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Association of α-synuclein seed-amplification kinetics with cognitive decline in idiopathic Parkinson's disease

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ABSTRACT

Introduction: Neurodegeneration and α-synuclein aggregates are pathological hallmarks of Parkinson's disease (PD). The α-synuclein seed-amplification assay (SAA) is a robust diagnostic tool for synucleinopathies. However, its binary readout limits its utility, and associations between semi-quantitative parameters and clinical outcomes remain inconsistent. This cohort study investigated whether baseline CSF α-synuclein SAA kinetic parameters are associated with longitudinal clinical trajectories in PD.

Methods: A total of 898 participants with idiopathic PD and positive α-synuclein SAA (24-h protocol) from the Parkinson's Progression Markers Initiative (PPMI) cohort were included.

Results: Linear mixed-effects models showed that higher maximum fluorescence, area under the fluorescence curve, and maximum slope at baseline visit were significantly associated with slower cognitive decline, both globally and across specific cognitive domains. In exploratory analyses, a similar, though less consistent, pattern was observed for motor progression. Results were consistent in sensitivity analyses restricted to participants with at least five years of follow-up ($n = 309$).

Conclusion: The main findings of this study suggest that higher baseline amplitude-related parameters on CSF α-synuclein SAA are associated with less pronounced long-term cognitive decline in a subset of PD patients. These results should be interpreted with caution, and further studies are needed to determine whether these observations reflect methodological limitations of the assay or biologically meaningful differences in α-synuclein aggregation.

1. Introduction

Neurodegeneration and α-synuclein (α-syn) aggregates are the pathological hallmarks of Parkinson's disease (PD) [1]. These aggregates are not only found in the nigrostriatal pathway but are also widely distributed throughout both the central and peripheral nervous systems [1]. While α-syn aggregates have traditionally been considered neurotoxic and a key pathogenic mechanism, the limited efficacy of clinical trials targeting α-syn has prompted the re-emergence of alternative hypotheses [2]. Although their pathogenic role remains debated, they are widely accepted as a useful diagnostic biomarker of PD and other synucleinopathies [3].

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Over the past decade, CSF α-syn seed-amplification assays (SAA) have emerged as a very sensitive and specific tool for the diagnosis of synucleinopathies [4]. α-syn SAAs involve the *in vitro* amplification of aggregated α-syn (seeds) present in patient samples, by incorporating α-syn monomers and applying fragmentation-elongation cycles through agitation or other methods [5]. A major limitation of the assay is, however, its categorical nature, typically classified as positive or negative based on whether the maximum fluorescence of the amplification curve exceeds a predefined threshold [5]. Nonetheless, several studies have explored the potential of kinetic parameters, such as time to threshold (TTT), maximum fluorescence (F_{max}), time to reach 50% of F_{max} (T50), maximum slope of the curve ($Slope_{max}$), time to reach $Slope_{max}$ (TS_{max}), or area under the fluorescence curve (AUFC), to semi-quantify the assay and investigate possible associations between amplification kinetics and clinical progression [6–17].

To date, findings in PD have been highly variable and inconsistent. Most studies have not found associations between kinetic parameters with cross-sectional or longitudinal clinical measures such as Movement Disorders Society-Unified PD Rating Scale (MDS-UPDRS), disease duration, or Montreal Cognitive Assessment (MoCA) [9,12–18]. In line with the hypothesis that CSF α-syn SAA reflects underlying Lewy-body pathology and potentially more severe neurodegeneration, some studies have reported limited associations, such as lower MoCA or *GBA* pathogenic variants with shorter TTT or TS_{max}, and higher Hoehn & Yahr, dysautonomia, or REM-sleep behavior disorder with lower T50, suggesting that faster aggregation might be associated with more severe disease [6–11]. Paradoxically, other studies have reported longer T50 associated with worse MoCA and higher MDS-UPDRS I, suggesting a more severe phenotype despite slower seeding [12].

Given these inconsistencies, further investigation in larger longitudinal cohorts is warranted. In addition, limited data are available regarding the relationship between CSF α-syn SAA kinetics with cognitive and neuropsychiatric symptoms. This study aimed to evaluate whether baseline kinetic parameters of CSF α-syn SAA predict the long-term clinical course in a large cohort of idiopathic PD patients, with particular focus on cognitive and neuropsychiatric symptoms. We analyzed data from the Parkinson's Progression Markers Initiative (PPMI) cohort, restricting analyses to samples generated using the 24-h SAA protocol, thereby avoiding the protocol heterogeneity present in prior PPMI-based studies [7,18].

2. Methods

2.1. Study design and selection of participants

Data used in the preparation of this cohort study were obtained on April 11, 2026, from the PPMI database (www.ppmi-info.org/access-dataspecimens/download-data), RRID:SCR 006431. PPMI is an ongoing longitudinal, observational, multi-center study assessing clinical, imaging, and biological features of PD across all stages of the disease. The PPMI protocol was approved by the Western Institutional Review Board (#20211908), as well as by the institutional review boards of all participating sites. Written informed consent was obtained from all participants. For up-to-date information, visit www.ppmi-info.org.

Patients from the *PD cohort* were included if they met the following criteria: diagnosis of idiopathic PD, positive CSF α-syn SAA ≤1 year after baseline, and at least one follow-up visit. Patients with monogenic forms of PD (*SNCA*, *LRRK2*, *PINK1*, or *PRKN*), as well as those from the *Prodromal*, *SWEDD*, or *Healthy Control cohorts*, were excluded from the analysis.

2.2. Clinical assessments

The following clinical variables were evaluated: MDS-UPDRS III for motor symptoms, MoCA for global cognitive performance, Hopkins Verbal Learning Test (HVLT) for memory, Letter Number Sequencing (LNS) for executive functions, Symbol Digit Modalities Test (SDMT) for attention and processing speed, animal fluency (AF) for language, Judgement of Line Orientation (JLO) for visuospatial abilities, MDS-UPDRS 1.2 for hallucinations, MDS-UPDRS 1.5 for apathy, 15-item Geriatric Depression Scale (GDS) for depression, State subscale of the State-Trait Anxiety Inventory (STAI state) for anxiety, and Questionnaire for Impulsive-Compulsive Disorders in PD (QUIP) for impulse-control disorder (ICD). For the exploratory subgroup analyses according to baseline cognitive status, participants were classified as PD-mild cognitive impairment (PD-MCI) if their baseline MoCA was <26 and as PD- normal cognition (PD-NC) if their baseline MoCA was ≥26.

2.3. CSF α-syn SAA

CSF collection, shipment, storage, and analysis were performed at each study site according to the protocols detailed in the PPMI biologics manual (http://www.ppmi-info.org/).

CSF α-syn SAA analyses were performed centrally by Amprion using standardized procedures and under blinded conditions as previously described [5]. Laboratory personnel performing the assays were independent from participant clinical assessments and from the statistical analyses conducted for the present study. All samples were run between January 3, 2023, and August 8, 2025. Only samples collected ≤1 year of the baseline visit were included in the present study. To ensure methodological consistency, only results generated using the Amprion 24-h protocol were considered; results from the Amprion 150-h protocol were excluded. Given that each sample was run in triplicate, kinetic parameters of the amplification curve (F_{max}, TTT, AUFC, TS_{max}, $Slope_{max}$) were averaged across the three replicates.

In brief, F_{max} represents the maximum fluorescence reached by the curve, TTT is the time at which fluorescence exceeds the predefined positivity threshold, AUFC corresponds to the area under the fluorescence curve, TS_{max} indicates the time to $Slope_{max}$, and $Slope_{max}$ refers to the inflection point of the curve (Supplementary Fig. 1).

2.4. Statistical analysis

Descriptive statistics were calculated for demographic, clinical, and CSF α-syn SAA variables at baseline. Since Kolmogorov-Smirnov tests indicated non-normal distributions for all continuous variables, these are reported as medians and interquartile ranges (IQR).

To explore bivariable associations between continuous variables, non-parametric tests were used, including the Mann-Whitney *U* test and Spearman's rank correlation coefficients. For associations between categorical variables, χ^2 tests were used. For the comparison between participants with <5 years and ≥5 years of follow-up, effect sizes were calculated for significant continuous comparisons and interpreted as small (≈0.1), medium (≈0.3), or large (≥0.5).

Linear mixed-effects models (LMMs) were used to examine the association between baseline SAA kinetic parameters and the longitudinal progression of clinical outcomes. In the primary analyses, all models included fixed effects for time (modeled as a continuous variable), the baseline kinetic parameter of interest, their interaction term, and covariates (specified in the *Results* section) selected *a priori* for each model. The LMM structure is detailed in the **Supplementary Methods**. The random-effects structure was determined by comparing random intercept-only and random slope models using maximum likelihood estimation and Akaike's Information Criterion (AIC). Models including random slopes for time provided a better fit and were therefore adopted as the primary specification. Models allowing correlation between random intercepts and slopes were also explored but showed numerical instability and convergence issues without meaningful improvement in model fit; therefore, a model with uncorrelated random slopes was used. Model parameters were estimated using restricted maximum likelihood (REML), and degrees of freedom were approximated using

Satterthwaite's method.

To further characterize the temporal pattern of associations, a *post hoc* analysis was conducted in which time was modeled as a categorical variable. In these models, only random intercepts were included, as random slopes were not estimable under this specification due to model complexity and convergence limitations. This approach allowed identification of the specific time points at which associations emerged. For illustrative purposes, kinetic parameters were additionally categorized into quartiles, and trajectories across kinetic parameter quartiles were compared using LMMs with time modeled as a categorical variable and random intercepts. The statistical significance of the quartile-time interaction was evaluated using a Type III Wald χ^2 test.

Multiple comparisons for the primary analyses were addressed using the Benjamini-Hochberg false discovery rate (FDR). Given the high intercorrelation among SAA kinetic parameters (Spearman's $\rho = 0.785$-0.998), results across kinetic parameters were interpreted as convergent rather than independent evidence.

A two-tailed p value < 0.05 was considered statistically significant for all tests. A significance threshold of $q < 0.05$ was applied for FDR-corrected results. All analyses were performed using R software (v4.4.2).

3. Results

3.1. Baseline characteristics

A total of 898 participants were included (Supplementary Fig. 2). The median age was 63.8 years (IQR 57-69.6), with a disease duration of 0.6 years (IQR 0.3-1.1), and MDS-UPDRS III of 21 at baseline (IQR 15-29). The median follow-up duration was 3 years (IQR 2-7), with 309 participants (34.4%) with ≥5 years of follow-up. Additional baseline characteristics are summarized in Table 1. The availability of variables at each timepoint is summarized in Supplementary Table 1.

As all kinetic parameters were derived from the mean of three replicate measurements, intra-assay variability was assessed across triplicates. The median coefficient of variation was 4.1% (IQR 2.6-6.4) for F_{max}, 7.7% (IQR 4.8-11.2) for TTT, 8.3% (IQR 5.0-14.6) for AUFC, 7.5% (IQR 4.6-11.0) for TS_{max}, and 6.3% (IQR 3.8-9.6) for $Slope_{max}$, indicating good reproducibility across replicate measurements.

Table 1
Baseline characteristics of idiopathic PD and positive CSF α-syn SAA cohort ($n = 898$).

Age, years	63.8 (57-69.6)
Sex, female	316 (35.2%)
Education level, years	16 (14-18)
Disease duration, years	0.6 (0.3-1.1)
MDS-UPDRS III	21 (15-29)
LEDD	0 (0-0)
MoCA	27 (26-29)
MDS-UPDRS 1.2 (hallucinations)	0 (0-0)
GDS	2 (0-3)
STAI state	61 (50-75)
MDS-UPDRS 1.5 (apathy)	0 (0-0)
QUIP total	0 (0-0)
GBA pathogenic variant	65 (7.2%)
APOE ε4	207 (23.1%)
F_{max}, value	146750 (127547-165692)
TTT, h	11.2 (9.2-12.7)
AUFC, value	5249666667 (4384083333-6060916667)
TS_{max}, h	11.7 (9.6-13.3)
$Slope_{max}$, value	42.3 (35.2-48.9)

Continuous variables are presented as median (interquartile range), categorical variables are presented as number (percentage).
AUFC: area under the fluorescence curve; F_{max}: maximum fluorescence; GDS: Geriatric Depression Scale; h, hours; LEDD: levodopa equivalent daily dose; MDS-UPDRS III: Movement Disorders Society-Unified Parkinson's Disease Rating Scale part III; MoCA: Montreal Cognitive Assessment; QUIP: Questionnaire for Impulsive-Compulsive disorders in Parkinson's disease; $Slope_{max}$: maximum slope value; STAI: State-Trait Anxiety Inventory; TS_{max}: time to reach maximum slope; TTT: time to threshold.

In the bivariate analysis between kinetic parameters and clinical-demographic features, age correlated with F_{max} ($\rho = -0.118$; $p < 0.001$), AUFC ($\rho = -0.068$; $p = 0.042$), and $Slope_{max}$ ($\rho = -0.130$; $p < 0.001$); female sex was associated with higher F_{max} ($p < 0.001$), AUFC ($p < 0.001$), and $Slope_{max}$ ($p < 0.001$); *GBA* carrier status was associated with lower $Slope_{max}$ ($p = 0.003$) and lower F_{max} ($p = 0.049$) (Supplementary Tables 2 and 3). As expected, amplitude-related measures (F_{max}, AUFC, and $Slope_{max}$; $\rho = 0.785$-0.853) and between time-related parameters (TTT and TS_{max}; $\rho = 0.998$) were highly intercorrelated. Consequently, findings across these parameter groups should be interpreted as convergent evidence from mathematically related measures rather than fully independent signals.

3.2. Baseline kinetic parameters and clinical progression

We conducted LMMs to assess the predictive value of baseline CSF α-syn SAA kinetic parameters on the longitudinal progression of clinical symptoms. All models were adjusted for age, sex, disease duration, *GBA* and *APOE ε4* carrier status; MoCA was included in all models except the MDS-UPDRS III and QUIP models; LEDD was included in the MDS-UPDRS III models; years of education was included in the MoCA models; and GDS was included in the MDS-UPDRS 1.5 (apathy) models.

All outcomes worsened over time when time was modeled as the sole predictor (Supplementary Table 4–8). When including the interaction between kinetic parameters and time, with time treated as a continuous variable and random slopes specified for time, higher baseline amplitude-related parameters (F_{max}, AUFC, and $Slope_{max}$) were significantly associated with a slower rate of MoCA decline (Table 2). Additionally, lower $Slope_{max}$, longer TTT and TS_{max} were significantly associated with higher QUIP scores over time. After FDR correction, only associations between amplitude parameters and MoCA progression remained significant ($q < 0.05$); all other associations were not significant.

To further examine the temporal dynamics of these associations, we conducted a complementary analysis in which time was modeled as a categorical variable with a random intercept (Supplementary Table 4–8). This analysis showed that the association between amplitude-related parameters and MoCA decline was not constant over follow-up, but became evident primarily from year six onwards, with effect sizes increasing progressively through year twelve, consistent with the main analysis. The number of participants contributing MoCA assessments decreased to 247 at year 6, 215 at year 7, 161 at year 8, 131 at year 9, 151 at year 10, 146 at year 11, and 134 at year 12 (Supplementary Table 1). Notably, for MDS-UPDRS III a similar progressive pattern emerged from year six onwards despite not reaching significance in the primary model, suggesting a late-emerging effect on motor progression that the continuous time variable plus random slopes model lacked sufficient power to detect. Results for MDS-UPDRS 1.2 (hallucinations), MDS-UPDRS 1.5 (apathy), GDS, and STAI state, were largely non-significant.

For illustrative purposes, the cohort was divided into quartiles based on baseline F_{max} (Q1, lowest values; Q4, highest values). The interaction between quartile and time was statistically significant for both MoCA ($\chi^2(36) = 124.6, p < 0.001$) and MDS-UPDRS III ($\chi^2(36) = 55.4, p = 0.020$), confirming that clinical trajectories diverged significantly across quartiles over the follow-up period (Fig. 1).

3.3. Baseline kinetic parameters and progression in cognitive domains

Given the observed association between MoCA progression and kinetic parameters, we further examined the longitudinal trajectories of individual cognitive domains in a *post hoc* exploratory analysis (Table 3). After FDR correction, higher baseline F_{max} was significantly associated with slower decline in HVLT retention, AF and JLO. Similarly, higher Slopemax was significantly associated with slower decline in HVLT

Table 2
LMMs exploring the association between baseline kinetic parameters and the progression of clinical scales.

	MDS-UPDRS III	MoCA	MDS-UPDRS 1.2 (hallucinations)	GDS	STAI state	MDS-UPDRS 1.5 (apathy)	QUIP
F_{max}	−0.134 (0.147)	**0.078 (0.003)***	−0.006 (0.158)	−0.008 (0.666)	0.031 (0.600)	−0.008 (0.100)	−0.004 (0.455)
TTT	−0.038 (0.680)	−0.026 (0.319)	0.002 (0.592)	0.008 (0.663)	−0.021 (0.724)	0.001 (0.885)	**0.012 (0.021)**
AUFC	−0.102 (0.271)	**0.059 (0.025)***	−0.005 (0.257)	−0.007 (0.731)	0.038 (0.509)	−0.007 (0.162)	−0.009 (0.069)
TS_{max}	−0.047 (0.605)	−0.026 (0.320)	0.002 (0.583)	0.006 (0.741)	−0.023 (0.696)	0.001 (0.851)	**0.012 (0.018)**
$Slope_{max}$	−0.093 (0.320)	**0.085 (0.001)***	−0.005 (0.195)	−0.014 (0.468)	0.020 (0.744)	−0.008 (0.114)	**−0.011 (0.032)**

Results are presented as estimate (*p* value). Statistically significant *p* values are shown in bold. *Results surviving FDR correction ($q < 0.05$).
AUFC: area under the fluorescence curve; F_{max}: maximum fluorescence; GDS: Geriatric Depression Scale; MDS-UPDRS III: Movement Disorders Society-Unified Parkinson's Disease Rating Scale part III; MoCA: Montreal Cognitive Assessment; QUIP: Questionnaire for Impulsive-Compulsive disorders in Parkinson's disease; $Slope_{max}$: maximum slope value; STAI: State-Trait Anxiety Inventory; TS_{max}: time to reach maximum slope; TTT: time to threshold.

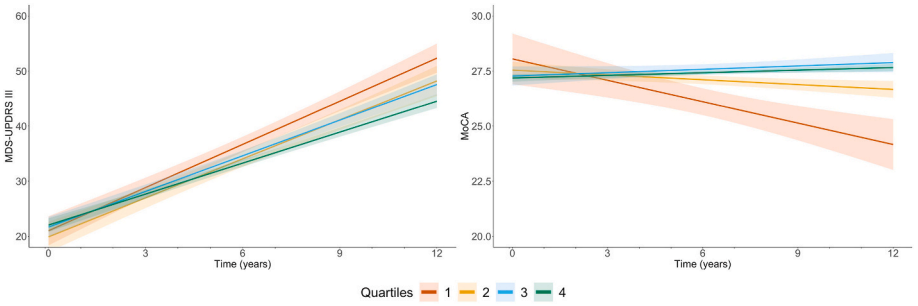

Fig. 1. LMMs exploring the association between baseline F_{max}-quartiles and the progression of MDS-UPDRS III and MoCA
MDS-UPDRS III: Movement Disorders Society-Unified Parkinson's Disease Rating scale part III; MoCA: Montreal Cognitive Assessment.

Table 3
LMMs exploring the association between baseline kinetic parameters and the progression of cognitive domains.

	HVLT imm	HVLT ret	HVLT del	LNS	SDMT	AF	JLO
F_{max}	**0.032 (0.048)**	**0.092 (0.010)***	0.003 (0.111)	0.036 (0.063)	**0.149 (0.035)**	**0.043 (0.011)***	**0.109 (0.002)***
TTT	0.015 (0.314)	−0.028 (0.425)	0.001 (0.531)	−0.001 (0.944)	**−0.183 (0.009)***	−0.01 (0.537)	−0.056 (0.112)
AUFC	0.012 (0.417)	0.063 (0.069)	0.001 (0.474)	0.018 (0.344)	**0.162 (0.021)**	0.031 (0.057)	**0.105 (0.002)***
TS_{max}	0.016 (0.286)	−0.026 (0.463)	0.001 (0.540)	−0.002 (0.925)	**−0.186 (0.007)***	−0.011 (0.491)	−0.055 (0.115)
$Slope_{max}$	0.018 (0.225)	**0.087 (0.014)***	0.002 (0.213)	0.034 (0.073)	**0.265 (<0.001)***	**0.046 (0.006)***	**0.112 (0.002)***

Results are presented as estimate (*p* value). Statistically significant *p* values are shown in bold. *Results surviving FDR correction ($q < 0.05$).
AF: Animal fluency; AUFC: area under the fluorescence curve; F_{max}: maximum fluorescence; HVLT del: HVLT delayed recall; HVLT imm: Hopkins Verbal Learning Test immediate recall; HVLT ret: HVLT retention; JLO: Judgement of Line Orientation; LNS: Letter Number Sequencing; SDMT: Symbol Digit Modalities Test; $Slope_{max}$: maximum slope value; TS_{max}: time to reach maximum slope; TTT: time to threshold.

retention, SDMT, AF, and JLO, while higher AUFC was associated with better longitudinal performance in JLO. In addition, shorter TTT and TS_{max} were associated with better longitudinal performance on the SDMT. Several additional nominal associations, including those between Fmax and HVLT immediate recall or SDMT and between AUFC and SDMT, did not survive FDR correction and should therefore be interpreted cautiously. Overall, these findings are consistent with those observed for the MoCA in the primary analysis.

3.4. Sensitivity analysis

The long follow-up period in this cohort resulted in a substantial amount of missing data over time. To assess the potential impact of this, participants were stratified into two subgroups for a sensitivity analysis: <5 years ($n = 589$) and ≥5 years of follow-up ($n = 309$). The median follow-up in the latter group was 10 years (IQR 7-12). Bivariate comparisons revealed statistically significant differences at baseline between the two subgroups in age, disease duration, education level, MDS-UPDRS III, LEDD, STAI state, QUIP, *GBA* carrier status, TTT, TS_{max} and $Slope_{max}$, with small effect sizes (Supplementary Table 9). Among participants with <5 years of follow-up, the shorter follow-up duration was primarily due to recent enrollment (88%) rather than withdrawal (12%).

In the sensitivity analysis restricted to participants with ≥5 years of follow-up, the primary findings were largely replicated (Supplementary Table 10). In the LMMs with time as a continuous variable and random slopes, the associations between amplitude-related parameters and cognitive decline on the MoCA remained statistically significant for F_{max} ($\beta = 0.083$, $p = 0.002$), and $Slope_{max}$ ($\beta = 0.060$, $p = 0.022$). For QUIP, the associations with AUFC ($\beta = -0.012$, $p = 0.035$) and $Slope_{max}$ ($\beta = -0.014$, p = 0.016) were also significant, whereas TTT ($p = 0.083$) and TS_{max} ($p = 0.079$) showed similar effect estimates but did not reach statistical significance in this subsample. Results for MDS-UPDRS III, MDS-UPDRS 1.2 (hallucinations), MDS-UPDRS 1.5 (apathy), GDS, and STAI-state remained non-significant. Associations with the progression of individual cognitive domains were largely unchanged (Supplementary Table 11).

Finally, we explored whether the association between kinetics and MoCA progression differed according to baseline cognitive status (PD-NC vs PD-MCI). A nominal interaction was observed for F_{max} ($\beta = -0.18$, $p = 0.016$), with a similar trend for AUFC ($\beta = -0.14$, $p = 0.056$), although none of these interactions remained significant after FDR correction (Supplementary Table 12).

4. Discussion

In this longitudinal study we observed that CSF α-syn SAA amplitude-related parameters, such as higher F_{max}, AUFC, and $Slope_{max}$,

at baseline visit were associated with a slower cognitive decline, as measured with MoCA and domain-specific tests, in idiopathic PD. In exploratory *post hoc* analyses, we also observed a more stable MDS-UPDRS III progression in individuals with higher baseline F_{max}. These findings were robust in sensitivity analyses restricted to participants with extended follow-up suggesting that, at least in a subset of idiopathic PD patients, baseline amplitude-related parameters may be linked to a more stable clinical progression.

α-syn SAA is a technique designed to infer the presence of seeding-competent forms of α-syn, typically fibrillar or proto-fibrillar with β-sheet structures [5]. As previously noted, despite providing a binary output, many studies, including the present one, have explored the potential of semi-quantitative parameters derived from the amplification curve. While time-related metrics such as TTT or TS_{max} are considered more reliable indicators of seed burden and overall seeding activity of the sample, amplitude-dependent metrics also capture aspects of seed burden and seeding dynamics, although they tend to be more variable and can be affected by saturation effects [19–22]. In our study, the strongest associations were observed between amplitude-related parameters (F_{max}, AUFC, and $Slope_{max}$), although we also found isolated associations between shorter TTT and TS_{max} with a better progression of QUIP, or SDMT. As these amplitude-related parameters are highly correlated and derived from the same amplification curve, their consistency should be interpreted as reflecting a shared assay characteristic rather than independent biological signals. Whether these findings reflect differences in the initial seed burden, distinct seeding dynamics driven by conformationally heterogeneous α-syn strains, or simply rapid plateau effects in patients with very high seed levels, remains to be determined [23,24]. In addition, technical and assay-related factors may also contribute to the observed variability in kinetic parameters and further complicate their biological interpretation. Although reproducibility across triplicates was good, kinetic parameters remain continuous measures derived from amplification curves and are therefore inherently more variable than the binary result. This residual variability may partially attenuate the strength of clinical associations and should be considered when interpreting the modest effect sizes observed.

In the past year, some studies using the same cohort as the present work have been published. Among them, only Orrú et al. reported a slightly increased risk of cognitive decline in patients with faster TTT, whereas other two studies did not identify associations between clinical symptoms and kinetic parameters [7,18,25]. Our dataset, obtained in April 2026, includes a larger cohort of participants assessed exclusively with the 24-h SAA protocol, rather than a mixture of 24- and 150-h protocols. Given that kinetic parameters have been shown to differ between these two protocols, this methodological variation may partly account for the discrepancies between our results and previous analyses from this cohort [25]. It is also worth noting that, although non-significant trends were observed during the initial years of follow-up, significant associations predominantly emerged after year six. This longer follow-up period may further account for differences with previous studies with shorter observation windows in a very early PD population [18,25].

The consistency in the direction of associations across multiple cognitive tests in this large multicenter cohort raises the question of whether patients with better outcomes truly represent individuals with greater intrinsic aggregation capacity, or whether the findings could instead reflect a saturation/plateau effect in those with worse progression, or limitations in the quantitative reliability of α-syn SAA, as suggested by recent studies [25]. In line with our results, a previous study also reported higher seeding activity parameters associated with a less severe clinical phenotype, an observation the authors described as *not expected* [12]. Furthermore, in the exploratory analyses of the present study, amplitude-related parameters were consistently associated with better longitudinal performance in memory, language, and visuospatial domains. Although these findings should be interpreted with caution, their convergence across domains typically linked to a more aggressive cognitive decline in PD may argue against a purely spurious association [26]. The ongoing debate regarding the pathogenic role of α-syn together with the emergence of alternative hypotheses, underscores the need for further studies to clarify these findings, and to better understand the SAA and the pathophysiological role of α-syn aggregates [2].

This study is not without limitations. First, both the MoCA and the MDS-UPDRS I are brief screening tools with limited range, which may not optimally capture changes over time. For cognitive assessment we were able to complement MoCA with domain-specific tests. Second, as is inherent to long-term prospective studies with multiple follow-up visits, there was a substantial amount of missing longitudinal data. As previously noted, most of the missing data among participants with <5 years of follow-up was due to their recent inclusion rather than withdrawal. To address this, we used LMMs, which are well-suited for handling missing data, and we also conducted a sensitivity analysis by re-running the LMMs in participants with ≥5 years of follow-up. This large subsample comprised 309 patients, in whom the findings were consistent with those of the full cohort. The number of participants contributing observations decreased at later follow-up visits; therefore, potential selection bias related to differential follow-up cannot be completely excluded. Although the generalizability of these findings to other PD cohorts warrants further investigation, they might be valid for a subset of PD patients. Third, although several associations were statistically significant, effect sizes were small (standardized $\beta < 0.2$), suggesting that their clinical utility may be limited. Nonetheless, these findings may still provide useful insights into the mechanistic basis of α-syn SAA and/or α-syn aggregation. Finally, the unclear biological role of fibrils and clinical significance of SAA kinetic parameters in PD, together with inconsistencies across different SAA protocols, complicate the interpretation of our findings. Nevertheless, several strengths of the study should be noted. These include the large sample size, the longitudinal design, the exclusive inclusion of idiopathic PD cases, and the use of a single SAA protocol for all participants. Furthermore, by modeling trajectories over time rather than relying on specific cut-offs, particularly for instruments with a narrow range, we provide a more reliable approach to capturing clinical progression [26].

In conclusion, these findings suggest that long-term cognitive decline may be less pronounced in a subset of individuals with idiopathic PD exhibiting higher baseline amplitude-related parameters in CSF α-syn SAA. Exploratory analyses further suggested a similar pattern for motor progression, although these findings were less consistent. These results should be interpreted with caution, and further studies are warranted to validate these findings and clarify the biological mechanisms underlying the observed associations.

Data availability

The datasets generated and analyzed during the current study are available in the PPMI repository, www.ppmi-info.org/access-dataspecimens/download-data.

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CRediT authorship contribution statement

Jon Rodriguez-Antiguedad: Conceptualization, Formal analysis, Writing – original draft. **Arnau Puig-Davi:** Formal analysis, Writing – review & editing. **Iñigo Ruiz-Barrio:** Conceptualization, Formal analysis, Writing – review & editing. **David Campo-Caballero:** Formal analysis, Writing – review & editing. **Elena Vacchi:** Supervision, Writing – review & editing. **Giorgia Melli:** Supervision, Writing – review & editing. **Lidia Bojtos:** Writing – review & editing. **Carla Franch-Marti:** Writing – review & editing. **Gonzalo Olmedo-Saura:** Writing –

review & editing. **Anna Vazquez-Oliver:** Writing – review & editing. **Saül Martinez-Horta:** Supervision, Writing – review & editing. **Jaime Kulisevsky:** Conceptualization, Supervision, Writing – review & editing. **Javier Pagonabarraga:** Conceptualization, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.parkreldis.2026.108856.

References

[1] B.R. Bloem, M.S. Okun, C. Klein, Parkinson's disease, Lancet 397 (2021) 2284–2303, https://doi.org/10.1016/S0140-6736(21)00218-X.
[2] A.J. Espay, J.A. Vizcarra, L. Marsili, A.E. Lang, D.K. Simon, A. Merola, K. A. Josephs, A. Fasano, F. Morgante, R. Savica, J.T. Greenamyre, F. Cambi, T. R. Yamasaki, C.M. Tanner, Z. Gan-Or, I. Litvan, I.F. Mata, C.P. Zabetian, P. Brundin, H.H. Fernandez, D.G. Standaert, M.A. Kauffman, M.A. Schwarzschild, S.P. Sardi, T. Sherer, G. Perry, J.B. Leverenz, Revisiting protein aggregation as pathogenic in sporadic Parkinson and Alzheimer diseases, Neurology 92 (2019) 329–337, https://doi.org/10.1212/WNL.0000000000006926.
[3] T.F. Outeiro, Alpha-synuclein in Parkinson's disease: the debate that must go on, Park. Relat. Disord. 136 (2025), https://doi.org/10.1016/j.parkreldis.2025.107850.
[4] A. Grossauer, G. Hemicker, F. Krismer, M. Peball, A. Djamshidian, W. Poewe, K. Seppi, B. Heim, α-Synuclein seed amplification assays in the diagnosis of synucleinopathies using cerebrospinal Fluid-A systematic review and meta-analysis, Mov. Disord. Clin. Pract. 10 (2023) 737–747, https://doi.org/10.1002/mdc3.13710.
[5] L. Concha-Marambio, S. Pritzkow, M. Shahnawaz, C.M. Farris, C. Soto, Seed amplification assay for the detection of pathologic alpha-synuclein aggregates in cerebrospinal fluid, Nat. Protoc. 18 (2023) 1179–1196, https://doi.org/10.1038/s41596-022-00787-3.
[6] K. Brockmann, S. Lerche, S. Baiardi, M. Rossi, I. Wurster, C. Quadalti, B. Roeben, A. Mammana, M. Zimmermann, A.-K. Hauser, C. Deuschle, C. Schulte, I. Liepelt-Scarfone, T. Gasser, P. Parchi, CSF α-synuclein seed amplification kinetic profiles are associated with cognitive decline in Parkinson's disease, npj Parkinson's Dis. 10 (2024) 24, https://doi.org/10.1038/s41531-023-00627-5.
[7] C.D. Orrú, D.P. Vaughan, N. Vijiaratnam, R. Real, A. Martinez-Carrasco, R. Fumi, M.T. Jensen, M. Hodgson, C. Girges, A.-L. Gil-Martinez, E.J. Stafford, L. Wu, S. Lerche, I. Wurster, B.R. Groveman, A.G. Hughson, O. Ansorge, A. Quaegebeur, K. S.J. Allinson, T.T. Warner, Z. Jaunmuktane, A. Misbahuddin, P.N. Leigh, B.C. P. Ghosh, K.P. Bhatia, A. Church, C. Kobylecki, M.T.M. Hu, J.B. Rowe, P. Parchi, K. Brockmann, T. Foltynie, H.R. Morris, B. Caughey, E. Jabbari, Diagnostic and prognostic value of α-synuclein seed amplification assay kinetic measures in Parkinson's disease: a longitudinal cohort study, Lancet Neurol. 24 (2025) 580–590, https://doi.org/10.1016/S1474-4422(25)00157-7.
[8] P. Grillo, L. Concha-Marambio, A. Pisani, G.M. Riboldi, U.J. Kang, Association between the amplification parameters of the α-Synuclein seed amplification assay and clinical and genetic subtypes of parkinson's disease, Mov. Disord. 40 (2025) 305–314, https://doi.org/10.1002/mds.30085.
[9] S. Bräuer, M. Rossi, J. Sajapin, T. Henle, T. Gasser, P. Parchi, K. Brockmann, B. H. Falkenburger, Kinetic parameters of alpha-synuclein seed amplification assay correlate with cognitive impairment in patients with Lewy body disorders, Acta Neuropathol. Commun. 11 (2023) 162, https://doi.org/10.1186/s40478-023-01653-3.
[10] G. Bellomo, F. Paolini Paoletti, L. Gaetani, A. Toja, Y. Ma, C.M. Farris, L. Concha-Marambio, L. Parnetti, Association of αS-SAA kinetics with clinical scores in the clinical spectrum of Parkinson's disease, J. Parkinsons Dis. (2025) 1877718X251342445, https://doi.org/10.1177/1877718X251342445.
[11] M. Shahnawaz, T. Tokuda, M. Waragai, N. Mendez, R. Ishii, C. Trenkwalder, B. Mollenhauer, C. Soto, Development of a biochemical diagnosis of parkinson disease by detection of α-Synuclein misfolded aggregates in cerebrospinal fluid, JAMA Neurol. 74 (2017) 163, https://doi.org/10.1001/jamaneurol.2016.4547.
[12] I. Poggiolini, V. Gupta, M. Lawton, S. Lee, A. El-Turabi, A. Querejeta-Coma, C. Trenkwalder, F. Sixel-Döring, A. Foubert-Samier, A. Pavy-Le Traon, G. Plazzi, F. Biscarini, J. Montplaisir, J.-F. Gagnon, R.B. Postuma, E. Antelmi, W.G. Meissner, B. Mollenhauer, Y. Ben-Shlomo, M.T. Hu, L. Parkkinen, Diagnostic value of cerebrospinal fluid alpha-synuclein seed quantification in synucleinopathies, Brain 145 (2022) 584–595, https://doi.org/10.1093/brain/awab431.
[13] L. Concha-Marambio, S. Weber, C.M. Farris, M. Dakna, E. Lang, T. Wicke, Y. Ma, M. Starke, J. Ebentheuer, F. Sixel-Döring, M.-L. Muntean, S. Schade, C. Trenkwalder, C. Soto, B. Mollenhauer, Accurate detection of α-Synuclein seeds in cerebrospinal fluid from isolated rapid eye movement sleep behavior disorder and patients with parkinson's disease in the DeNovo parkinson (DeNoPa) cohort, Mov. Disord. 38 (2023) 567–578, https://doi.org/10.1002/mds.29329.
[14] L.M. Chahine, T.G. Beach, C.H. Adler, M. Hepker, A. Kanthasamy, S. Appel, S. Pritzkow, M. Pinho, S. Mosovsky, G.E. Serrano, C. Coffey, M.C. Brumm, L.M. A. Oliveira, J. Eberling, B. Mollenhauer, Systemic synuclein sampling study, central and peripheral α-synuclein in Parkinson disease detected by seed amplification assay, Ann. Clin. Transl. Neurol. 10 (2023) 696–705, https://doi.org/10.1002/acn3.51753.
[15] U.J. Kang, A.K. Boehme, G. Fairfoul, M. Shahnawaz, T.C. Ma, S.J. Hutten, A. Green, C. Soto, Comparative study of cerebrospinal fluid α-synuclein seeding aggregation assays for diagnosis of Parkinson's disease, Mov. Disord. 34 (2019) 536–544, https://doi.org/10.1002/mds.27646.
[16] C.D. Orrù, T.C. Ma, A.G. Hughson, B.R. Groveman, A. Srivastava, D. Galasko, R. Angers, P. Downey, K. Crawford, S.J. Hutten, U.J. Kang, B. Caughey, A rapid α-synuclein seed assay of Parkinson's disease CSF panel shows high diagnostic accuracy, Ann. Clin. Transl. Neurol. 8 (2021) 374–384, https://doi.org/10.1002/acn3.51280.
[17] A. Mastrangelo, A. Mammana, S. Hall, E. Stomrud, C. Zenesini, M. Rossi, S. Janelidze, A. Ticca, S. Palmqvist, F. Magliocchetti, S. Baiardi, N. Mattsson-Carlgren, O. Hansson, P. Parchi, Alpha-synuclein seed amplification assay longitudinal outcomes in lewy body disease spectrum, Brain 148 (2025) 2038–2048, https://doi.org/10.1093/brain/awae405.
[18] J.G. Schumacher, X. Zhang, E.A. Macklin, J. Wang, A. Bayati, J.M. Dijkstra, H. Watanabe, M.A. Schwarzschild, M. Cortese, X. Zhang, X. Chen, Baseline α-synuclein seeding activity and disease progression in sporadic and genetic Parkinson's disease in the PPMI cohort, EBioMedicine 119 (2025) 105866, https://doi.org/10.1016/j.ebiom.2025.105866.
[19] Y. Ma, C.M. Farris, S. Weber, S. Schade, H. Nguyen, A. Pérez-Soriano, D.M. Giraldo, M. Fernández, M. Soto, A. Cámara, C. Painous, E. Muñoz, F. Valldeoriola, M. J. Martí, J. Clarimon, P. Kallunki, T.C. Ma, R.N. Alcalay, B.F. Gomes, K. Blennow, H. Zetterberg, J. Constantinescu, D. Mengel, V. Kadam, P. Parchi, K. Brockmann, T. F. Tropea, A. Siderowf, M. Synofzik, U.J. Kang, Y. Compta, P. Svenningsson, B. Mollenhauer, L. Concha-Marambio, Sensitivity and specificity of a seed amplification assay for diagnosis of multiple system atrophy: a multicentre cohort study, Lancet Neurol. 23 (2024) 1225–1237, https://doi.org/10.1016/S1474-4422(24)00395-8.
[20] J.A. Wiseman, C.P. Turner, R.L.M. Faull, G.M. Halliday, B.V. Dieriks, Refining α-synuclein seed amplification assays to distinguish Parkinson's disease from multiple system atrophy, Transl. Neurodegener. 14 (2025) 7, https://doi.org/10.1186/s40035-025-00469-6.
[21] G. Bellomo, S. Paciotti, L. Gatticchi, D. Rizzo, F.P. Paoletti, M. Fragai, L. Parnetti, Seed amplification assays for diagnosing synucleinopathies: the issue of influencing factors, Front. Biosci. 26 (2021) 1075, https://doi.org/10.52586/5010 (Landmark Ed).
[22] A. Mammana, S. Baiardi, M. Rossi, C. Quadalti, A. Ticca, F. Magliocchetti, A. Bernhardt, S. Capellari, P. Parchi, Improving protocols for α-synuclein seed amplification assays: analysis of preanalytical and analytical variables and identification of candidate parameters for seed quantification, Clin. Chem. Lab. Med. 62 (2024) 2001–2010, https://doi.org/10.1515/cclm-2023-1472.
[23] M. Shahnawaz, A. Mukherjee, S. Pritzkow, N. Mendez, P. Rabadia, X. Liu, B. Hu, A. Schmeichel, W. Singer, G. Wu, A.-L. Tsai, H. Shirani, K.P.R. Nilsson, P.A. Low, C. Soto, Discriminating α-synuclein strains in Parkinson's disease and multiple system atrophy, Nature 578 (2020) 273–277, https://doi.org/10.1038/s41586-020-1984-7.
[24] S. Bräuer, I. Schniewind, E. Dinter, B.H. Falkenburger, Recursive seed amplification detects distinct α-synuclein strains in cerebrospinal fluid of patients

with Parkinson's disease, Acta Neuropathol. Commun. 13 (2025) 13, https://doi.org/10.1186/s40478-024-01923-8.

[25] D. Belete, C. Mattjie, B. Huxford, J.P. Bestwick, A.J. Noyce, C. Simonet, Evaluating the role of α-Synuclein seed amplification as a disease progression marker: evidence and uncertainties, Mov. Disord. Clin. Pract. (2025) mdc3.70434, https://doi.org/10.1002/mdc3.70434.

[26] J. Rodríguez-Antigüedad, S. Martínez-Horta, A. Puig-Davi, A. Horta-Barba, J. Pagonabarraga, T. de Deus Fonticoba, S. Jesús, M. Cosgaya, J. García Caldentey, M.A. Ávila-Rivera, N. Caballol, I. Legarda, J. Hernández Vara, I. Cabo, L. López Manzanares, I. González Aramburu, V. Gómez Mayordomo, J. González Ardura, J. Dotor García-Soto, C. Borrué, B. Solano Vila, M. Álvarez Sauco, L. Vela, S. Escalante, E. Cubo, Z. Mendoza, I. Pareés, P. Sánchez Alonso, M.G. Alonso Losada, N. López Ariztegui, I. Gastón, J. Ruíz Martínez, M.T. Buongiorno, C. Ordás, C. Valero, V. Puente, M. Kurtis, M. Blázquez Estrada, P. Martínez-Martín, P. Mir, D. Santos-García, C. Study Group, J. Kulisevsky, Heterogeneity of cognitive progression and clinical predictors in Parkinson's disease-subjective cognitive decline, J. Neurol. 272 (2025) 246, https://doi.org/10.1007/s00415-024-12808-0.