Diagnostic and prognostic value of α-synuclein seed amplification assay kinetic measures in Parkinson's disease: a longitudinal cohort study

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Lancet Neurol 2025; 24: 580–90

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Summary

Background α-synuclein seed amplification assay (SAA) positivity has been proposed as a diagnostic biomarker for Parkinson's disease. However, studies of the prognostic value of this biomarker have been limited to small, single-centre studies over short follow-up periods. We aimed to assess the diagnostic and prognostic value of quantitative CSF α-synuclein SAA kinetic measures in Parkinson's disease.

Methods In this longitudinal cohort study, we collected and analysed data from participants with Parkinson's disease, progressive supranuclear palsy, and healthy controls enrolled in three cohorts: the UK parkinsonism cohort, the Parkinson's Progression Markers Initiative (PPMI) international observational study, and the Tübingen Parkinson's disease cohort. Baseline CSF α-synuclein SAA data and longitudinal clinical data were collected between Jan 1, 2005, and Nov 1, 2023. The following seeding kinetic measures were calculated from the α-synuclein SAA curve for each SAA-positive sample: time to threshold (TTT) for a positive SAA result; maximum Thioflavin T fluorescence during the reaction time (MaxThT); and area under the fluorescence curve during the reaction time (AUC). We compared seeding kinetic measures between sporadic Parkinson's disease and progressive supranuclear palsy, and between sporadic Parkinson's disease and monogenic Parkinson's disease. We used time-to-event analyses to assess the ability of α-synuclein SAA kinetic measures to predict an unfavourable outcome in Parkinson's disease, adjusting for sex, age, and disease duration at SAA testing.

Findings We analysed data from 1631 participants: newly generated data from the UK parkinsonism cohort (Parkinson's disease, n=66; progressive supranuclear palsy, n=52; controls, n=9) and previously generated data from the PPMI (Parkinson's disease, n=1036; controls, n=239) and Tübingen (Parkinson's disease, n=229) cohorts. In the UK parkinsonism cohort, α-synuclein SAA was positive in 63 (96%) of 66 Parkinson's disease samples and eight (15%) of 52 progressive supranuclear palsy samples, with six (75%) of eight positive progressive supranuclear palsy samples having distinct low and slow seeding kinetics (low MaxThT and high TTT) as a marker of Lewy body co-pathology. TTT was faster in *GBA1*-associated Parkinson's disease compared with sporadic Parkinson's disease in both the PPMI (p=0·04) and Tübingen (p=0·01) cohorts. In the PPMI cohort, after excluding individuals who had an unfavourable outcome at the time of baseline SAA testing, an unfavourable outcome was observed in 593 (73%) of 810 participants with α-synuclein SAA-positive Parkinson's disease during a median follow-up period of 4·5 years (IQR 2–9). TTT at baseline predicted only cognitive decline (Montreal Cognitive Assessment score ≤21) as a component of an unfavourable outcome in Parkinson's disease in both the PPMI (n=824, hazard ratio [HR] 2·36 [95% CI 1·60–3·46], p=0·001) and Tübingen (n=135, 2·17 [1·07–4·41], p=0·03) cohorts. TTT also predicted cognitive decline in a subgroup of participants with Parkinson's disease in the PPMI cohort who were Alzheimer's disease biomarker negative (n=355, HR 1·80 [95% CI 1·03–3·18], p=0·04).

Interpretation Assessing α-synuclein SAA kinetic measures might aid in the diagnostic differentiation of Parkinson's disease from progressive supranuclear palsy with Lewy body co-pathology. Furthermore, faster seeding kinetics are found in *GBA1*-Parkinson's disease and predict cognitive decline in Parkinson's disease independently of Alzheimer's disease co-pathology.

Funding Medical Research Council, PSP Association.

Research in context

Evidence before this study

We searched PubMed for articles on the diagnostic and prognostic value of CSF α-synuclein seed amplification assays (SAAs) in Parkinson's disease, with no language restrictions, from database inception up to April 1, 2025, using the following search string: "Parkinson's disease" AND "CSF seed amplification assay", "diagnosis" OR "prognosis". Previous studies, including those involving the Parkinson's Progression Markers Initiative (PPMI) cohort, have reported on the diagnostic value of α-synuclein SAA status in sporadic and monogenic forms of Parkinson's disease. However, the effect of co-pathologies and genetic status on α-synuclein seeding kinetics has not been explored. Similarly, previous analyses of the prognostic value of α-synuclein SAA seeding kinetics have been limited to small, single-centre studies over short follow-up periods.

Added value of this study

To the best of our knowledge, this is the first study to show distinct low and slow seeding kinetics in α-synuclein SAA-positive CSF samples from individuals with progressive supranuclear palsy as a marker of Lewy body co-pathology. We also found that the α-synuclein SAA kinetic measure, time to threshold (TTT), was faster in *GBA1*-Parkinson's disease compared with sporadic Parkinson's disease, in line with *GBA1*-Parkinson's disease being a faster progressing form of Parkinson's disease. Furthermore, our comprehensive time-to-event analyses in the PPMI cohort have shown that baseline TTT predicts cognitive decline in both sporadic and monogenic Parkinson's disease, and this finding was independently replicated in the Tübingen Parkinson's disease cohort. Importantly, we also highlight that the association between TTT and cognitive decline in Parkinson's disease is independent of Alzheimer's disease co-pathology. This suggests that fast TTT is a reflection of more aggressive α-synuclein seeding that increases the rate of Lewy body pathology spread to the cortex.

Implications of all the available evidence

α-Synuclein SAA positivity with distinct low and slow kinetics in a subset of progressive supranuclear palsy samples is a marker of Lewy body co-pathology, which highlights the diagnostic value of quantitative seeding kinetic measures. This result also reinforces the need for the development of four-repeat tau SAA to be used in combination with α-synuclein SAA for greater diagnostic accuracy in clinical practice. We have also shown that genetic status in Parkinson's disease determines the profile of α-synuclein seeding kinetics. Furthermore, TTT could be used as a prognostic biomarker and clinical trial stratification tool to recruit Parkinson's disease trial cohorts with homogeneous disease progression trajectories.

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Introduction

Parkinsonian disorders include Parkinson's disease, progressive supranuclear palsy, corticobasal degeneration, multiple system atrophy, and dementia with Lewy bodies. Each of these disorders is characterised pathologically by the prion-like spread of distinct structures of misfolded proteins in specific brain regions, leading to neurodegeneration and progressive neurological impairment.[1] Misfolded neuronal α-synuclein in the form of Lewy body pathology is the hallmark of Parkinson's disease and dementia with Lewy bodies, whereas multiple system atrophy comprises α-synuclein pathology in the form of glial cytoplasmic inclusions.[2] By contrast, progressive supranuclear palsy and corticobasal degeneration are characterised by the presence of neuronal and astrocytic four-repeat tau pathology.[3]

In the absence of objective biomarkers, achieving an accurate diagnosis in patients presenting with symptoms suggestive of an underlying parkinsonian disorder is challenging, especially in the early stage of symptomatic disease when there is a high degree of clinical overlap between Parkinson's disease, progressive supranuclear palsy, multiple system atrophy, corticobasal syndrome, and dementia with Lewy bodies. This diagnostic challenge has been highlighted in previous cohort studies of patients who initially fulfil Parkinson's disease clinical diagnostic criteria where up to 10% of patients later have revised clinical diagnoses including progressive supranuclear palsy and multiple system atrophy.[4]

Furthermore, considerable variability exists in the rate of clinical disease progression in Parkinson's disease. Certain motor and cognitive features have been shown to predict Parkinson's disease progression subtypes,[5] but the reporting of such clinical features is subjective and varies between patients.

Ultimately, the combination of clinical diagnostic uncertainty and variable progression rates leads to a lack of homogeneous groups for disease-modifying clinical trials.[6]

There is emerging evidence that positivity in α-synuclein seed amplification assays (SAAs), which detect seeding-competent α-synuclein aggregates, is a marker of Lewy body pathology. The large-scale application of the Amprion 150 h α-synuclein SAA in the Parkinson's Progression Markers Initiative (PPMI) cohort showed high rates of assay positivity in sporadic Parkinson's disease and high sensitivity and specificity of the assay to reliably differentiate Parkinson's disease from healthy controls. Monogenic forms of Parkinson's disease had distinct rates of positivity in α-synuclein SAA with high positivity rates in *GBA1*-Parkinson's disease samples, whereas only 68% of *LRRK2*-Parkinson's disease samples were α-synuclein SAA-positive, suggesting pathological heterogeneity. Furthermore, α-synuclein SAA might be reliable in detecting pathology in the prodromal phase of Parkinson's

disease and in differentiating Parkinson's disease from multiple system atrophy.[7–9] As such, there are now proposed biologically defined criteria for neuronal α-synuclein disease and Parkinson's disease to enable future research studies.[10,11]

For the **PPMI database** see www.ppmi-info.org/access-dataspecimens/download-data

We aimed to assess the diagnostic and prognostic value of quantitative seeding kinetic measures derived from α-synuclein SAA by assaying the UK parkinsonism cohort and analysing previously generated data from the PPMI and Tübingen Parkinson's disease cohorts. Specifically, the aims of this study were to assess whether the profile of α-synuclein SAA kinetic measures can distinguish Parkinson's disease from progressive supranuclear palsy with Lewy body co-pathology in the setting of α-synuclein SAA positivity; whether α-synuclein SAA kinetic measures differ in sporadic and monogenic forms of Parkinson's disease; and whether α-synuclein SAA kinetic measures at baseline predict the rate of clinical progression in Parkinson's disease.

Methods

Study design and participants

In this longitudinal cohort study, we collected and analysed data from three cohorts.

The UK parkinsonism cohort consisted of participants with Parkinson's disease or progressive supranuclear palsy as well as healthy controls who had provided written informed consent. Participants with Parkinson's disease who fulfilled Queen Square Brain Bank clinical diagnostic criteria[12] and were recruited to the Exenatide-PD3 clinical trial between Jan 1, 2020, and April 1, 2022, were included in the cohort.[13] The trial received research ethics committee (initial date of approval Oct 15, 2019, research ethics committee reference number 19/SC/0447) and regulatory approvals (Medicines and Healthcare products Regulatory Agency clinical trial authorisation 20363/0406/001-0001), and is registered with the ISRCTN Registry (14552789), EudraCT (2018-003028-35) and ClinicalTrials.gov (NCT04232969). Only baseline (pre-drug or pre-placebo) CSF samples were used as part of this study. People with progressive supranuclear palsy fulfilling possible or probable clinical diagnostic criteria[14] who were recruited to the PROSPECT-UK study's natural history and longitudinal cohorts (Queen Square Research Ethics Committee 14/LO/1575) between Sept 1, 2015, and Nov 1, 2023, were also included in the UK parkinsonism cohort.[15] Age-matched and sex-matched healthy controls who were recruited to the study were also included in the cohort. Post-mortem evaluation was conducted on a subset of participants with progressive supranuclear palsy.

See **Online** for appendix

PPMI is an international observational study recruiting patients with Parkinson's disease from outpatient neurology practices at academic centres in Austria, Canada, France, Germany, Greece, Israel, Italy, the Netherlands, Norway, Spain, the UK, and the USA.[7] The study was approved by the institutional review board at each site and is registered with ClinicalTrials.gov (NCT01141023). Clinical and α-synuclein SAA data, which were collected between July 1, 2010, and July 1, 2023, and used in the preparation of the current study, were obtained on Nov 1, 2024, from the PPMI database (RRID:SCR_006431).

The Tübingen Parkinson's disease cohort consists of 229 participants with Parkinson's disease fulfilling Queen Square Brain Bank clinical diagnostic criteria[12] who were recruited to the cohort between Jan 1, 2005, and Jan 1, 2020. The study was approved by the Ethics Committee of the University of Tübingen (26/2007BO1, 404/2010BO1, 199/2011BO1, and 702/2013BO1).

Participants from each cohort provided written informed consent at the time of enrolment into each respective study, which included the use of anonymised data and samples in related studies such as this one.

Procedures

Baseline CSF samples from participants in the PPMI cohort were previously analysed with the Amprion 24 h or 150 h α-synuclein SAAs, as described previously.[7] Baseline CSF samples from participants in the Tübingen cohort were previously analysed with an α-synuclein SAA from the Institute of Neurological Science of Bologna (ISNB), as described previously.[16]

CSF sampling methods and application of the Rocky Mountain Laboratories (RML; Hamilton, MT, USA) α-synuclein SAA to baseline Parkinson's disease, progressive supranuclear palsy, and age-matched and sex-matched healthy control CSF samples from the UK parkinsonism cohort are outlined in the appendix (p 2).

Outcomes

Across all cohorts, in samples assigned a positive α-synuclein SAA result at baseline, the following quantitative measures of α-synuclein seeding kinetics were derived from the Thioflavin T (ThT) fluorescence curves by calculating mean values across positive replicates: time to threshold (TTT) for a positive SAA result; maximum Thioflavin T fluorescence during the reaction time (MaxThT); and area under the fluorescence curve during the reaction time (AUC).

In each of the studied cohorts, the following clinical variables and measures were collected from participants with Parkinson's disease: sex; age at symptom onset; age and disease duration at baseline assessment; and baseline and follow-up scores for the Movement Disorder Society-Unified Parkinson's Disease Rating Scale part III (MDS-UPDRS-III) in the off state, Montreal Cognitive Assessment (MoCA), Schwab and England Activities of Daily Living (SEADL), and Hoehn and Yahr (H&Y) clinical scales. Additionally, in participants with progressive supranuclear palsy from the UK parkinsonism cohort, we noted the dominant progressive supranuclear palsy clinical subtype and the progressive supranuclear palsy rating scale (PSPRS) score. Further

details are provided in the appendix (p 3). Ethnicity data were not available in all of the included cohorts so they were not reported in this study.

Methods used for CSF Alzheimer's disease biomarker testing in the PPMI cohort and genetic stratification of all the studied cohorts are outlined in the appendix (p 3). In the PPMI cohort, participants with Parkinson's disease and healthy controls were stratified according to whether CSF samples were assessed with the Amprion 24 h or 150 h α-synuclein SAA.

To assess the diagnostic value of α-synuclein SAA, we obtained α-synuclein SAA positivity rates in Parkinson's disease, progressive supranuclear palsy, and control groups in the UK parkinsonism cohort. We also assessed the profile of α-synuclein SAA seeding kinetic measures in α-synuclein SAA-positive Parkinson's disease and progressive supranuclear palsy samples.

In the PPMI cohort, we assessed whether α-synuclein SAA kinetic measures differed between sporadic and monogenic Parkinson's disease groups.

To assess the prognostic value of α-synuclein SAA, we used a time-to-event analysis to assess whether baseline α-synuclein SAA kinetic measures predict an unfavourable outcome in sporadic and monogenic Parkinson's disease participants from the PPMI cohort with a positive α-synuclein SAA result.

Statistical analysis

The study size was determined by including all participants from each of the cohorts as long as baseline α-synuclein SAA and clinical data were available.

In all cohorts, group comparisons of clinical measures were done with Fisher's exact test for categorical variables and linear regression (adjusting for sex, age, and disease duration at baseline) for continuous variables. For these comparisons, statistical significance was set at p values less than 0·05.

To explore the diagnostic value of α-synuclein SAA kinetic measures in the UK parkinsonism cohort, we created a plot of MaxThT versus TTT values for all CSF α-synuclein SAA-positive Parkinson's disease and progressive supranuclear palsy samples. We then highlighted samples with a less aggressive kinetic profile (ie, low and slow kinetics), which we defined as samples that fell within both the first quartile of MaxThT values (low) and the fourth quartile of TTT values (slow) in all α-synuclein SAA-positive samples.

In the PPMI cohort, comparisons of raw α-synuclein SAA kinetic measures in the sporadic and monogenic Parkinson's disease groups were done with linear regression, adjusting for sex, age, and disease duration at baseline, with statistical significance set at p values less than 0·05.

In the assessment of the prognostic value of α-synuclein SAA kinetic measures in participants with Parkinson's disease from the PPMI cohort, we built on previous

	Parkinson's disease (n=66)	Progressive supranuclear palsy (n=52)	Healthy controls (n=9)
Sex			
Female	14 (21%)	21 (40%)	8 (89%)
Male	52 (79%)	31 (60%)*	1 (11%)†
Progressive supranuclear palsy clinical subtype at baseline assessment			
Richardson syndrome	..	31 (60%)	..
Parkinsonism	..	10 (19%)	..
Progressive gait freezing	..	3 (6%)	..
Corticobasal syndrome overlap	..	3 (6%)	..
Frontal	..	3 (6%)	..
Speech and language disorder	..	2 (4%)	..
Mean age at symptom onset, years (SD)‡	52·6 (9·5)	64·4 (6·8)*	..
Mean age at baseline assessment, years (SD)‡	59·3 (8·6)	68·4 (6·7)*	66·2 (3·9)*
Mean disease duration, years (SD)‡	6·6 (3·6)	4·0 (2·1)*	..
Mean progressive supranuclear palsy rating scale score (SD)§	..	34·3 (13·8)	..
Mean MDS-UPDRS III score (SD)§	33·5 (8·9)	36·0 (14·0)	..
Mean MoCA score (SD)§	28·3 (1·5)	22·2 (4·5)*	28·8 (1·3)¶
CSF α-synuclein SAA positive, n (%; 95% CI)	63/66 (96%; 87–99)	8/52 (15%; 7–28)	0/9; 0%
Participants deceased at censoring (n), mean disease duration from symptom onset to death, years (SD)	..	n=26; 7·2 (3·0)	..
Primary pathological diagnosis in participants undergoing post-mortem evaluation (n)	..	Progressive supranuclear palsy, n=5; corticobasal degeneration, n=1	..

Data are n, n (%), or mean (SD), unless otherwise stated. SAA=seed amplification assay. MDS-UPDRS III=Movement Disorder Society-Unified Parkinson's Disease Rating Scale part III. MoCA=Montreal Cognitive Assessment. Fisher's exact test used for group comparisons of sex distributions. Group comparisons of continuous variables were done with linear regression. CSF α-synuclein SAA positive 95% CI calculated with the binomial formula for all proportions >0% and <100%. *p<0·05 versus Parkinson's disease group. †p<0·05 versus progressive supranuclear palsy and Parkinson's disease groups. ‡Unadjusted for sex, age, and disease duration at baseline. §Adjusted for sex, age, and disease duration at baseline (group comparisons involving controls were only adjusted for sex and age at baseline). ¶p<0·05 versus progressive supranuclear palsy group.

Table 1: **Baseline clinical and CSF α-synuclein SAA status of the UK parkinsonism cohort**

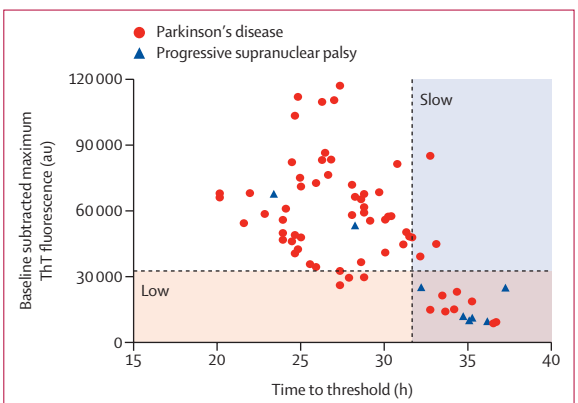

Figure 1: **Low and slow kinetics of α-synuclein SAA-positive CSF samples in the UK parkinsonism cohort**

Cutoff points for the low and slow group defined by the first quartile of maximum ThT values and the fourth quartile of time to threshold values in all α-synuclein SAA-positive samples. ThT=Thioflavin T fluorescence value. SAA=seed amplification assay.

	Sporadic Parkinson's disease (n=764)	*LRRK2*-Parkinson's disease (n=166)	*GBA1*-Parkinson's disease (n=76)	*SNCA*-Parkinson's disease (n=12)	*PRKN*-Parkinson's disease (n=18)	Controls (n=239)
Sex						
Female	260 (34%)	85 (51%)	29 (38%)	7 (58%)	7 (39%)	86 (36%)
Male	504 (66%)	81 (49%)*†	47 (62%)	5 (42%)	11 (61%)	153 (64%)
Mean age at symptom onset, years (SD)‡	61·0 (9·7)	58·1 (9·8)*	58·0 (10·6)*	44·0 (7·5)*	51·3 (16·3)	..
Mean age, years (SD)‡	63·2 (9·5)	63·1 (8·7)	61·9 (10·2)	47·6 (8·0)*†	57·3 (12·6)	61·7 (11·8)
Mean disease duration, years (SD)‡	2·2 (1·9)	5·0 (5·2)*	3·8 (3·1)*	3·6 (2·2)*	5·9 (6·6)*	..
Mean MDS-UPDRS-III score (SD)§	22·5 (9·6)†	19·1 (9·2)*†	24·6 (11·9)†	16·7 (13·7)†	18·9 (10·3)†	1·3 (2·2)*
Mean MoCA score (SD)§	27·0 (2·4)†	26·2 (3·0)*†	26·5 (2·4)†	25·4 (6·1)*†	26·7 (2·4)	28·0 (1·5)*
Median SEADL (IQR)	95 (90–100)	90 (90–100)	90 (85–100)	80 (70–90)	98 (90–100)	100 (100–100)
Median H&Y (IQR)	2 (1–2)	2 (1–2)	2 (1–2)	2 (1–2)	2 (1–2)	0 (0–0)
CSF α-synuclein SAA positive, n (%; 95% CI)	701/764 (92%; 90–94)	109/166 (66%; 58–73)	70/76 (92%; 84–97)	12/12 (100%)	11/18 (61%; 36–83)	20/239 (8%; 5–13)

Data are n (%), mean (SD), or median (IQR), unless otherwise stated. PPMI=Parkinson's Progression Markers Initiative. SAA=seed amplification assay. MDS-UPDRS III=Movement Disorder Society-Unified Parkinson's Disease Rating Scale part III. MoCA=Montreal Cognitive Assessment. SEADL=Schwab and England Activities of Daily Living Scale. H&Y=Hoehn and Yahr stage. Fisher's exact test used for group comparisons of sex distributions. Group comparisons of continuous variables were done with linear regression. *p<0·05 versus sporadic Parkinson's disease group, †p<0·05 versus controls group. ‡Unadjusted for sex, age, and disease duration at baseline. §Adjusted for sex, age, and disease duration at baseline (group comparisons involving controls were only adjusted for sex and age at baseline). CSF α-synuclein SAA-positive 95% CI calculated with the binomial formula for all proportions greater than 0% and less than 100%.

Table 2: **Baseline clinical profile and CSF α-synuclein SAA status of the PPMI cohort**

studies,[4,17–19] by defining an unfavourable outcome as the development of any of the following: cognitive decline (MoCA ≤21), postural instability (H&Y ≥3), motor progression (≥5 point increase in the MDS-UPDRS-III score relative to the baseline score) or dependency (SEADL <80) during the study follow-up period; or death at any point from baseline SAA testing up until the date of censoring (Nov 1, 2024). To harmonise the two subcohorts within the PPMI cohort that had been analysed with the Amprion 24 h and 150 h assays, we assessed the three SAA kinetic measures separately in each assay subcohort and divided α-synuclein SAA-positive participants into two groups as follows: the fourth quartile of MaxThT values were defined as the high MaxThT group, the fourth quartile of AUC values were defined as the high AUC group, and the first quartile of TTT values were defined as the fast TTT group; the first, second, and third quartile of MaxThT and AUC values were defined as the low MaxThT and low AUC groups, and the second, third, and fourth quartile of TTT values were defined as the slow TTT group. SAA kinetic measure groups from both assay subcohorts were then combined for a whole cohort Cox proportional hazards time-to-event analysis to assess whether each baseline α-synuclein SAA kinetic measure predicts an unfavourable outcome, adjusting for sex, age, and disease duration at baseline. We also repeated the analysis for each of the individual clinical events that comprised an unfavourable outcome as outlined above. For this analysis, a Bonferroni-adjusted p value significance threshold was used. Based on our primary finding from this analysis, we then carried out two exploratory analyses using the PPMI cohort data with statistical significance set at p values less than 0·05: whether baseline α-synuclein SAA kinetic measures predict dementia through criteria based on the MDS Taskforce definition of Parkinson's disease dementia[20] (appendix p 3); and whether baseline α-synuclein SAA kinetic measures predict both cognitive decline (MoCA ≤21) and Parkinson's disease dementia in participants with Parkinson's disease who are Alzheimer's disease biomarker-negative. For each of the above Cox proportional hazards analyses, we concluded that the proportional hazards assumption was met if the Schoenfeld residuals were not time dependent (p>0·05).

Missing clinical scale data at specific timepoints were included in the time-to-event analyses as censored data, so for the missing clinical scale it was assumed that the associated unfavourable outcome had not been reached at that specific timepoint.

To replicate the primary findings from the PPMI cohort, we sought out independent Parkinson's disease cohorts that had at least 100 participants with a median follow-up time of at least 4 years; and CSF α-synuclein SAA data generated with one of the Amprion assays or a research-based assay that has previously had post-mortem validation of results. Using these criteria, we accessed previously generated data from the Tübingen Parkinson's disease cohort to assess whether α-synuclein SAA kinetic measures differ between sporadic and monogenic forms of Parkinson's disease, and whether baseline α-synuclein SAA kinetic measures predict cognitive decline (MoCA ≤21) in Parkinson's disease, with statistical significance set at p values less than 0·05.

	Sporadic Parkinson's disease	*LRRK2*-Parkinson's disease	*GBA1*-Parkinson's disease	*SNCA*-Parkinson's disease	*PRKN*-Parkinson's disease	Controls
Amprion 24 h assay PPMI cohort						
Number of participants	380	26	23	1	1	14
Mean MaxThT, value (SD)	140 909·8 (23 274·0)	144 358·6 (22 930·0)	117 429·9 (20 979·0)*†	135 485·3 (NA)	135 906·7 (NA)	137 810·3 (18 237·1)
Mean TTT, h (SD)	10·1 (2·0)	9·6 (1·9)	9·2 (2·4)*†	7·7 (NA)	7·9 (NA)	11·2 (2·8)
Mean AUC, value (SD)	5 195 143 859·6 (979 812 982·0)	5 388 230 769·3 (692 078 125·0)	4 667 639 681·1 (1 222 145 164·0)*	5 815 333 333·3 (NA)	5 565 666 667·0 (NA)	4 737 968 690·5 (1 322 221 503·0)
Amprion 150 h assay PPMI cohort						
Number of participants	321	83	47	11	10	6
Mean MaxThT, value (SD)	85 175·6 (25 912·1)	83 432·5 (24 209·0)	89 786·9 (23 043·4)	91 649·8 (30 839·5)	70 493·7 (25 804·9)	88 443·7 (22 577·7)
Mean TTT, h (SD)	65·6 (10·9)†	70·2 (14·4)*	63·9 (11·1)†	54·3 (4·9)*†	66·3 (14·4)	81·5 (20·3)*
Mean AUC, value (SD)	26 332 577·3 (3 719 112·5)†	24 397 007·8 (4 841 043·6)*	26 739 389·9 (3 774 203·8)†	30 142 730·3 (2 398 036·6)*†	25 694 147·3 (4 443 572·2)	20 898 245·0 (6 635 823·3)*
Tübingen cohort						
Number of participants	98	7	83	2	0	10
Mean MaxThT, value (SD)	68·1 (13·7)	74·0 (8·5)	71·4 (12·6)	66·9 (5·4)	NA	64·3 (14·1)
Mean TTT, h (SD)	21·2 (3·0)	20·2 (2·8)	19·8 (2·9)*	21·2 (3·5)	NA	22·1 (2·8)
Mean AUC, value (SD)	718·1 (234·2)	797·6 (197·2)	812·4 (229·9)*	687·6 (168·4)	NA	655·8 (236·0)

Data are mean (SD), unless otherwise indicated. SAA=seed amplification assay. PPMI=Parkinson's Progression Markers Initiative. MDS-UPDRS III=Movement Disorder Society-Unified Parkinson's Disease Rating Scale part III. MoCA=Montreal Cognitive Assessment. SEADL=Schwab and England Activities of Daily Living Scale. MaxThT=maximum Thioflavin T fluorescence value. TTT=time to threshold. AUC=area under the curve. Group comparisons of continuous variables were done with linear regression that was adjusted for sex, age, and disease duration at baseline (group comparisons involving controls were only adjusted for sex and age at baseline). *p<0·05 versus sporadic Parkinson's disease group. †p<0·05 versus controls group.

Table 3: **Baseline kinetic profile of a-synuclein SAA positive samples in the PPMI and Tübingen cohorts**

All statistical analyses were performed in Stata, version 18, and GraphPad Prism 9.1.1.

Role of the funding source

The funders of the study had no role in study design, data collection, data analysis, data interpretation, writing of the report, or the decision to submit the manuscript for publication.

Results

66 participants with Parkinson's disease from the Exenatide-PD3 trial as well as 52 participants with progressive supranuclear palsy and nine healthy, age-matched and sex-matched controls from the PROSPECT-UK study were included in the UK parkinsonism cohort (table 1). Only one Parkinson's disease sample from the entire cohort tested positive for the *LRRK2* Gly2019Ser variant.

We found that samples from 63 (96%) of 66 participants with clinically diagnosed Parkinson's disease and from eight (15%) of 52 participants with clinically diagnosed progressive supranuclear palsy were α-synuclein SAA-positive (table 1). Of the six participants with clinically diagnosed progressive supranuclear palsy who went on to have post-mortem evaluation, one had primary progressive supranuclear palsy pathology and Braak stage 1 Lewy body co-pathology and was CSF α-synuclein SAA-positive; one had primary corticobasal degeneration pathology and Braak stage 3 Lewy body co-pathology and was CSF α-synuclein SAA-negative; and four had primary progressive supranuclear palsy pathology and no Lewy body co-pathology and were CSF α-synuclein SAA-negative.

We found low and slow α-synuclein SAA kinetics in eight (13%) of 63 positive Parkinson's disease samples and six (75%) of eight positive progressive supranuclear palsy samples, including the sample of the participant with post-mortem diagnosed progressive supranuclear palsy and Braak stage 1 Lewy body co-pathology (figure 1). Relative to unequivocally α-synuclein SAA-positive Parkinson's disease samples, α-synuclein SAA-positive Parkinson's disease samples with low and slow kinetics included individuals who were: older (aged >65 years) at symptom onset with a faster rate of motor progression; or younger (aged <40 years) at symptom onset with a similar rate of cognitive and motor progression (appendix pp 4–5).

In total, publicly available data for 1275 participants from the PPMI cohort were studied (1036 with Parkinson's disease and 239 controls), with CSF samples previously analysed with the Amprion 24 h or 150 h α-synuclein SAA (table 2). Genetic status was available for 273 (46%) of 596 participants in the 24 h Amprion α-synuclein SAA subcohort and 679 (100%) of 679 participants in the 150 h Amprion α-synuclein SAA subcohort. Participants with Parkinson's disease without genetic data available were included in the sporadic Parkinson's disease group. The pathogenic variants detected in participants with

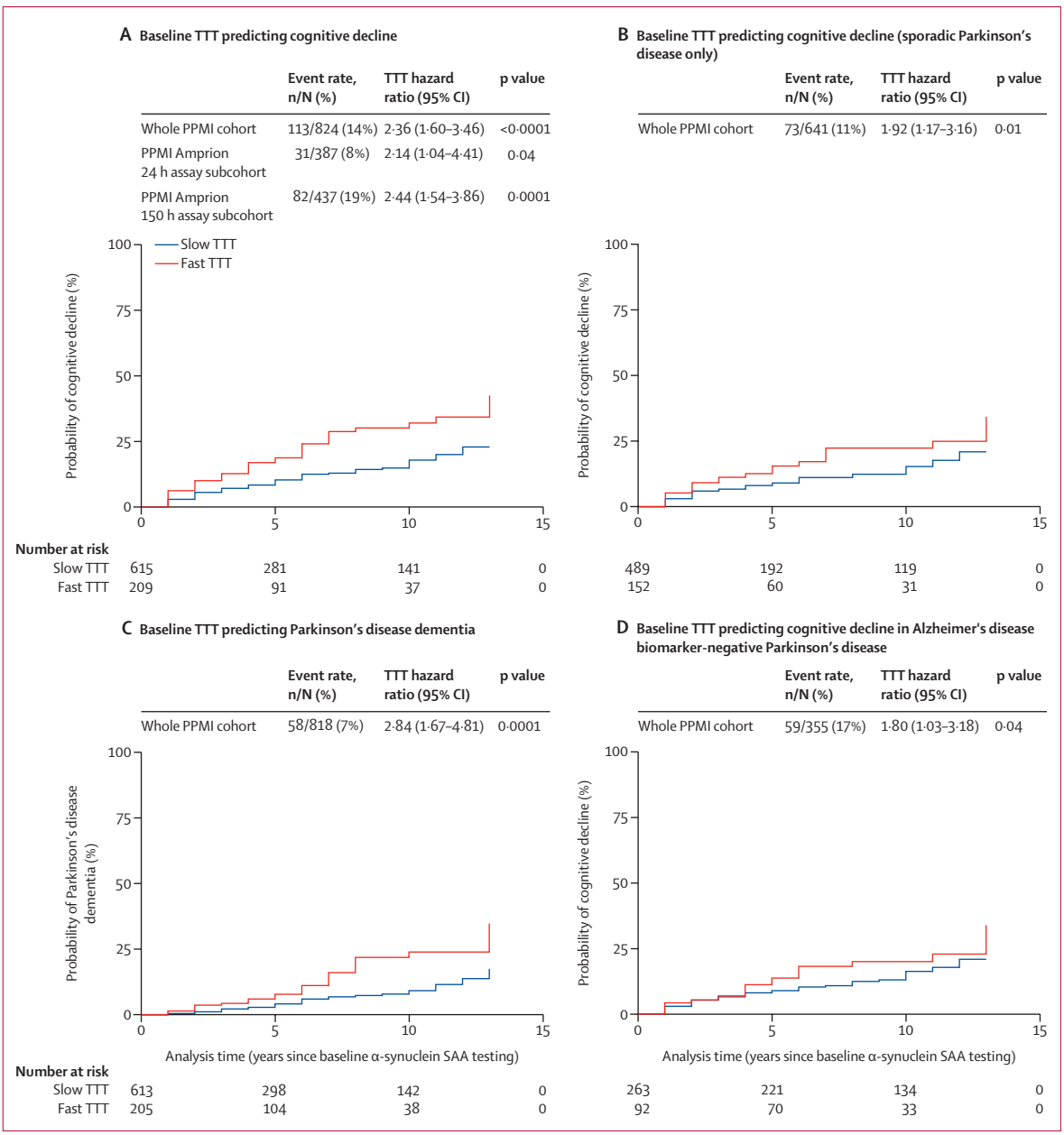

Figure 2: **Time-to-event analyses in the PPMI cohort, stratified by baseline CSF α-synuclein SAA time to threshold**
(A) Cognitive decline in sporadic and monogenic Parkinson's disease. (B) Cognitive decline in sporadic Parkinson's disease only. (C) Parkinson's disease dementia in sporadic and monogenic Parkinson's disease. (D) Cognitive decline in Alzheimer's disease biomarker negative sporadic and monogenic Parkinson's disease. Associated summary statistics in each plot generated by a Cox proportional hazards time-to-event analysis, adjusting for sex, age, and disease duration at baseline. PPMI=Parkinson's Progression Markers Initiative. SAA=seed amplification assay. TTT=time to threshold.

monogenic Parkinson's disease within the PPMI cohort are summarised in the appendix (p 6).

We explored potential differences in α-synuclein SAA seeding kinetics in α-synuclein SAA-positive monogenic versus sporadic Parkinson's disease samples in the PPMI cohort. In this analysis, we found that *GBA1*-Parkinson's disease samples in the 24 h assay subcohort had a faster TTT relative to sporadic Parkinson's disease samples (p=0·04). By contrast, *LRRK2*-Parkinson's disease samples in the 150 h assay subcohort had a slower TTT relative to sporadic Parkinson's disease samples (p=0·001; table 3).

In the PPMI cohort, we did a time-to-event analysis to assess whether baseline α-synuclein SAA kinetic measures predict an unfavourable outcome in Parkinson's disease. In all of the following analyses, the Schoenfeld residuals were not time dependent (p>0·05), confirming that the proportional hazards assumption was met.

After excluding individuals who had an unfavourable outcome at the time of baseline SAA testing, an unfavourable outcome was observed in 593 (73%) of 810 sporadic and monogenic Parkinson's disease participants with a positive α-synuclein SAA result during a median follow-up period of 4·5 years (IQR 2–9). Cox proportional hazards model analyses showed that none of the kinetic measures reached the Bonferroni significance threshold (p<0·003) in predicting an unfavourable outcome (appendix p 7). We then analysed each component of an unfavourable outcome separately and found that TTT predicted only cognitive decline (MoCA ≤21) during the study follow-up period (HR 2·36 [95% CI 1·60–3·46], p<0·0001; figure 2A; appendix p 7). With regard to this result, we found statistically significant differences (p<0·05) in both subcohorts of the PPMI cohort despite the relatively short follow-up period in the Amprion 24 h assay subcohort (median 2 years; IQR 1–3) compared with the Amprion 150 h assay subcohort (median 7 years; IQR 5–11; figure 2A). In the whole PPMI cohort, TTT also predicted cognitive decline in participants with sporadic Parkinson's disease after excluding monogenic Parkinson's disease samples (HR 1·92 [95% CI 1·17–3·16], p=0·01; figure 2B).

Parkinson's disease dementia was observed in 58 (7%) of 818 sporadic and monogenic Parkinson's disease participants with a positive α-synuclein SAA result. After excluding individuals who had developed Parkinson's disease dementia at the time of baseline SAA testing, we found that TTT predicted the development of Parkinson's disease dementia during the study follow-up period (HR 2·84 [95% CI 1·67–4·81], p=0·0001; figure 2C). Furthermore, in the subset of sporadic and monogenic Parkinson's disease participants with a positive α-synuclein SAA result who underwent Alzheimer's disease biomarker testing, 56 (13%) of 424 fulfilled criteria for Alzheimer's disease biomarker positivity. Using Alzheimer's disease biomarker status as the predictor variable in the same Cox proportional hazards model analyses as above, we found that Alzheimer's disease biomarker positivity predicted both cognitive decline (HR 3·15 [95% CI 1·96–5·08], p<0·0001) and Parkinson's disease dementia (5·99 [3·28–10·93], p<0·0001) during the study follow-up period. In the Alzheimer's disease biomarker-negative subgroup, we found that TTT predicted both cognitive decline (HR 1·80 [95% CI 1·03–3·18], p=0·04; figure 2D) and Parkinson's disease dementia (2·70 [95% CI 1·17–6·24], p=0·02) during the study follow-up period.

To independently replicate the primary findings from the PPMI cohort, we accessed data from the Tübingen Parkinson's disease cohort consisting of 229 patients with Parkinson's disease (appendix p 8), with CSF samples previously analysed with the ISNB α-synuclein SAA. Genetic status was available for 229 (100%) of 229 participants. The pathogenic variants detected in participants with monogenic Parkinson's disease within the Tübingen Parkinson's disease cohort are summarised in the appendix (pp 9–10). As in the PPMI cohort, we found that *GBA1*-Parkinson's disease samples had a faster TTT relative to sporadic Parkinson's disease samples (p=0·01; table 3).

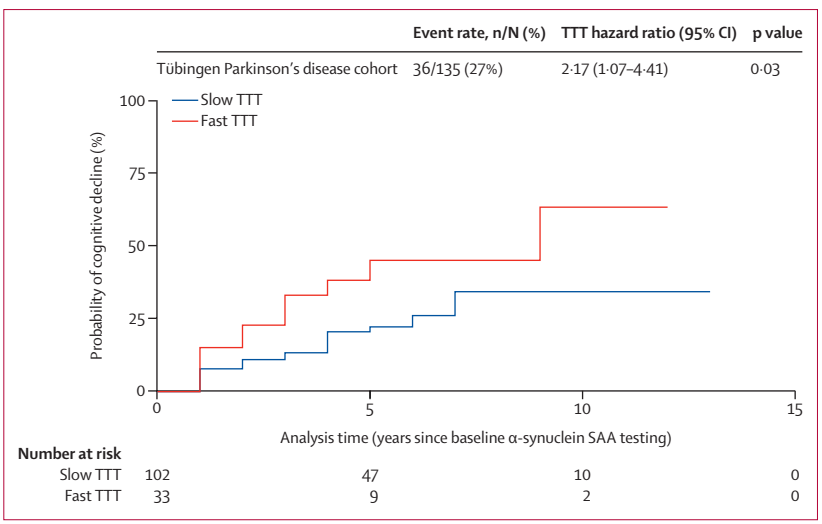

***Figure 3*: Baseline CSF α-synuclein SAA time to threshold predicting cognitive decline in the Tübingen Parkinson's disease cohort**

Associated summary statistics generated by a Cox proportional hazards time-to-event analysis, adjusting for sex, age, and disease duration at baseline. SAA=seed amplification assay. TTT=time to threshold.

After excluding individuals who had developed cognitive decline (MoCA ≤21) at the time of baseline SAA testing, cognitive decline was observed in 36 (27%) of 135 sporadic and monogenic Parkinson's disease participants with a positive α-synuclein SAA result during a median follow-up period of 4 years (IQR 3–7). In this cohort we found that TTT predicted cognitive decline during the study follow-up period (HR 2·17 [95% CI 1·07–4·41], p=0·03; figure 3).

Discussion

In this study we comprehensively assessed the diagnostic and prognostic value of quantitative α-synuclein SAA kinetic measures in independent cohorts, including the large PPMI cohort of sporadic and monogenic forms of Parkinson's disease.

In the UK parkinsonism cohort, our finding of α-synuclein SAA positivity in 15% of CSF samples from participants with clinically diagnosed progressive supranuclear palsy is in line with recent studies.[21,22] We hypothesise that this represents the presence of Lewy body co-pathology. Furthermore, our α-synuclein SAA positivity rate is similar to the rates of Lewy body co-pathology observed in post-mortem studies of progressive supranuclear palsy.[23,24] Alternatively, α-synuclein SAA positivity in progressive supranuclear palsy samples could be due to tau aggregation causing the misfolding of α-synuclein, and the resulting low levels of α-synuclein pathology might not be detectable via conventional neuropathological analysis. Ultimately,

further studies of patient cohorts with post-mortem validation are required to explore these hypotheses.

The presence of α-synuclein SAA positivity with distinct low and slow kinetics in the majority of α-synuclein SAA-positive progressive supranuclear palsy samples might reflect low-level Lewy body co-pathology, noting that one of the α-synuclein SAA-positive samples with low and slow kinetics was from a patient who had post-mortem confirmation of primary progressive supranuclear palsy pathology and Braak stage 1 Lewy body co-pathology. This finding highlights that multiple proteinopathies can co-exist in individual patients and can be detectable during their lifetime. However, it also emphasises that it is insufficient to rely on α-synuclein SAA positivity in isolation to support a diagnosis of Parkinson's disease. Refinement of the analysis of α-synuclein SAA kinetics might improve the performance of the assay in distinguishing Parkinson's disease from progressive supranuclear palsy with Lewy body co-pathology. Furthermore, future studies of Parkinson's disease cohorts that include α-synuclein SAA and biomarkers of pathologies other than α-synuclein will enable us to interpret the significance of low and slow α-synuclein SAA kinetics in Parkinson's disease samples. In line with this, the ongoing development of four-repeat tau SAA[25,26] is a major priority that will allow combined testing with α-synuclein SAA for more accurate diagnosis of patients with early-stage parkinsonism that is clinically indeterminate.

In both the PPMI and Tübingen cohorts, relative to sporadic Parkinson's disease, we found more aggressive seeding kinetics (faster TTT) in *GBA1*-Parkinson's disease. This observation suggests that genetic status determines the profile of α-synuclein seeding kinetics in Parkinson's disease, and is in line with previous studies showing a more aggressive disease course in *GBA1*-Parkinson's disease.[27]

In the time-to-event analyses in the PPMI cohort, which included individuals with up to 13 years of follow-up data, we found robust evidence of fast TTT predicting cognitive decline in Parkinson's disease. This was apparent in monogenic Parkinson's disease, including *GBA1* and *SNCA* variants (which are known genetic risk factors for dementia), but also in sporadic Parkinson's disease. We also observed an association between TTT and dementia based on the MDS Task Force criteria for Parkinson's disease dementia, which supports our findings on cognitive decline. By contrast, our results show that baseline α-synuclein SAA kinetic measures do not predict a 5-point or greater increase in the MDS-UPDRS-III score as a marker of motor progression in Parkinson's disease (appendix p 7). Similarly, baseline TTT did not predict death, but the death rate in the PPMI cohort was low (7%) and it is unclear whether the cause of death in each case was due to Parkinson's disease or an alternative non-neurological cause (appendix p 7). Future studies with detailed mortality data are needed to explore whether TTT predicts overall survival in Parkinson's disease. We independently replicated our primary finding of TTT predicting cognitive decline in the Tübingen Parkinson's disease cohort. Notably, our analysis of the Tübingen cohort used longer follow-up data and a more conservative definition of cognitive decline compared with previous α-synuclein SAA analyses of this cohort.[28] Furthermore, our main finding of TTT predicting cognitive decline in Parkinson's disease is supported by a recent study showing that PPMI participants with prodromal Parkinson's disease and fast TTT had higher rates of phenoconversion than participants with prodromal Parkinson's disease and slow TTT.[29]

In the PPMI cohort, Alzheimer's disease biomarker positivity was a strong predictor of cognitive decline in Parkinson's disease, as reported in previous studies.[30] However, importantly, in our Alzheimer's disease biomarker negative subgroup, we showed that TTT predicted cognitive decline independently of Alzheimer's disease co-pathology. We hypothesise that fast TTT reflects high seeding capacity, which translates to a faster rate of cortical spread of Lewy body pathology and subsequent cognitive decline. This suggests that α-synuclein seeding drives pathological and clinical disease progression and is therefore a justifiable disease-modifying target in Parkinson's disease.

Although different α-synuclein SAAs were used in the three cohorts, we are reassured by the fact that the Amprion, RML, and ISNB α-synuclein SAAs are well-validated assays. Moreover, previous work has shown high concordance of results between the Amprion 150 h assay and the RML assay in Parkinson's disease CSF samples.[31] Furthermore, the ISNB assay has previously shown high sensitivity (95%) and specificity (98%) for detecting Lewy body pathology in a series of ante-mortem CSF samples from individuals referred to the laboratory of neuropathology at ISNB for dementia of various causes in which the presence of Lewy body pathology was evaluated at post-mortem examination.[9]

Our study has some limitations that need to be considered. Post-mortem confirmation of neuropathology was only available in a small subset of participants with progressive supranuclear palsy so we cannot definitively comment on the sensitivity, specificity, positive predictive value, and negative predictive value of α-synuclein SAA. Linked to this, a priority for future studies is to explore the performance of α-synuclein SAA in ethnically diverse populations. The UK parkinsonism cohort was limited to genetic testing for only the *LRRK2*-Gly2019Ser variant, and comprehensive genetic status was established in only 46% of the PPMI Amprion 24 h assay subcohort, so it is likely that the sporadic Parkinson's disease groups in these two cohorts will contain a small number of unidentified monogenic Parkinson's disease cases, which could have influenced our results.

In summary, our study has advanced our understanding of the clinical and research applications of α-synuclein SAA by highlighting the diagnostic and prognostic value of quantitative α-synuclein SAA kinetic measures. The presence of α-synuclein SAA positivity with distinct low and slow kinetics in a subset of progressive supranuclear palsy samples reinforces the need for the development of four-repeat tau SAA to enable combined testing with α-synuclein SAA for greater diagnostic accuracy in clinical practice. We have also shown that genetic status in Parkinson's disease determines the profile of α-synuclein seeding kinetics. Furthermore, our results suggest that TTT can be used as a prognostic biomarker and trial stratification tool to recruit Parkinson's disease trial cohorts with homogeneous disease progression trajectories.

Contributors

CDO and EJ directly accessed and verified the data reported in this manuscript. DV, NV, RR, RF, MTJ, MH, AM, PNL, KPB, BCPG, AC, CK, MTMH, JBR, TF, HRM, and EJ carried out study assessments for participants in the UK parkinsonism cohort. OA, AQ, KSJA, TTW, and ZJ carried out post-mortem assessments on participants with progressive supranuclear palsy in the UK parkinsonism cohort. CDO, DV, NV, RR, AMC, RF, MTJ, MH, CG, ALGM, EJS, LW, BRG, AGH, CB, TF, HRM, BC, and EJ contributed to the acquisition and analysis of data. SL, IW, PP, and KB generated clinical and α-synuclein SAA data in the Tübingen Parkinson's disease cohort. EJ conceptualised and supervised the study; CDO and EJ drafted the manuscript. All authors had full access to all the data in the study and had final responsibility for the decision to submit the manuscript for publication.

Declaration of interests

BCPG's salary is paid by University Hospital Southampton. In the past 12 months BCPG has received honoraria from the neurology masterclass and GEC, grants from the PSP Association, and paid consultancy from ImmunoBrain. BCPG serves as a trustee and is on the research committee for the PSP Association. CK is employed by Northern Care Alliance NHS Foundation Trust. In the past 12 months CK has received speaker honoraria from Neurology Academy, Ipsen, and Britannia Pharmaceuticals. JBR is employed by Cambridge University with academic grants from AstraZeneca, Lilly, GSK, and Janssen, and paid consultancy for Asceneuron, Astex, Astronautx, Alector, Booster Therapeutics, Clinical Ink, Curasen, CumulusNeuro, Eisai, Ferrer, ICG, Invicro, Prevail, in the past 12 months unrelated to the current work. HRM is employed by UCL. In the past 12 months HRM reports paid consultancy from Roche, Aprinoia, AI Therapeutics and Amylyx; lecture fees or honoraria from BMJ, Kyowa Kirin, and the Movement Disorders Society. TF has served on advisory boards for Peptron, Treefrog, AbbVie, Bluerock, Bayer, and Bial, and has received honoraria for talks sponsored by Bayer, Bial, Profile Pharma, Boston Scientific, and Novo Nordisk. HRM is a co-applicant on a patent application related to C9ORF72 (Method for diagnosing a neurodegenerative disease; PCT/GB2012/052140). BC, CDO, BRG, and AGH are inventors on a patent pertaining the RML α-synuclein SAA (RT-QuIC) technology. All other authors declare no competing interests.

Data sharing

De-identified data that support the findings and a data dictionary will be made available upon reasonable request via email to the corresponding author.

Acknowledgments

DPV is supported by CBD Solutions. RR is supported by Aligning Science Across Parkinson's. AQ is supported by Aligning Science Across Parkinson's. CK is supported by the Multiple System Atrophy Trust and Parkinson's UK. JBR is supported by the Medical Research Council (MC_UU_00030/14; MR/T033371/1), the PSP Association, Cambridge Centre for Parkinson-plus, the NIHR Clinical Research Network, and the NIHR Cambridge Biomedical Research Centre (NIHR203312: the views expressed are those of the authors and not necessarily those of the National Institute for Health and Care Research [NIHR] or the Department of Health and Social Care). TF is supported by the NIHR, Edmond J Safra Foundation, Michael J Fox Foundation, John Black Charitable Foundation, Cure Parkinson's, Parkinson's UK, Gatsby Foundation, Innovate UK, Janet Owens Research Fellowship, Rosetrees Trust, Van Andel Research Institute, and Defeat MSA. HRM is supported by Parkinson's UK, Cure Parkinson's Trust, PSP Association, Medical Research Council, and The Michael J. Fox Foundation. EJ is supported by Medical Research Council Clinician Scientist (UKRI1388) and Clinical Research Training (MR/S000992/1) fellowships, and grants from the PSP Association (PSPA2023/PROJECTGRANT001) and CurePSP (681-2022/06). The other authors have no funding declarations. This work was supported in part by the Division of Intramural Research of the NIAID. Parkinson's Progression Markers Initiative (PPMI), a public–private partnership, is funded by the Michael J. Fox Foundation for Parkinson's Research and funding partners, including 4D Pharma, AbbVie, AcureX, Allergan, Amathus Therapeutics, Aligning Science Across Parkinson's, AskBio, Avid Radiopharmaceuticals, BIAL, BioArctic, Biogen, Biohaven, BioLegend, BlueRock Therapeutics, Bristol-Myers Squibb, Calico Labs, Capsida Biotherapeutics, Celgene, Cerevel Therapeutics, Coave Therapeutics, DaCapo Brainscience, Denali, Edmond J. Safra Foundation, Eli Lilly, Gain Therapeutics, GE HealthCare, Genentech, GSK, Golub Capital, Handl Therapeutics, Insitro, Jazz Pharmaceuticals, Johnson & Johnson Innovative Medicine, Lundbeck, Merck, Meso Scale Discovery, Mission Therapeutics, Neurocrine Biosciences, Neuron23, Neuropore, Pfizer, Piramal, Prevail Therapeutics, Roche, Sanofi, Servier, Sun Pharma Advanced Research Company, Takeda, Teva, UCB, Vanqua Bio, Verily, Voyager Therapeutics, the Weston Family Foundation, and Yumanity Therapeutics.

References

1 Condello C, Westaway D, Prusiner SB. Expanding the prion paradigm to include Alzheimer and Parkinson diseases. *JAMA Neurol* 2024; **81:** 1023–24.

2 Koga S, Sekiya H, Kondru N, Ross OA, Dickson DW. Neuropathology and molecular diagnosis of synucleinopathies. *Mol Neurodegener* 2021; **16:** 83.

3 Kovacs GG, Lukic MJ, Irwin DJ, et al. Distribution patterns of tau pathology in progressive supranuclear palsy. *Acta Neuropathol* 2020; **140:** 99–119.

4 Williams-Gray CH, Mason SL, Evans JR, et al. The CamPaIGN study of Parkinson's disease: 10-year outlook in an incident population-based cohort. *J Neurol Neurosurg Psychiatry* 2013; **84:** 1258–64.

5 Dadu A, Satone V, Kaur R, et al. Identification and prediction of Parkinson's disease subtypes and progression using machine learning in two cohorts. *NPJ Parkinsons Dis* 2022; **8:** 172.

6 Vijiaratnam N, Foltynie T. How should we be using biomarkers in trials of disease modification in Parkinson's disease? *Brain* 2023; **146:** 4845–69.

7 Siderowf A, Concha-Marambio L, Lafontant DE, et al. Assessment of heterogeneity among participants in the Parkinson's Progression Markers Initiative cohort using α-synuclein seed amplification: a cross-sectional study. *Lancet Neurol* 2023; **22:** 407–17.

8 Concha-Marambio L, Pritzkow S, Shahnawaz M, Farris CM, Soto C. Seed amplification assay for the detection of pathologic alpha-synuclein aggregates in cerebrospinal fluid. *Nat Protoc* 2023; **18:** 1179–96.

9 Rossi M, Candelise N, Baiardi S, et al. Ultrasensitive RT-QuIC assay with high sensitivity and specificity for Lewy body-associated synucleinopathies. *Acta Neuropathol* 2020; **140:** 49–62.

10 Simuni T, Chahine LM, Poston K, et al. A biological definition of neuronal α-synuclein disease: towards an integrated staging system for research. *Lancet Neurol* 2024; **23:** 178–90.

11 Höglinger GU, Adler CH, Berg D, et al. A biological classification of Parkinson's disease: the SynNeurGe research diagnostic criteria. *Lancet Neurol* 2024; **23:** 191–204.

12 Gibb WR, Lees AJ. The relevance of the Lewy body to the pathogenesis of idiopathic Parkinson's disease. *J Neurol Neurosurg Psychiatry* 1988; **51:** 745–52.

13 Vijiaratnam N, Girges C, Auld G, et al. Exenatide once weekly over 2 years as a potential disease-modifying treatment for Parkinson's disease: protocol for a multicentre, randomised, double blind, parallel group, placebo controlled, phase 3 trial: the 'Exenatide-PD3' study. *BMJ Open* 2021; **11:** e047993.

14 Höglinger GU, Respondek G, Stamelou M, et al, and the Movement Disorder Society-endorsed PSP Study Group. Clinical diagnosis of progressive supranuclear palsy: the movement disorder society criteria. *Mov Disord* 2017; **32:** 853–64.

15 Jabbari E, Holland N, Chelban V, et al. Diagnosis across the spectrum of progressive supranuclear palsy and corticobasal syndrome. *JAMA Neurol* 2020; **77:** 377–87.

16 Rossi M, Baiardi S, Teunissen CE, et al. Diagnostic value of the CSF α-synuclein real-time quaking-induced conversion assay at the prodromal MCI stage of dementia with Lewy bodies. *Neurology* 2021; **97:** e930–40.

17 McGhee D, Parker A, Fielding S, Zajicek J, Counsell C. Using 'dead or dependent' as an outcome measure in clinical trials in Parkinson's disease. *J Neurol Neurosurg Psychiatry* 2015; **86:** 180–85.

18 Hu MTM, Szewczyk-Królikowski K, Tomlinson P, et al. Predictors of cognitive impairment in an early stage Parkinson's disease cohort. *Mov Disord* 2014; **29:** 351–59.

19 Horváth K, Aschermann Z, Ács P, et al. Minimal clinically important difference on the Motor Examination part of MDS-UPDRS. *Parkinsonism Relat Disord* 2015; **21:** 1421–26.

20 Dubois B, Burn D, Goetz C, et al. Diagnostic procedures for Parkinson's disease dementia: recommendations from the movement disorder society task force. *Mov Disord* 2007; **22:** 2314–24.

21 Vaughan DP, Fumi R, Theilmann Jensen M, et al. Evaluation of cerebrospinal fluid α-synuclein seed amplification assay in progressive supranuclear palsy and corticobasal syndrome. *Mov Disord* 2024; **39:** 2285–91.

22 Anastassiadis C, Martinez-Valbuena I, Vasilevskaya A, et al. CSF α-synuclein seed amplification assay in patients with atypical parkinsonian disorders. *Neurology* 2024; **103:** e209818.

23 Jecmenica Lukic M, Kurz C, Respondek G, et al. Copathology in progressive supranuclear palsy: does it matter? *Mov Disord* 2020; **35:** 984–93.

24 Robinson JL, Yan N, Caswell C, et al. Primary tau pathology, not copathology, correlates with clinical symptoms in PSP and CBD. *J Neuropathol Exp Neurol* 2020; **79:** 296–304.

25 Saijo E, Metrick MA 2nd, Koga S, et al. 4-Repeat tau seeds and templating subtypes as brain and CSF biomarkers of frontotemporal lobar degeneration. *Acta Neuropathol* 2020; **139:** 63–77.

26 Martinez-Valbuena I, Tartaglia MC, Fox SH, Lang AE, Kovacs GG. Four-repeat tau seeding in the skin of patients with progressive supranuclear palsy. *JAMA Neurol* 2024; **81:** 1228–30.

27 Stoker TB, Camacho M, Winder-Rhodes S, et al. Impact of *GBA1* variants on long-term clinical progression and mortality in incident Parkinson's disease. *J Neurol Neurosurg Psychiatry* 2020; **91:** 695–702.

28 Brockmann K, Lerche S, Baiardi S, et al. CSF α-synuclein seed amplification kinetic profiles are associated with cognitive decline in Parkinson's disease. *NPJ Parkinsons Dis* 2024; **10:** 24.

29 Coughlin DG, Shifflett B, Farris CM, et al. α-synuclein seed amplification assay amplification parameters and the risk of progression in prodromal Parkinson disease. *Neurology* 2025; **104:** e210279.

30 Siderowf A, Xie SX, Hurtig H, et al. CSF amyloid β 1-42 predicts cognitive decline in Parkinson disease. *Neurology* 2010; **75:** 1055–61.

31 Russo MJ, Orrú CD, Concha-Marambio L, et al. High diagnostic performance of independent alpha-synuclein seed amplification assays for detection of early Parkinson's disease. *Acta Neuropathol Commun* 2021; **9:** 179.