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RESEARCH ARTICLE

Association of CSF α-synuclein seed amplification assay positivity with disease progression and cognitive decline: A longitudinal Alzheimer's Disease Neuroimaging Initiative study

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Data used in preparation of this article were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (adni.loni.usc.edu). As such, the investigators within the ADNI contributed to the design and implementation of ADNI and/or provided data but did not participate in the analysis or writing of this report.

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Abstract

INTRODUCTION: Cerebrospinal fluid (CSF) α-synuclein (α-syn) seed amplification assay (SAA) is a sensitive and specific tool for detecting Lewy body co-pathology in Alzheimer's disease.

METHODS: A total of 1637 cross-sectional and 407 longitudinal CSF samples from the Alzheimer's Disease Neuroimaging Initiative (ADNI) were tested with SAA. We examined longitudinal dynamics of amyloid beta (Aβ), α-syn seeds, and phosphorylated tau181 (p-tau181), along with global and domain-specific cognition in stable SAA+, stable SAA−, and those who converted to SAA+ from SAA−.

RESULTS: SAA+ individuals had faster cognitive decline than SAA−, notably in mild cognitive impairment, and presented with earlier symptom onset. SAA+ conversion was associated with CSF Aβ42 positivity but did not impact the progression of either CSF Aβ42 or CSF p-tau181 status. CSF Aβ42, p-tau181, and α-syn SAA were all strong predictors of clinical progression, particularly CSF Aβ42. In vitro, CSF α-syn SAA kinetic parameters were associated with participant demographics, clinical profiles, and cognitive decline.

DISCUSSION: These results highlight the interplay between amyloid and α-syn and their association with disease progression.

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KEYWORDS
Alzheimer's disease, cognitive decline, co-pathology, Lewy body, seed amplification assay

Highlights

- Seed amplification assay (SAA) positivity was associated with greater cognitive decline and earlier symptom onset.
- Thirty-four Alzheimer's Disease Neuroimaging Initiative (ADNI) individuals progressed from SAA− to SAA+, that is, ≈ 5% conversion.
- SAA conversion was associated with amyloid beta (Aβ) pathology and greater cognitive decline.
- SAA status did not impact the progression of either CSF Aβ42 or phosphorylated tau181 biomarkers.
- Change in clinical diagnosis was associated with both Alzheimer's disease biomarkers and SAA.
- SAA kinetic parameters were associated with clinical features and progression.

1 | INTRODUCTION

Alzheimer's disease (AD) and Lewy body disease (LBD), characterized by the pathological deposition of amyloid beta (Aβ) and alpha synuclein (α-syn), respectively, are commonly identified at autopsy. Up to half (25%–50%) of autopsy cases exhibit Lewy body (LB) co-pathology in sporadic early- and late-onset AD,[1–4] familial/inherited AD,[5] and Down syndrome AD cases.[6] Pathological coexistence implies a potential interplay between amyloid and α-syn in the human brain. Despite the unique stereotypical progression of each pathology,[7–9] evidence suggests that these pathways potentially may overlap at later disease stages,[1–4] implicating a synergistic process known as "crosstalk."

Crosstalk has been observed in neurodegenerative diseases and can occur by impaired cellular clearance processes; impaired protein homeostasis; synergy of disease-related pathways; or when amyloidogenic proteins such as amyloid, tau, and α-syn interact and cause aggregation.[10–13] Each pathologic deposition occurs in distinct, observable locations in the brain: amyloid plaques are extracellular, tau neurofibrillary tangles (NFTs) are intracellular, and LB aggregates are in vesicles and exosomes.[14,15] Despite this, the interaction of these proteins may intersect at later disease stages, potentially exacerbating disease progression. Our understanding of crosstalk in living organisms, particularly in humans, remains limited. As such, the mechanism, the dynamics of interaction between amyloid and α-syn, and the subsequent impact on disease progression are areas of active research.

With the recently developed cerebrospinal fluid (CSF) α-syn seed amplification assay (SAA) technology, there is now in vivo evidence that individuals with both LB (α-syn) and AD (amyloid and tau) pathology exhibit faster cognitive decline than those with only LB or AD pathology.[16–19] Evidence from autopsy studies showed that the amyloid and tau pathologies only account for a portion of the observed variance in cognitive decline[20] while co-pathologies lower the threshold for clinical symptoms of AD.[21] Accordingly, while current treatments with anti-amyloid antibodies have been shown to slow cognitive decline, their impact is relatively modest, reducing the rate of decline by ≈ 25% to 40%.[22–24] This underscores the possibility that additional pathologies may play a critical role. Given that α-syn is the most commonly observed co-pathology in AD,[25] the presence of α-syn pathology could help explain the variability in cognitive decline that is not accounted for by amyloid and tau alone.

Recently, analysis of cross-sectional CSF samples from the Alzheimer's Disease Neuroimaging Initiative (ADNI) study using SAA demonstrated an association between the presence of misfolded α-syn and various factors such as age, disease stage, burden of AD pathology, and rates of longitudinal cognitive decline.[19] We recently expanded the ADNI CSF α-syn SAA analysis by incorporating longitudinal time points, aiming for a better understanding of longitudinal downstream effects resulting from amyloid and α-syn pathologies in ADNI participants. To our knowledge, this is the first study to incorporate longitudinal AD biomarker data with longitudinal SAA data in an AD cohort in which extensive longitudinal follow-up allowed us to identify individuals who progressed from SAA negative (SAA−) to SAA positive (SAA+).

Here, in the context of AD co-pathologies, we hypothesize that SAA positivity would correspond to greater rates of cognitive decline and earlier onset of cognitive impairment. Further, we postulate that the emergence of α-syn pathology is dependent on pre-existing AD amyloid pathology, and the apolipoprotein E (*APOE*) ε4 allele exerts a significant influence over this interplay as ε4 has been increasingly recognized as a common genetic risk factor for both AD and LBD.[26–29] *APOE* contributes to progression and cognitive decline in Parkinson's disease (PD),[30] and ε4 has been shown to worsen α-syn pathology in AD+LB brains,[31] suggesting a role in modulating crosstalk. Last, the dichotomous outcome derived from the qualitative

SAA approach poses a limitation. To address this, we investigate the utility of SAA kinetic parameters as quantitative indicators for the burden of α-syn seeds in CSF or the propagation of LB pathology. Thus, we assess the association of the quantitative SAA kinetic parameters with clinical characteristics, biomarker data, and cognitive outcome measures.

2 | METHODS

2.1 | Study design and participants

Data was obtained from the ADNI database (https://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 AD. For up-to-date information, see www.adni-info.org. This study used a longitudinal examination of biomarker, demographic, clinical, and cognitive data. The sample included all ADNI 1–3 cohort participants who had available CSF samples (N = 1637). The participant pool consisted of cognitively unimpaired (CU) individuals, individuals with MCI, and individuals clinically diagnosed with dementia due to AD.

In summary, at the time of enrollment, participants in the ADNI study were aged between 55 and 90 years, had a study partner to provide an independent evaluation of functioning, and were proficient in English or Spanish. For CU individuals, enrollment criteria included a Mini-Mental State Examination (MMSE) score between 24 and 30; a Clinical Dementia Rating (CDR) of 0; and absence of depression, MCI, and dementia.

MCI participants were required to have MMSE scores between 24 and 30, a subjective memory complaint, objective memory loss (adjusted for education) as measured by the Wechsler Memory Scale Logical Memory II, a CDR of 0.5, no significant impairment in other cognitive domains, essentially preserved daily living activities, and no dementia.

Participants diagnosed with dementia due to AD met the criteria with MMSE scores between 20 and 26, a CDR of 0.5 or 1.0, and fulfilled the National Institute of Neurological and Communicative Disorders and Stroke/Alzheimer's Disease and Related Disorders Association criteria for probable AD.

Exclusion criteria at the time of ADNI study enrollment included significant neurological disease apart from AD (including PD and dementia with Lewy bodies [DLB]), contraindications to neuroimaging or other ADNI protocols; neuroimaging evidence of infection, infarction, lacunes, or other focal lesions; psychiatric disorders, including psychotic features; alcohol abuse; significant systemic illness or unstable medical condition; laboratory abnormalities that could interfere with the study; use of certain psychoactive medications; and participation in other clinical trials.

RESEARCH IN CONTEXT

1. **Systematic review**: We reviewed the literature using traditional sources (e.g., PubMed, Google Scholar). While cerebrospinal fluid α-synuclein seed amplification assay (SAA) has demonstrated high sensitivity and specificity in detecting Lewy bodies concurrent with Alzheimer's disease (AD), the interactions between amyloid beta (Aβ) and α-synuclein, and their combined effect on the progression of the disease, remain an active field of study.
2. **Interpretation**: SAA+ was independently associated with greater cognitive decline. Conversion to SAA+ was associated with the presence of amyloid pathology, accelerated cognitive decline, and earlier symptom onset. SAA status did not impact progression of either amyloid or tau biomarkers, offering new insights into how Lewy body pathology interacts with AD pathology and AD clinical progression.
3. **Future directions**: The study emphasizes the potential interaction between amyloid and α-synuclein and their association with the disease course. Future work should aim to determine how the emergence of these pathologies influences the progression of clinical symptoms over time.

2.2 | CSF α-syn SAA processing

CSF samples were initially gathered into collection tubes provided to every participating ADNI site. These were then transferred to polypropylene tubes and frozen on dry ice within an hour of collection. The samples were subsequently shipped overnight, still on dry ice, to the ADNI Biomarker Core laboratory at the University of Pennsylvania Medical Center. Upon their arrival at the ADNI Biomarker Core laboratory, the CSF samples were thawed and aliquoted into 0.5 mL cryotubes for long-term storage at −80°C.

The CSF α-syn SAA testing was carried out by the Amprion Clinical Laboratory (CLIA ID No. 05D2209417; CAP No. 8168002) using a method clinically validated and compliant with Clinical Laboratory Improvement Amendment (CLIA) standards.

For the analysis, each pristine aliquot of CSF was tested in three technical replicates within a 96-well plate. The 100 μL reaction mixture was composed of 100 mM PIPES pH 6.5, 0.5 M NaCl, 0.1% sarkosyl, 10 μM ThT, 0.3 mg/mL recombinant α-syn, 40 μL CSF, and two silicon nitride beads.[18] Positive and negative quality control samples were included in each plate to ensure assay accuracy.

The plates were sealed with an optical adhesive film and placed into a BMG LABTECH FLUOstar Ω Microplate Reader. They were incubated at 42°C, with cycles of 1 minute of shaking followed by 14 minutes of rest. Fluorescence was recorded after each shake, using an excitation wavelength of 440 nm and an emission wavelength of

490 nm. After a total incubation period of 20 hours, the maximum fluorescence (Fmax) intensity for each well was recorded. An algorithm was then applied to the triplicate reading to categorize the result.

CSF samples were classified as follows: "PD/DLB-like Detected" if α-syn aggregates were identified with an aggregation profile consistent with Type 1 seeds observed in PD and DLB, "MSA-like Detected" if α-syn aggregates matched Type 2 seeds typically seen in multiple system atrophy (MSA), or "Not Detected" if no α-syn aggregates were detected. Samples that did not yield a definitive result after two tests were classified as "Indeterminate."

The processing of ADNI CSF α-syn SAA was done in two phases. Phase 1 data were processed in Q4 2023 and included 1637 CSF samples from the latest CSF sample collection time point for each participant. Phase 2 data were processed in Q1 2024 and incorporated CSF samples from earlier collection time points specifically focusing on participants showing detectable seeding activity from Phase 1. These earlier time points for the Phase 2 CSF α-syn SAA processing were from 222 participants. This sample was selected based on the availability of longitudinal CSF samples. Specifically, we included participants whose most recent CSF samples, processed during our Phase 1 cross-sectional CSF α-syn SAA study, were positive for α-syn seeds, that is, classified as either "PD/DLB-like Detected" or "MSA-like Detected." Additionally, we included samples from participants classified as "Indeterminate" and those with *post mortem* neuropathological confirmation as special cases. For participants whose most recent CSF samples were classified as "Not Detected," we did not process earlier CSF samples unless these participants were part of the autopsy cohort. This decision was supported by data from prior studies indicating that earlier CSF samples from these participants would also likely be classified as "Not Detected." This approach led to a total of 407 CSF samples from 222 unique participants, ensuring the inclusivity of all qualifying samples based on the specified criteria.

For the in vitro assay of SAA, the following five kinetic parameters (illustrated in Figure S1 in supporting information) were estimated for each SAA+ replicate: (1) time to threshold (TTT, [hours])—time in hours when the fluorescence signal reaches the lower patient classification threshold (1000 RFU [relative fluorescence units]); (2) Fmax (RFU)—a maximum of the reaction signal in RFU; (3) AUC-Fluoro (RFU, [seconds])—area under the signal versus time reaction curve in RFU; (4) Maximum Slope (Smax, [RFU, seconds])—maximum of the derivative of the signal/time reaction curve in RFU/seconds; and (5) Time to Smax (TSmax, [hours])—the time in hours when the Smax occurs.

All CSF α-syn SAA analyses were performed with the analysts blinded to the participants' demographic details, clinical profiles, and AD biomarker data. The integrity of the blinding was maintained by using unique specimen identifiers for randomly assigned sample shipments.

2.3 | Assessments of CSF AD biomarkers

Pristine aliquots of CSF were examined using the Elecsys CSF Aβ42, CSF phosphorylated tau181 (p-tau181), and CSF total-tau electrochemiluminescence immunoassays (ECLIA) on a fully automated Elecsys cobas e 601 instrument, using a single lot of reagents for each biomarker. The Roche Elecsys CSF immunoassays were used in accordance with a Roche Study Protocol at the ADNI Biomarker Laboratory, following the kit manufacturer's instructions.

The analyses were carried out in a series of runs, with each sample run once (in singlicate) for each biomarker test, from November 17, 2016 to June 22, 2022. This process followed a standard new lot rollover protocol from the manufacturer, which involved repeated analyses of quality control samples.

CSF biomarkers were restricted to Aβ42 and p-tau181 due to the limited availability of other AD-related pathophysiological biomarkers (e.g., neurofilament light chain [NfL], glial fibrillary acidic protein [GFAP], Aβ42/40 ratio) in the ADNI samples.

The analyte measuring ranges from the lower technical limit to the upper technical limit for each biomarker were: 200 to 1700 pg/mL for the Elecsys CSF Aβ42 immunoassay, and 8 to 120 pg/mL for the Elecsys CSF p-tau181 immunoassay.

The AD CSF biomarker positivity was defined as "Aβ42+" if CSF Aβ42 was < 980 pg/mL, and "p-tau181+" if CSF p-tau181 was > 24 pg/mL.

Participant ages at the time of phenoconversion to CSF p-tau181/Aβ42 positivity (i.e., estimated amyloid onset age [EAOA]) were estimated using the sampled iterative local approximation (SILA) method on all ADNI participants with available CSF Aβ42 and CSF p-tau181 data, as described elsewhere.[32] Records falling outside the technical limits of the Elecsys CSF Aβ42 assay (lower limit of 200 pg/mL and upper limit of 1700 pg/mL) and the Elecsys CSF p-tau181 assay (lower limit of 8 pg/mL and upper limit of 120 pg/mL) were excluded from the EAOA modeling. The performance of the EAOA model was evaluated using a subset of ADNI participants ($N = 63$) who phenoconverted from CSF p-tau181/Aβ42− to CSF p-tau181/Aβ42+ (defined as a p-tau181 to Aβ42 ratio > 0.025). Specifically, their actual ages at phenoconversion were calculated as the weighted average of their ages at the last CSF p-tau181/Aβ42− and first CSF p-tau181/Aβ42+ samples, with weights given by the CSF p-tau181/Aβ42 levels at these time points. Pearson correlation and linear regression were used to assess the fit between the EAOA and the actual age at phenoconversion.

Amyloid time of each biomarker and clinical measure assessed in this study is defined as the difference between the chronological age at the time of that measure collection and EAOA.

2.4 | Cognitive assessments

The global cognitive assessments included the CDR Sum of Boxes (CDR-SB), the Alzheimer's Disease Assessment Scale Cognitive subscale 11-item (ADAS-Cog11), MMSE based on a 30-point questionnaire, and the Preclinical Alzheimer's Cognitive Composite (PACC) score. The domain-specific cognitive assessments included the composite measures of memory, executive function, and language.[33] Observations in domain-specific measures were excluded if the standard error of measurement for a given observation exceeded 0.6.

2.5 | Statistical analysis

All statistical analyses and data preparation were conducted in R (version 4.4.0). The Holm–Bonferroni method was used to correct for multiple comparisons, when applicable.

Participants with any MSA-like samples were excluded from all analyses because of the small sample size in this group, as detailed in Section 3. Indeterminate CSF α-syn SAA samples were discarded for all primary analyses. Samples were designated as SAA– ("Not Detected") if no α-syn aggregates were detected, and SAA+ ("PD/DLB-like Detected") if α-syn aggregates conformed to Type 1 seeds, typically observed in PD and DLB. Participants who were SAA– after baseline were inferred to have been SAA– at all prior observations, as detailed in Section 3.

Participants were classified as "phenoconverters" in each measure if they were negative in a measure and later were positive in that measure and remained positive at all subsequent observations. The phenoconversion date was estimated as the midpoint between the last biomarker-negative date and the first biomarker-positive date. Participants who were positive in a measure and were then negative at any subsequent observations were classified as reverse phenoconverters and were excluded from analyses.

Participants were classified as "Stable SAA–" if they had multiple CSF samples and were SAA– at their last observation, "Stable SAA+" if they were SAA+ at two or more observations and were not SAA– at any observation. Participants who only had one CSF observation did not have enough information to sort into these groups and were excluded from analyses that involved these groupings.

Demographic, CSF biomarker, and cognitive measures were compared cross-sectionally for participants across the Stable SAA–, SAA Converter, and Stable SAA+ groups listed, with observations before and after phenoconversion included for SAA Converters. The most recent observation with SAA data available was used for both the Stable SAA– and Stable SAA+ groups.

Pairwise group differences were assessed. Binary variables for SAA Converters were compared before and after phenoconversion using a McNemar test, continuous variables using paired *t* tests, and diagnosis using paired sign tests. For all other group comparisons, categorical comparisons between groups were performed using chi-squared tests when all categories had enough observations, and Fisher tests when one or more did not. All cognitive measures and continuous CSF Aβ42 were compared between groups with analyses of covariance (ANCOVAs) adjusted for age, sex, years of education, diagnosis, and *APOE* ε4 genotype. Continuous CSF p-tau181 was compared between groups with ANCOVAs adjusted for age, sex, years of education, diagnosis, *APOE* ε4 genotype, and CSF Aβ42 status. Logistic regressions, adjusted for age, sex, years of education, and *APOE* ε4 genotype, were performed to compare group differences in CSF biomarker positivity.

Generalized additive mixed-effects models (GAMMs) with penalized cubic regression spline were fit to assess the changes in longitudinal cognitive outcome measures, as a function of amyloid time. GAMMs were fit separately for Stable SAA– and Stable SAA+ groups while accounting for differences in age, sex, years of education, and *APOE* ε4 genotype. Amyloid time at which Stable SAA– and Stable SAA+ groups reached a cognitive performance threshold defined as two standard deviations below the mean of CSF Aβ42– CU participants were estimated via bootstrap resampling.

To evaluate the effects of transitioning to SAA positivity among SAA Converters, we first identified a reference group of Stable SAA– individuals. We matched their cognitive assessment time points with those of SAA Converters at their last SAA– evaluation. The matching criteria included age, sex, years of education, *APOE* ε4 genotype, and amyloid time, using a 2-to-1 genetic matching approach.[34,35] The duration of cognitive assessments before the last SAA– evaluation for SAA Converters was matched to the duration before the corresponding matched time point for the Stable SAA– reference group. Matching was stratified by clinical diagnostic groups (i.e., CU, MCI, and Dementia). We then applied piecewise mixed-effects regression models to the longitudinal cognitive data, setting a predefined breakpoint at $t = 0$. This breakpoint represents the estimated SAA phenoconversion time for the SAA Converters and the midpoint between the matched time point and the subsequent assessment for the Stable SAA– reference group. The estimated cognitive decline rates before ($t < 0$) and after ($t > 0$) the breakpoint were compared between the SAA Converters and the Stable SAA– reference groups.

Survival analysis was conducted for phenoconversion in four outcomes: CSF SAA positivity, CSF Aβ42 positivity, CSF p-tau181 positivity, and clinical diagnosis progression (i.e., CU to MCI/Dementia or MCI to Dementia). Time for all outcomes was measured from baseline. Kaplan–Meier survival curves, stratified by the status of other outcomes at baseline and *APOE* ε4 genotype, were estimated for all outcomes. Cox proportional hazard models were fit for each outcome separately. Person-period coding was used to reflect change in outcome measures. CSF SAA status was included as a time-variant predictor in models for CSF AD positivity and clinical diagnosis progression models. Similarly, CSF Aβ42 and p-tau181 statuses were included as time-variant predictors in models for CSF SAA positivity and clinical diagnosis progression models. Age at baseline, sex, and *APOE* ε4 genotype were included in all models.

Next, we evaluated the extent to which the SAA kinetic parameters are associated with disease characteristics and risk factors, with a particular focus on the stable SAA+ and SAA Converter cohorts. We assessed the independent effects of age, sex, *APOE* ε4 status, clinical diagnosis, and CSF Aβ42 positivity on SAA kinetic parameters, in a full model including all of these factors. The association of SAA kinetic parameters with CSF Aβ42 and p-tau181 levels was assessed using linear regression models adjusted for age, sex, *APOE* ε4 status, and clinical diagnosis, in which the p-tau181 model was further adjusted for CSF Aβ42 positivity. Similarly, the relationship between SAA kinetic parameters and cognitive outcome scores cross-sectionally at the first CSF time point was assessed using linear regression models adjusted for age, sex, *APOE* ε4 status, and clinical diagnosis. To determine how changes in SAA kinetic parameters are associated with the progression of cognitive decline, we used linear mixed-effects models (LMM), with cognitive measure of interest as the outcome variable and time since the initial CSF sample collection, the SAA kinetic parameter, and their

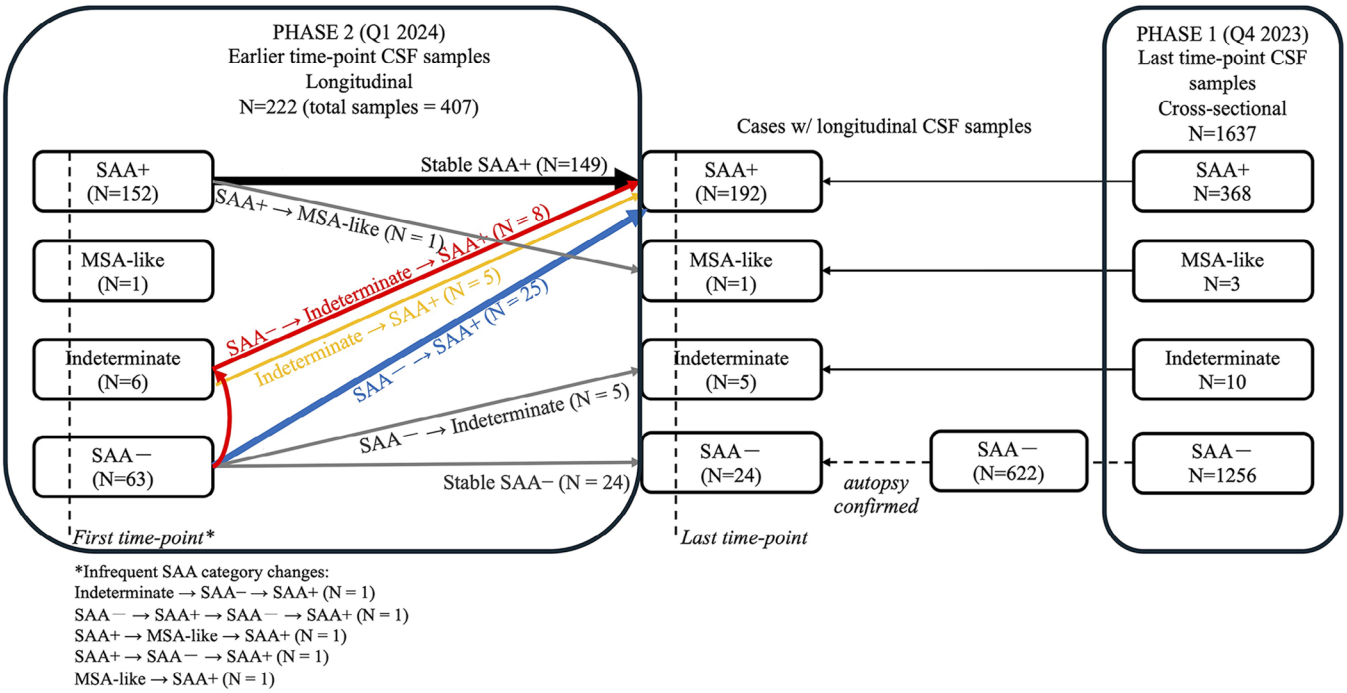

FIGURE 1 Graphical overview of the ADNI CSF α-syn SAA workflow. On the right, Phase 1 included SAA measurement of all ADNI 1–3 participants' most recent time points, previously published.[19] As a follow-up in Phase 2, we included all previous CSF time points available from groups identified as (1) SAA+, (2) MSA-like, and (3) indeterminate in Phase 1 processing, and (4) autopsy cohort, totaling 222 participants with 407 samples. ADNI, Alzheimer's Disease Neuroimaging Initiative; α-syn, α-synuclein; CSF, cerebrospinal fluid; MSA, multiple system atrophy; SAA, seed amplification assay

interaction as predictor variables. We adjusted these models for age, sex, education years, and *APOE* ε4 status, incorporating random intercepts and slopes to account for correlations within participants. We conducted these analyses for each SAA kinetic parameter and within each clinical diagnostic category independently. Finally, leveraging longitudinal kinetic data from the SAA Converter group, we repeated the association between changes in SAA kinetic parameters and cognitive decline rates, using time since the initial SAA+ measurement in the longitudinal models.

In all SAA kinetic parameter association analyses, we included the total CSF protein concentration as a surrogate measurement for lipoproteins as it was shown to affect the kinetics of α-syn seed amplification in a concentration-dependent manner.[18]

3 | RESULTS

3.1 | ADNI SAA study cohort characteristics

The initial set of CSF α-syn SAA analysis (Phase 1; Figure 1) comprised the latest CSF specimens from 1637 participants who were part of the ADNI 1–3 studies. Of these, 368 (22.5%) specimens exhibited PD/DLB-like α-syn seeding activity (i.e., SAA+), while 3 showed MSA-like α-syn seeding activity. No α-syn seeding aggregation was observed in 1256 samples, which were thus classified as SAA−. The SAA outcomes were indeterminate for 10 samples. These findings from the cross-sectional CSF α-syn SAA have been previously reported in detail.[19]

The samples for the second set of longitudinal CSF α-syn SAA analysis (Phase 2; Figure 1) were selected from those with available CSF samples from previous study time points, totaling 819. This selection was narrowed to include only those identified as SAA+ (192 individuals), those with MSA-like α-syn seeding (1 individual), or those with indeterminate seeding activity (5 individuals) as determined by the initial cross-sectional analysis at their last CSF collection (Phase 1). Additionally, Phase 2 also included samples from all the participants from the ADNI autopsy subcohort, which led to inclusion of an additional 24 individuals who were identified as SAA− at their last CSF collection. In total, Phase 2 comprised 407 longitudinal CSF samples from 222 distinct participants.

3.2 | CSF p-tau181/Aβ42 SILA model for estimation amyloid onset age

Figure S2 in supporting information displays the observed CSF p-tau181/Aβ42 values by age on the left, and the modeled CSF p-tau181/Aβ42 patterns as a function of estimated time to the CSF p-tau181/Aβ42 > 0.025 threshold on the right. The EAOA showed a linear correlation with the actual age of CSF p-tau181/Aβ42 phenoconversion (r = 0.96; P < 0.0001), with no bias observed in the

regression analysis (regression line fit: EAOA = 0.89 + 0.99 x actual age at phenoconversion; intercept $P = 0.77$; slope $P < 0.0001$).

3.3 | Assessment of longitudinal CSF α-syn SAA profiles

As depicted in Figure 1, the CSF samples from participants who were classified as SAA− at their final CSF collection (totaling 24 individuals) consistently tested SAA− at all preceding time points as well, suggesting stability of the SAA− findings retrospectively. Consequently, for those individuals who were determined to be SAA− at their most recent CSF collection according to Phase 1 analysis but were not selected for the longitudinal Phase 2 processing ($N = 598$), their CSF samples from earlier time points were deemed SAA−. Including the 24 SAA− samples from the longitudinal Phase 2 analysis, these individuals ($N = 622$) were collectively classified as having a Stable SAA− status.

Among the 222 participants included in the longitudinal Phase 2 analysis, 63 were initially classified as SAA− at their first CSF time point. Of these, 24 remained consistently SAA−, thus described earlier as Stable SAA−. In contrast, 25 individuals progressed from SAA− to SAA+ by their final CSF time point. Additionally, there were eight participants who, while initially SAA−, had an interim CSF time point yielding indeterminate SAA results, but ultimately were found to be SAA+ by their final CSF sample. Another five began as SAA− and maintained this status up to their last CSF collection, which ended with an indeterminate SAA result. As one of the infrequent instances of fluctuating SAA categories, there was one participant who initially tested SAA−, subsequently transitioned to SAA+, reverted to SAA−, and then returned to being SAA+ by the subsequent assessments.

Out of the six participants who initially presented with indeterminate SAA results, five were classified as SAA+ by the time of their last CSF collection. As one of the infrequent instances of fluctuating SAA categories, there was one participant who initially had an indeterminate SAA result, subsequently tested SAA−, and then transitioned to being SAA+ by the subsequent assessments. In all the analyses described below, CSF samples with indeterminate SAA results were excluded.

Among the 222 participants in the longitudinal Phase 2 study, 152 were initially classified as SAA+ at their first CSF collection. Of these, 149 consistently tested SAA+ across all study time points, that is, Stable SAA+. However, one participant changed from the SAA+ category to exhibiting MSA-like seeding patterns in their final CSF collection. The CSF specimen with MSA-like seeding was visibly discolored, likely due to blood contamination. Two rare cases of fluctuating SAA categories included one individual transitioning from SAA+ to MSA-like and then back to SAA+, and another who moved from SAA+ to SAA−, and then reverted to SAA+.

Additionally, there was a single case of a participant who initially presented with an MSA-like seeding pattern and subsequently tested SAA+ in their final CSF sample. The CSF specimen with MSA-like seeding was visibly discolored, likely due to blood contamination. For the analyses that follow, any individual who showed MSA-like α-syn seed aggregation at any time point, regardless of their initial or final CSF SAA status, was excluded. Moreover, the two individuals demonstrating the patterns SAA− → SAA+ → SAA− → SAA+ and SAA+ → SAA− → SAA+ were also excluded from the study analyses. In total 5 out of 222 (2%) of the participants in the longitudinal Phase 2 study were excluded from the study analysis.

In total, 34 individuals presented with the SAA− → SAA+ pattern. SAA Converters averaged 2.5 ± 1.6 years between their last visit with a SAA− result and their first visit with a SAA+ result, with a minimum of 0.9 years and a maximum of 6.1 years between those visits. The midpoint between their last visit with a SAA− result and their first visit with a SAA+ result was considered the SAA conversion time, due to the binary nature of the CSF α-syn SAA assay. As a result, the phenoconversion time estimates have a wide uncertainty window, which limits our ability to precisely assess the speed of α-syn co-pathology progression.

Among participants with a SAA− sample at baseline and follow-up CSF samples, the proportions of SAA Converters within baseline diagnosis groups of CU, MCI, and Dementia were 3.7% (11 out of 297), 6.7% (20 out of 299), and 5.1% (3 out of 59), respectively. Among SAA Converters, 1 out of 11 (9%) participants who were CU at baseline progressed to MCI at their first visit after SAA conversion, and 10 out of 20 (50%) participants who were MCI at baseline were diagnosed with dementia at their first visit after SAA conversion. One converter (5%) who was MCI at baseline was diagnosed as CU at their first visit after SAA conversion.

There were no significant differences in CSF levels of total protein, white blood cell count, and red blood cell count between groups with CSF samples categorized as SAA+, SAA−, and Indeterminate.

3.4 | Cohort characteristics of Stable SAA−, SAA Converters, and Stable SAA+

Next, we assessed the demographic, biomarker, and clinical characteristics of SAA groups, including Stable SAA− group ($N = 622$), SAA Converter group ($N = 34$), and Stable SAA+ group ($N = 149$), as shown in Table 1.

The Stable SAA+ group, compared to Stable SAA− individuals, had a higher proportion of cognitively impaired individuals, lower levels of CSF Aβ42, and poorer scores in all global and domain-specific cognitive measures assessed in this study. The Stable SAA+ group did not include any participants with Hispanic/Latino ethnic background and a greater frequency of Asian racial background but a lower frequency of Black/African American racial background compared to the Stable SAA−. Although these racial and ethnic differences between Stable SAA+ and Stable SAA− groups were significant ($P = 0.04$), these results should be interpreted with caution given the low ethnoracial diversity in this ADNI cohort.

SAA Converters exhibited a higher level of educational attainment compared to individuals who remained consistently Stable SAA− or Stable SAA+ throughout the ADNI study. A greater frequency of *APOE* ε4 homozygotes was observed among SAA Converters compared to the Stable SAA− group. SAA Converters at their last visit with an

TABLE 1 Demographic, clinical, and biomarker characteristics of the individuals with CSF α-syn SAA− stable over time (Stable SAA−), individuals with CSF α-syn SAA+ stable over time (Stable SAA+), and individuals progressing from CSF α-syn SAA− to CSF α-syn SAA+ (SAA Converters).

Characteristic	i. Stable SAA−, *N* = 622	ii. SAA Converters (before), *N* = 34	iii. SAA Converters (after), *N* = 34	iv. Stable SAA+ *N* = 149	*P* i vs. ii	*P*[g] ii vs. iii	*P* iii vs. iv	*P* i vs. iv
[a]Diagnosis					0.40	0.81	0.59	<0.001
CU	269 (43%)	11 (32%)	11 (32%)	36 (24%)				
MCI	213 (34%)	15 (44%)	10 (29%)	52 (35%)				
Dementia	140 (23%)	8 (24%)	13 (38%)	61 (41%)				
[b]Age	76 (71, 81)	76 (66, 79)	78 (70, 83)	78 (73, 81)	0.08	<0.001	0.67	0.15
[a]Male	316 (51%)	21 (62%)	21 (62%)	87 (58%)	0.29	N/A	0.89	0.10
[c]Ethnicity					0.71	N/A	N/A	0.04
Hispanic or Latino	18 (2.9%)	0 (0%)	0 (0%)	0 (0%)				
Not Hispanic or Latino	599 (96%)	34 (100%)	34 (100%)	149 (100%)				
Unknown	5 (0.8%)	0 (0%)	0 (0%)	0 (0%)				
[c]Race					0.13	N/A	0.01	0.04
Asian	4 (0.6%)	0 (0%)	0 (0%)	4 (2.7%)				
Black or African American	24 (3.9%)	1 (2.9%)	1 (2.9%)	4 (2.7%)				
More than one race	13 (2.1%)	3 (8.8%)	3 (8.8%)	0 (0%)				
White	581 (93%)	30 (88%)	30 (88%)	141 (95%)				
[b]Years of education	16.00 (14.00, 18.00)	18.00 (16.00, 20.00)	18.00 (16.00, 20.00)	16.00 (14.00, 18.00)	0.03	N/A	0.02	0.37
[c]*APOE* ε4 genotype					0.03	N/A	0.41	0.15
Non-carrier	370 (59%)	15 (44%)	15 (44%)	80 (54%)				
Heterozygotes	202 (33%)	12 (35%)	12 (35%)	50 (34%)				
Homozygotes	50 (8.1%)	7 (21%)	7 (21%)	19 (13%)				
[d]CSF Aβ42	849 (591, 1387)	732 (580, 1024)	638 (516, 1098)	690 (495, 1211)	0.14	0.21	0.65	0.02
[f]CSF Aβ42+	338 (57%)	25 (74%)	23 (70%)	97 (66%)	0.13	>0.9	0.41	0.53
[e]CSF p-tau181	24 (17, 35)	27 (21, 38)	27 (21, 43)	24 (18, 34)	0.04	>0.9	0.01	0.56
[f]CSF p-tau181+	287 (49%)	20 (59%)	22 (67%)	72 (50%)	0.41	0.37	0.10	0.13
[d]ADAS-Cog11	13 (7, 22)	15 (10, 23)	21 (10, 29)	19 (11, 30)	0.16	<0.001	0.90	<0.001
[d]CDR-SB	1.0 (0.0, 3.0)	1.0 (0.5, 3.4)	2.0 (0.5, 4.9)	2.0 (0.5, 5.0)	0.37	<0.01	0.36	<0.001
[d]PACC	−3 (−10, 1)	−6 (−10, −1)	−10 (−12, −1)	−8 (−15, −2)	<0.01	<0.01	0.50	<0.001
[d]MMSE	28.0 (25.0, 30.0)	28.0 (25.0, 29.0)	26.0 (23.0, 29.0)	26.0 (22.0, 29.0)	0.10	<0.01	0.88	<0.001
[d]Executive function	0.47 (0.01, 0.94)	0.36 (−0.23, 0.65)	0.26 (−0.06, 0.62)	0.10 (−0.55, 0.64)	0.05	0.22	0.21	<0.001
[d]Memory	0.45 (−0.35, 1.10)	0.13 (−0.35, 0.75)	−0.40 (−0.65, 0.64)	−0.11 (−0.83, 0.64)	0.06	<0.001	0.77	<0.001
[d]Language	0.51 (0.08, 0.82)	0.54 (−0.09, 0.81)	0.44 (−0.17, 0.76)	0.16 (−0.30, 0.62)	>0.9	0.04	0.25	<0.001

Note: For SAA Converters, cohort characteristics are provided before and after conversion time points (i.e., last time point with CSF α-syn SAA− and first time point with CSF α-syn SAA+, respectively). *n* (%) are provided for dichotomized and categorical variables, median (IQR) for continuous variables. Missing data counts and percentages for clinical and biomarker data are provided in Table S1 in supporting information.

Abbreviations: Aβ, amyloid beta; ADAS-Cog11, Alzheimer's Disease Assessment Scale Cognitive subscale 11-item; ANCOVA, analyses of covariance; ANOVA, analysis of variance; *APOE*, apolipoprotein E; CDR-SB, Clinical Dementia Rating Sum of Boxes; CSF, cerebrospinal fluid; CU, cognitively unimpaired; MCI, mild cognitive impairment; MMSE, Mini-Mental State Examination; PACC, Preclinical Alzheimer's Cognitive Composite; p-tau181, phosphorylated tau181; SAA, seed amplification assay.

[a]Pearson's chi-squared test.

[b]One-way ANOVA.

[c]Fisher exact test.

[d]ANCOVA adjusted for age, sex, education, diagnosis, and *APOE*.

[e]ANCOVA adjusted for age, sex, education, *APOE*, diagnosis, and CSF Aβ42 status.

[f]Logistic regression adjusted for age, sex, education, diagnosis, and *APOE*.

[g]Paired *t* test: all continuous variables; McNemar test: all binary variables; paired sign test: diagnosis.

SAA− result had increased levels of CSF p-tau181 and poorer performance on the PACC and executive function composite score relative to the Stable SAA− group. In addition, SAA Converters at their first visit with a SAA+ result presented greater levels of CSF p-tau181 compared to the stable SAA+ group. Furthermore, SAA Converters were more likely to be from non-White racial groups than those who were consistently Stable SAA+, though this result should be interpreted with caution due to the small sample size.

Last, between their last SAA− assessment and the subsequent SAA+ result, five SAA Converters who were previously diagnosed with MCI advanced to a clinical diagnosis of dementia due to AD. Three individuals from the MCI SAA Converter group developed dementia after their SAA+ conversion, while one individual initially classified as MCI SAA Converter later reverted to CU, as detailed in Table S2 in supporting information. Relative to their cognitive performance at their last SAA− time point, SAA Converters demonstrated greater impairment in all global and domain-specific cognitive measures except the executive function composite measures at their initial SAA+ time point.

3.5 | Association of SAA positivity with the rates of cognitive decline

After accounting for differences in age, sex, years of education, *APOE* ε4 genotype, and CSF Aβ42 and p-tau181 levels, Stable SAA+ CU participants, compared to their Stable SAA− counterparts, experienced faster increases in ADAS-Cog11 and a steeper decline in PACC memory and executive function composite scores (Table 2). Similarly, Stable SAA+ MCI participants showed more rapid declines across all global and domain-specific cognitive measures assessed, relative to Stable SAA− MCI participants, with a medium Cohen f^2 effect size of 0.15 to 0.21.[36] Only SAA positivity associated rapid cognitive decline in ADAS-Cog11, PACC, memory, executive function, and language measures within MCI survived Holm–Bonferroni correction.

We next modeled the longitudinal trajectories of cognitive outcome measures as a function of amyloid time, while adjusting for age, sex, years of education, and *APOE* ε4 genotype (Figure 2). We estimated the relative amyloid time for the SAA+ and SAA− groups to reach a cognitive performance threshold defined as two standard deviations below the mean of CSF Aβ42− CU participants. For the SAA− group, the time from CSF p-tau181/Aβ42 positivity (amyloid time) to reach the cognitive performance threshold was as follows: 2.0 ± 0.7 years for ADAS-Cog11, −1.4 ± 0.7 years for MMSE, −2.3 ± 0.6 years for the PACC, 8.7 ± 0.4 years for memory function, 10.5 ± 0.6 years for executive function, and 13.8 ± 0.6 years for language function. In contrast, the SAA+ individuals reached the same cognitive performance thresholds 4.7 to 9.3 years earlier than their SAA− counterparts. The amyloid time to reach the cognitive performance threshold for the SAA+ group and significance of the differences compared to SAA− were as follows: −6.1 ± 1.9 years for ADAS-Cog11 ($P < 0.0001$), −8.6 ± 1.1 years for MMSE ($P < 0.0001$), −11.5 ± 1.0 years for PACC ($P < 0.0001$), 0.4 ± 1.5 years for memory function ($P < 0.0001$), 5.8 ± 3.1 years for executive function ($P = 0.14$), and 7.1 ± 1.8 years for language function($P = 0.0005$).

Next, we compared SAA Converters ($N = 34$) to a matched group of Stable SAA− individuals (Reference group) in a 2:1 ratio. Our aim was to assess their cognitive decline rates both before and after the critical phenoconversion point, denoted as $t = 0$ (Figure 3). For SAA Converters, $t = 0$ represents the approximate time of phenoconversion to SAA+, while for the Reference group, it aligns with the point at which they were matched to the SAA Converters based on age, sex, years of education, *APOE* ε4 status, amyloid time, and clinical diagnosis. After Holm–Bonferroni correction, MCI SAA Converters compared to the MCI Reference group showed a significantly accelerated decline in PACC ($z = 4.10$, $P < 0.0001$) and memory function ($z = 4.52$, $P < 0.0001$) after their estimated time of SAA conversion.

3.6 | SAA conversion time relative to CSF Aβ time

The timing of the CSF p-tau181/Aβ42 pathology relative to SAA phenoconversion is illustrated in Figure 4. Among the 34 SAA Converters, 70% ($N = 24$; 7 out of 8 Dementia, 13 out of 15 MCI, and 4 out of 11 CU) were CSF p-tau181/Aβ42 positive before their SAA phenoconversion time point. On average, amyloid time (i.e., time from CSF p-tau181/Aβ42 positivity) for Dementia and MCI SAA Converters was 14.9 years and 8.6 years prior to their SAA phenoconversion time, respectively, while amyloid time for CU SAA Converters was on average coincided with their SAA conversion time (i.e., 0.2 years after EAOA).

3.7 | Risk for CSF α-syn SAA and AD biomarker conversion and change in clinical diagnosis

Risks for CSF α-syn SAA, Aβ42, and p-tau181 biomarker phenoconversion, as well as change in clinical diagnosis (from CU to MCI/Dementia or from MCI to Dementia), were assessed through Cox proportional hazards regression survival analyses.

The survival analysis indicated a significant association of being CSF Aβ42 positive (hazard ratio [HR]: 2.44; 95% confidence interval [CI]: 1.05–5.68) with SAA conversion risk (Figure 5; Figure S3 in supporting information). When EAOA and amyloid time were used in a repeated survival analysis instead of CSF Aβ42 positivity, the associations remained consistent (data not shown).

In contrast, the risk for CSF Aβ42 phenoconversion was associated with older age (HR: 1.06; 95% CI: 1.02–1.10) and *APOE* ε4 genotype (heterozygotes: HR: 3.05; 95% CI: 1.73–5.37; homozygotes: HR: 12.48; 95% CI: 4.19–37.15; Figure 5; Figure S4 in supporting information). Similarly, the risk for CSF p-tau181 phenoconversion was associated with older age (HR: 1.04; 95% CI: 1.00–1.08), and *APOE* ε4 genotype (heterozygotes: HR: 2.65; 95% CI: 1.46–4.80; homozygotes: HR: 4.88; 95% CI: 1.84–12.95; Figure 5; Figure S5 in supporting information).

The likelihood of change in clinical diagnosis, whether from CU to MCI/Dementia or MCI to Dementia, was associated with CSF Aβ42

TABLE 2 Rates of change in cognitive outcome measures for Stable SAA− and Stable SAA+ groups.

Diagnosis	Measure	Stable SAA−	Stable SAA+	Cohen f^2 effect size	*P*
CU	ADAS-Cog11	0.11 (0.12)	0.16 (0.14)	0.14	0.0096
	CDR-SB	0.028 (0.056)	0.021 (0.049)	0.035	0.64
	MMSE	−0.041 (0.040)	−0.42 (0.034)	0.011	0.85
	PACC	−0.096 (0.14)	−0.14 (0.11)	0.091	0.040
	Memory composite	−0.015 (0.027)	−0.025 (0.020)	0.12	0.017
	Executive function composite	0.026 (0.026)	−0.037 (0.027)	0.13	0.024
	Language composite	−0.028 (0.015)	−0.033 (0.020)	0.087	0.14
MCI	ADAS-Cog11	0.57 (0.55)	0.81 (0.54)	0.18	<0.001*
	CDR-SB	0.15 (0.21)	0.23 (0.21)	0.15	0.0078
	MMSE	−0.23 (0.29)	−0.34 (0.26)	0.15	0.0070
	PACC	−0.51 (0.62)	−0.76 (0.59)	0.17	0.0012*
	Memory composite	−0.084 (0.094)	−0.12 (0.087)	0.17	0.0015*
	Executive function composite	−0.041 (0.044)	−0.064 (0.054)	0.21	<0.001*
	Language composite	−0.054 (0.038)	−0.074 (0.036)	0.21	<0.001*
Dementia	ADAS-Cog11	3.90 (2.90)	4.40 (2.90)	0.067	0.24
	CDR-SB	1.60 (1.20)	1.70 (0.97)	0.034	0.63
	MMSE	−1.90 (1.60)	−2.30 (1.70)	0.10	0.098
	PACC	−2.70 (1.80)	−2.90 (2.00)	0.056	0.33
	Memory composite	−0.30 (0.15)	−0.33 (0.14)	0.095	0.076
	Executive function composite	−0.30 (0.12)	−0.27 (0.14)	0.098	0.40
	Language composite	−0.36 (0.30)	−0.40 (0.31)	0.058	0.26

Note: Rates of change in each cognitive outcome measure were separately modeled for all groups. Estimated rates were compared across groups after adjusting for age, sex, gender, *APOE* genotype, and CSF Aβ42 and p-tau181 levels. Rates are listed as mean (SD).
*Significance survived Holm–Bonferroni correction.
Abbreviations: Aβ, amyloid beta; ADAS-Cog11, Alzheimer's Disease Assessment Scale Cognitive subscale 11-item; *APOE*, apolipoprotein E; CDR-SB, Clinical Dementia Rating Sum of Boxes; CSF, cerebrospinal fluid; CU, cognitively unimpaired; MCI, mild cognitive impairment; MMSE, Mini-Mental State Examination; PACC, Preclinical Alzheimer's Cognitive Composite; p-tau181, phosphorylated tau181; SAA, seed amplification assay; SD, standard deviation.

positivity (HR: 3.18; 95% CI: 2.39–4.24), CSF p-tau181 positivity (HR: 2.33; 95% CI: 1.82–2.98), CSF α-syn SAA positivity (HR: 1.49; 95% CI: 1.15–1.93), older age (HR: 1.02; 95% CI: 1.01–1.04), and *APOE* ε4 genotype (heterozygotes: HR: 1.35; 95% CI: 1.04–1.75; homozygotes: HR: 1.48; 95% CI: 1.02–2.14) as shown in Figure 5 and Figure S6 in supporting information.

3.8 | Association of SAA kinetic parameters with cohort characteristics and cognitive decline

Within the SAA+ group ($N = 368$), we assessed the independent association of demographic (age, sex, and *APOE* ε4 status) and clinical factors (clinical diagnosis and CSF Aβ42 positivity) with the SAA kinetic parameters (Fmax, Smax, TTT, and TSmax) in a full model including all these factors as well as the total CSF protein concentration. The kinetic parameter analyses were repeated with continuous CSF Aβ42 and p-tau181 levels, cognitive outcome measures, as well as for the associations between change in cognition and change in SAA kinetic parameter, all adjusted for age, sex, *APOE* ε4 status, clinical diagnosis, and total CSF protein concentration.

Although the uncorrected association between SAA kinetic parameters and various demographic (age, sex) and clinical (diagnosis, CSF Aβ42 positivity, CSF p-tau181 levels, ADAS-Cog11, CDR-SB, and language function) factors were observed as illustrated in Figure S7 in supporting information, only a few survived Holm–Bonferroni correction. Specifically, less steep Smax values were associated with older age ($\beta = -0.040$; $P = 0.0013$) and MCI diagnosis compared to being CU or having Dementia ($\beta = -0.74$; $P = 0.0013$).

Next, we investigated whether the SAA kinetic parameters were associated with the follow-up time, as an indicator of an association with the duration of α-syn pathology, using data from individuals who transitioned to SAA+ from SAA−. Longitudinal kinetic parameters from SAA Converters were aligned at the time of their SAA phenoconversion (i.e., $t = 0$; Figure 6). After the SAA phenoconversion time, both TTT and TSmax significantly decreased over the subsequent years ($P < 0.01$), converging to the levels observed within the stable SAA+ participants. In contrast, Fmax and Smax remained constant over time, at the level

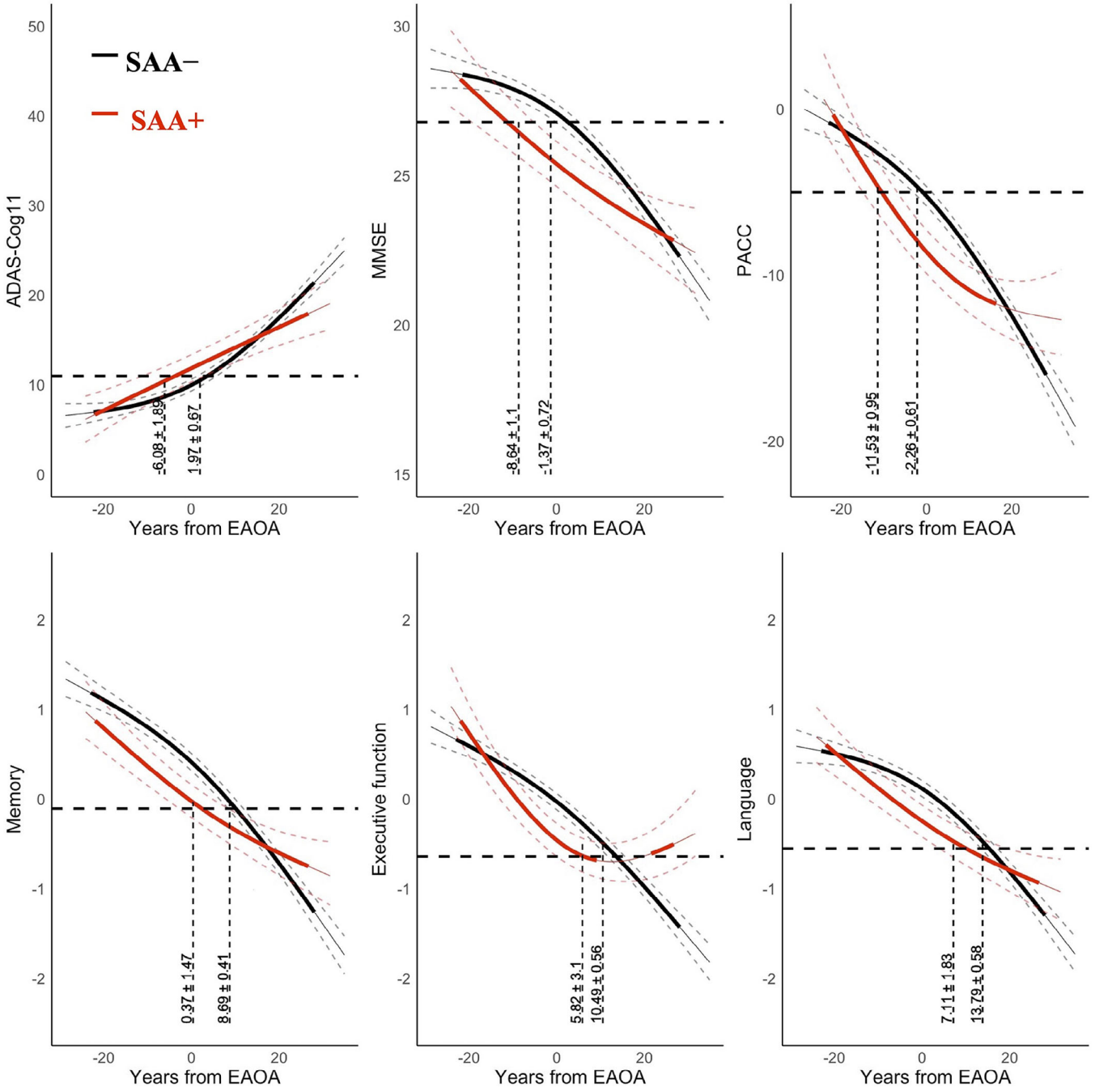

FIGURE 2 The longitudinal trajectories of cognitive outcome measures as a function of amyloid time, while adjusting for age, sex, years of education, and *APOE* ε4 genotype. Amyloid time at the cognitive assessment time was measured relative to the SILA-estimated age at CSF p-tau181/Aβ42 positivity (EAOA). Horizontal dashed lines indicate the cognitive performance threshold defined as two standard deviations below the mean of CSF Aβ42– CU participants. Aβ, amyloid beta; ADAS-Cog 11, Alzheimer's Disease Assessment Scale Cognitive subscale 11-item; *APOE*, apolipoprotein E; CSF, cerebrospinal fluid; CU, cognitively unimpaired; EAOA, estimated amyloid onset age; MMSE, Mini-Mental State Examination; PACC, Preclinical Alzheimer's Cognitive Composite; p-tau181, phosphorylated tau181; SILA, sampled iterative local approximation

observed within the stable SAA+ participants. This constancy, combined with the decreasing TTT and TSmax, as expected resulted in a significant increase in the AUC-Fluoro ($P = 0.03$) in years after the SAA conversion time. When repeated within each diagnostic group (i.e., CU, MCI, and Dementia) separately, similar longitudinal profiles were observed (Figure S8 in supporting information).

4 | DISCUSSION

We recently applied CSF α-syn SAA to the latest available CSF samples from the ADNI cohort (Phase 1), examining the prevalence of LB pathology (SAA positivity) and its correlation with AD biomarkers and cognitive function.[19] Expanding upon this, we incorporated earlier CSF

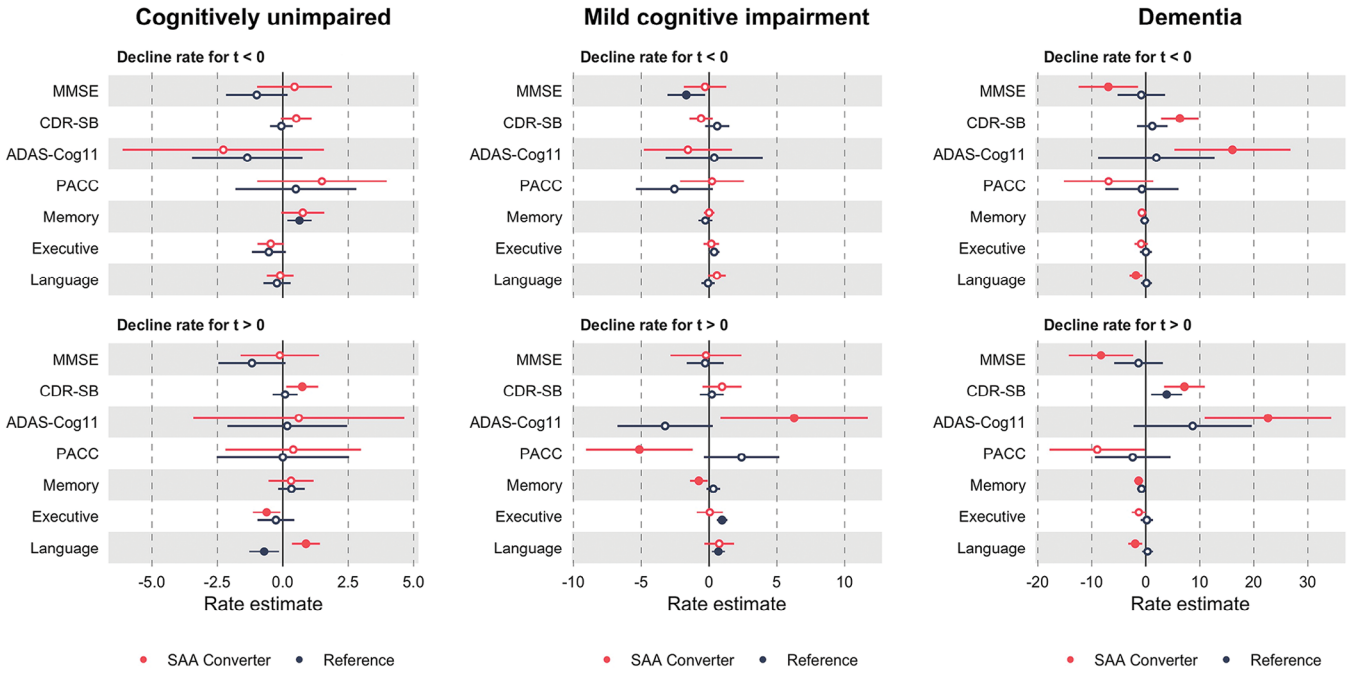

FIGURE 3 Cognitive decline rates for SAA Converters ($N = 34$) and a matched group of Stable SAA− individuals (Reference group). For SAA Converters, $t = 0$ represents the approximate time of phenoconversion to SAA+, while for the Reference group, it aligns with the point at which they were matched to the SAA Converters based on age, sex, years of education, *APOE* ε4 status, amyloid time, and clinical diagnosis in a 2:1 ratio. Closed and open circles indicate $P \leq 0.05$ and $P > 0.05$, respectively, for the estimated cognitive decline rates. *APOE*, apolipoprotein E; ADAS-Cog 11, Alzheimer's Disease Assessment Scale Cognitive subscale 11-item; CDR-SB, Clinical Dementia Rating Sum of Boxes; MMSE, Mini-Mental State Examination; PACC, Preclinical Alzheimer's Cognitive Composite; SAA, seed amplification assay

samples (Phase 2) with particular focus on individuals who were SAA+ in Phase 1 (Figure 1). This allowed us to track the progression of CSF Aβ42, α-syn seeds, and p-tau181, along with comprehensive cognitive assessments, in three groups: those with consistent SAA positivity (Stable SAA+), those with consistent SAA negativity (Stable SAA−), and those who phenoconverted from SAA− to SAA+ status (SAA Converters). The major findings of this study were: (1) Stable SAA+ individuals exhibited a more rapid cognitive decline compared to Stable SAA− individuals, particularly during the MCI stage. Stable SAA+ participants reached a cognitive performance threshold—defined as two standard deviations below the mean of CSF Aβ42− CU individuals—4.7 to 9.3 years earlier than their Stable SAA− counterparts. (2) In the subset with longitudinal CSF data, 34 individuals (≈ 5%) transitioned from SAA− to SAA+ status by their final CSF collection. These "SAA Converters" experienced a more pronounced cognitive decline post-conversion than a matched cohort of Stable SAA− individuals. (3) The risk of converting to SAA+ status was linked to CSF Aβ42 positivity. However, the SAA status itself did not influence the likelihood of becoming positive for either CSF Aβ42 or p-tau181 biomarkers. (4) The positivity in all three CSF biomarkers—Aβ42, p-tau181, and α-syn SAA—independently was associated with greater risk for a change in clinical diagnosis (CU to MCI/Dementia or MCI to Dementia). Of these, CSF Aβ42 positivity was the strongest risk indicator. (5) The SAA kinetic parameter of Smax was associated with age and MCI diagnosis.

The relationship between SAA status and cognitive trajectories versus change in clinical diagnosis presents a nuanced aspect of AD clinical progression. Most importantly, SAA+ was associated with more rapid cognitive decline in a fully adjusted model, predominantly during the MCI stage. We also observed that after SAA phenoconversion, individuals experienced an accelerated decline in cognitive performance. Interestingly, the onset of cognitive impairment, defined as two standard deviations below the mean of CU individuals, for Stable SAA+ compared to Stable SAA− was 4.7 to 9.3 years earlier for both global and domain-specific cognitive measures. This observation was consistent with previous studies reporting earlier age of symptom onset in AD patients with LB co-pathology.[37,38] Our observation that SAA positivity was a significant risk factor for change in clinical diagnosis also aligns with findings from these studies. As expected, in the context of AD co-pathologies, CSF Aβ42 positivity as a marker of amyloid pathology was the strongest risk factor for change in clinical diagnosis.

Our findings provide insights into the interactions particularly of amyloid and α-syn within the AD framework. CSF Aβ42 positivity significantly increased the likelihood of SAA phenoconversion, supporting the hypothesis that α-syn co-pathology may not arise independently but is rather facilitated by existing amyloid pathology, especially in symptomatic individuals. Interestingly, a notable proportion (55%) of CU exhibited SAA positivity before Aβ42 positivity, challenging the linear progression model of AD and suggesting that the temporal sequence and chronicity of AD pathologies may hold greater implications for cognitive decline and clinical diagnosis than the mere presence of multiple pathological entities.

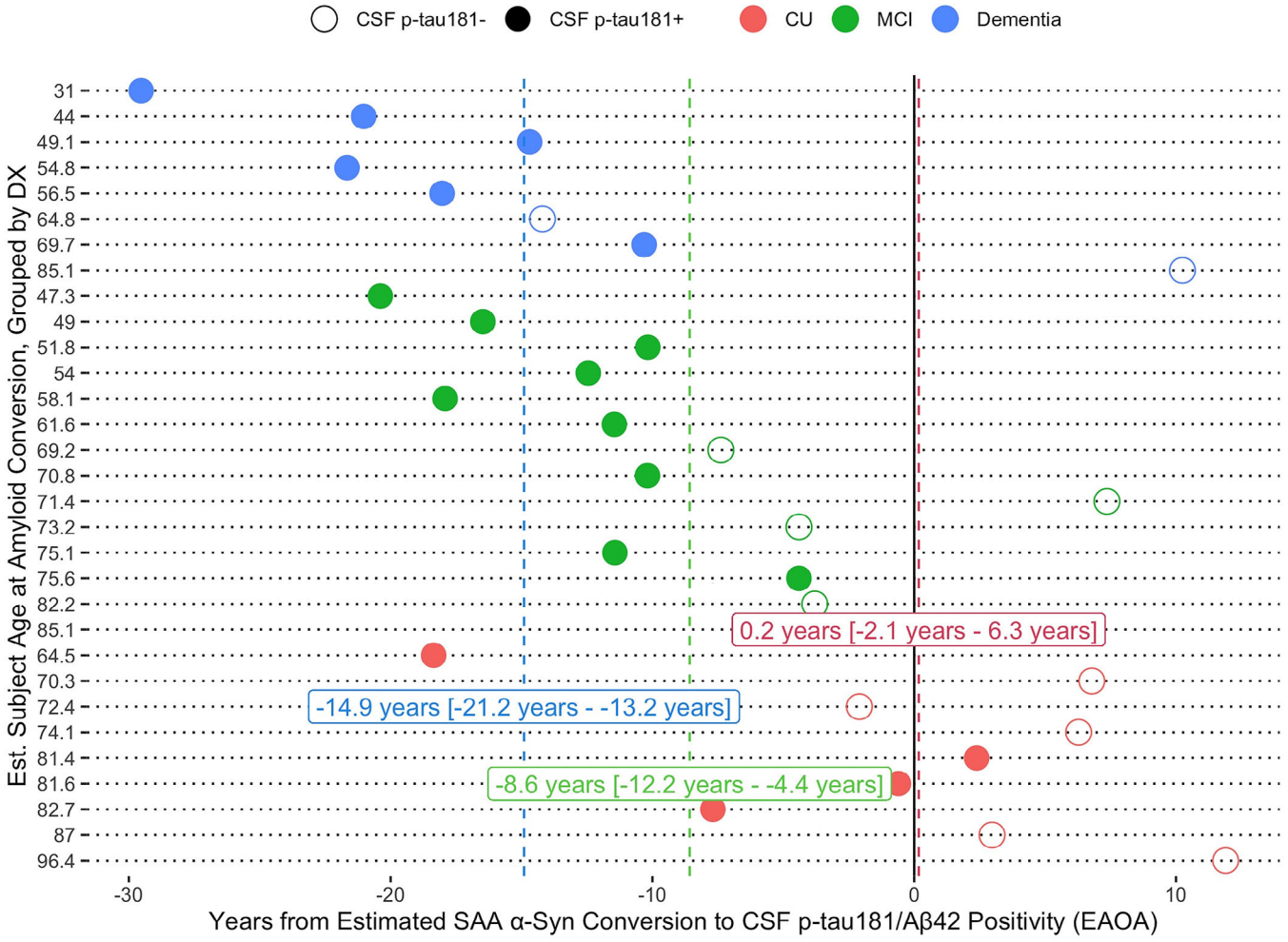

FIGURE 4 Timing of the CSF p-tau181/Aβ42 positivity (i.e., EAOA) relative to CSF α-syn SAA phenoconversion. Three out of 34 SAA Converters had CSF Aβ42 levels above the upper technical limit of 1700 pg/mL, therefore missing SILA EAOA. Closed and open circles indicate CSF p-tau181 positive and negative participants at the time of SAA conversion, respectively. Vertical dashed lines represent the median (interquartile range) for the timing of the CSF p-tau181/Aβ42 positivity relative to CSF α-syn SAA phenoconversion time within each diagnostic group. Aβ, amyloid beta; α-syn, α-synuclein; CSF, cerebrospinal fluid; CU, cognitively unimpaired; EAOA, estimated amyloid onset age; MCI, mild cognitive impairment; p-tau181, phosphorylated tau181; SAA, seed amplification assay; SILA, sampled iterative local approximation

Nevertheless, supporting the hypothesis of amyloid's influence on α-syn, our findings show that the advent of α-syn pathology appears to be influenced by pre-existing amyloid deposits, with its onset further modulated to some extent by *APOE* ε4 homozygosity ($P = 0.078$). However, it is important to note that our results do not indicate a significant impact of SAA positivity on its own in the conversion to biomarker positivity for CSF Aβ42 or p-tau181. This suggests that the effect of amyloid on α-syn is likely unidirectional, without evidence of a reciprocal relationship.

Consistent with neuropathological evidence from autopsy studies,[2,5,6,39] which show a higher prevalence of α-syn changes in brains with abundant neuritic plaques but not necessarily correlating with the severity of NFTs, our findings did not identify a strong association between SAA phenoconversion and CSF p-tau181 positivity as a tau biomarker. We previously reported an inverse relationship between SAA+ prevalence and CSF p-tau181 levels and flortaucipir PET burden in the dementia stage of the disease.[19] Consistently, although it did not reach significance due to limited sample size ($P = 0.12$; Figure S9 in supporting information), the risk for SAA phenoconversion within CSF Aβ42+ Dementia was marginally associated with lower levels of CSF p-tau181 (HR: 0.21; 95% CI: 0.03–1.50). An important limitation of our study is the use of CSF p-tau181 as a surrogate biomarker for tau pathology, given its closer relationship to soluble tau fragments that may reflect a reaction to amyloid plaques or to soluble amyloid species in the plaque penumbra. Tau PET imaging was available for only a limited number of participants, making it difficult to thoroughly investigate the association between SAA and tau burden. In particular, although only 37% of Stable SAA+, 34% of Stable SAA−, and 41% of SAA Converters had cross-sectional tau PET data, the longitudinal availability was even more limited (18% of Stable SAA+, 21% of Stable SAA−, and 26% of SAA Converters). While augmenting the tau PET data is not feasible, there are novel plasma biomarkers that show great promise in capturing the presence and, to some extent, the burden of tau pathology. These promising

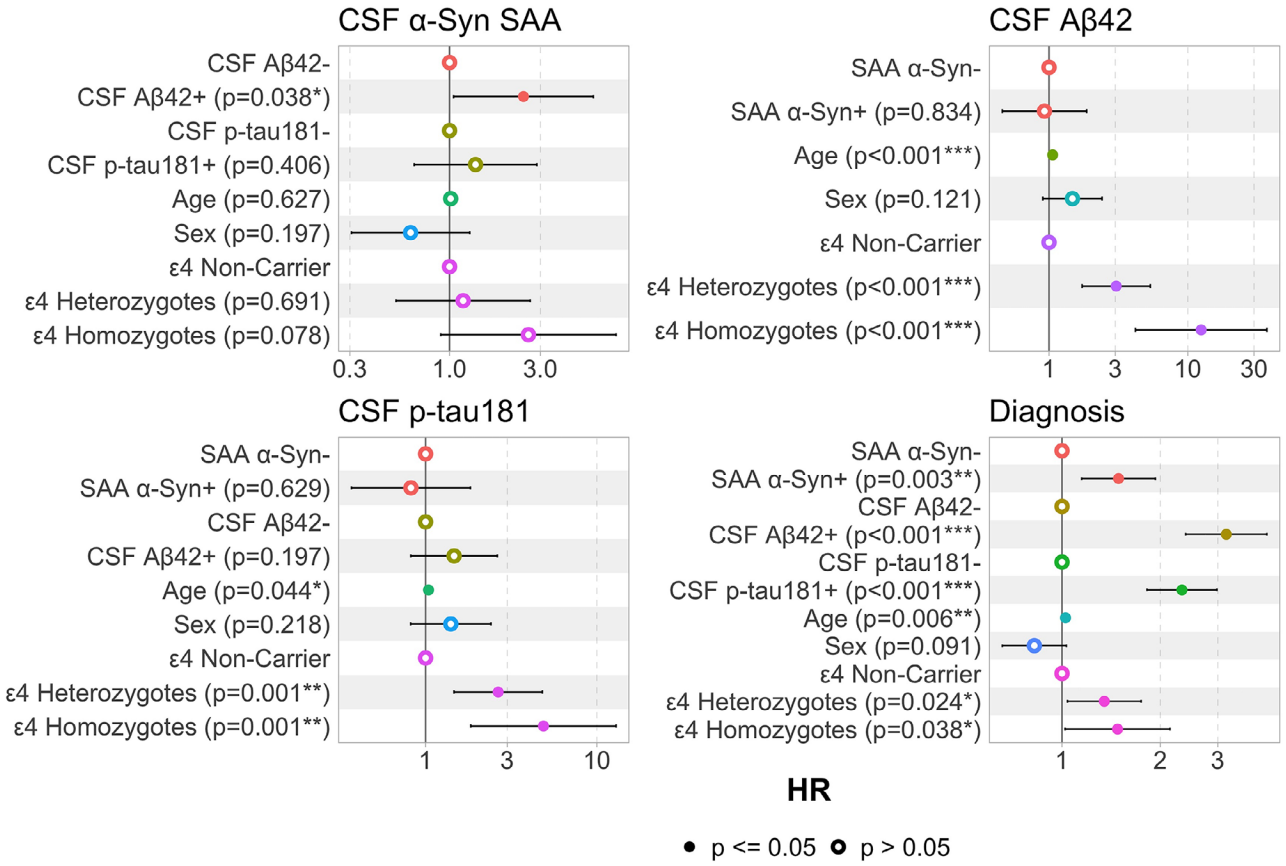

FIGURE 5 Hazard ratios for predictors from adjusted Cox regression models predicting conversion in CSF α-syn SAA positivity, CSF Aβ42 positivity, CSF p-tau181 positivity, and change in clinical diagnosis (whether from CU to MCI/Dementia or MCI to Dementia). Aβ, amyloid beta; α syn, α-synuclein; CSF, cerebrospinal fluid; CU, cognitively unimpaired; HR, hazard ratio; MCI, mild cognitive impairment; p-tau181, phosphorylated tau181; SAA, seed amplification assay

novel plasma biomarkers for tau pathology will be considered in future studies as they become available. Nevertheless, immunostaining of brain tissues for tau and α-syn antibodies has revealed a higher burden of α-syn pathology in AD with LBs (AD+LB) compared to PD dementia cases. Interestingly, the pathological tau load was found to be similar or even slightly lower in AD+LB compared to AD alone, with co-localization of phosphorylated tau and α-syn within astrocytes in the middle temporal gyrus.[40] In contrast, autopsy literature on PD frequently reports concurrent deposition of α-syn and tau, reflecting a complex interplay that may differ from AD pathology. Reviews[38] posit that α-syn may interact more significantly with tau than with amyloid, emphasizing the importance of protein species, whether soluble or insoluble, in the early seeding events of these pathologies. This suggests a potential divergence in the pathophysiological mechanisms underlying these proteinopathies in different neurodegenerative disease presentations.

Taken together, our findings suggest possible interactions between AD and LB pathologies, potentially involving crosstalk mechanisms and genetic predispositions such as the *APOE* ε4 allele. These interactions may be influenced by compromised proteostasis, raising the question of whether α-syn preferentially engages with one pathological species over another, or if it is merely the timing of the emergence of these pathologies that dictates their interrelationship. It is important to note that our study was not designed or powered to understand the mechanism by which amyloid and α-syn as well as tau pathologies interact and coexist in the brain of AD cases. Nevertheless, prior work has suggested that interaction between amyloid and α-syn may reduce protein clearance, activate inflammatory processes, increase tau phosphorylation, and enhance aggregation of each other.[41] Notably, the biomarkers currently used for detecting these pathological changes may have inherent limitations, such that measurable changes in CSF Aβ42 levels may precede alterations in p-tau levels.

Our results show that changes in the onset of α-syn seeds in CSF within phenoconverters are associated with changes in seed amplification parameters. Although this does not mean that the onset of α-syn seeds in CSF can be accurately predicted on the individual level, it suggests that SAA might respond to early changes in CSF that should be further studied. One interpretation of our findings could be a potential association between amplification time at the emergence of LB pathology in the context of co-pathologies while in later stages SAA features remain stable over time. The faster aggregation kinetics with time from the initial SAA positivity might also be related to many factors including changing number of α-syn aggregates over time, changes in the biophysical properties of the seeds, as well as the presence of lipids,

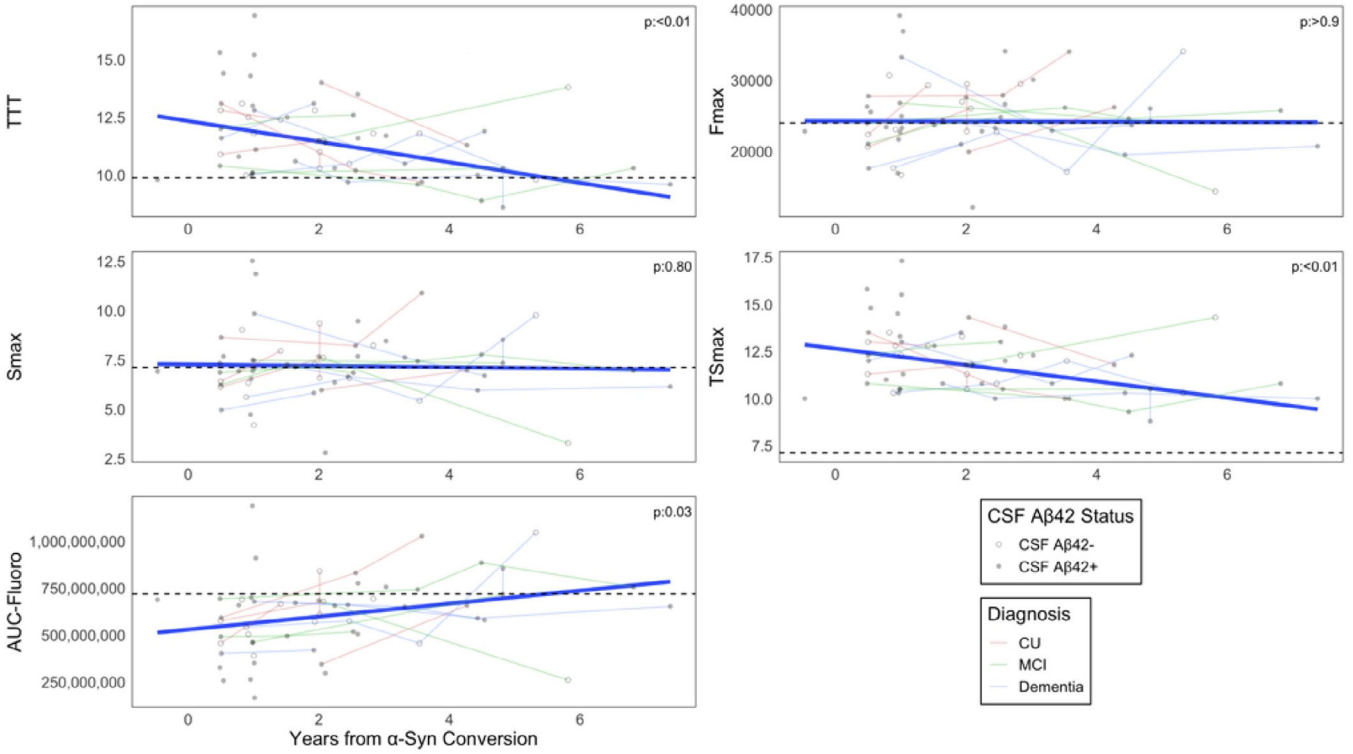

FIGURE 6 Change in SAA kinetic parameters over years after the SAA phenoconversion time. Horizontal dashed lines represent the average levels of kinetic parameters within stable SAA+ participants. Aβ, amyloid beta; AUC-Fluoro, area under the signal versus time reaction curve in relative fluorescence units; CSF, cerebrospinal fluid; CU, cognitively unimpaired; Fmax, maximum fluorescence; MCI, mild cognitive impairment; SAA, seed amplification assay; Smax, maximum slope; TSmax, time to Smax; TTT, time to threshold

proteins, or other compounds in the CSF. There is no clear evidence that levels of α-syn seeds increase in CSF mirroring accumulation in the brain and that amplification parameters correlate with the number of α-syn seeds. In fact, the evidence suggests that the CSF milieu influences the speed of amplification.[18] Thus, the objective of the substudy focusing on the kinetic parameters was to assess clinically meaningful associations. Accordingly, recent studies suggest that SAA kinetic parameters, particularly TTT and TSmax, were associated with clinical and cognitive characteristics of PD and DLB patients, measured by the Unified Parkinson's Disease Rating Scale part (UPDRS) III and Montreal Cognitive Assessment.[42,43]

Limitations of the study include the exclusion of individuals with prominent DLB clinical features from ADNI, the lack of measures associated with PD/DLB clinical features (e.g., UPDRS and smell tests), as well as the limited ancestral diversity in the available ADNI cohorts, and SAA kinetic parameters not quantified for SAA− participants. In addition, amyloid PET and tau PET imaging, gold-standard biomarkers for AD amyloid and tau pathologies, were only available for a limited number of study participants (51% and 28%, respectively).

5 | CONCLUSION

In summary, our results highlight the potential for interplay between amyloid and α-syn and their impact on disease progression, emphasizing the importance of further investigation into their underlying mechanisms in the context of co-pathologies of AD. The longitudinal tracking of CSF α-syn SAA positivity alongside other biomarkers prompts consideration of differential diagnosis between AD and other neurodegenerative conditions, especially DLB. Moving forward, it is imperative to broaden the detection of LB pathology in diverse cohorts to enhance our understanding of the causes and triggers of AD and LB co-pathologies.

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CONFLICT OF INTEREST STATEMENT

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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

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

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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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