


α -Synuclein Real-Time Quaking-Induced Conversion in the Cerebrospinal Fluid of Uncertain Cases of Parkinsonism

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A reliable biomarker is needed for accurate and early differentiation between Parkinson disease and the various forms of atypical parkinsonism. We used a novel real-time quaking-induced conversion (RT-QuIC) assay to detect α -synuclein (α -syn) aggregates in cerebrospinal fluid (CSF) of 118 patients with parkinsonism of uncertain clinical etiology and 52 controls. Diagnostic accuracy to distinguish α -synucleinopathies from non- α -synucleinopathies and controls was 84% (sensitivity = 75%, specificity = 94%, area under the curve = 0.84, 95% confidence interval = 0.78–0.91, $p < 0.0001$, positive predictive value = 93%). CSF α -syn RT-QuIC could be a useful diagnostic tool to help clinicians differentiate α -synucleinopathies from other forms of parkinsonism when the clinical picture is uncertain.

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There is currently no reliable objective test to discriminate Parkinson disease (PD) from the various forms of atypical parkinsonism (AP), such as multiple system atrophy (MSA), progressive supranuclear palsy (PSP), dementia with Lewy bodies (DLB), corticobasal syndrome (CBS), and vascular parkinsonism (VaP) during a lifetime. When confronted with a straightforward and unequivocal clinical picture, biomarkers are not needed. However, the clinical picture can often be puzzling, especially early in the disease course, when symptoms overlap, and then reliable biomarkers are needed for accurate and early differentiation between PD and AP.

Recently, a novel assay has been developed to detect minute amounts of α -synuclein (α -syn) aggregates in cerebrospinal fluid (CSF) using real-time quaking-induced conversion (RT-QuIC)¹ with reported high sensitivity and specificity (95–100%).^{1–4} However, this test has thus far only been evaluated in clear-cut clinical cases and/or neuropathologically confirmed cases, whereas clinicians would rather use the test in

ambiguous cases. Therefore, we evaluated the α -syn RT-QuIC assay in CSF from patients with suspicion of, but an as yet uncertain clinical diagnosis of, parkinsonism at the time of lumbar puncture. Importantly, in this prospective observational cohort study, patients were routinely followed for a long period of time and extensively re-examined after 3 and 12 years of follow-up.

Patients and Methods

Patient group

CSF samples were obtained from a prospective observational cohort of 118 patients with parkinsonism and an uncertain clinical diagnosis upon inclusion.⁵ Patients were recruited consecutively from the outpatient department of the Radboud University Medical Center movement disorder center between January 2003 and December 2006. All patients underwent a structured interview, detailed and standardized neurological examination, and, within 6 weeks after the initial visit, lumbar puncture. The protocol was approved by our local medical ethics committee, and written informed consent was obtained from every subject. The study design, methods, and patient population have been extensively described.⁵ Three and 12 years after inclusion, the clinical condition was re-evaluated by a repeated structured interview and extensive neurological examination (Fig). After 3 years, a clinical diagnosis was established by consensus of 2 movement disorder specialists. In 2018, all clinical diagnoses were evaluated again and updated according to the most recent clinical criteria,^{6–8} disease course, and neuropathological examination whenever available.

Control Group

CSF samples were obtained from 52 control patients who underwent a lumbar puncture to exclude a neurological disease. In none of these cases was the suspected disease, parkinsonism, or any other neurodegenerative disease present. Leukocyte

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count, glucose, total protein, blood pigments, lactate, and oligoclonal IgG bands were all normal in the CSF.

CSF α -syn RT-QuIC

CSF α -syn RT-QuIC was performed as previously described.¹ Each sample was run in duplicate. A positive response was defined as a relative fluorescence unit value of >2 standard deviations above the mean of the negative controls at 120 hours in both of the CSF duplicates. If only 1 of 2 CSF samples was positive, the analysis was repeated in quadruplicate. A positive signal in 2 or more of the replicates was considered positive. Results were considered equivocal either because of a long lag phase (>80 hours vs \pm 60 hours in the truly positive samples) or if 1 in 4 wells reacted.

Neurofilament Light Chain in CSF

Elevated CSF neurofilament light chain (NFL) concentrations have been reported in patients with MSA, PSP, and CBS compared to PD.^{9–12} We evaluated whether CSF NFL concentrations (previously described^{5,11,13}) could hint toward the correct diagnosis in cases where the RT-QuIC test had an unexpected result (ie, negative in an α -synucleinopathy case or positive in a non- α -synucleinopathy case). A concentration >2,700 ng/l was considered to be consistent with a diagnosis of MSA or tauopathy (PSP, CBD) and was based on the p90 of 43 non-neurological controls aged >50 years.

Results

The study population is shown in Table 1, and the main results are shown in Table 2. Diagnostic accuracy of the CSF α -syn RT-QuIC to distinguish α -synucleinopathies from non- α -synucleinopathies and controls was 84%. The positive predictive value was 93%, and the negative predictive value was 77%.

Unexpected negative results were found in 21 of 85 α -synucleinopathy cases (8/53 PD, 11/17 MSA, and 2/11 α -synucleinopathies with overlapping vasculopathy). Remarkably, all 4 MSA cases with predominant cerebellar features (MSA-C) were negative, and 7 of 13 MSA cases with predominant parkinsonian features (MSA-P) were negative as well. The only neuropathologically confirmed MSA-P case had a positive result. Of the 11 MSA cases with a negative RT-QuIC result, 9 had a high CSF NFL concentration (>2,700 ng/l), consistent with a diagnosis of MSA (3/4 MSA-C, 6/7 MSA-P). Of 8 PD cases with a negative RT-QuIC test, 7 had a low NFL concentration, as expected in PD.

Unexpected positive test results were found in 5 of 79 cases (1/8 PSP, 3/9 VaP, 1/53 controls). The only positive PSP patient had a high NFL concentration.

There was no significant difference in baseline characteristics (age, disease duration, disease stage, motor and cognitive function scores), or RT-QuIC responses (relative

TABLE 1. Baseline Characteristics of Study Population

Characteristic	Patients, n = 118	Controls, n = 52	p
Men, n (%)	76 (64)	30 (58)	0.41 ^a
Age, yr (\pm SD)	61 \pm 10	64 \pm 9	0.19 ^b
Disease duration, mo (IQR)	29 (18–48)	NA	
Hoehn and Yahr stage, n (%)			
0–1.5	26 (22)	NA	
2–2.5	57 (48)	NA	
3	24 (20)	NA	
4	11 (9)	NA	
5	0 (0)	NA	
UPDRS-III total score \pm SD	30 \pm 14	NA	
ICARS total score (IQR)	4 (1–11)	NA	
MMSE total score (IQR)	29 (27–30)	NA	

Data are presented as numbers (percentages), means (\pm SD), or medians (IQR).

^aAnalyzed using chi-squared test.

^bAnalyzed using Mann–Whitney *U* test for comparison of 2 groups.

ICARS = International Cooperative Ataxia Rating Scale for cerebellar symptoms; IQR = interquartile range; MMSE = Mini-Mental State Examination; NA = not applicable; SD = standard deviation; UPDRS-III = Unified Parkinson's Disease Rating Scale part III.

fluorescence value or lag phase) between subgroups with expected and unexpected test results.

Diagnostic Certainty

In most cases (98%), a clinical diagnosis was established, whereas a definite diagnosis was established in 1 neuropathologically confirmed MSA (RT-QuIC positive), 1 PSP (RT-QuIC negative), and 1 *PARK2* mutation (RT-QuIC negative) patient.

Discussion

Our study yielded good sensitivity (75%) and area under the curve (0.80–0.86) and very high specificity (85–98%) and positive predictive values (93%) for the CSF α -syn RT-QuIC assay. The high specificity and positive predictive values indicate that the vast majority of patients with an undefined diagnosis of parkinsonism and a positive α -syn RT-QuIC will have an underlying α -synucleinopathy.

TABLE 2. Cerebrospinal Fluid α -syn RT-QuIC and NFL Results and α -syn RT-QuIC Test Characteristics per Diagnosis

Diagnosis (n)	α -syn RT-QuIC Results	α -syn RT QuIC Test Characteristics		NFL Results
	+/- (equivocal)	Sensitivity	AUC (95% CI)	+/- (missing) ^a
α -Synucleinopathies (85)	62/21 (2)	75%		16/67 (2)
PD (53)	43/8 (2)	84%		1/50 (2)
MSA (17)	6/11	35%		11/6
DLB (1)	1/0	100%		0/1
α -Synucleinopathy with vasculopathy (11)	9/2	82%		4/7
α -Synucleinopathy of uncertain origin (3)	3/0	100%		0/3
		Specificity		
Non- α -synucleinopathies (26)	4/22	89%	0.80 (0.70–0.89) ^b	12/14
PSP (8)	1/7	88%		6/2
Tauopathy of uncertain origin (2)	0/2	100%		2/0
VaP (9)	3/6	67%		4/5
Other diagnosis (7) ^c	0/7	100%		0/7
Diagnosis undetermined (7)	4/3			4/3
α -Synucleinopathy or tauopathy (4)	2/2			3/1
α -Synucleinopathy or other diagnosis (3)	2/1			1/2
Controls (52)	1/50 (1)	98%	0.86 (0.80–0.93) ^b	0/0 (52)
Total (170)	71/56 (3)	94%	0.84 (0.78–0.91) ^b	32/84 (54)

Data are presented as numbers.

^aNFL concentration >2,700 ng/l is reported as positive and concentration <2,700 ng/l as negative.

^b $p < 0.0001$.

^cIdiopathic late onset cerebellar ataxia (n = 1), hereditary ataxia (n = 1), functional tremor (n = 2), medication-induced parkinsonism (n = 1), unilateral resting tremor (n = 1), and superficial hemosiderosis (n = 1).

AUC = area under the curve; CI = confidence interval; DLB = dementia with Lewy bodies; MSA = multiple system atrophy; NFL = neurofilament light chain; PD = Parkinson disease; PSP = progressive supranuclear palsy; RT-QuIC = real-time quaking-induced conversion; VaP = vascular parkinsonism; α -syn = α -synuclein.

In a few previous α -syn RT-QuIC studies, sensitivity (89–95%) and specificity (up to 100%) were even higher.^{1–4} However, in these studies, selected CSF samples or brain homogenates of clinically straightforward or neuropathologically confirmed cases were used. Unlike these studies, our study is the first to examine the diagnostic value of CSF α -syn RT-QuIC in a prospectively collected series of patients with an unclear diagnosis of parkinsonism at the time of inclusion. The final diagnosis was made after a median follow-up of 7 years (interquartile range = 2–12 years) after the original lumbar puncture, and such a long follow-up helps to considerably reduce the clinical uncertainty.^{14,15}

Unexpected negative results were found in 21 of 85 α -synucleinopathy cases, with a remarkably high number of negative MSA cases (11/17). In a previous study, sensitivity for (clinically diagnosed) MSA was also lower than for other α -synucleinopathies (80%), but not as low as in our study (35%).² Misdiagnosis seems unlikely, as we followed our patients for many years, and 2 experienced movement disorder neurologists established the diagnosis during consensus sessions, according to international criteria. Moreover, 9 of 11 cases with a negative RT-QuIC result had a positive CSF NFL result, consistent with a diagnosis of MSA.¹⁶ Several explanations exist for these divergent test results: (1) differences

in the nature of the underlying α -syn pathology between PD and DLB on the one hand (ie; neuronal α -syn inclusions) and MSA on the other hand (ie, glial α -syn inclusions), (2) variability in the extent of the underlying neuropathology across MSA cases, and (3) variability because the clinical picture had not yet fully matured in all cases.

The RT-QuIC test result was negative in our PARK2 PD case. Because there was only 1 genetic case in our cohort, we could only speculate that the CSF α -syn RT-QuIC test might not be suitable for detecting all patients with a genetic form of PD, as autopsy reports of genetic (including PARK2) PD cases have shown that Lewy bodies may be either present or absent.¹⁷

Unexpected positive test results were found in 5 of 79 cases. The 1 PSP patient with a positive test result was a patient who, at disease onset, had clinical MSA-like features with prominent autonomic features (unusual for PSP), but at follow-up had clinical features that were more PSP-like (including supranuclear gaze palsy). The CSF α -syn RT-QuIC result may therefore prompt the clinician to reconsider the clinical diagnosis once more.

Three of 9 patients with VaP had a positive CSF α -syn RT-QuIC result. Although all VaP patients had unambiguous vascular magnetic resonance imaging abnormalities combined with a clinical presentation suggestive of VaP (eg, lower body parkinsonism), we cannot exclude that these patients had a mixed α -synucleinopathy and vasculopathy, as described previously for PD patients.^{18,19} Finally, the positive test result in 1 control subject remains unexplained, as detailed review of this subject's clinical chart did not reveal any signs of prodromal parkinsonism.

This study is not without limitations. First, the final diagnosis at the end of follow-up, which was used as a reference to measure the diagnostic value, was a clinical diagnosis and in the majority of cases (98%) not neuropathologically confirmed. Therefore, it cannot be excluded that some patients received an incorrect clinical diagnosis. However, a meta-analysis of longitudinally followed subjects with autopsy-confirmed diagnoses showed that the clinical diagnosis is the best available surrogate for the neuropathological diagnosis when established (1) by a movement disorder expert, (2) after several years of follow-up, and (3) according to the international diagnostic criteria.²⁰ We followed all of these recommendations in our study. Second, the diagnostic value of this test is limited because it can only identify patients with an α -synucleinopathy and cannot differentiate between PD and MSA. In clinical practice, these particular diseases can be very challenging to differentiate from each other, especially in early disease stages when symptoms overlap. Probably a combination of biomarkers will be needed to yield an optimal differentiation of parkinsonian syndromes. CSF NFL analysis may be part of such a combination, as a positive

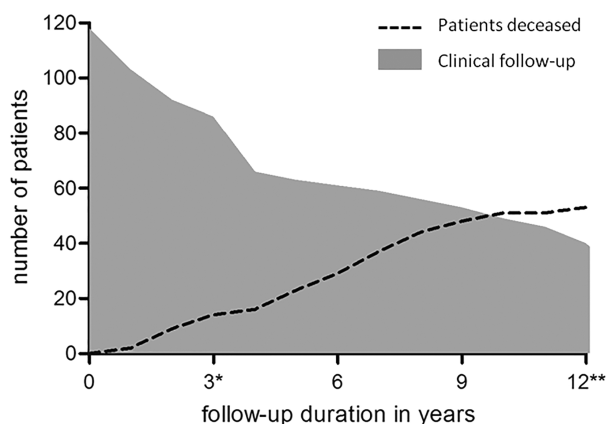


FIGURE 1: Clinical follow-up and mortality. Patients with parkinsonism and an uncertain clinical diagnosis underwent a structured interview, extensive neurological examination, and lumbar puncture upon inclusion (n = 118). *Three years after inclusion, the clinical condition was re-evaluated by a repeated structured interview and extensive neurological examination (n = 81). **Twelve years after inclusion, the clinical condition was re-evaluated by a repeated structured interview and extensive neurological examination (n = 34).

CSF α -syn RT-QuIC combined with an increased CSF NFL is likely to be associated with MSA, whereas a positive CSF α -syn RT-QuIC combined with a normal CSF NFL is associated with PD or DLB. Likewise, a negative α -syn RT-QuIC with an increased NFL hints toward CBS or PSP, whereas if both biomarkers are negative, a neurodegenerative form of parkinsonism is unlikely.^{1,2,16} Future systematic studies will have to reveal the diagnostic values of a combined analysis of CSF α -syn RT-QuIC and NFL to differentiate between patients with various parkinsonisms. Nevertheless, our study suggests that CSF α -syn RT-QuIC has the potential to be a useful diagnostic tool to help clinicians differentiate α -synucleinopathies from other forms of parkinsonism when the clinical picture is puzzling.

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Author Contributions

B.R.B., R.A.J.E., and M.M.V. contributed to the conception and design of the study. A.v.R., A.J.E.G., G.F., R.A.J.E., and B.R.B. contributed to the acquisition and analysis of the data. A.J.E.G., A.v.R., and G.F. contributed to the data. A.v.R., A.J.E.G., and M.M.V. contributed to drafting the text and preparing the figure 1.

Potential Conflicts of Interest

A.J.E.G. and G.F. have a patent pending for the RT-QuIC assay that was used in this study (Alpha-Synuclein Detection Assay PCT/GB2017/051988). The other authors have nothing to report.

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