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RESEARCH ARTICLE

Associations between misfolded alpha-synuclein aggregates and Alzheimer's disease pathology in vivo

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Abstract

INTRODUCTION: We examined the relations of misfolded alpha synuclein (α-synuclein) with Alzheimer's disease (AD) biomarkers in two large independent cohorts.

METHODS: We included Biomarkers for Identifying Neurodegenerative Disorders Early and Reliably Two (BioFINDER-2) and Alzheimer's Disease Neuroimaging Initiative (ADNI) participants ($n = 2315$, cognitively unimpaired, mild cognitive impairment, AD dementia) who had cross-sectional cerebrospinal fluid (CSF) α-synuclein measurement from seed-amplification assay as well as cross-sectional and longitudinal amyloid beta (Aβ) and tau levels (measured in CSF and/or by positron emission tomography). All analyses were adjusted for age, sex, and cognitive status.

RESULTS: Across cohorts, the main biomarker associated with α-synuclein positivity at baseline was higher levels of Aβ pathology (all p values ≤ 0.02), but not tau. Looking at longitudinal measures of AD biomarkers, α-synuclein –positive participants had a statistically significant faster increase of Aβ load, although of modest magnitude (1.11 Centiloid/year, $p = 0.02$), compared to α-synuclein –negative participants in BioFINDER-2 but not in ADNI.

Alexa Pichet Binette and Angela Mammana contributed equally to this work as first authors.

Oskar Hansson and Piero Parchi contributed equally to this work as senior authors.

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DISCUSSION: We showed associations between concurrent misfolded α-synuclein and Aβ levels, providing in vivo evidence of links between these two molecular disease pathways in humans.

KEYWORDS
amyloid beta, co-pathology, Lewy body, neurodegenerative diseases, seed-amplification assay, tau

Highlights:

- Amyloid beta (Aβ), but not tau, was associated with alpha-synuclein (α-synuclein) positivity.
- Such association was consistent across two cohorts, beyond the effect of age, sex, and cognitive status.
- α-synuclein–positive participants had a small, statistically significant faster increase in Aβ positron emission tomography levels in one of the two cohorts.

1 | INTRODUCTION

Aggregates of misfolded alpha-synuclein (α-synuclein) proteins result in the formation of Lewy bodies and Lewy neurites in the brain.[1] Lewy bodies and neurites are the core pathology of neuronal α-synuclein disease (i.e., Parkinson's disease [PD] and dementia with Lewy bodies [DLB]).[2] They co-occur frequently with Alzheimer's disease (AD) pathology in brain tissue from patients with the clinical diagnosis of either AD dementia[3–5] or neuronal α-synuclein disease.[6,7] Further, many experimental and animal models suggest potential synergistic interaction between α-synuclein and AD pathology, that is, amyloid beta (Aβ) or tau, although such interactions have yet to be investigated in humans in vivo.[8,9] Until recently, biomarkers that could measure α-synuclein pathology in vivo with high accuracy and sensitivity were lacking. However, with recent improvement in seed-amplification assays (SAAs), it is now possible to indirectly detect misfolded α-synuclein aggregates in cerebrospinal fluid (CSF), providing a biomarker specific to Lewy body disease.[10] The availability of such novel assays thus enables us to better understand the clinicopathological relations between α-synuclein and AD pathologies. We have shown previously that cognitively unimpaired participants who had both AD and neuronal α-synuclein pathologies had faster memory and executive function decline compared to those with AD pathology alone or neuronal α-synuclein pathology alone.[11] Here, we aimed to examine the associations between misfolded α-synuclein aggregates measured with SAA and concomitant Aβ and tau levels cross-sectionally, as well as with accumulation of Aβ and tau levels over time in two large cohorts covering the AD spectrum.

2 | MATERIALS AND METHODS

2.1 | Participants—BioFINDER-2 cohort

We included all participants from the ongoing prospective Swedish Biomarkers for Identifying Neurodegenerative Disorders Early and Reliably Two (BioFINDER-2) cohort (NCT03174938) who had CSF α-SAA measurement, as well as measures of Aβ and tau available at baseline; who were > 50 years old; and were cognitively unimpaired (CU), had mild cognitive impairment (MCI), or AD dementia.

Analyses focused on positron emission tomography (PET) were restricted to CU and participants with MCI, given that as per the cohort protocol, patients with dementia undergo tau PET but not Aβ PET, for a total of 1074 individuals. Analyses focused on CSF measures of AD biomarkers included the full spectrum of AD, for a total of 1273 individuals. The main inclusion and exclusion criteria are described in supporting information and in Palmqvist et al.[12] All CU participants had a Mini-Mental State Examination (MMSE) between 27 and 30 or 26 and 30 depending on age. MCI diagnosis was established as performance below 1.5 standard deviation of normative score on at least one cognitive domain from an extensive neuropsychological battery and all had a MMSE between 24 and 30. Diagnosis of AD fulfilled the Diagnostic and Statistical Manual of Mental Disorders Fifth edition criteria for mild or major neurocognitive disorder due to AD, as well as criteria for Aβ positivity, as per the National Institute on Aging–Alzheimer's Association and the International Working Group criteria. Participants with non-AD neurodegenerative diseases, such as Lewy body diseases, were not included in the main analyses, to match sample selection with the Alzheimer's Disease Neuroimaging Initiative (ADNI) study. However, in a complementary analysis to compare associations in the AD continuum to patients with Parkinsonian disorders, we conducted logistic regressions in a subpopulation of BioFINDER-2 with such disorders ($n = 177$; 7 corticobasal syndrome, 48 DLB, 15 multiple system atrophy, 75 PD, and 32 progressive supranuclear palsy). Overall, α-synuclein status, and Aβ and tau biomarkers (either from PET or CSF) were all taken from the baseline visit. Aβ and tau PET were also available longitudinally, all prospective to the baseline visit. The study was approved by the regional ethics committee in Lund, Sweden. All data for the current study were acquired between January 2017 and May 2023.

2.2 | Participants—ADNI cohort

We selected ADNI participants who had a measure of CSF α-synuclein based on SAA, as well as at least one Aβ PET scan available. ADNI is a multi-site study launched in 2003 as a public–private partnership. The primary goal of ADNI has been to test whether serial magnetic resonance imaging (MRI), PET, other biological markers, and clinical and neuropsychological assessment can be combined to measure the progression of MCI and early AD. For up-to-date information, see www.adni-info.org. The last lumbar puncture available was selected for the CSF α-synuclein assay by the ADNI investigators. We then selected the Aβ PET closest to the α-synuclein measure for cross-sectional analysis, allowing a maximum 2-year interval between CSF α-synuclein and PET (average time difference between measures of −0.02 ± 0.29 year) to maximize the sample size (see Figure S1 in supporting information for a flow chart of ADNI participants selection). The overlap of participants with an α-synuclein assay and tau PET done within 2 years was less than one third of the sample, and thus tau PET was not included in the ADNI analyses. All data were collected between December 2008 and December 2022. Overall, we included a sample of 1242 participants, composed of CU individuals, and individuals with MCI and AD dementia. CU participants had a Clinical Dementia Rating score of 0 and participants with MCI and AD were diagnosed according to standard criteria.[13]

RESEARCH IN CONTEXT

1. **Systematic review**: We reviewed the literature on misfolded alpha-synuclein (α-synuclein) aggregates and Alzheimer's disease (AD) pathology in living humans using traditional sources like PubMed. This is a new area given the only recent availability of cerebrospinal fluid (CSF) seed-amplification assays for α-synuclein aggregates. The relevant studies are appropriately cited.
2. **Interpretation**: We found co-occurrence of α-synuclein positivity and elevated amyloid beta (Aβ) levels, but not tau (both measured either in CSF or by positron emission tomography), across two large cohorts spanning the AD spectrum. Our results are overall consistent with previous *post mortem* studies.
3. **Future directions**: We provide novel evidence for in vivo associations between Aβ and α-synuclein in the AD continuum. Future studies should investigate if there are potential additive or synergistic effects of abnormal α-synuclein and Aβ accumulation in the brain and the molecular mechanisms that are at play.

2.3 | α-synuclein

α-synuclein SAAs in CSF, which detect if α-synuclein aggregates are present or not, were used in both cohorts. The output is a binary variable indicating if the CSF sample is positive or negative for aggregates. α-synuclein seeding activity measured by SAAs is expressed in relative fluorescence units (RFUs). The assay's positivity or negativity, indicating the presence or absence of Lewy body pathology, is based on the number of replicates showing fluorescent curves exceeding a given threshold, which is calculated based on the background fluorescent signal recorded in negative controls.

The α-synuclein SAA testing of the BioFINDER-2 cohort was performed by the Neuropathology Laboratory at IRCCS-ISNB (Italy) with

a validated method initially developed by Byron Caughey's lab (United States).[14] For this analysis, each CSF sample was tested in quadruplicate. CSF samples were classified as positive if at least three replicates (maximum fluorescence signal above 30% of the median iMax of the positive controls) and negative if none of the replicates exceeded the threshold. Samples showing one or two positive replicates were repeated three times and classified as positive if at least 4 of the total 12 replicates exceeded the threshold. The protocol is described in more detail in supporting information and in recent publications.[11,15]

The α-syn SAA testing in ADNI was conducted by Amprion Clinical Laboratory (United States; CLIA ID No. 05D2209417; CAP No. 8168002) with a validated method. For the analysis, each CSF sample was tested in triplicate. CSF samples were classified as positive if all three replicates showed an aggregation profile consistent with Type 1 seeds (maximum fluorescence signal, iMAX, > 45,000 RFUs) observed in PD and DLB or "not detected" if no or only one replicate exceeded the threshold.[16] The detailed protocol, provided by Amprion, is available in the ADNI database) (Amprion synuclein seeding assay). In ADNI, 1252 participants had an Aβ PET within 2 years of the α-synuclein assay out of a total of 1641 participants. We further excluded 10 participants who had either a result that was "indeterminate" (i.e., determination could not be made after the sample was tested twice) or detected Type 2 seeds, which are seen in multiple system atrophy, for the final sample of 1242 participants mentioned above. Outcomes were thus consistent in both cohorts, that is, only negative samples and those with Type 1 seeds seen in PD and Lewy body dementia (positive samples) were retained.

In both cohorts, only cross-sectional measurements of α-synuclein were available and assays were performed blinded to diagnosis and demographics. Overall the two methods have shown similar accuracy in neuropathologically verified cohorts[17,18] and to distinguish between early PD patients and controls.[19] An overview of the critical variables in the two SAAs in BioFINDER-2 and ADNI is provided in Table S1 in supporting information.

2.4 | PET imaging and processing

In BioFINDER-2, Aβ and tau PET images were acquired on digital GE Discovery MI scanners. Acquisition for Aβ PET was done 90 to 110 minutes post injection of ≈ 185 MBq [18F]Flutemetamol and 70 to 90 minutes post injection of ≈ 370 MBq [18F]RO948 for tau PET. Images were processed according to our pipeline described previously.[20] Briefly, PET images were attenuation corrected, motion corrected, summed, and registered to the closest T1-weighted MRI image processed through the longitudinal pipeline of FreeSurfer version 6.0. Structural T1-weighted images were acquired from a magnetization-prepared rapid gradient echo (MPRAGE) sequence with 1 mm isotropic voxels on a Siemens 3T MAGNETOM Prisma scanner. Standardized uptake value ratio (SUVR) images were created using the whole cerebellum as reference region for [18F]Flutemetamol[21] and the inferior cerebellar gray matter for [18F]RO948. For Aβ PET, the main region of interest was the average SUVR from a global neocortical region including prefrontal, lateral temporal, parietal, anterior cingulate, and posterior cingulate/precuneus (same regions as in ADNI; scans > 1.03 SUVR were considered Aβ PET positive[21]). Centiloids were also calculated using calibrating images from the Global Alzheimer's Association Interactive Network website. For tau PET, the main region of interest was the average SUVR temporal composite region of the entorhinal cortex, amygdala, parahippocampal gyrus, inferior temporal, and middle temporal gyri.[22] Based on a previously established cutoff of 1.36 SUVR in this temporal meta-region of interest (ROI),[20] 28% of the sample would be tau PET positive. On average, 47% of the participants also had longitudinal PET data available (n = 567 for Aβ and n = 596 for tau), with two to four follow-up scans. The average follow-up time was 2.6 ± 1.0 years. In complementary analyses, we also performed the main analyses in BioFINDER-2 using regional Aβ or tau SUVR from all neocortical regions (as well as the amygdala for tau).

In ADNI, Aβ PET acquisition included two tracers ([18F]florbetapir with acquisition 50 to 70 minutes post injection and [18F]florbetaben with acquisition 90 to 110 minutes post injection). Briefly, frames were realigned, averaged, resliced to a common voxel size, smoothed to 8 mm^3, and registered to the closest T1 MPRAGE image that had been processed through FreeSurfer 7.1. Whole cerebellum was used as the reference region to generate SUVR and the ROI was the global neocortical region as described above. To determine Aβ PET positivity, a cutoff of 1.11 SUVR for florbetapir and 1.08 SUVR for florbetaben were used, defined by the ADNI PET Core. Given that two tracers were used, our main measure of interest was the Centiloid value, taken from the ADNI database and based on the most updated methods (see Amyloid PET Processing Methods version 2; revised June 29, 2023). Thirty-eight percent of the participants (n = 473) also had longitudinal Aβ PET scans prospective to the time point at which the α-synuclein status was determined (average follow-up time of 3.74 ± 2.22 years, 2 to 6 scans). In the main analyses for longitudinal Aβ PET in ADNI, we included these scans prospective to α-synuclein status to be in line with the longitudinal BioFINDER-2 data (in which all longitudinal data is prospective from the baseline visit). In a sensitivity analysis, we extended our selection to PET scans both retrospective or prospective to the α-synuclein measurement, to maximize the length of the Aβ PET trajectories and ensure that results were consistent across different data selection. Cross-sectional analyses were conducted when merging the two cohorts (using Centiloids) and were validated separately in each cohort.

2.5 | CSF biomarkers of Aβ and phosphorylated tau

We also investigated the cross-sectional relations between α-synuclein and AD pathology measured with CSF. In both cohorts, Aβ42 and phosphorylated tau181 (p-tau181) levels were measured using the Elecsys immunoassays in pg/mL.[23] Lower levels of Aβ42 in CSF correspond to higher Aβ pathology and higher levels of p-tau181 in CSF correspond to higher tau pathology. Results related to CSF Aβ were inverted to be easier to interpret and to follow the same direction as p-tau181 and PET results. In BioFINDER-2, CSF biomarkers were

available across the full spectrum of the disease (n = 1273 including CU, MCI, and AD). Additionally, in BioFINDER-2, Aβ40 was also available measured with the same assay, which allowed us to derive the Aβ42/40 ratio, which is preferred over Aβ42 alone. We used this ratio to determine Aβ positivity in BioFINDER-2, in which individuals with a value > 0.08 were considered Aβ positive.[24] In CSF analyses performed in BioFINDER-2 only, we used Aβ42/40 as our measure of interest. In analyses merging both BioFINDER-2 and ADNI, Aβ42 alone was used. In ADNI, concentrations of Aβ42 and p-tau181 were available for 70% of the sample who had Aβ PET (n = 864/1242; all details in the UPENN CSF Biomarkers Roche Elecsys Methods in LONI). For Aβ42, in ADNI, values above the upper level of detection (1700 pg/mL) were imputed at 1700 (n = 150 out 864), while in BioFINDER-2, for samples above the detection limit (n = 436 out of 1273), Aβ42 values were extrapolated from the standard curve. As done with PET, cross-sectional analyses were conducted when merging the two cohorts and were validated separately in each cohort. The analyses in the separate individual cohort used the full dataset available in each cohort. To merge the CSF concentrations across the two cohorts, harmonization of Aβ42 needed to be performed, using an approach previously described to harmonize such values between ADNI and BioFINDER data.[25] Importantly, in the merged dataset, participants with Aβ42 values equal to or higher than the highest detection limit were removed to ensure harmonization across values in a similar range (total n = 1550 in the merged dataset with CSF). We calculated the percent mean difference between the two cohorts and multiplied the value for a given cohort by this coefficient, if the mean difference between cohorts was significant. For Aβ42, the ADNI values were on average 8.51% (95% confidence interval [CI]: 8.07–8.96, p value < 0.001) lower than in BioFINDER-2 (ADNI average: 865.66 [95% CI: 839.05–892.27], BioFINDER-2 average: 1026.62 [95% CI: 1004.28–1048.96]), likely because BioFINDER-2, but not ADNI, uses LoBind tubes for collection and storage of CSF, which results in higher Aβ42 levels.[26,27] As such, Aβ42 concentrations in ADNI were multiplied by 1.085 to reach the harmonized Aβ42 concentrations. There was no significant difference in the mean concentrations of p-tau181, with a percent mean difference of 1.40% (95% CI: 1.40–1.42, p value = 0.34), and thus no harmonization needed to be performed (ADNI average = 27.95 [26.81, 29.10]; BioFINDER-2 average = 27.18 [26.07, 28.28]). Longitudinal, prospective CSF measures after the α-synuclein measurement were not available in either cohort.

2.6 | Statistical analysis

Characteristics between α-synuclein–positive and –negative participants were compared using t tests for continuous variables and chi-square for categorical variables. We used logistic regressions to investigate cross-sectional associations between α-synuclein status as outcome (positive or negative) and measures of AD pathology (using PET or CSF) as predictors. The main analysis investigating the effect of Aβ PET was done by grouping both cohorts using Centiloids. Similarly, the main analysis investigating the effect of CSF Aβ42 and p-tau181 was done by grouping both cohorts. Analyses were also conducted in each individual cohort. In BioFINDER-2 alone, in which both Aβ-PET and tau-PET were analyzed, global Aβ and temporal meta-ROI tau-PET SUVR were added as independent variables in the same model. In ADNI alone, Aβ Centiloids was the main independent variable. All models were adjusted for age, sex, and cognitive status (cognitively unimpaired or impaired) by adding these variables as additional predictors in the logistic regression, to account for their impact on the different proteinopathies. The models combining both BioFINDER-2 and ADNI were also further adjusted for the cohort. In sensitivity analyses, we also further adjusted for apolipoprotein E (*APOE*) ε4 genotype (carriers [having at least one ε4 allele] vs. non-carriers), given its strong link to Aβ. We also repeated the same models with fewer covariates to evaluate if results remained the same: we fitted the same logistic regressions when (1) adjusting only for age and sex or (2) having only AD biomarkers as independent variables and no covariates. Across all models, multicollinearity was low (with variance inflation factors of maximum 1.7) and no outlier influenced the results.

We also wanted to better understand whether the presence of α-synuclein influenced the trajectories of AD biomarkers over time, to complement associations detected with cross-sectional data only. To evaluate the effect of α-synuclein status on longitudinal AD biomarkers, we fitted separate linear mixed effect models with longitudinal PET measures (global Aβ SUVR and temporal meta-ROI tau-PET SUVR in BioFINDER-2 [to use the raw values], Aβ Centiloids in ADNI) as outcome, including random slopes and intercepts with restricted maximum likelihood from lme4 v1.1-35.1. The α-synuclein status x time was the main variable of interest and all models included age, sex, and cognitive status as covariates. Time was operationalized as years from the α-synuclein measurement, where α-synuclein measurement was considered t = 0. All covariates were taken from t = 0, only PET measurements were time varying. p values ≤ 0.05 were considered significant. Analyses were performed in R v4.3.2 and plots were generated with ggplot2 v3.4.4.

3 | RESULTS

3.1 | Demographics and α-synuclein positivity

We included 1074 participants from BioFINDER-2 and 1242 participants from ADNI (Table 1), all of whom had CSF α-synuclein status and Aβ PET available. In BioFINDER-2, 10% of the cohort was α-synuclein positive, compared to 20% in ADNI. Similarly, the proportion of Aβ-positive participants was slightly higher in ADNI: 52% compared to 41% in BioFINDER-2. In this sample with Aβ PET, there were no participants with AD dementia in BioFINDER-2, whereas these participants constituted 21% of the ADNI sample. ADNI participants were, on average, almost 5 years older than BioFINDER-2 participants (74.0 vs. 69.6 years old). The two cohorts were balanced in terms of sex. The breakdown of participants by α-synuclein status in each cohort is also provided in Tables S2-S3 in supporting information. The characteristics of participants in the BioFINDER-2 and ADNI who had CSF measures

TABLE 1 Demographics.

	BioFINDER-2 ($n = 1074$)	ADNI ($n = 1242$)
Age (years)	69.6 ± 9.9	74.0 ± 7.6
Sex F *n* (%F)	563 (52%)	607 (49%)
Education (years)[1]	12.8 ± 3.8	16.4 ± 2.5
MMSE	28.3 ± 1.7	27.2 ± 3.4
α-synuclein status *n* positive (% positive)	112 (10%)	252 (20%)
***APOE* ε4 carriers *n* (%)**[2]	466 (50%)	525 (44%)
Cognitive status Unimpaired: MCI: AD dementia (%)	732: 342: 0 (68: 32: 0%)	476: 508: 257 (38: 41: 21%)
Aβ status *n* positive (% positive)	439 (41%)	640 (52%)

Note: Data are presented as mean ± standard deviation unless specified otherwise. α-synuclein status was determined using seed assay amplification in CSF. Aβ status was determined based on CSF Aβ42/40 ratio in BioFINDER-2 and on Aβ PET in ADNI.

Abbreviations: Aβ, amyloid beta; AD, Alzheimer's disease; ADNI, Alzheimer's Disease Neuroimaging Initiative; *APOE*, apolipoprotein E; BioFINDER-2, Biomarkers for Identifying Neurodegenerative Disorders Early and Reliably Two; F, female; MCI, mild cognitive impairment; MMSE, Mini-Mental State Examination; PET, positron emission tomography; ROI, region of interest; SUVR, standardized uptake value ratio.

[1]Missing for 17 participants in BioFINDER-2 and 49 in ADNI.

[2]Missing for 143 participants in BioFINDER-2 and 49 in ADNI.

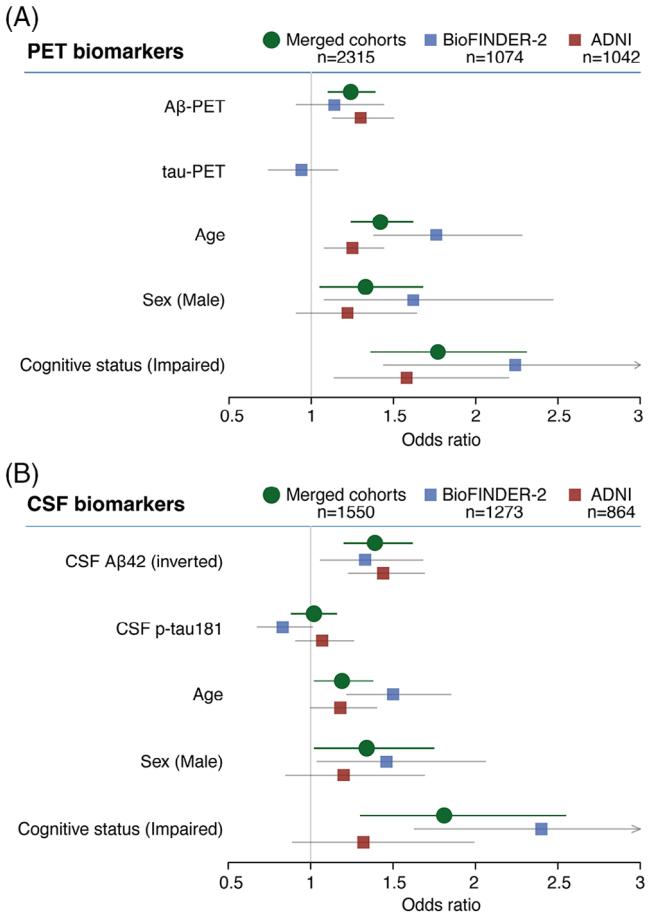

FIGURE 1 Odds ratio from logistic regressions assessing α-synuclein positivity. Odds ratio (95% CI) from logistic regression assessing α-synuclein status as outcome are reported. All listed variables were included as independent variables simultaneously in the same model. Main predictors were based on PET measures of AD pathology (A) or CSF measures (B). Logistic regressions were conducted in the merged cohorts, in BioFINDER-2 alone or ADNI alone. α-synuclein, alpha synuclein; Aβ, amyloid beta; AD, Alzheimer's disease; ADNI, Alzheimer's Disease Neuroimaging Initiative; BioFINDER-2, Biomarkers for Identifying Neurodegenerative Disorders Early and Reliably Two; CI, confidence interval; CSF, cerebrospinal fluid; PET, positron emission tomography.

of AD pathology was highly similar to those who underwent PET, with the exception that it included patients with AD dementia in BioFINDER (Table S4 in supporting information).

3.2 | Cross-sectional associations between α-synuclein and AD biomarkers

In the whole sample combining BioFINDER-2 and ADNI, the variables associated with α-synuclein positivity were Aβ, age, male sex, and cognitive impairment. The odds ratio ranged from 1.24 (95% CI: 1.10–1.39) for Aβ PET (Figure 1A) to 1.39 (95% CI: 1.20–1.62) for CSF Aβ42 (Figure 1B). Covariates included in the same model (age, sex, cognitive impairment) were all significant, as shown in Figure 1. The area under the curve (AUC) was 0.69 for the model with PET and 0.66 for the model with CSF biomarkers. All statistical details are reported in Table S5 in supporting information. Further including *APOE* ε4 genotype as a covariate did not change the results in the models based on PET or CSF AD biomarkers (Table S5).

Centiloids Aβ PET and CSF Aβ42 levels by α-synuclein status in each cohort are also displayed in Figure 2 for visualization purposes. Across cohorts and Aβ measures, α-synuclein–positive participants consistently had higher Aβ burden, with effect sizes ranging between 0.37 and 40, suggesting a small effect.

Logistic regressions were also tested in each cohort separately, using measures of AD pathology from PET and CSF, which all yielded consistent results from models based on the merged cohorts. Results are displayed in Figure 1 and the statistical details of all models are reported in Table S6 in supporting information. In all models based on CSF measures of AD, Aβ42 (odds ratio of 1.28 [95% CI: 1.05–1.60] in BioFINDER-2 and 1.44 [95% CI: 1.23–1.69] in ADNI) but not p-tau181 was significantly associated with α-synuclein positivity, beyond the effect of age, sex, and cognitive status. The AUC was 0.72 in BioFINDER-2 and 0.64 in ADNI. Results were similar in models based on PET measures of AD. In ADNI, greater Centiloids Aβ was associated with being α-synuclein positive (odds ratio: 1.30 [95% CI: 1.13–1.50]). In BioFINDER-2, logistic regression models included both Aβ- and tau PET as independent variables in the same model, along with age, sex, and cognitive status. Tau PET levels were was not significantly associated with α-synuclein status. The effect of Aβ PET was not significant

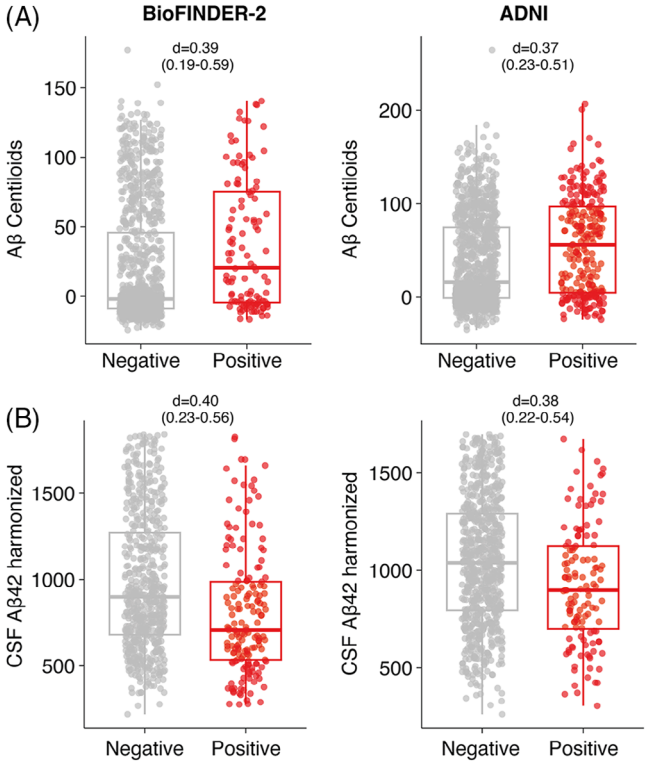

FIGURE 2 Levels of Aβ PET and CSF Aβ42 by α-synuclein status. Levels of Aβ Centiloids (A) in BioFINDER-2 and in ADNI as well as CSF Aβ42 after harmonization (B) in α-synuclein–negative and –positive participants. Effect size (95% confidence interval) between the groups is reported on top of each graph. α-synuclein, alpha synuclein; Aβ, amyloid beta; AD, Alzheimer's disease; ADNI, Alzheimer's Disease Neuroimaging Initiative; BioFINDER-2, Biomarkers for Identifying Neurodegenerative Disorders Early and Reliably Two; CSF, cerebrospinal fluid; PET, positron emission tomography.

beyond cognitive impairment in this sample (odds ratio 1.14, 95% CI: 0.91–1.44, $p = 0.25$ [Figure 1A, Table S6] vs. 1.27, 95% CI: 1.01–1.58, $p = 0.04$ when cognitive status is not included as covariate). The AUC was 0.70 in BioFINDER-2 and 0.62 in ADNI. Repeating the same analyses using regional SUVR, there were no individual regions where PET uptake was associated with α-synuclein positivity (all $p_{FDR} > 0.05$). In BioFINDER-2 the discrepancy in the results between the PET and CSF samples is likely explained by the difference in sample size and participants, given that patients with AD dementia do not undergo Aβ PET. Results across each cohort and the merged cohorts were also highly consistent when repeating logistic regression without any covariates or when only including age and sex (Table S7 in supporting information). Across all analyses, a higher Aβ load was consistently associated with α-synuclein positivity, whereas tau measures were not significant. The odds ratio of Aβ was also slightly higher in the models without or with fewer covariates compared to the main models. Results were different in the BioFINDER-2 subpopulation with Parkinsonian disorders, in which two thirds were α-synuclein positive ($n = 118/177$). In this group Aβ or tau proteinopathies were not associated with α-synuclein status (Table S8 in supporting information).

3.3 | Associations between α-synuclein and longitudinal AD biomarkers

Next, we investigated whether the α-synuclein groups differed in terms of accumulation of AD pathology over time. In BioFINDER-2, the α-synuclein–positive participants had a statistically significant, albeit small, faster increase in Aβ PET levels compared to the α-synuclein–negative group (α-synuclein status x time standardized $\beta = 0.037$, standard error [SE] = 0.014, $p = 0.01$; Figure 3A, statistical details in Table S9 in supporting information). Given that we found that α-synuclein–positive participants had a higher prevalence of Aβ positivity, we further adjusted the linear mixed effect model for the Aβ status at baseline (based on CSF Aβ42/40), to ensure that the significant accumulation Aβ pathology was not mainly driven by a high proportion of Aβ positivity in the α-synuclein–positive group. The interaction between α-synuclein status and time remained similar (standardized $\beta = 0.034$, SE = 0.014, $p = 0.02$), suggesting an effect of α-synuclein on change in Aβ PET SUVR beyond the fact that α-synuclein–positive participants have higher Aβ load. However, when investigating such association only in Aβ-negative participants or only in Aβ-positive participants, there was no difference in increase of Aβ PET levels between the two α-synuclein groups (all statistical results in Table S10 in supporting information). To better contextualize the results, we calculated the effects using Aβ Centiloids: the α-synuclein–positive participants accumulated 1.11 (SE = 0.44) Centiloid more per year than the α-synuclein–negative group. In the same group of participants, there was no apparent difference in longitudinal tau-PET trajectories between the α-synuclein groups (α-synuclein status x time standardized $\beta = 0.020$, SE = 0.031, $p = 0.51$; Figure 3B, all statistical details in Table S9). Repeating the same analyses using regional SUVR, in line with the widespread deposition of Aβ, there were 15 individual regions where Aβ SUVR accumulation was faster in the α-synuclein positive group compared to the negative group (Figure S2 in supporting information, all $p_{FDR} \leq 0.05$). No individual region was significant with longitudinal tau PET. In ADNI, there was no difference between the two α-synuclein groups on their longitudinal accumulation of Aβ levels prospectively to the α-synuclein measurement (α-synuclein status x time standardized $\beta = -0.030$, SE = 0.031, $p = 0.33$, Figure 3C, Table S9). Results were also unchanged if we included both retrospective and prospective longitudinal Aβ PET with respect to the α-synuclein measurement in ADNI (Figure S3 in supporting information).

4 | DISCUSSION

In two independent and large cohorts covering the AD continuum, we found that α-synuclein positivity was associated with elevated Aβ pathology, but not tau in cross-sectional analyses. Using longitudinal measures of AD biomarkers, we found a small statistically significant faster increase of Aβ pathology in α-synuclein–positive participants in one cohort only. The cross-sectional results support the abundant literature suggesting frequent co-occurrence of AD pathology and neuronal α-synuclein pathology based on *post mortem* brain tissue,

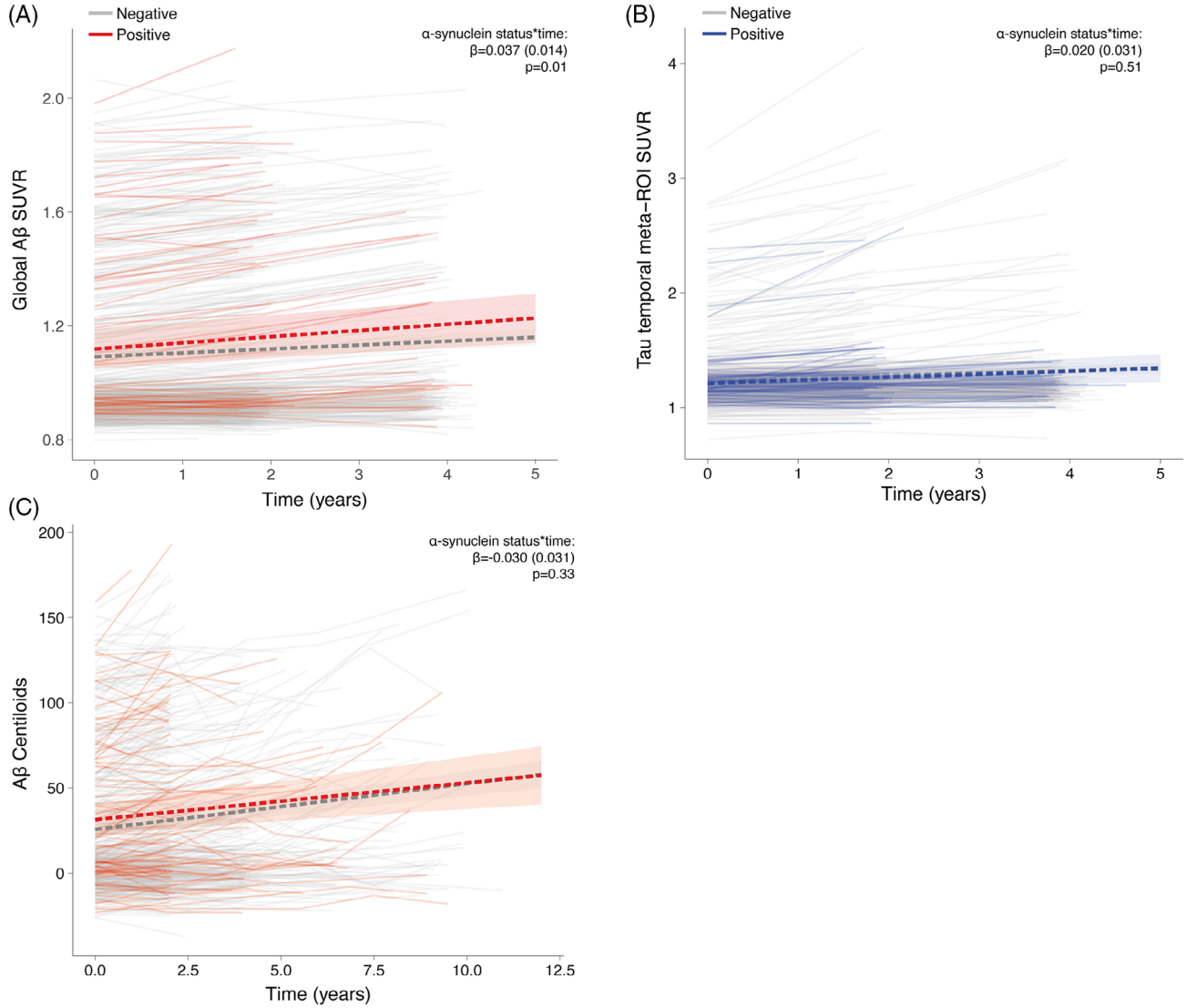

FIGURE 3 Effect of α-synuclein status on longitudinal Aβ- and tau PET. Linear mixed effect models investigating the effect of α-synuclein status on longitudinal global neocortical Aβ SUVR ($n = 567$) (A) and temporal tau PET SUVR ($n = 596$) (B) in BioFINDER-2, and Aβ Centiloids in ADNI ($n = 473$) (C). Linear mixed effect models with random slope and intercept were fitted, including age and sex as covariates. Standardized beta coefficient (standard error) and *p* value of α-synuclein status x time is reported on each graph. Negative/positive indicates CSF α-synuclein status. α-synuclein, alpha synuclein; Aβ, amyloid beta; AD, Alzheimer's disease; ADNI, Alzheimer's Disease Neuroimaging Initiative; BioFINDER-2, Biomarkers for Identifying Neurodegenerative Disorders Early and Reliably Two; CSF, cerebrospinal fluid; PET, positron emission tomography; SUVR, standardized uptake value ratio.

reported in patients with AD dementia as well as in patients with neuronal α-synuclein disease (aka Lewy body disease).[28,29]

Associations between neuronal α-synuclein pathology and tau pathology are also commonly reported in *post mortem* tissue and are not unique to Aβ pathology. Still, we should note that in some cohorts, correlations were seen only between α-synuclein and Aβ and not with tau.[4] Given that in vivo Aβ often starts to deposit years before neocortical tau pathology in the AD pathophysiological cascade, it is perhaps not surprising that we found greater co-occurrence of α-synuclein and Aβ compared to tau along the AD spectrum, in participants ranging from CU older adults to patients with AD dementia. Such results are also in line with a recent study in ADNI only where no relations were found between p-tau and α-synuclein positivity.[16] Earlier reports observed that neuronal α-synuclein pathology was also frequent in brains of patients with familial AD, all of whom had mutations causing overproduction of Aβ,[30,31] potentially further linking Aβ proteinopathy to the development of concomitant α-synuclein aggregates.

It has been proposed that the presence of widespread, pathological accumulation of Aβ might act as a facilitator or catalyzer for the subsequent aggregation of other proteinopathies and thus induce accumulation of misfolded α-synuclein, as well as tau neurofibrillary tangles (see Spires-Jones et al. and Kotzbauer et al. for reviews).[32,33] In AD, the amygdala and entorhinal cortex are the most common

regions where neuronal α-synuclein pathology is found, which are the same regions prone to early tau aggregates. This pattern contrasts with α-synuclein pathology in classical neuronal α-synuclein disease (PD and DLB), where a more widespread deposition is found across subcortical areas such as the brainstem, hypothalamus, and basal forebrain.[7,33] In the context of AD, we thus speculate that potentially similar mechanisms might explain aggregation of tau and α-synuclein in the presence of widespread Aβ. We do not exclude additive or perhaps synergistic effects that might also occur between α-synuclein and tau in vivo in humans, which have been reported in several experimental animal models,[8,9] but we could not detect any significant associations between these two pathologies in the current study. One explanation for the lack of association with tau pathology might be the generally low tau burden in patients with AD and Lewy body pathologies. Evidence indicates that the presence of co-pathologies lowers the threshold for meeting clinical criteria for a diagnosis of dementia. Therefore, the association between α-synuclein and Aβ pathology leads to an earlier presentation of symptoms than in patients with pure AD and can explain the lower tau burden. In other words, the possible interaction between α-synuclein and tau pathology, which has been suggested in experimental studies, might be more difficult to document in vivo because of its later occurrence in the disease course compared to Aβ and α-synuclein interaction. Age and cognitive status are also other important factors in the presence of concomitant pathologies. We also found that older age and having cognitive impairment were both associated with greater odds of being α-synuclein positive across cohorts, to a similar or greater magnitude than Aβ. It is likely that both AD-dependent and independent mechanisms exert their effects on α-synuclein pathology in aging and neurodegenerative diseases.

To better elucidate the relations among all pathologies, longitudinal α-synuclein measurements will be crucial and are not yet available. Longitudinal measurements of α-synuclein in the future will allow us to further clarify the timing between the different pathological changes and how they relate to one another. In one cohort we found a small faster increase of Aβ over time in the α-synuclein–positive group, but to start to understand if one pathology might "drive" or "trigger" the other, the reverse association will need to be tested, that is, assessing α-synuclein over time in Aβ-positive and -negative participants. As such, we do not imply directionality or causality from the present results, and potential bidirectional effects are likely to be at play.[9,34] The longitudinal effect reported here was found in BioFINDER-2 and not in ADNI and will also need to be investigated further in other cohorts. Still, it is also important to note some differences between the two cohorts, which might explain in part the discrepant result. We used stringent criteria to define α-synuclein positivity in BioFINDER-2, which might result in more participants with focal pathology in ADNI compared to BioFINDER-2. Such participants would be less likely to show an interaction with Aβ because the Lewy body pathology has not reached the cerebral cortex. Overall, there were up to 10% more α-synuclein–positive participants in ADNI compared to BioFINDER-2, including a greater proportion of participants with cognitive impairment and an older population. Patients with advanced MCI or dementia are also more likely to start showing slower accumulation of Aβ pathology, which might also explain part of the different longitudinal trajectories. Further, in BioFINDER-2, patients with dementia do not undergo Aβ PET. Another limitation is the binary measure of α-synuclein but α-synuclein SAAs development has not yet reached the stage of providing accurate continuous α-synuclein seed levels. More quantitative measures will be important to evaluate the links between continuous levels of the different pathologies in the future. For instance, the time to reach the positivity threshold in the SAA (kinetic parameter lag phase) is a promising marker currently being investigated.[35,36]

The current results also have potential immediate and future clinical implications. First, our results suggest that patients showing cognitive decline and Aβ positivity with limited associated tau pathology are at significant risk of having AD and Lewy body copathology. The results are also relevant in light of the recently approved anti-amyloid therapies: we can speculate that, given the association between Aβ and α-synuclein, removing Aβ could have a downstream effect of lowering α-synuclein pathology. Of course the opposite could also be true, that is, facilitating removal of α-synuclein would also lower Aβ levels, and it would be an important pathway to test when such therapies emerge.

Overall, along the AD spectrum, we found evidence for concomitant α-synuclein and Aβ and interaction between the two pathologies in two large cohorts. Future studies should investigate if there are potential additive or synergistic effects of abnormal α-synuclein and Aβ accumulation in vivo and the molecular mechanisms that are at play.

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CONFLICT OF INTEREST STATEMENT

OH has acquired research support (for the institution) from AVID Radiopharmaceuticals, Biogen, C2N Diagnostics, Eli Lilly, Eisai, Fujirebio, GE Healthcare, and Roche. In the past 2 years, he has received consultancy/speaker fees from AC Immune, Alzpath, BioArctic, Biogen, Bristol Meyer Squibb, Cerveau, Eisai, Eli Lilly, Fujirebio, Merck, Novartis, Novo Nordisk, Roche, Sanofi, and Siemens. RS has received a speaker fee from Roche. SP has acquired research support (for the institution) from ki elements / ADDF. In the past 2 years, he has received consultancy/speaker fees from Bioartic, Biogen, Lilly, and Roche. The remaining authors declare no competing interests. Author disclosures are available in the supporting information.

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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