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RESEARCH ARTICLE

Misfolded α-synuclein co-occurrence with Alzheimer's disease proteinopathy

Erin M. Jonaitis[1,2,3] | Karen MacLeod[4] | Jennifer Lamoureux[4] | Beckie Jeffers[1,2,3] | Rachel L. Studer[1] | John Middleton[4] | Rachael E. Wilson[2] | Nathaniel A. Chin[3] | Ozioma C. Okonkwo[2,3] | Barbara B. Bendlin[2,3] | Sanjay Asthana[2,3] | Cynthia M. Carlsson[1,2,3] | Catherine L. Gallagher[5] | Bruce Hermann[5] | Sean McEvoy[4] | Gwendlyn Kollmorgen[6] | Henrik Zetterberg[2,7,8,9,10,11] | Luis Concha-Marambio[4] | Sterling C. Johnson[1,2] | Russ M. Lebovitz[4] | Rebecca E. Langhough[1,2,3]

[1]Wisconsin Alzheimer's Institute, School of Medicine and Public Health, University of Wisconsin–Madison, Madison, Wisconsin, USA

[2]Wisconsin Alzheimer's Disease Research Center, School of Medicine and Public Health, University of Wisconsin–Madison, Madison, Wisconsin, USA

[3]Department of Medicine, Division of Geriatrics and Gerontology, School of Medicine and Public Health, University of Wisconsin–Madison, Madison, Wisconsin, USA

[4]Amprion Inc., San Francisco, California, USA

[5]Department of Neurology, School of Medicine and Public Health, University of Wisconsin–Madison, Madison, Wisconsin, USA

[6]Roche Diagnostics GmbH, Penzberg, Germany

[7]Department of Psychiatry and Neurochemistry, Institute of Neuroscience and Physiology, the Sahlgrenska Academy at the University of Gothenburg, Mölndal, Sweden

[8]Clinical Neurochemistry Laboratory Hus V3, Sahlgrenska University Hospital, Mölndal, Sweden

[9]Department of Neurodegenerative Disease, UCL Institute of Neurology, London, UK

[10]UK Dementia Research Institute at UCL, London, UK

[11]Hong Kong Center for Neurodegenerative Diseases, Hong Kong, China

Correspondence
Erin M. Jonaitis, Wisconsin Alzheimer's Institute, School of Medicine and Public Health, University of Wisconsin–Madison, 600 Highland Avenue CSC K6/440 MC4670 Madison, WI 53792, USA.
Email: jonaitis@wisc.edu

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Abstract

INTRODUCTION: Multi-etiology dementia necessitates in vivo markers of copathologies including misfolded α-synuclein (syn). We measured misfolded syn aggregates (syn-seeds) via qualitative seed amplification assays (synSAA) and examined relationships with markers of Alzheimer's disease (AD).

METHODS: Cerebrospinal fluid (CSF) was obtained from 420 participants in two AD risk cohorts (35% male; 91% cognitively unimpaired; mean [standard deviation] age, 65.42 [7.78] years; education, 16.17 [2.23]) years). synSAA results were compared to phosphorylated tau (T), amyloid beta (A), and clinical outcomes. Longitudinal cognition was modeled with mixed effects.

RESULTS: Syn positivity (synSAA+) co-occurred with T (in synSAA+ vs. synSAA–, 36% vs. 20% T+; P$p = 0.011$) and with cognitive impairment (10% vs. 7% mild cognitive impairment; 10% vs. 0% dementia; $p = 0.00050$). synSAA+ participants' cognitive

performance declined ≈ 40% faster than synSAA− for Digit Symbol Substitution, but not other tests.

DISCUSSION: Findings support prevalent syn copathology in a mostly unimpaired AD risk cohort. Relationships with progression should be evaluated once more have declined.

KEYWORDS

α-synuclein, Alzheimer's disease, amyloid, cerebrospinal fluid, Lewy body dementia, seed amplification assay, tau

Highlights

- In a middle-aged sample, misfolded α-synuclein (syn) co-occurred with phosphorylated tau181 (T).
- syn+/T+ status was linked with higher levels of other cerebrospinal fluid biomarkers.
- syn+ individuals were more likely than syn− to be cognitively impaired.
- syn+ status was linked to faster decline on an executive function task.

1 | INTRODUCTION

Post mortem neuropathological investigations of patients with neurodegenerative disorders, including Alzheimer's disease (AD), have revealed the coexistence of different proteinopathies. This profile, referred to as mixed dementia, may relate to patients' presenting and evolving symptom complex, and may also have implications for treatment.[1] Identifying the underlying pathologies in persons with dementia or in those at risk is of high priority, both to understand the biological disease processes and to select individuals for preventive and therapeutic clinical trials. Although much progress has been made in identifying biomarkers for amyloid beta (A) and tau (T), tests for other proteinopathies have lagged.

Recently an α-synuclein (syn) seed amplification assay (synSAA) has been developed to identify individuals with underlying Lewy body disease (LBD) by detection of misfolded syn aggregates (syn-seeds) in cerebrospinal fluid (CSF).[2,3] Reported concordance between synSAA results and neocortical neuropathology was high in two studies: 97% to 100% of the true cases with diffuse syn pathology were detected by the assay (synSAA+), and 97% to 98.1% of the cases without syn pathology were correctly identified as negative (synSAA−) when using either an early version of the validated test (≈ 7 day incubation)[4] or more recently validated rapid test conditions (≈ 20 hour incubation).[5] Other studies have shown that synSAA combined with other biomarker tests can be used to predict rate of decline in patients with and without AD co-pathology.[6,7] In fact, synSAA positivity has been shown to increase from mild cognitive impairment (MCI; A−T−) to AD dementia (A+T+), and to associate with the AD phenotypical presentation.[5,8]

In this study, we investigated the relationship between syn-seeds and concurrent AD biomarkers, clinical characteristics, and cognitive trajectories in predominantly unimpaired community-based cohorts enriched for AD risk. Specifically, we examined whether synSAA status was associated with elevated prevalence of concurrent CSF AD biomarker abnormalities or cognitive status, physical symptoms associated with Parkinson's disease (PD) or dementia with Lewy bodies (DLB), or other concurrent characteristics. In addition, we examined whether syn-seeds, with or without comorbid AD biomarkers, were associated with faster cognitive decline.

2 | METHODS

2.1 | Participants

Data were obtained and combined from participants in the Wisconsin Alzheimer's Disease Research Center (WADRC), a community-based longitudinal cohort of individuals across the AD continuum,[9] and the Wisconsin Registry for Alzheimer's Prevention (WRAP), a similar community cohort of individuals who were non-demented at baseline.[10] Both cohorts are enriched by design for a positive parental family history of AD. Participants were eligible for inclusion if they had had a lumbar puncture (LP) collected under a standard centerwide LP protocol, between December 5, 2018, and June 30, 2022; had consented to sample reuse; and had adequate samples available for the present analysis.

2.2 | CSF collection

CSF collection is described in detail elsewhere.[11] Briefly, CSF was obtained via gentle extraction using a Sprotte 24- or 25-gauge atraumatic spinal needle. Samples were gently mixed and centrifuged at

2000 × g for 10 minutes at 4 ° C within 30 minutes of extraction. After centrifugation, samples were aliquoted and stored in low-binding 0.5 mL tubes at −80 ° C.

2.3 | synSAA

CSF samples were analyzed by the Amprion Clinical Laboratory using a rapid qualitative synSAA validated for clinical use under Clinical Laboratory Improvement Amendments (CLIA)/College of American Pathologists certifications. Based on information available on the manufacturer's website, using pathology as a comparator, the diagnostic sensitivity of the test for detection of syn aggregates in CSF is 100% in patients with diffuse neocortical and brainstem predominant Lewy pathology (95% confidence interval [CI] = 82.4%–100.0%) and 64.7% in patients with limbic, amygdala predominant, or olfactory bulb Lewy pathology (95% CI = 38.3%–85.8%); specificity is 97.0% (95% CI = 84.2%–99.9%). Each blinded sample was analyzed in triplicate (40 μ L CSF per well) in a 96-well plate with a final reaction volume of 100 μ L. The reaction mixture consisted of 0.3 mg/mL rec-syn in 100 mM PIPES pH 6.50, 0.44 M NaCl, 10 μ M ThT, and 0.1% Sarkosyl, with two 1/8-inch silicon nitride beads per well. Plates were sealed using an Optical Adhesive Film, placed in a BMG LABTECH FLUOStar Ω Microplate Reader, and incubated at 42 ° C with intermittent shaking (800 rpm orbital shaking for 1 minute followed by 14 minutes of rest). Fluorescence readings (excitation wavelength, 440 nm; emission wavelength, 490 nm) were performed after every shaking cycle. After 20 hours of shaking/incubation, the maximum relative fluorescence unit (RFU; Fmax) of each well was determined, and CSF samples were classified based on a pre-established proprietary algorithm.[8] Based on this algorithm, if all three replicates return a positive result, the sample is classified as Detected; if 0 or 1 replicates return a positive result, the sample is classified as Not Detected; and if 2 replicates return a positive result the sample is classified as Indeterminate. The Detected category is further subdivided on the basis of Fmax into Detected-1 (syn seeding aggregates detected; amplification profile consistent with that found predominantly in subjects with neuronal synuclein disease) and Detected-2 (syn seeding aggregates detected; amplification profile consistent with that found predominantly in patients with multiple system atrophy). This clinical version of the assay was performed according to standard operating procedures in accordance with CLIA regulations.

2.4 | AD and NeuroToolKit biomarker assays

AD biomarkers were assayed at the Clinical Neurochemistry Laboratory in Gothenburg, Sweden, using fully automated electrochemiluminescent immunoassays. CSF levels of amyloid beta 42 (Aβ42) and phosphorylated tau 181 (p – tau_{181}) were measured using the in vitro diagnostic (IVD) Elecsys β-amyloid(1–42) CSF (second-generation) and Phospho-Tau(181P) CSF assays, and Aβ_{40} levels were measured using the robust prototype assay Elecsys β-amyloid(1–40). (Note that in the 2024 revision of the AD biomarker framework, CSF p-tau181 is considered a marker of early rather than late disease; see further commentary in the Discussion section.) These three measurements were taken on a Cobas e 601 analyzer (Roche Diagnostics International Ltd). CSF levels of neurofilament light chain (NfL), glial fibrillary acidic protein (GFAP), neurogranin (Ng), soluble triggering receptor expressed on myeloid cells (sTREM2), and chitinase-3-like protein 1 (YKL-40) were measured using the NeuroToolKit (NTK), a panel of exploratory robust prototype assays, on a Cobas e 411 analyzer (Roche Diagnostics International Ltd). Derivation of cutpoints for AD biomarker positivity has been described elsewhere.[12] Briefly, thresholds for two ratios, Aβ42/40 and p – tau_{181}/Aβ42, were determined by fitting receiver operating characteristic curves against an amyloid positron emission tomography PET criterion. The first of these ratios is often used as an indicator of amyloid positivity; the second has been variously construed as an amyloid positivity marker[12] or as a single-dimensional marker of overall AD pathology.[13] The threshold for p – tau_{181} was defined at the upper bound of a 95% CI on the distribution of p – tau_{181} levels in a subset of 223 young (mean age = 55), cognitively unimpaired (CU), CSF amyloid-negative participants.

RESEARCH IN CONTEXT

1. **Systematic review**: Using PubMed, we searched for other publications mentioning qualitative α-synuclein (syn) seed amplification assays in both the abstract and the references section. Although the assay is new, we found several recent publications applying this assay to cohorts with both unimpaired and impaired participants and have cited them appropriately.
2. **Interpretation**: Multi-etiology neurological disorders are a critical challenge in clinical care and trial design. A novel assay for misfolded syn was associated with several variables of interest, including Alzheimer's disease, other cerebrospinal fluid biomarkers, cognitive impairment, and executive function decline in a late-middle-aged cohort. Similarities and differences between our findings and others are discussed.
3. **Future directions**: Once more of the cohort has reached clinical endpoints, future analyses will assess the prognostic value of mid-life syn aggregates for clinical status. In addition, cognitive decline patterns in continuous outcomes can be used to inform clinical trial design.

2.5 | Cognitive assessment

Participants in each cohort complete neuropsychological tests every 1 to 2 years. The WRAP battery has been described in detail elsewhere.[10] The WADRC battery includes several tests that are

core to the WRAP battery, as well as those specified in the National Alzheimer's Coordinating Center Uniform Data Sets.[14] For the present study, we used three cognitive tests that measure aspects of executive function, including visuomotor speed: the Trail-Making Test, Parts A and B, and their difference;[15] Wechsler Adult Intelligence Scale-Digit Span Backward;[16,17] and Digit Symbol Substitution.[16] We also included a cognitive composite that is sensitive to cognitive decline associated with AD, a three-test Preclinical Alzheimer Cognitive Composite (PACC-3)[18,19] comprising the sum of learning trials on the Rey Auditory Verbal Learning Test,[20] delayed performance on a story recall task,[14,21] and Letter Fluency from the Controlled Oral Word Association Test.[22] Scores were equated using published crosswalks where available, and internal equipercentile mappings otherwise.[23,24]

Cognitive status was assigned at each neuropsychological assessment via a multidisciplinary clinical consensus conference. Consensus review procedures are similar across cohorts; however, due to the high prevalence of CU individuals in the WRAP cohort, the WRAP consensus process begins with an algorithmic screening in which only those who fall at least 1.5 standard deviations below robust internal norms and those whose raw test scores or study partner questionnaire responses exceed thresholds for concern are reviewed in detail. The remainder are assigned a status indicating no clinically significant impairment.[25]

2.6 | Self-reported health variables

Along with cognitive assessment, participants complete detailed health questionnaires every 1 to 2 years. Participant physical activity was estimated in metabolic equivalent hours per week based on responses to questions about mild, moderate, and vigorous activity and walking outside the home. Recent-onset depression was defined as any first report of depression at a visit after baseline and within 5 years of LP.

2.7 | Clinical evaluation

Clinical symptoms were assessed by a nurse practitioner following the standard procedures associated with the National Alzheimer's Coordinating Center Uniform Data Set version 3, forms B8 and B9. The presence of a symptom at any visit was counted as a positive sign.

2.8 | Statistical methods

Primary analyses examined relationships between binary synSAA status and other variables. synSAA results of Detected-1 were coded as synSAA+, Not Detected as synSAA−, and both Detected-2 and Indeterminate as missing values. The co-occurrence in CSF of syn-seeds (based on positive/negative status as detected via synSAA) with various AD biomarkers (based on positive/negative status for amyloid beta, Aβ42/40 and p − tau$_{181}$/Aβ42, and phosphorylated tau, p − tau$_{181}$) and clinical symptoms (any neurological finding; Parkinsonian signs; rapid eye movement behavior disorder; recent-onset depression; clinically significant cognitive impairment as adjudicated by consensus conference) was examined with chi-square tests. Confidence intervals for proportions were estimated using Agresti–Coull intervals.[26] Group differences in cognition and exercise were examined with *t* tests and Mann–Whitney *U* tests, respectively. For these analyses, missing data were excluded on a pairwise basis. Sensitivity analyses assessed the dependence of our findings on age and cognitive status. Chi-square tests were repeated in subsets stratified by age into younger (< 65) and older (≥ 65), and in the subset who were CU at the time of LP. In addition, we used logistic regression to model binary synSAA (synSAA+ = 1) as a function of these biomarkers and clinical predictors after controlling for age.

Exploratory analyses compared levels of NTK biomarkers between groups defined on the basis of joint synSAA status and T status. Continuous biomarker outcomes were modeled using separate one-way analyses of variance (synSAA−/T−; synSAA−/T+; synSAA+/T−; synSAA+/T+). Initial examination revealed an extreme outlier on NfL (result > 35 times the median result); this data point was removed for a post hoc analysis.

Cognitive trajectories were estimated using linear mixed-effects models of Trail-Making Test B, the Trail-Making Test difference score (B − A), Backward Digit Span Backward, Digit Symbol Substitution, and a PACC-3. All models included subject-level random intercepts and age slopes with unstructured covariance. The fixed effects of age were modeled by centering age and including up to a quadratic term. Covariates included sex, education, and number of prior cognitive assessments per protocol (to adjust for practice effects). For each outcome, we examined whether last known synSAA status modified age trajectories (models 1A–4A). In a second set of models (1B–4B), we further examined syn pathology effects after adjusting for separate age interactions with binarized Aβ42/40 and p − tau$_{181}$ statuses. Finally, an exploratory set of models (1C–4C) used p − tau$_{181}$/Aβ42 as a single-dimensional indicator of AD pathology to ask whether AD biomarker status and synSAA status interact with age or each other to predict cognition. For this analysis, pairwise contrasts of simple slopes in all four biomarker groups were estimated using Tukey adjustment for multiple comparisons. In all three sets of models, non-significant interaction terms were removed ($p > 0.1$). Biomarker negative status was the reference category for each marker. For these analyses, participants missing data on any predictor were excluded on a listwise basis to facilitate nested model comparisons within outcomes (i.e., comparisons of models A and B for a given outcome). However, all participants with at least one observation on any outcome were included in analyses of that outcome. Sensitivity analyses repeated these procedures in the subgroup who were CU at the time of LP.

Statistical analyses were conducted in R.[27] Mixed effects models were fit with the lmerTest package[28] and marginal effects were estimated using the packages ggeffects[29] and emmeans.[30]

3 | RESULTS

3.1 | Participants

Five hundred forty-three LPs were performed during the relevant period, of which 515 samples on 420 participants had sufficient volume remaining for synSAA analysis. This included 214 participants from WADRC (at visit closest to LP, 183 CU; 19 MCI; 6 dementia) and 206 participants from WRAP (195 CU; 11 MCI; 0 dementia). Two participants had a synSAA result of Indeterminate, and one had a result of Detected-2, and were excluded from further analyses. Among those with synSAA results meeting criteria for inclusion, 12% were identified as synSAA+. Participant characteristics for those with includable synSAA results are shown in Table 1, overall and by synSAA status. The overall sample had more women than men (35% male), but the sex balance differed by synSAA status (synSAA+, 49% male; synSAA−, 33% male). Further, synSAA+ participants tended to be older at LP (synSAA+, 69.04 [7.47]; synSAA−, 64.93 [7.70]). No differences in education or apolipoprotein E genotype were seen ($p > 0.05$).

3.2 | synSAA and AD biomarkers

Proportions of overall AD biomarker positivity among the 417 participants observed with includable results for synSAA are shown in Table 1. Cross-tabs of A and T results in the full sample were as follows: A−/T−, 255 (63%); A−/T+, 23 (6%); A+/T−, 66 (16%); A+/T+, 61 (15%). Proportions in the CU-only sensitivity subset were similar, but featured relatively fewer A+/T+ (A−/T−, 246 [68%]; A−/T+, 19 [5%]; A+/T−, 60 [17%]; A+/T+, 38 [10%]). In the full set, 23 (6%; 95% CI, 4%–8%) were positive for syn-seeds and any of the three AD CSF markers; 26 (6%; 4%–9%) were positive for syn-seeds only, and not any of the AD markers; 132 (32%; 27%–36%) were positive for any of the AD biomarkers, but negative for syn-seeds; and 236 (57%; 52%–61%) were negative for all four biomarkers. Among 92 participants with more than one synSAA analysis, none were discordant, suggesting medium-term stability of this measure (range of inter-LP intervals observed: 0–3.1 years).

synSAA status was associated with p – tau_{181} status ($p = 0.011$), and weakly with p – tau_{181}/Aβ42 status ($p = 0.067$), but not with Aβ42/40 status ($p = 0.17$). Among p – tau_{181}+ participants, 17/88 (19%; 12%–29%) were also synSAA+, compared to 30/322 (9%; 7%–13%) p – tau_{181}− participants. For p – tau_{181}/Aβ42+ and p – tau_{181}/Aβ42−, the figures were 19/118 (16%; 10%–24%) and 27/287 (9%; 7%–13%), respectively. However, sensitivity analyses suggest that these relationships between synSAA and other biomarkers may be affected by age: after stratifying into older and younger subgroups, chi-square tests are no longer significant in either group (all $p > 0.1$). The reduction of N and the resulting loss of power may have influenced this shift; nevertheless, logistic regression models controlling for age produce similar findings, with no p values reaching statistical significance. In a similar vein, relationships with AD biomarkers were attenuated to non-significance in the CU-only group. p values for primary and sensitivity analyses are in Table S1 in supporting information; a forest plot of overall, age-stratified, age-adjusted, and CU-only confidence intervals for synSAA status by AD biomarker group is shown in Figure S1 in supporting information.

3.3 | synSAA and exploratory biomarkers

All markers except for NfL exhibited differences between some pairs of groups ($p < 0.0001$). GFAP levels were lower in synSAA−/T− participants than in either of the T+ groups. Ng and YKL-40 levels were lower in all T− groups than in all T+ groups, irrespective of synSAA status. sTREM2 levels were lower in synSAA−/T− participants than in either of the T+ groups, and were lower in synSAA+/T− participants than in synSAA+/T+ participants. Examination of the NfL data revealed that one NfL observation had a value > 35 times as high as the median; a post hoc comparison removing this outlier revealed a pattern of group means for NfL similar to Ng and YKL-40. Group comparisons excluding the outlier are shown in Table 2 and Figure 1.

3.4 | synSAA, clinical impairment, and health

Compared to synSAA− participants, synSAA+ had a higher incidence of cognitive impairment as judged by consensus conference ($p = 0.00050$; synSAA+, MCI = 5/49 [10%; 4%–22%], dementia = 5/49 [10%; 4%–22%]; synSAA−, MCI = 25/362 [7%; 5%–10%], dementia = 1/362 [0%; 0%–2%]). Relationships between synSAA and other clinical variables are described in the supporting information. p values for primary and sensitivity analyses are in Table S1; a forest plot of overall and age-stratified confidence intervals for synSAA status by these groupings is shown in Figure S1.

Self-reported physical activity did not differ significantly between synSAA+ and synSAA− participants (synSAA+, median [range] = 12.00 [0–39.75]); synSAA−, median [range] = 16.00 [0–83.00]; $p = 0.099$).

3.5 | Syn-seeds and cognitive trajectories

Availability of cognitive follow-up depended on the outcome, with the least for Trail-Making Test difference (N_{sub}= 387; N_{obs}= 2675) and Digit Symbol Substitution (N_{sub}= 388; N_{obs}= 2079), and the most for Digit Span Backward (N_{sub}= 403; N_{obs}= 2972). Cognitive statuses at baseline in the primary analysis set were as follows: CU, 375; MCI, 22; dementia, 4.

Full results of cognitive trajectory analyses are shown in Tables 3 and 4 by each of the five cognitive outcomes and two model sets: Table 3, model A set: covariates + age + age^2 + synSAA + (synSAA × age) + (synSAA × age^2); Table 4, model B set: covariates + age + age^2 synSAA + (synSAA × age) + (synSAA × age^2) + Aβ42/40 + (Aβ42/40 × age) + (Aβ42/40 × age^2) + pTau_{181} + (pTau_{181} × age) + (pTau_{181} × age^2). Each table contains the results from both primary (all participants) and sensitivity (CU only) analyses. Simple age slopes for synSAA− and synSAA+ estimated from the model B sets

TABLE 1 Sample characteristics of participants, overall and by synSAA status.

Variable	Overall	synSAA−	synSAA+	*p*
N	417	368/417 (88%; 85%–91%)	49/417 (12%; 9%–15%)	–
Demographics				
Sex, male, *N* (%)	146/417 (35%; 31%-40%)	122/368 (33%; 29%–38%)	24/49 (49%; 36%–63%)	0.038
URG member, yes, *N* (%)	48/417 (12%; 9%–15%)	46/368 (12%; 9%–16%)	2/49 (4%; 0%–14%)	0.090
Education, years, mean (SD)	16.17 (2.23)	16.14 (2.21)	16.39 (2.39)	0.50
APOE ε4+, *N* (%)	164/360 (46%; 40%–51%)	140/315 (44%; 39%–50%)	24/45 (53%; 39%–67%)	0.33
Age at LP, years, mean (SD)	65.42 (7.78)	64.93 (7.70)	69.04 (7.47)	0.00064
Time between LP and cognition, years, mean (SD)	0.026 (0.49)	0.030 (0.52)	0.0012 (0.0033)	0.30
AD biomarkers				
Aβ42/40 positive, *N* (%)	127/412 (31%; 27%–35%)	108/364 (30%; 25%–35%)	19/48 (40%; 27%–54%)	0.17
p − tau_{181}/Aβ42 positive, *N* (%)	118/405 (29%; 25%–34%)	99/359 (28%; 23%–32%)	19/46 (41%; 28%–56%)	0.067
p − tau_{181} positive, *N* (%)	88/410 (21%; 18%–26%)	71/363 (20%; 16%–24%)	17/47 (36%; 24%–51%)	0.011
Cognitive status at nearest assessment				0.00050
Cognitive status, CU, *N* (%)	375/411 (91%; 88%–94%)	336/362 (93%; 90%–95%)	39/49 (80%; 66%–89%)	
Cognitive status, MCI, *N* (%)	30/411 (7%; 5%–10%)	25/362 (7%; 5%–10%)	5/49 (10%; 4%–22%)	
Cognitive status, dementia, *N* (%)	6/411 (1%; 1%–3%)	1/362 (0%; 0%–2%)	5/49 (10%; 4%–22%)	
Other health variables				
Exercise, MET hours per week, mean (SD)	15.75 (0–83.00)	16.00 (0–83.00)	12.00 (0–39.75)	0.099

Note: Three participants were excluded ($N = 1$ with results suggesting multiple system atrophy; $N = 2$ with indeterminate results).
Abbreviations: Aβ42/40, positive amyloid beta 42/40 status; *APOE* ε4, carriage of at least one apolipoprotein E ε4 allele; CU, cognitively unimpaired; LP, lumbar puncture; MCI, mild cognitive impairment; MET hours, metabolic equivalent hours per week; p − tau_{181}, positive phosphorylated tau-181 status; pTau_{181}/Aβ42, positive p-tau to amyloid ratio; REM, rapid eye movement; SD, standard deviation; synSAA, α-synuclein seed amplification assay status; URG, underrepresented racial group membership.

TABLE 2 Comparison of robust prototype biomarkers from the NKT in groups defined by synSAA status and T status.

Variable	synSAA−/T−	synSAA+/T−	synSAA−/T+	synSAA+/T+	*p*	Contrasts
NfL	120.45 (216.74)	117.30 (44.02)	166.42 (77.44)	187.49 (66.97)	<0.0001	synSAA−/T− < synSAA−/T+; synSAA−/T− < synSAA+/T+; synSAA+/T− < synSAA−/T+; synSAA+/T− < synSAA+/T+
GFAP	9.50 (3.55)	10.86 (3.21)	12.38 (3.89)	13.60 (6.12)	<0.0001	synSAA−/T− < synSAA−/T+; synSAA−/T− < synSAA+/T+
Ng	798.81 (229.94)	822.77 (268.41)	1466.87 (375.48)	1522.33 (549.18)	<0.0001	synSAA−/T− < synSAA−/T+; synSAA−/T− < synSAA+/T+; synSAA+/T− < synSAA−/T+; synSAA+/T− < synSAA+/T+
sTREM2	8.79 (2.78)	9.42 (2.56)	11.06 (3.41)	12.96 (3.88)	<0.0001	synSAA−/T− < synSAA−/T+; synSAA−/T− < synSAA+/T+; synSAA+/T− < synSAA+/T+
YKL-40	159.85 (56.43)	171.38 (63.45)	233.55 (75.09)	269.47 (93.81)	<0.0001	synSAA−/T− < synSAA−/T+; synSAA−/T− < synSAA+/T+; synSAA+/T− < synSAA−/T+; synSAA+/T− < synSAA+/T+

Note: One NfL observation whose value was > 35 times the median value was removed for comparisons; if included, NfL comparisons are non-significant, $p > 0.10$.

Abbreviations: GFAP, glial fibrillary acidic protein; NfL, neurofilament light chain; Ng, neurogranin; NKT, NeuroToolKit; sTREM2, soluble triggering receptor expressed on myeloid cells 2; synSAA, α-synuclein seed amplification assay status; T, phosphorylated tau 181 status; YKL-40, chitinase-3-like protein 1.

are depicted for each outcome in Figure 2 and Figure S2 in supporting information, paneling each figure by A/T statuses.

3.5.1 | Trail-Making Test Part B

In model 1A, the interaction estimates marginally more age-related worsening in synSAA+ than synSAA− ($\beta_{synSAA\times age} = 0.66$ seconds per year, $p = 0.076$). However, once AD biomarkers and their interactions with age terms are added in model 1B, $\beta_{synSAA\times age}$ is attenuated ($p > 0.1$), while both A+ and T+ statuses are associated with significantly worse age-related change ($\beta A\beta 42/40 \times age = 0.61$, $p = 0.032$; $\beta_{pTau181\times age^2} = 0.06$, $p = 0.0021$). Sensitivity analyses in the CU subgroup were broadly similar, with no moderation of age effects by synSAA status in either A or B model. In the CU-only models, the moderating effect of A+ status was also attenuated ($\beta A\beta 42/40 \times age = 0.52$, $p = 0.054$), although the effects of T+ status on age-related change remained similar ($\beta_{pTau181\times age^2} = 0.05$, $p = 0.016$).

3.5.2 | Trail-Making Test difference

In model 2A, the interaction estimates marginally more age-related worsening in synSAA+ than synSAA− ($\beta_{synSAA\times age} = 0.58$ seconds per year, $p = 0.092$). However, once AD biomarkers and their interactions with age terms are added in model 2B, $\beta_{synSAA\times age}$ is attenuated ($p > 0.1$), while both A+ status (but not T+ status) is associated with significantly greater acceleration in age-related change ($\beta A\beta 42/40 \times age^2 = 0.05$, $p = 0.0010$). Sensitivity analyses in the CU subgroup were broadly similar, with no moderation of age effects by synSAA status in either A or B model, but effects of A+ status remaining similar ($\beta A\beta 42/40 \times age^2 = 0.04$, $p = 0.028$).

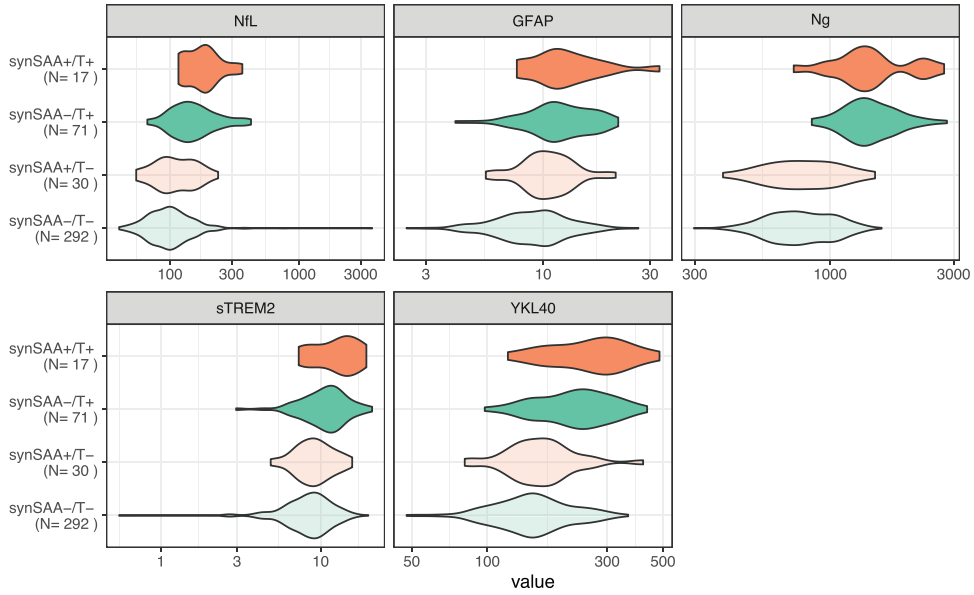

FIGURE 1 Violin plots of robust prototype biomarkers from the NTK in groups defined by synSAA status and T status. One NfL observation whose value was > 35 times the median value was removed to aid in visualization. GFAP, glial fibrillary acidic protein; NfL, neurofilament light chain; Ng, neurogranin; NTK, NeuroToolKit; sTREM2, soluble triggering receptor expressed on myeloid cells 2; synSAA, α-synuclein seed amplification assay status; T, phosphorylated tau 181 status; YKL-40, chitinase-3-like protein 1.

3.5.3 | Digit Span Backward

No significant differences in age-related change are seen by synSAA status in either model 3A or 3B. In model 3B, faster declines are seen for A+ versus A− (βAβ42/40 × age = −0.03, p = 0.050), but not for T+ versus T−. Sensitivity analyses with CU only show similar results for model 3A, but in model 3B, the moderating effect of A+ status on decline is not observed (βAβ42/40 × age = −0.03, p = 0.11) and so has been removed from the final sensitivity model.

3.5.4 | Digit Symbol Substitution

In model 4A, faster age-related declines were observed in synSAA+ compared to synSAA− ($\beta_{\text{synSAA×age}}$ = −0.24 points per year, p = 0.021). This effect was preserved after adjusting for AD biomarkers and their interactions with age in model 4B ($\beta_{\text{synSAA×age}}$ = −0.23 seconds per year, p = 0.026; at age 60, $\hat{\beta}_{\text{age|synSAA−}}$ = −0.57, CI = −0.69 to −0.44; $\hat{\beta}_{\text{age|synSAA+}}$ = −0.8, CI = −1.01 to −0.58). In addition, in Model 3B, faster age-related declines were seen in A+ versus A− ($\beta_{\text{synSAA×age}}$ = −0.21 points per year, p = 0.0039). Taken together, synSAA−/A− individuals showed the least decline per year on this test, while synSAA+/A+ individuals declined the fastest (at age 60, $\hat{\beta}_{\text{age|SAA−,A−}}$ = −0.46, CI = −0.59 to −0.33; $\hat{\beta}_{\text{age|SAA−,A+}}$ = −0.67, CI = −0.83 to −0.52; $\hat{\beta}_{\text{age|SAA+,A−}}$ = −0.69, CI = −0.91 to −0.47; $\hat{\beta}_{\text{age|SAA+,A+}}$ = −0.9, CI = −1.14 to −0.67). In sensitivity analyses, the moderating effect of synSAA+ status on age-related decline is virtually unchanged, both in models A ($\beta_{\text{synSAA×age}}$ = −0.25 points per year, p = 0.022) and B ($\beta_{\text{synSAA×age}}$ = −0.24 seconds per year, p = 0.025). Similarly, restricting to CU did not alter the moderating effect of A+ status on decline ($\beta_{\text{synSAA×age}}$ = −0.19 points per year, p = 0.015).

3.5.5 | PACC-3

No significant differences in age-related change are seen by synSAA status in either model 5A or 5B. In model 5B, faster declines are seen for T+ versus T− ($\beta_{\text{pTau}_{181}\text{×age}}$ = −0.7, p = 0.000031), but not for A+ versus A−. In sensitivity analyses within the CU only group, the pattern of effects of A and T reverses, with faster declines seen for A+ versus A− (βAβ42/40 × age = −0.33, p = 0.039), but not T+ versus T− ($\beta_{\text{pTau}_{181}\text{×age}}$ = −0.28, p = 0.097), which was then removed from the final sensitivity model.

In an exploratory analysis, we reparametrized the above models to use binary p − tau_{181}/Aβ42 as a single indicator of AD biomarker positivity, and created a four-level variable representing combined status on this variable with synSAA status. As in previous models, we tested the main effects of this variable and its interactions with both age terms. The patterns were largely similar to those in A and B models. However, sensitivity analyses often showed somewhat different patterns, with larger models being retained for two of the five variables. For Trail-Making Test Part B (Model 1C), the four-level interaction term with quadratic age was significant, so the full age structure was retained (4way × age^2, p = 0.017). Pairwise contrasts of the instantaneous age trends at selected ages indicated differences between the synSAA−/AD− group and the synSAA−/AD+ and synSAA+/AD+ groups, but only at older ages. Findings for the Trail-Making Test difference score (Model 2C) were very similar. Digit Span Backward results indicated no significant interactions with age (Model 3C). For Digit Symbol Substitution, only the linear age interaction was significant (4way × age, p = 0.0073), and pairwise trend contrasts once more indicated differences between the synSAA−/AD− group and the synSAA−/AD+ and synSAA+/AD+ groups (Model 4C), although after

TABLE 3 Results of nested linear mixed-effects models of cognitive tests associated with executive function (Trail-Making Test Part B; Trail-Making Test Parts B–A difference score; Digit Span Backward; Digit Symbol Substitution Test) and a global Preclinical Alzheimer Cognitive Composite (PACC-3).

	1A: All		1A: CU		2A: All		2A: CU		3A: All		3A: CU		4A: All		4A: CU		5A: All		5A: CU	
Predictors	**Est**	***p***	**Est**	***p***	**Est**	***p***	**Est**	***p***	**Est**	***p***	**Est**	***p***	**Est**	***p***	**Est**	***p***	**Est**	***p***	**Est**	***p***
Intercept	103.87	**<0.001**	101.46	**<0.001**	72.78	**<0.001**	70.10	**<0.001**	4.08	**<0.001**	3.87	**<0.001**	43.44	**<0.001**	44.94	**<0.001**	68.56	**<0.001**	68.26	**<0.001**
Sex (male)	7.34	**0.008**	4.96	0.052	5.05	**0.024**	3.44	0.103	−0.13	0.484	−0.05	0.808	−5.04	**<0.001**	−5.05	**<0.001**	−11.63	**<0.001**	−10.42	**<0.001**
Education (years)	−2.87	**<0.001**	−2.74	**<0.001**	−2.44	**<0.001**	−2.30	**<0.001**	0.20	**<0.001**	0.21	**<0.001**	0.95	**<0.001**	0.88	**<0.001**	2.08	**<0.001**	2.12	**<0.001**
Number of prior tests	−0.66	0.077	−0.59	0.116	−0.24	0.479	−0.10	0.769	0.11	**<0.001**	0.10	**<0.001**	0.36	**0.002**	0.35	**0.007**	2.30	**<0.001**	2.21	**<0.001**
Age (years), linear	0.70	**<0.001**	0.60	**0.001**	0.65	**<0.001**	0.54	**0.001**	−0.03	**0.031**	−0.02	0.125	−0.55	**<0.001**	−0.52	**<0.001**	−0.70	**<0.001**	−0.58	**<0.001**
Age (years), quadratic	0.06	**<0.001**	0.04	**<0.001**	0.05	**<0.001**	0.03	**<0.001**	−0.00	**<0.001**	−0.00	**0.012**	−0.01	**<0.001**	−0.01	**<0.001**	−0.04	**<0.001**	−0.03	**<0.001**
Alpha synuclein (synSAA+)	2.77	0.510	−1.55	0.688	0.32	0.925	−3.64	0.256	−0.13	0.651	−0.11	0.713	−0.69	0.663	−0.22	0.894	1.01	0.642	3.11	0.146
Age × synSAA+	0.66	0.075			0.58	0.091							−0.24	**0.020**	−0.25	**0.022**				
Random Effects																				
σ^2	228.07		196.43		226.31		195.18		1.66		1.63		13.04		12.95		46.01		42.84	
τ_{00}	540.63 $_{\text{Reggieid}}$		410.02 $_{\text{Reggieid}}$		350.56 $_{\text{Reggieid}}$		267.86 $_{\text{Reggieid}}$		3.02 $_{\text{Reggieid}}$		2.96 $_{\text{Reggieid}}$		80.38 $_{\text{Reggieid}}$		78.49 $_{\text{Reggieid}}$		140.67 $_{\text{Reggieid}}$		121.78 $_{\text{Reggieid}}$	
τ_{11}	3.02 $_{\text{Reggieid.age\_c60}}$		2.39 $_{\text{Reggieid.age\_c60}}$		2.62 $_{\text{Reggieid.age\_c60}}$		2.02 $_{\text{Reggieid.age\_c60}}$		0.01 $_{\text{Reggieid.age\_c60}}$		0.01 $_{\text{Reggieid.age\_c60}}$		0.19 $_{\text{Reggieid.age\_c60}}$		0.18 $_{\text{Reggieid.age\_c60}}$		0.97 $_{\text{Reggieid.age\_c60}}$		0.62 $_{\text{Reggieid.age\_c60}}$	
ρ_{01}	0.16 $_{\text{Reggieid}}$		−0.04 $_{\text{Reggieid}}$		0.41 $_{\text{Reggieid}}$		0.23 $_{\text{Reggieid}}$		−0.39 $_{\text{Reggieid}}$		−0.34 $_{\text{Reggieid}}$		−0.07 $_{\text{Reggieid}}$		−0.06 $_{\text{Reggieid}}$		0.06 $_{\text{Reggieid}}$		0.15 $_{\text{Reggieid}}$	
ICC	0.77		0.74		0.72		0.67		0.66		0.67		0.88		0.87		0.82		0.80	
N	387 $_{\text{Reggieid}}$		347 $_{\text{Reggieid}}$		387 $_{\text{Reggieid}}$		347 $_{\text{Reggieid}}$		403 $_{\text{Reggieid}}$		361 $_{\text{Reggieid}}$		388 $_{\text{Reggieid}}$		347 $_{\text{Reggieid}}$		401 $_{\text{Reggieid}}$		359 $_{\text{Reggieid}}$	
Observations	2680		2457		2675		2452		2972		2719		2079		1905		2389		2183	
Marginal R^2/conditional R^2	0.139/0.804		0.087/0.759		0.128/0.756		0.084/0.700		0.056/0.683		0.0470.684		0.266/0.908		0.204/0.897		0.292/0.876		0.239/0.845	
AICc	23572.190		21175.175		23333.869		20967.821		11174.375		10185.733		12821.265		11700.473		17444.100		15677.889	

Note: Model set A examined the effect of binary synSAA, synSAA × age (centered at 60), and synSAA × age^2, controlling for sex, education, and prior exposure to the battery. In this table, each model in Set A is presented alongside a version that was fit on a subset of observations including only those participants who were cognitively unimpaired at the time of assessment, using the same model fitting procedure. Non-significant interaction terms ($p > 0.1$) were removed.

p values falling below 0.05 are bolded.

Abbreviation: AIC, Akaike information criterion; CU, cognitively unimpaired; ICC, intra-class correlation; synSAA, α-synuclein seed amplification assay status.

TABLE 4 Results of nested linear mixed-effects models of cognitive tests associated with executive function (Trail-Making Test Part B; Trail-Making Test Parts B–A difference score; Digit Span Backward; Digit Symbol Substitution Test) and a global Preclinical Alzheimer Cognitive Composite (PACC-3).

	1B: All		1B: CU		2B: All		2B: CU		3B: All		3B: CU		4B: All		4B: CU		5B: All		5B: CU	
Predictors	**Est**	***p***	**Est**	***p***	**Est**	***p***	**Est**	***p***	**Est**	***p***	**Est**	***p***	**Est**	***Pp***	**Est**	***Pp***	**Est**	***p***	**Est**	***p***
Intercept	102.77	**<0.001**	101.28	**<0.001**	72.22	**<0.001**	70.37	**<0.001**	4.04	**<0.001**	3.84	**<0.001**	43.58	**<0.001**	45.36	**<0.001**	68.34	**<0.001**	67.98	**<0.001**
Sex (male)	7.46	**0.008**	4.90	0.057	5.39	**0.017**	3.56	0.094	−0.16	0.414	−0.07	0.723	−4.84	**<0.001**	−4.88	**<0.001**	−11.55	**<0.001**	−10.40	**<0.001**
Education (years)	−2.86	**<0.001**	−2.74	**<0.001**	−2.42	**<0.001**	−2.30	**<0.001**	0.20	**<0.001**	0.21	**<0.001**	0.96	**<0.001**	0.87	**<0.001**	2.10	**<0.001**	2.12	**<0.001**
Number of prior tests	−0.60	0.117	−0.61	0.115	−0.19	0.590	−0.12	0.723	0.10	**<0.001**	0.10	**<0.001**	0.31	**0.009**	0.33	**0.012**	2.28	**<0.001**	2.21	**<0.001**
Age (years), linear	0.53	**0.012**	0.48	**0.017**	0.52	**0.006**	0.42	**0.018**	−0.01	0.286	−0.02	0.116	−0.46	**<0.001**	−0.46	**<0.001**	−0.57	**<0.001**	−0.52	**<0.001**
Age (years), quadratic	0.04	**<0.001**	0.03	**<0.001**	0.02	**0.018**	0.02	**0.033**	−0.00	**0.001**	−0.00	**0.011**	−0.01	**<0.001**	−0.01	**0.002**	−0.03	**<0.001**	−0.03	**<0.001**
Alpha synuclein (synSAA+)	2.65	0.532	−1.64	0.673	−0.89	0.796	−3.75	0.245	−0.09	0.751	−0.09	0.767	−0.63	0.689	−0.29	0.861	1.09	0.616	2.95	0.172
Amyloid beta (A+)	0.66	0.837	0.49	0.867	−1.86	0.479	−1.53	0.531	0.37	0.110	0.21	0.353	−0.90	0.450	−1.30	0.291	−0.01	0.995	0.73	0.643
Phosphorylated tau181 (T+)	3.17	0.387	0.89	0.802	3.19	0.284	1.11	0.704	−0.36	0.145	−0.24	0.385	0.13	0.920	0.81	0.580	0.40	0.832	0.65	0.738
Age x A+	0.61	**0.031**	0.52	0.053	0.32	0.194	0.28	0.247	−0.03	**0.049**			−0.21	**0.004**	−0.19	**0.014**			−0.33	**0.039**
Age x T+	−0.33	0.341	−0.49	0.164													−0.70	**<0.001**		
$Age^2 \times T+$	0.06	**0.002**	0.05	**0.015**																
$Age^2 \times A+$					0.05	**0.001**	0.04	**0.028**											0.02	0.075
Age × synSAA+													−0.23	**0.026**	−0.24	**0.024**				
Random effects																				
σ^2	227.63		196.02		225.52		194.66		1.66		1.63		13.06		12.96		46.15		42.67	
τ_{00}	548.58 $_{Reggieid}$		412.92 $_{Reggieid}$		354.37 $_{Reggieid}$		269.81 $_{Reggieid}$		3.03 $_{Reggieid}$		2.97 $_{Reggieid}$		80.76 $_{Reggieid}$		78.68 $_{Reggieid}$		141.01 $_{Reggieid}$		122.93 $_{Reggieid}$	
τ_{11}	2.88 $_{Reggieid.age\_c60}$		2.36 $_{Reggieid.age\_c60}$		2.55 $_{Reggieid.age\_c60}$		2.03 $_{Reggieid.age\_c60}$		0.01 $_{Reggieid.age\_c60}$		0.01 $_{Reggieid.age\_c60}$		0.18 $_{Reggieid.age\_c60}$		0.17 $_{Reggieid.age\_c60}$		0.87 $_{Reggieid.age\_c60}$		0.63 $_{Reggieid.age\_c60}$	
ρ_{01}	0.16 $_{Reggieid}$		−0.03 $_{Reggieid}$		0.42 $_{Reggieid}$		0.24 $_{Reggieid}$		−0.40 $_{Reggieid}$		−0.34 $_{Reggieid}$		−0.09 $_{Reggieid}$		−0.08 $_{Reggieid}$		0.06 $_{Reggieid}$		0.15 $_{Reggieid}$	
ICC	0.77		0.74		0.72		0.67		0.66		0.67		0.87		0.87		0.82		0.80	
N	387 $_{Reggieid}$		347 $_{Reggieid}$		387 $_{Reggieid}$		347 $_{Reggieid}$		403 $_{Reggieid}$		361 $_{Reggieid}$		388 $_{Reggieid}$		347 $_{Reggieid}$		401 $_{Reggieid}$		359 $_{Reggieid}$	
Observations	2680		2457		2675		2452		2972		2719		2079		1905		2389		2183	
Marginal R^2/conditional R^2	0.173/0.812		0.099/0.763		0.157/0.764		0.097/0.706		0.063/0.686		0.048/0.685		0.276/0.909		0.214/0.898		0.315/0.876		0.238/0.847	
AICc	23565.812		21174.917		23325.438		20968.025		11183.134		10190.579		12816.179		11697.243		17428.333		15684.188	

Note: Model set B examined the effect of binary synSAA, synSAA × age (centered at 60), and synSAA × age^2, along with binary Aβ42/40 and p − tau_{181} and their interactions with age and age^2. In this table, each model in Set B is presented alongside a version that was fit on a subset of observations including only those participants who were cognitively unimpaired at the time of LP, using the same model fitting procedure. As in Model set A, all models controlled for sex, education, and prior exposure to the battery. Non-significant interaction terms ($p > 0.1$) were removed.

p values falling below 0.05 are bolded.

Abbreviation: Aβ, amyloid beta; AIC, Akaike information criterion; CU, cognitively unimpaired; ICC, intra-class correlation; LP, lumbar puncture; p-tau, phosphorylated tau; synSAA, α-synuclein seed amplification assay status.

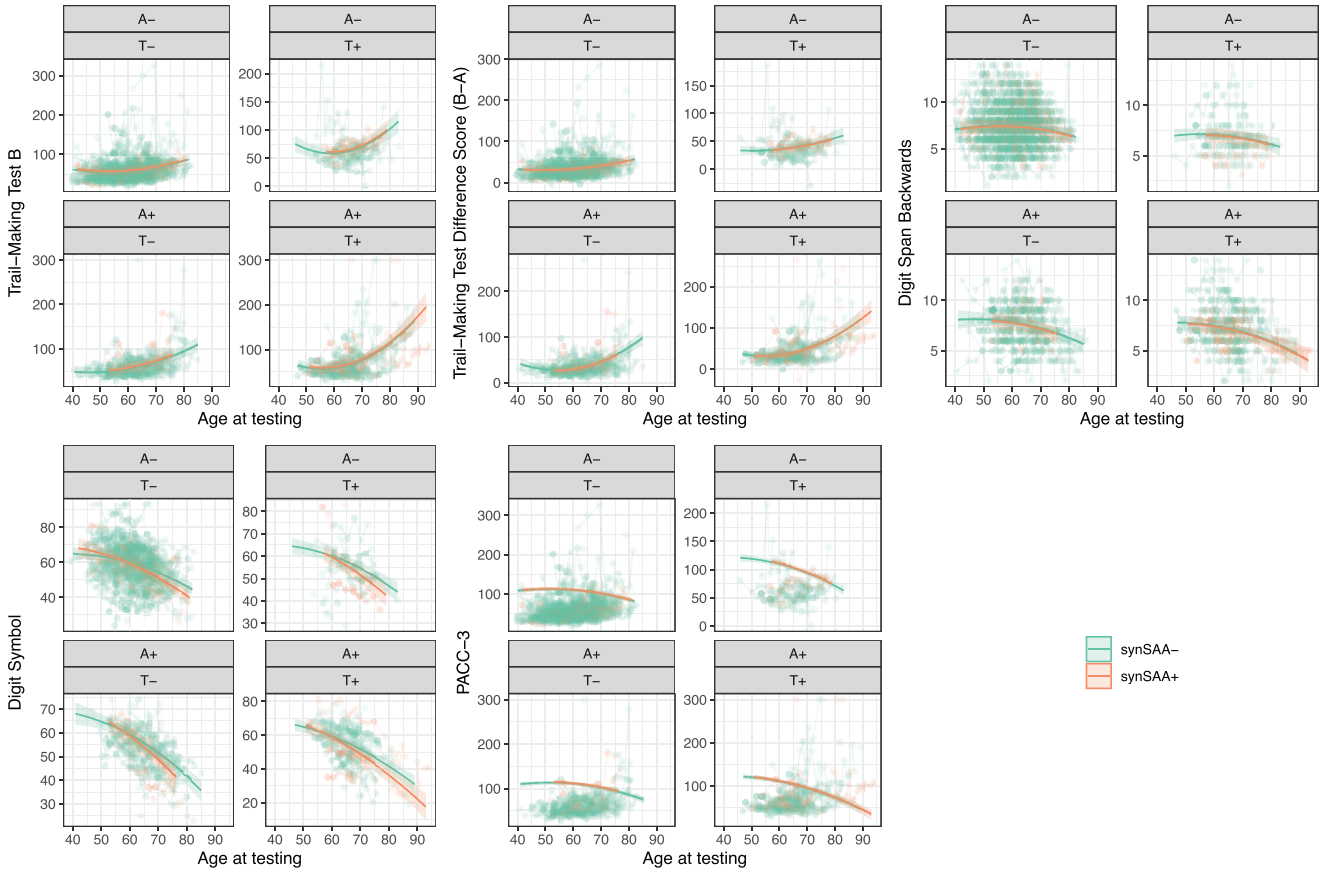

FIGURE 2 Results of nested linear mixed-effects models of cognitive tests associated with executive function (Trail-Making Test Part B; Trail-Making Test Parts B–A difference score; Digit Span Backward; Digit Symbol Substitution Test) and a global Preclinical Alzheimer Cognitive Composite (PACC-3). Model-predicted values and confidence bands derived from final models represented in Table 2. Predictors not shown directly in the graph have been set to their average value. The largest model examined the effect of binary synSAA, synSAA × age (centered at 60), and synSAA × age^2, controlling for sex, education, and prior exposure to the battery, alongside binary Aβ42/40 and p – tau$_{181}$ and their interactions with age and age^2. From this largest model, non-significant interaction terms ($p > 0.1$) were removed. The spaghetti plot layer beneath represents individual participants' measurements over time. Aβ, amyloid beta; p-tau, phosphorylated tau; synSAA, α-synuclein seed amplification assay status.

adjustment for multiplicity these did not reach statistical significance ($0.05 < p < 0.10$). Finally, for PACC-3, again only the linear age interaction was significant (4way × age, $p = 0.0000050$), and pairwise trend contrasts revealed differences between the synSAA–/AD+ group and the synSAA–/AD– and synSAA+/AD– groups (Model 5C). Full results of this analysis and the parallel version including only CU individuals are shown in Table S2 and Figures S3 and S4 in supporting information. Estimated marginal trends for each of the four groups are shown at three different ages (50, 60, 70) in Figure S5 in supporting information, with non-overlapping arrows reflecting significant pairwise contrasts after Tukey correction.

4 | DISCUSSION

Results of the present study of a cohort of predominantly CU, late-middle-aged participants showed syn, A, and T prevalence of 12%, 31%, and 21%, respectively. Observed co-occurrence of these markers suggests significant syn and AD copathology, specifically based on p – tau$_{181}$, but this relationship attenuates in magnitude and significance after adjusting for age, as well as when limiting the sample to those who were CU at the time of LP. Several other cohorts have recently shown evidence of copathology, but the specifics have differed. In a cohort representing multiple etiologies, Bellomo et al. found increased occurrence of syn-seeds with increasing progression along the AD clinical continuum, even after adjusting for age.[8] Two other cohorts have established prevalent synSAA copathology with A, but not T. In BioFINDER, syn prevalence was higher among unimpaired, A+ individuals with and without adjusting for age,[6] but among impaired participants, no significant syn and AD copathology was found after adjusting for age, although a marginal link between syn and T was observed.[7] However, the T marker used in BioFINDER was, variously, CSF p – tau$_{217}$ or tau PET, whereas the present analysis used only CSF p – tau$_{181}$. More recently, a study in the Alzheimer's Disease Neuroimaging Initiative (ADNI) relating synSAA to CSF Aβ42 and p – tau$_{181}$ suggested higher prevalence of syn among A+, but *lower*

prevalence among T+,[5] in both impaired and unimpaired cohorts. This study also explored the frequency of AD neuropathological correlates in a subset that were positive for syn at autopsy, but the relationship of Lewy bodies to A and T specifically was not reported. One possible resolution for these apparently conflicting reports is that CSF p – tau_{181} is better understood as an early stage (Core 1) biomarker, not a true indicator of T positivity.[31] Indeed, an exploratory analysis of a smaller subset with tau PET imaging taken within 1 year of LP supports this view, as we saw no relationship between synSAA and this Core 2 marker (data not shown). Taken together, emerging work suggests that syn copathology occurs as aged individuals progress along the AD clinical and biomarker spectrum. Further longitudinal follow-up will be needed to understand whether co-occurrence of AD and syn pathology reflects a mechanistic connection or is an epiphenomenon of age-related processes, and to explore the prognostic value of such copathology for development of LBD.

In addition to synSAA and AD biomarkers, our deeply phenotyped cohort also had NTK results, allowing us to examine the effects of joint pathophysiology on these exploratory outcomes. Significant group differences were seen across markers, but the pattern of differences related most directly to T, with pairwise contrasts between groups that differed only on synSAA status generally proving non-significant. Across all NTK markers, mean levels were highest in the synSAA+/T+ group. To our knowledge this is the first study of NTK markers in a cohort characterized for syn, and future work will need to replicate this finding.

Relationships between the synSAA and cognitive status as adjudicated by clinical consensus suggest that the assay may be predictive of overall impairment in this population, which also agrees broadly with other recent work. However, we did not see strong relationships with specific neurological findings related to LBD. Findings from BioFINDER were somewhat similar to ours. In those without dementia, no relationship was observed between synSAA and motor symptoms.[6] Among memory clinic patients with MCI or dementia, a relationship between synSAA and motor symptoms was seen, but only among those who were synSAA+/AD–.[7] Similarly, in ADNI, no relationship was seen between synSAA and either sleep difficulties or hallucinations.[5] In our largely middle-aged sample that was targeted to be enriched for AD risk specifically, statistical power to detect co-occurrence of syn-seeds with relatively rare LBD signs and symptoms was low. Additionally, clinical exams were performed by nurse practitioners rather than neurologists and did not include full assessments for PD and related disorders, and this imprecision may have reduced our assessment accuracy and statistical power further.

We observed steeper age-related decreases in Digit Symbol Substitution performance among synSAA+ individuals. Additionally, trajectories on both Trail-Making Test Part B and the Trail-Making Test difference score worsened slightly faster among synSAA+ individuals, although this difference was marginal and attenuated to non-significant once AD biomarkers were added to the model. Importantly, the synSAA finding held when restricting the sample to those who were CU at the time of LP. These findings were not corrected for multiple comparisons across outcomes and should be interpreted with caution. However, if replicated, it would suggest that preclinical cognitive change, analogous to Stage 2 decline in the A/T/N framework for AD, can be observed in the LBD spectrum as well, on executive function tasks expected to show early change. Palmqvist et al. likewise found evidence for preclinical cognitive change associated with syn-seeds, but in contrast to the present work, this difference was observed across all cognitive outcomes.[6] Tosun et al. found evidence of steeper decline among AD+/SAA+ only on global assessments including Alzheimer's Disease Assessment Scale Cognitive subscale and PACC, but not on executive function.[5] Differences between these studies may be due to the fact that both BioFINDER and ADNI have larger cohorts, or to differences in assessments; in particular, BioFINDER's assessment of executive function made use of a test similar to our Digit Symbol Substitution task, but other assessments were different in substantive ways.

The study has some limitations that should be noted. First, our sample is a largely White convenience sample that has been upweighted for AD risk. Additional studies are needed in population-based samples, especially where absolute proportions of synSAA positivity are concerned. Second, because LPs were not generally available at the same time as the cognitive baseline, we have relatively few years of cognitive follow-up after the biomarker measurement, making a prospective design impractical. The design we chose instead was to use status at the most recent available LP to predict the full longitudinal trajectory, a largely retrospective design, which has inferential drawbacks. Third, because the original focus of the cohort studies was AD, our clinical assessments were not designed with Lewy body disorders in mind. Both cohorts are now exploring ways to broaden our portfolio of assessments in the hopes of getting a clearer clinical picture of this dementia etiology in our samples. Finally, because both of our cohorts are upweighted by design for preclinical disease, our estimates of synSAA+ in MCI and dementia in this cohort should be taken as preliminary. Future work in these cohorts will reexamine this question, and the related question of synSAA+ as a predictor of cognitive progression.

In summary, this study provides additional insight into the co-occurrence of syn and AD pathology. The utility of a qualitative SAA for syn pathology is apparent and expected to significantly enhance the ability to understand the contribution of multiple proteinopathies to cognitive decline and mixed dementia. Development of methods to measure proteinopathies in vivo (and early) will provide greater precision for preventing and treating dementia.

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CONFLICT OF INTEREST STATEMENT

E.M.J. serves on a data monitoring committee (K01 AG073587; PI: Albert) and her spouse receives salary and stock options from Epic Systems Corporation. K.M., J.L., J.M., S.M., L.C.-M., and R.M.L. are employees of Amprion Inc. N.A.C. has served as a consultant for New Amsterdam Pharma. O.C.O. receives funding from the National Institutes of Health and serves on the board of directors of the International Neuropsychological Society. B.B.B. has served on scientific advisory boards and/or as a consultant for Weston Family Foundation, New Amsterdam, Cognito Therapeutics, and Merry Life Biomedical; she has received support from the National Institute on Aging (R01AG062285, P30AG062715, R01AG037639, R01AG054059, RF1AG057784, R01AG070973, R01AG070883, and R01AG059312). S.A. receives funding from the National Institutes of Health and is an editor of a textbook on geriatrics and gerontology for which he receives royalties from McGraw-Hill. C.M.C. receives funding from the National Institutes of Health, the Alzheimer's Association, and the Veterans Administration; has received study drugs from Armarin Corporation; has served on the data and safety monitoring boards for three clinical trials; has served on the US Health and Human Services Advisory Council on Alzheimer's Research, Care, and Services, and on the Wisconsin Dementia State Plan Steering Committee; and has been paid for lectures and travel by the Indiana Neurological Society, the American Medical Association, Northwestern University, and the Clinical Trials in Alzheimer's Disease conference. B.H. receives funding from the National Institutes of Health. C.L.G. has performed minor consulting for Medtronic and Destum Partners and has received funding from the Department of Veterans Affairs Clinical Sciences R&D (I01-CX000555-01-10). G.K. is a full-time employee of Roche Diagnostics GmbH, Penzberg, Germany. H.Z. has served on scientific advisory boards and/or as a consultant for Abbvie, Acumen, Alector, Alzinova, ALZPath, Amylyx, Annexon, Apellis, Artery Therapeutics, AZTherapies, Cognito Therapeutics, CogRx, Denali, Eisai, LabCorp, Merry Life, Nervgen, Novo Nordisk, Optoceutics, Passage Bio, Pinteon Therapeutics, Prothena, Red Abbey Labs, reMYND, Roche, Samumed, Siemens Healthineers, Triplet Therapeutics, and Wave; has given lectures in symposia sponsored by Alzecure, Biogen, Cellectricon, Fujirebio, Lilly, Novo Nordisk, and Roche; and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program (outside submitted work). S.C.J. serves as a consultant to ALZPath and to Enigma Biomedical. Authors B.J., R.L.S., R.E.W., and R.E.L. have no competing interests to declare. Author disclosures are available in the supporting information.

CONSENT STATEMENT

All participants provided 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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