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Short Communication

Seed Amplification Assay as a Diagnostic Tool in Newly-Diagnosed Parkinson's Disease

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Abstract. Seed amplification assays (SAA) are the first credible molecular assay for Parkinson's disease (PD). However, the value of SAA to support the clinicians' initial diagnosis of PD is not clear. In our study, we analyzed cerebrospinal fluid samples from 121 PD patients recruited through population screening methods and taken within a median delay of 38 days from diagnosis and 51 normal controls without neurodegenerative disease. SAA yielded a sensitivity of 82.6% (95% CI, 74.7%–88.9%) and specificity of 88.2% (95% CI, 76.1%–95.6%). These results highlight the potential of SAA to support the initial diagnosis of PD in clinical practice and research.

Keywords: Seed amplification assay, alpha-synuclein, biomarker, cerebrospinal fluid, Parkinson's disease, diagnosis

INTRODUCTION

Aggregation of α-synuclein is the pathological hallmark of Parkinson's disease (PD). α-synuclein seed amplification assays (SAA), including real-time quaking-induced conversion (RT-QuIC), can detect synucleinopathies in cerebrospinal fluid (CSF) samples with high sensitivity and specificity, especially in cohorts of autopsy-confirmed or advanced PD [1–5]. Patients with incident PD often present with mild or unclear clinical symptoms, making early accurate diagnosis challenging and highlighting the need for an objective molecular marker to support diagnosis. The ability of SAA to detect PD at the earliest clinical disease stages is promising [6]. Here we applied this assay to a PD cohort recruited from the general population at the time of initial clinical diagnosis and with available clinical follow-up data for up to ten years.

MATERIALS AND METHODS

Participants

PD patients were included from the Norwegian ParkWest study of incident PD [7]. Between Novem-

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ber 1, 2004 and August 31, 2006, all residents of Southwestern Norway with suspected incident PD were screened and invited to the study. Patients underwent rigorous diagnostic procedures [7] and were followed prospectively every six months (by experienced movement disorders neurologists) for up to 10 years. All met the UKBB PD criteria [8] at their latest or final clinical visit. Of 190 eligible patients, 121 consented to lumbar puncture at the time of clinical diagnosis. The control group were without known brain disease and underwent elective neurological examination or orthopedic surgery at Stavanger University Hospital. All participants provided informed written consent. The study was approved by the Regional Committee for Medical and Health Research Ethics of Western Norway.

Cerebrospinal fluid samples

Lumbar puncture was performed after overnight fasting according to standardized procedures [9]. All CSF samples were immediately cooled on ice, followed by centrifugation at 2000× *g* for 10 min at 4°C. Thereafter, samples were frozen at –80°C in polypropylene tubes. Samples were subjected to one freeze-thaw event for aliquotation purposes.

In-house SAA assay

This assay was developed at the Stavanger University Hospital. Reactions were performed in black 96-well plates (# 655906, Greiner Bio-One). Recombinant wild-type alpha-synuclein (#S-1001-2, rPeptide) was filtered with Ultracel filtration units (#MRCFOR100, Merck Millipore). For each reaction, six silica beads (#BMBG 800-200-1, OPS Diagnostics), a final concentration of 50 mM 2-(N-morpholino)ethanesulfonic acid (MES) buffer pH 5.5 (#M1503, Duchefa Biochemie), 10 μM Thioflavin-T (#ab120751, Abcam), 5 mM zinc sulfate (#1.08883, Sigma-Aldrich), 0.1 mg/ml alpha-synuclein, and 15 μl CSF were added in a total volume of 100 μl. Plates were sealed and incubated in a Synergy H1 m multimode reader (BioTek) at 37°C with repeated shake and rest: 1 min double orbital shaking (3 mm, 425 cpm) followed by 3 min rest. Fluorescence (Ex: 435 nm, Em: 480 nm) was read at the end of every fourth cycle.

Samples were analyzed in triplicates and blinded to the experimenter. A replicate was defined as positive if it passed the threshold of 4000 relative fluorescence units (RFU) within 48 h. The test result for a sample was defined as positive if two or three replicates were positive, and as negative if no replicate was positive. Samples that initially gave one positive replicate were retested and the final test result defined as positive when ≥ two of then six replicates were positive and else as negative.

CSF total protein content was measured using the Pierce BCA assay (#23227; Thermo Fisher Scientific).

Statistical analysis

Continuous variables are presented as means with standard deviations (mean ± SD) and categorical variables as counts and percentages. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated using the MedCalc online tool [10]. Between-group differences were analyzed using independent samples *t*-test, Mann-Whitney U test, or Chi square as appropriate. Normality was assessed using Shapiro-Wilk and Q-Q plots. Kendall tau was used to assess correlation between clinical variables and CSF protein content and time-to-threshold in SAA-positive PD cases.

RESULTS

A total of 172 CSF samples from 121 PD patients from the ParkWest cohort and 51 control participants were included. The median delay between the time of PD diagnosis and lumbar puncture was 38 days. PD patients were followed-up for median 9.9 (IQR 8.6–10.0) years and had clinically confirmed PD diagnosis at their latest visit. Demographic and clinical characteristics are presented in Table 1.

α-synuclein seeding activity in newly diagnosed PD patients was detected with 82.6% sensitivity (95% CI 74.7%–88.9%) and 88.2% specificity (95% CI 76.1%–95.6%) against the control group. The positive predictive value of the assay was 94.3% (95% CI: 88.7%–97.3%) and the negative predictive value was 68.2% (95% CI: 58.9%–76.2%). Twenty-one PD patients scored negative in SAA but did not differ in demographic or clinical characteristics at the time of diagnosis from the 100 patients with positive SAA score (Table 1). There were no significant correlations between time-to-threshold and Age, MMSE, UPDRS-III, Hoehn and Yahr score, or CSF total protein content.

Table 1
Demographics and clinical data of the participants

	Controls	All PD	*p*	RT-QuIC positive PD	RT-QuIC negative PD	*p*
N	51	121		100	21	
Age, y	64.4 ± 12.1	67.1 ± 9.3	0.151[a]	66.8 ± 9.3	68.8 ± 9.7	0.364[a]
Sex male, n (%)	22 (43.1%)	78 (64.5%)	0.011[b]	64 (64.0%)	14 (66.7%)	1.000[b]
Education, y	10 (8–13)	11 (9–13)	0.371[c]	11 (9–14)	9 (8–12)	0.225[c]
MMSE	29 (28–30)	29 (27–29)	0.007[c]	29 (27–29)	28 (26–29)	0.475[c]
UPDRS-III, total score	–	20 (15–29)	–	20 (15–29)	19 (14–27)	0.845[c]
H&Y	–	2 (1.5–2)	–	2 (1.5–2)	2 (1.5–2)	0.745[c]
Follow-up, y	–	9.9 (8.6–10.0)	–	9.9 (8.7–10.0)	10.0 (7.8–10.0)	0.748[c]
Time from diagnosis to lumbar puncture, days	–	38 (20.5–57–5)	–	39.5 (21–58)	34 (16.5–56)	0.445[c]
SAA, pos./neg. (% positive of total)	6/45 (11.8%)	100/21 (82.6%)				

Values are presented as mean ± SD or Median (P25-P75). [a]Independent samples *t*-test, two-sided p, [b]Chi-Square test, two-sided p; [c]Mann Whitney U Test, two-sided p. MMSE, Mini-Mental State Examination; UPDRS, Unified Parkinson's Disease Rating Scale; H&Y, Hoehn and Yahr; SAA, seed amplification assay.

DISCUSSION

These results show that SAA can identify newly-diagnosed PD patients recruited through population screening methods with high sensitivity and specificity. In a recent study, sensitivity and specificity to detect de-novo-PD with SAA was up to 94.6% and 98%, respectively [6]. The differences in performance might be due to technical differences of the employed assays or due to different patient recruitment strategies. The greatest need to improve the diagnosis of PD is in the earliest clinical stages when symptoms overlap with other neurodegenerative conditions and misdiagnosis and non-diagnosis are common [11]. Indeed, SAA was shown to have good sensitivity and specificity to identify an underlying α-synucleinopathy in cases referred to a tertiary movement disorder center with parkinsonism, but with an initially uncertain diagnosis [12, 13].

Similar to previous studies in more advanced PD, we found a small subset of patients with negative results [4]. We cannot exclude that this is due to the presence of inhibitory components in the CSF, like lipoproteins [14] or that these cases represent restricted α-synuclein pathology not affecting the cortex, which have shown lower SAA sensitivity before [15]. Interestingly, these patients were clinically indistinguishable from those with a positive SAA score. Given the relatively small numbers of patients with no detectable α-synuclein seeding activity, larger longitudinal studies including biomarkers of PD, neurodegeneration or related disorders are needed to understand if these (initially) negative cases represent a persistent subtype of PD or turn positive later during the disease course.

Our study has strengths and limitations. Since there was no follow-up data for the controls, we cannot exclude that some were in the prodromal stages of an α-synucleinopathy which might have impacted the specificity of the assay. Indeed, incidental α-synuclein pathology is found in about 10% of individuals older than 60 years [16] and α-synuclein seeding activity can be detected up to 9 years before a clinical diagnosis [17]. As strengths, all incident PD cases were recruited from a population-based cohort using strict clinical diagnostic criteria and had been followed prospectively with a comprehensive clinical program for up to ten years ensuring high diagnostic accuracy, and all samples were analyzed blinded for diagnosis.

SAA showed high sensitivity and specificity for the earliest stages of PD and may aid in clinical practice to support accurate early diagnosis and improved disease management, and for selection of patients eligible for clinical trials of disease-modifying therapies.

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ern Norway Regional Health Authority (911218), the Norwegian Parkinson's Research Foundation, and Rebergs Legacy.

CONFLICTS OF INTEREST

O-BT, GA: None.

LO, JMG, JL: Hold a Swedish provisional patent application (No. 2251247-9) on the employed SAA method on which LO, JMG, and JL are named as inventor.

DATA AVAILABILITY

The data supporting the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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