern Sweden (Skåne University Hospital and the Hospital of Ängelholm) as previously described.[10] The inclusion criteria for controls were: (1) ages 40–100 years; (2) absence of cognitive symptoms as assessed by a physician specialized in cognitive disorders; (3) MMSE score of 27-30 points (40–65 years of age) or 26–30 points (66–100 years of age) at screening visit; (4) do not fulfill the criteria for mild or major neurocognitive disorder (MCI or dementia) according to DSM-5[11]; and (5) fluent in Swedish. The recruitment process of neurologically and cognitively healthy controls was designed to build a study population with 50% *APOE* ε4 carriers. Inclusion criteria for patients with subjective cognitive decline (SCD) or MCI were: (1) age 40–100 years; (2) referred to the memory clinics due to cognitive symptoms; (3) MMSE score of 24–30 points; (4) does not fulfill the criteria for any dementia (major neurocognitive disorder) according to DSM-5; and (5) fluent in Swedish. Participants were classified as having MCI if

[1] $A\beta^+$, subjects with positive Aβ-PET; $A\beta^-$, subjects with negative Aβ-PET; AD, Alzheimer's disease; ADAS-cog, cognitive subscale from the Alzheimer's Disease Assessment Scale; CSF, cerebrospinal fluid; CU, cognitively unimpaired individuals; IP-MS, immunoprecipitation mass-spectrometry; IQR, interquartile range; MCI, mild cognitive impairment; MMSE, Mini-Mental State Examination; mPACC, modified Preclinical Alzheimer's Cognitive Composite; MPRAGE, magnetization-prepared rapid gradient echo; ROI, region of interest; SCD, subjective cognitive decline; SUVR, standardized uptake value ratio

they performed worse than −1.5 standard deviation (SD) in any cognitive domain according test norms adjusted for age and education. Those patients that were not classified as MCI were considered to have SCD. According to the updated NIA-AA criteria for AD, cognitively healthy controls and participants with SCD were considered as CU.[12] Inclusion criteria for patients with AD dementia were: (1) age 40–100 years; (2) referred to the memory clinics due to cognitive symptoms; (3) MMSE score of ≥12 points; (4) fulfill the DSM-5 criteria for dementia (major neurocognitive disorder) due to AD; and (5) fluent in Swedish. Exclusion criteria for both controls and patients were: (1) significant unstable systemic illness that makes it difficult to participate in the study; (2) current significant alcohol or substance misuse; (3) refusing lumbar puncture, MRI, or PET. Study participants were classified as $A\beta^-$ or $A\beta^+$ based on Aβ-PET status when available (all CU and MCI, 22 AD dementia) and using CSF Aβ42/Aβ40 for the 63 patients in the AD dementia group where Aβ-PET was not performed by design, using cutoffs described below. We included 109 CU individuals ($A\beta^+$, $N = 61$; $A\beta^-$, $N = 48$), 36 $A\beta^+$ patients with MCI and 85 $A\beta^+$ patients with AD dementia. Cognitive function was assessed with MMSE and mPACC. The mPACC was calculated as the average of five z-scores for tests of global cognition (MMSE), memory (the word list delayed recall test from the cognitive subscale from the Alzheimer's Disease Assessment Scale [ADAS-cog], counted twice in order to preserve the weight on memory from the original PACC), executive function (Trail Making Test A) and verbal ability (animal fluency).

The study was approved by the Regional Ethics Committee in Lund, Sweden.

2.2 | Plasma β-synuclein and CSF Aβ analysis

EDTA-plasma and CSF samples were collected and handled according to established protocols.[10]

CSF Aβ42 and Aβ40 were quantified using Elecsys electrochemiluminescence immunoassays on a fully automated cobas e 601 instrument (Roche Diagnostics International Ltd., Rotkreuz, Switzerland) or Lumipulse G (Fujirebio) immunoassays. Abnormal CSF Aβ42/Aβ40 was defined using a previously described threshold of 0.080 (Elecsys) or 0.072 (Lumipulse G[13,14]).

Plasma β-synuclein was measured by immunoprecipitation mass-spectrometry (IP-MS) as previously described[3,9] using 490 μL of plasma per sample and including quality control samples in all runs to monitor assay performance. Intra- and interassay CV was 3%–17% and 15%.

2.3 | Image acquisition and processing

Out of 230 participants, 168, 226, and 225 had Aβ-PET, tau-PET, and MRI, respectively. The average time intervals between plasma collection and Aβ-PET, tau-PET, and MRI were 0.47 (0.72) years, 0.47 (0.72) years, and 0.54 (0.79) years, respectively.

RESEARCH IN CONTEXT

1. **Systematic Review**: Recent data support plasma β-synuclein as an easily accessible biomarker to study synaptic degeneration in Alzheimer's disease (AD). We searched the PubMed database but there is no information if plasma β-synuclein is already changed in preclinical AD and how it relates to amyloid and tau deposition in the brain.
2. **Interpretation**: Plasma β-synuclein levels are higher in early AD, already during the preclinical phase, supporting that synaptic degeneration belongs to the very early events in AD and preceding tau pathology. This supports plasma β-synuclein as an easily accessible biomarker that might be used to track synaptic dysfunction, even during preclinical stages of AD.
3. **Future Directions**: Longitudinal studies are needed to confirm the temporal estimations on synaptic degeneration suggested by our study. Plasma β-synuclein might be used to detect positive effects of novel disease-modifying therapies on synaptic dysfunction in AD and to further study synaptic degeneration in the pathophysiology of AD.

PET imaging was performed on a digital GE Discovery MI scanner as previously described.[10] For Aβ-PET, scans were acquired 90–110 min after the injection of ~185 MBq [^{18}F]Flutemetamol. For tau-PET, the acquisition was done 70–90 min post injection of ~370 MBq [^{18}F]RO948. Images were processed according to our pipeline described previously.[15] Briefly, PET images were attenuation corrected, motion corrected, summed and registered to the closest T1-weighted MRI processed through the longitudinal pipeline of FreeSurfer version 6.0. Standardized uptake value ratio (SUVR) images were created using inferior cerebellar gray matter as reference region for [^{18}F]RO948, and the cerebellum for [^{18}F]flutemetamol. [^{18}F]flutemetamol SUVR were calculated for a composite neocortical region of interest (ROI) including the caudal anterior cingulate, frontal, lateral parietal, and lateral temporal gyri.[16] [^{18}F]RO948 SUVRs were obtained for a temporal meta-ROI composed of entorhinal cortex, inferior and middle temporal cortices, fusiform gyrus, parahippocampal cortex, and amygdala that corresponding to Braak I-IV regions.[17]

Aβ-PET and tau-PET data was binarized using cutoffs determined using mixture modelling (Aβ-PET, 1.033; tau-PET, 1.36[15,18]).

MRI was performed using a Siemens 3T MAGNETOM Prisma scanner (Siemens Medical Solutions). Structural T1-weighted MRI images were acquired from a magnetization-prepared rapid gradient echo (MPRAGE) sequence with 1 mm isotropic voxels. FreeSurfer version 6.0 was used to extract cortical thickness within a meta-ROI encompassing temporal regions with known susceptibility in AD (AD

TABLE 1 Demographic and clinical characteristics.

	CU Aβ−	CU Aβ+	MCI Aβ+	AD dementia	*p*-Value
Age, years	78.0 (66.5–81.7)	74.9 (68.2–79.9)	73.9 (70.7–77.0)	76.8 (70.6–80.3)	0.347
	N = 61	*N* = 48	*N* = 36	*N* = 85	
Sex, F/M	33/28	25/23	17/19	56/29	0.185
APOE ε4 positivity, *n (%)*	21 (34.4)	32 (66.7)	23 (63.9)	59 (69.4)	0.0001
Education, years	12.0 (9.0–15.0)	11.0 (9.0–14.3)	12.5 (9.0–15.3)	12.0 (9.0–15.0)	0.694
	N = 60	*N* = 46	*N* = 34	*N* = 80	
MMSE	29.0 (28.0–30.0)	29.0 (28.0–30.0)	27.0 (25.0–29.0)	18.0 (13.0–22.0)	3.0e−29
	N = 60	*N* = 48	*N* = 33	*N* = 74	
mPACC	0.05 (−0.35 to 0.57)	−0.4 (−1.05 to 0.3)	−1.72 (−2.20 to −1.06)	−4.03 (−6.22 to −3.31)	1.2e−25
	N = 59	*N* = 47	*N* = 30	*N* = 52	
Plasma β-synuclein. pg/mL	8.5 (7.0–10.3)	9.2 (7.5–12.6)	10.1 (7.8–14.3)	12.8 (9.1–16.7)	4.5e−08
	N = 61	*N* = 48	*N* = 36	*N* = 85	
Aβ-PET	0.92 (0.90–0.96)	1.35 (1.23–1.47)	1.47 (1.30–1.56)	1.58 (1.35–1.73)	1.7e−25
	N = 61	*N* = 48	*N* = 36	*N* = 23	
tau-PET SUVR	1.18 (1.13–1.21)	1.22 (1.15–1.31)	1.26 (1.15–1.75)	1.92 (1.49–2.55)	1.8e−24
	N = 61	*N* = 48	*N* = 36	*N* = 81	
Temporal cortical thickness (mm)	2.69 (2.62–2.79)	2.65 (2.57–2.75)	2.58 (2.42–2.67)	2.43 (2.28–2.54)	2.2e−17
	N = 60	*N* = 47	*N* = 36	*N* = 82	

Note: Data are shown as median (interquartile range) unless otherwise specified. Differences between the groups were tested using the Kruskal-Wallis test and chi-squared test (sex and APOE).
Abbreviations: Aβ, β-amyloid; AD, Alzheimer's disease; CU, cognitively unimpaired; F, female; M, male; MCI, mild cognitive impairment; MMSE, Mini Mental State Examination; mPACC, modified Preclinical Alzheimer's Disease Cognitive Composite; NA, not available; PET, positron emission tomography; SUVR, standardized uptake value ratio.

cortex: mean thickness in bilateral entorhinal, inferior temporal, middle temporal, and fusiform cortices).[19]

2.4 | Statistical analysis

SPSS version 28 (IBM) was used for statistical analysis. Demographic and clinical data were compared using the Kruskal-Wallis and Chi-squared tests. Correlation between plasma β-synuclein and age were studied using the Spearman correlation test and linear regression model adjusting for AD status. Difference between men and women and between *APOE* ε4 carriers and non-carriers were examined with the Mann-Whitney test as well as univariate general linear models adjusted for age and AD status. Univariate general linear models adjusted for age and sex were also used to assess differences in plasma β-synuclein levels between diagnostic groups. Associations of plasma β-synuclein with Aβ-PET and tau-PET SUVR and MRI measures of AD signature cortical thickness were examined with linear regression models including age and sex as covariates. Associations with cognitive scores on MMSE and mPACC were also examined with linear regression models but including age, sex, and additionally duration of education as covariates. Log-transformed β-synuclein data were used in all regression models. Associations between plasma β-synuclein and Aβ-PET or tau-PET status were tested by ROC curve analysis.

3 | RESULTS

3.1 | Participants

The study included 230 participants with the mean (SD) age of 72.3 (9.2) years of whom 131 (57.0%) were women. Demographic and clinical characteristics of CU participants (Aβ+, *N* = 61; Aβ−, *N* = 48) and patients with MCI (Aβ+, *N* = 36) and AD dementia (Aβ+, *N* = 85) are shown in Table 1. There were no differences in age, sex and years of education between the groups. The rate of *APOE* ε4 positivity, cognitive sores (MMSE, mPACC), and imaging biomarkers (Aβ-PET, tau-PET, and AD signature cortical thickness) were increasingly abnormal in CU Aβ+, MCI Aβ+, and AD dementia compared with CU Aβ−.

3.2 | Association between plasma β-synuclein and demographics

Correlations between plasma β-synuclein and age showed a trend toward statistical significance (Rs = 0.116, $p = 0.079$). Plasma levels of β-synuclein were higher in women (median, 11.1; interquartile range [IQR], 8.4–16.3) than in men (median, 9.0; IQR, 7.3–11.9; $p < 0.001$), whereas there was no difference between *APOE* ε4 carriers (median, 10.5; IQR, 7.9–14.5) and non-carriers (median, 9.4; IQR, 8.1–13.1;

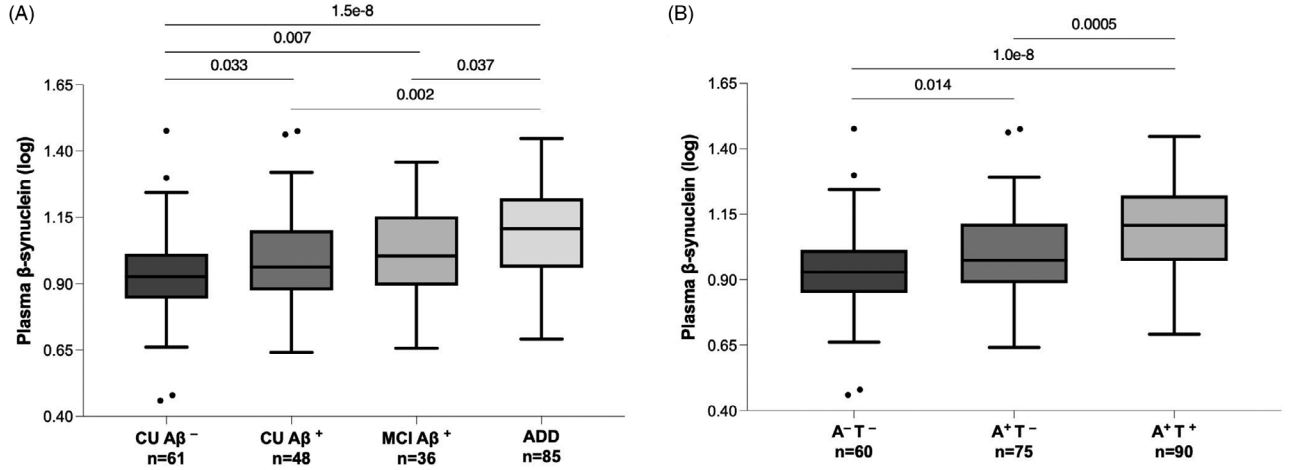

FIGURE 1 Plasma β-synuclein by diagnostic groups and Aβ and tau-PET status. Plasma concentration of β-synuclein was compared between the (A) CU Aβ^-, CU Aβ^+, MCI Aβ^+, and AD dementia groups and (B) between A^-T^-, A^+T^-, and A^+T^+ subjects. All p-values are from univariate general linear models with log-transformed plasma β-synuclein as an independent variable including age and sex as covariates. Aβ, β-amyloid; AD, Alzheimer's disease; CU, cognitively unimpaired; MCI, mild cognitive impairment; PET, positron emission tomography.

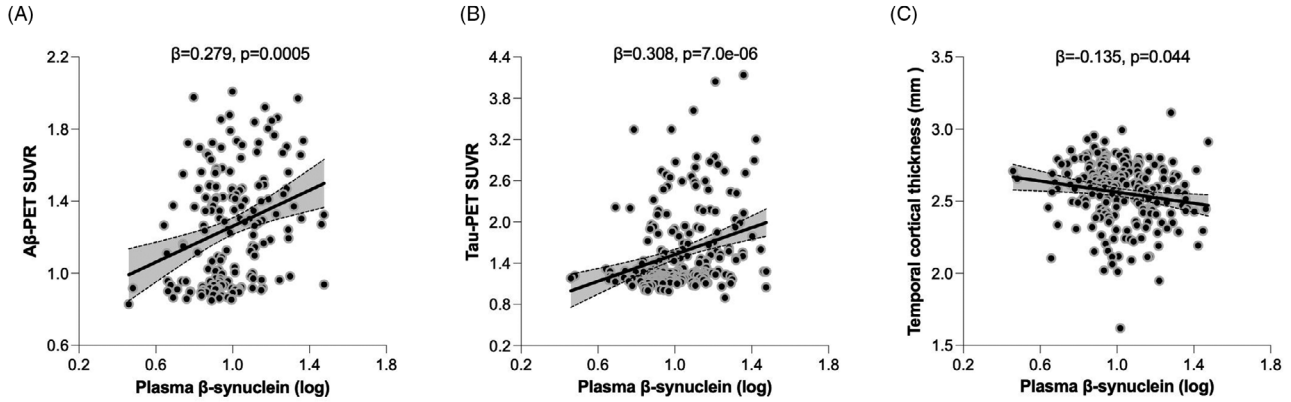

FIGURE 2 Associations between plasma β-synuclein and PET measures of Aβ and tau pathologies. Associations of plasma β-synuclein with Aβ-PET (A), tau-PET (B), and AD signature cortical thickness (C). Data are shown as β (standardized coefficient) and p value from linear regression models with log-transformed plasma β-synuclein as an independent variable including age and sex as covariates. Aβ, β-amyloid; PET, positron emission tomography; SUVR, standardized uptake value ratio.

$p = 0.334$). Differences in β-synuclein levels between women and men remained significant in the models adjusting for age and AD status ($F(1, 224) = 16.4$; $p < 0.001$) whereas association with age was not significant when accounting for the effects of AD status (standardized coefficient β, 0.101; $p = 0.10$).

3.3 | Plasma β-synuclein concentration across diagnostic groups

Plasma concentration of β-synuclein varied between the CU Aβ^-, CU Aβ^+, MCI Aβ^+, and AD dementia groups ($F(3, 224) = 11.8$; $p < 0.001$) (Figure 1). The levels were higher in CU Aβ^+ ($p = 0.033$), MCI Aβ^+ ($p = 0.007$), and AD dementia ($p < 0.001$) compared with CU Aβ^- and higher in AD dementia than in CU Aβ^+ ($p = 0.002$) and MCI Aβ^+ ($p = 0.037$) (Table 1, Figure 1A). Moreover, there were significant differences between participants when stratified by both Aβ (A) and tau-PET (T) status ($F(2, 220) = 18.3$; $p < 0.001$). β-synuclein levels were higher in both A^+T^- ($p = 0.014$) and A^+T^+ ($p < 0.001$) compared with A^-T^- and even higher in the A^+T^+ group compared with A^+T^- group ($p < 0.001$) (Figure 1B). Only one case had normal Aβ-PET and abnormal tau-PET scans; therefore, A^-T^+ group was not considered in this analysis.

3.4 | Association of plasma β-synuclein with imaging biomarkers and cognition

Higher plasma concentration of β-synuclein were associated with increased Aβ-PET SUVR (β, 0.279; $p < 0.001$; $n = 168$) and tau-PET SUVR (β, 0.308; $p < 0.001$; $n = 226$) and decreased AD signature cortical thickness (β, −0.135; $p = 0.044$; $n = 225$) (Figure 2). Associations with tau-PET SUVR were significant in Aβ^+ (β, 0.235; $p = 0.004$;

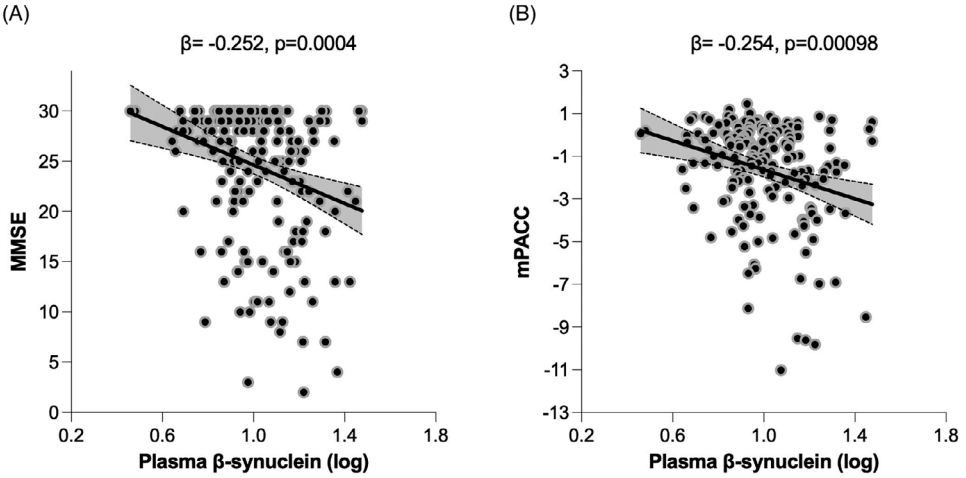

FIGURE 3 Associations between plasma β-synuclein and cognition. Associations of plasma β-synuclein with MMSE (A) and mPACC (B). Data are shown as β (standardized coefficient) and p value from linear regression models with log-transformed plasma β-synuclein as an independent variable including age, sex, and years of education as covariates. Aβ, β-amyloid; MMSE, Mini Mental State Examination; mPACC, modified preclinical Alzheimer's Disease Cognitive Composite.

TABLE 2 Accuracy of plasma β-synuclein to identify individuals with abnormal tau-PET and Aβ-PET.

	AUC (95% CI)	Threshold	Sensitivity	Specificity
Tau-PET	0.712 (0.644–0.780)	9.44	0.747	0.570
Aβ-PET	0.653 (0.569–0.736)	10.85	0.453	0.823

Note: Data are from ROC curve analyses with tau-PET or Aβ-PET status as outcome.

Abbreviations: Aβ, β-amyloid; AUC, area under the curve; CI, confidence interval; PET, positron emission tomography; ROC, receiver operating characteristic curve.

$n = 165$) but not in Aβ^- participants (β, −0.065; $p = 0.60$; $n = 62$). When studying the accuracy of plasma β-synuclein to identify individuals with abnormal Aβ-PET scans ($N = 106$, 46.1%) or tau-PET scans ($N = 91$, 39.6%), higher areas under the curve (AUCs) were seen for tau-PET (AUC, 0.712; 95% confidence interval [CI] 0.644–0.780; $p < 0.001$) than Aβ-PET (AUC, 0.653; 95% CI 0.569–0.736; $p = 0.001$; Table 2).

Higher plasma concentrations of β-synuclein were also associated with worse performance on MMSE (β, −0.252; $p < 0.001$; $n = 206$) and mPACC (β, −0.254; $p < 0.001$; $n = 179$) (Figure 3). Associations with MMSE (β, −0.176; $p = 0.046$; $n = 147$) were significant in Aβ^+ participants with a trend for mPACC (β, −0.188; $p = 0.054$; $n = 121$).

4 | DISCUSSION

In this study with 230 participants, we showed higher plasma β-synuclein levels already in the preclinical phase of AD with even higher levels in the MCI and AD dementia stages. Plasma β-synuclein levels were related to tau and Aβ pathology and associated to temporal cortical thinning and cognitive impairment. Temporal estimations suggest that rise of β-synuclein levels precedes tau pathology as determined with tau-PET.

The time course of synaptic degeneration during the AD continuum and its temporal relation to amyloid and tau pathology is unclear to date and fluid biomarkers of synaptic degeneration in blood and CSF, such as β-synuclein, are a promising tool to study these associations in humans. We could show that plasma β-synuclein levels are already higher in preclinical AD providing evidence that synaptic degeneration belongs to the earliest events in AD pathogenesis. Our observation is supported by a recent study also showing higher CSF levels of β-synuclein in preclinical AD subjects.[6] Other synaptic markers in CSF show early increase in AD as well, including both sporadic AD[20] and autosomal dominant AD mutation carriers.[21] Another piece of evidence comes from a study in Down syndrome (DS) subjects, a genetic form of AD, where blood levels of β-synuclein are already higher in subjects without clinical signs of AD and even more in DS patients with dementia.[22] Yet it is unclear whether the higher β-synuclein levels in DS are a sole result of the AD pathology or a combined effect of AD- and DS-related changes, but it is nevertheless in agreement with an early start of synaptic degeneration in AD as indicated by our data in sporadic AD.

The consistent results in the present and our previous studies[3,9] are of high clinical relevance because they indicate that with blood β-synuclein levels we might, for the first time, have an easily accessible and scalable biomarker that can be used to track synaptic dysfunction in AD and, therefore, fill an important gap. This includes the detection of positive effects from novel disease-modifying therapies or life style factors on synaptic degeneration, but also to further decipher the role of synaptic degeneration in AD in basic clinical research. Indeed, the recent breakthrough with lecanemab in the treatment of AD, for the first time showing a significantly reduced disease progression, supports synaptic markers as sensitive read-outs for positive effects. This is due to CSF levels of the synaptic marker neurogranin being

significantly reduced by lecanemab treatment and being more sensitive than neurofilaments as a general neurodegeneration marker.[23]

We assessed amyloid deposition and tau pathology in patients using PET imaging and investigated the relationship with plasma β-synuclein levels. Both Aβ-PET and tau-PET significantly correlated with β-synuclein levels, indicating that both processes are related to synaptic degeneration. However, the only moderate association of plasma β-synuclein levels with quantitative PET data indicates that the longitudinal dynamics of the reflected pathophysiological mechanisms are different. We here investigated subjects in different stages of AD and amyloid pathology was already present in the preclinical AD cases (by definition). Plasma β-synuclein values gradually increased from preclinical AD to AD dementia. Tau pathology measured by tau-PET showed the strongest changes between the MCI and AD dementia stage. In addition to the clinical AD staging, we stratified subjects according to their Aβ- and tau-PET status. Here, we observed higher plasma β-synuclein levels in tau-negative but Aβ-positive subjects compared with A^-T^- and a further increase in tau- and Aβ-positive subjects. Both the clinical and pathological stratification of the subjects indicates that synaptic degeneration starts early after amyloid deposition and precedes tau pathology within the AD continuum. A recent study measuring different pre- and postsynaptic markers in CSF also concluded that synaptic changes occur before tau-related alterations.[24] Longitudinal studies will be required to prove this hypothesis and our study supports the use of blood β-synuclein levels as an easily accessibly tool for such investigations.

Plasma β-synuclein levels were associated with AD-typical temporal cortical atrophy thereby confirming our previous observations[9] and suggesting most pronounced synaptic degeneration in this part of the brain. The correlation of β-synuclein levels with cognitive performance especially using the mPACC, a test designed to detect first cognitive changes in preclinical AD, is further in support of the early synaptic alterations in AD.

The longitudinal assumptions in our report might be limited by the cross-sectional design of our study. However, our study included subjects at different AD stages with a rigorous clinical, neuroimaging and biomarker-based characterization thereby reflecting different phases of the AD continuum. Also, there is no reference standard for synaptic degeneration available in our study that we could use to compare the β-synuclein results against. PET imaging of synaptic density could be such a reference marker and future studies are required to investigate the association of plasma β-synuclein with synaptic PET.

In conclusion, our data show that plasma β-synuclein levels are already higher during the preclinical phase of AD, supporting that synaptic degeneration belongs to the very early events in sporadic AD and preceding tau pathology. The results support plasma β-synuclein as an easily accessible and scalable biomarker that might be used to detect positive effects of novel disease-modifying therapies on synaptic dysfunction in AD and to further study synaptic degeneration in the pathophysiology of AD. Longitudinal studies are needed to confirm the temporal estimations on synaptic degeneration suggested by our study.

AUTHOR CONTRIBUTIONS

Conception and design of the study: Patrick Oeckl, Shorena Janelidze, Markus Otto, and Oskar Hansson. Acquisition and analysis of data: Patrick Oeckl, Shorena Janelidze, Steffen Halbgebauer, Erik Stomrud, Sebastian Palmqvist, Markus Otto, and Oskar Hansson. Drafting a significant portion of the manuscript or figures: Patrick Oeckl, Shorena Janelidze, Markus Otto, and Oskar Hansson.

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CONFLICT OF INTEREST STATEMENT

Patrick Oeckl received research support from the Michael J. Fox Foundation for Parkinson's Research (Grant ID: MJFF-010349) and Alzheimer Forschung Initiative e.V. (20059CB). Markus Otto received research support from German Federal Ministry of Education and Research (FTLDc 01GI1007A), the EU Joint Programme-Neurodegenerative Diseases networks Genfi-Prox (01ED2008A), the EU (MOODMARKER 01EW2008), the German Research Foundation/DFG (SFB1279), the foundation of the state Baden-Württemberg (D.3830), Boehringer Ingelheim Ulm University BioCenter (D.5009), and the Thierry Latran Foundation. Markus Otto, Steffen Halbgebauer, and Patrick Oeckl are co-inventors of a patent application for using β-synuclein measurement in blood. Sebastian Palmqvist has served on scientific advisory boards and/or given lectures in symposia sponsored by Bioartic, Biogen, Cytox, Eli Lilly, Geras Solutions, and Roche. Oskar Hansson has acquired research support (for the institution) from ADx, AVID Radiopharmaceuticals, Biogen, Eli Lilly, Eisai, Fujirebio, GE Healthcare, Pfizer, and Roche. In the past 2 years, he has received consultancy/speaker fees from AC Immune, Amylyx, Alzpath, BioArctic, Biogen, Cerveau, Eisai, Fujirebio, Genentech, Novartis, Novo Nordisk, Roche, and Siemens. Shorena Janelidze and Erik Stomrud report no conflicts of interest. The authors report no disclosures relevant to

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CONSENT STATEMENT

All participants provided written informed consent.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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