Brain and Cerebrospinal Fluid -Synuclein Real-Time Quaking-Induced Conversion Identifies Lewy Body Pathology in LRRK2-PD

BRIEF REPORT

Brain and Cerebrospinal Fluid α-Synuclein Real-Time Quaking-Induced Conversion Identifies Lewy Body Pathology in LRRK2-PD

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ABSTRACT: Background: The neuropathology of Parkinson's disease (PD) associated with leucine-rich repeat kinase 2 (LRRK2) mutations (LRRK2-PD) is heterogeneous and varies with the type of mutation. There are only a few studies evaluating seeding aggregation assays to detect α-synuclein (α-syn) in patients with LRRK2-PD.

Objective: We aimed to investigate whether α-syn real-time quaking induced conversion (RT-QuIC) is a sensitive biomarker of synucleinopathy in LRRK2-PD.

Methods: We studied α-syn RT-QuIC in brain tissue and postmortem ventricular cerebrospinal fluid (CSF) of LRRK2-PD cases with and without Lewy-type pathology.

Results: The accuracy of α-syn RT-QuIC in substantia nigra and CSF samples of patients with LRRK2-PD was 100%. The test also obtained 100% sensitivity to detect misfolded α-syn in substantia nigra of cases with idiopathic PD and was negative in the substantia nigra of all the control brains without Lewy-type pathology.

Conclusions: Substantia nigra and ventricular CSF RT-QuIC discriminates with high sensitivity and specificity LRRK2 cases with Lewy-type pathology from those without it. RT-QuIC assay could be of particular interest in the selection of cases for clinical trials in this genetic form of PD. © 2022 The Authors. *Movement Disorders* published by Wiley Periodicals LLC on behalf of International Parkinson and Movement Disorder Society.

Key Words: Parkinson's disease; LRRK2; α-synuclein; RT-QuIC; Lewy body

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Introduction

Mutations in leucine-rich repeat kinase 2 (LRRK2) cause late-onset parkinsonism, which is clinically indistinguishable from idiopathic Parkinson's disease (iPD).[1-6] A pleiomorphic pathology has been reported since the first descriptions of LRRK2-PD,[7-10] and although many patients with PD with G2019S LRRK2 mutations exhibit α-synuclein (α-syn)-positive Lewy-type pathology (LTP),[10,11] nearly half of the remaining cases are Lewy body negative.[10,11] In patients carrying non-G2019S variants, LTP is even rarer, and nigral degeneration without abnormal protein deposition or associated to tau, Aβ, ubiquitin, TDP-43, or α-syn glial-cytoplasmic deposits is found.[8,10-19]

Seeding amplification assays (SAAs) such as real-time quaking-induced conversion (RT-QuIC) have shown in cerebrospinal fluid (CSF) and other tissues sensitivity and specificity values greater than 85% to identify a synucleinopathy.[21-24]

We aimed to investigate in a series of LRRK2-PD autopsy cases whether α-syn RT-QuIC can accurately separate LTP from non-LTP cases in postmortem brain tissue and CSF.

Subjects and Methods

Brain and postmortem ventricular CSF samples were obtained from brain bank cases of six subjects diagnosed

during life as LRRK2-PD, seven cases with histological diagnosis of synucleinopathy (six with premortem diagnosis of iPD and one case without known parkinsonism during life), and five cases without LTP (with a clinical diagnosis of frontotemporal dementia in one patient, vascular dementia in one, and three donors without a known neurodegenerative disease), matched for age and sex, at the Neurological Tissue Bank of the Biobank-Hospital Clinic–Institut d'Investigacions Biomèdiques August Pi i Sunyer (BTN-IDIBAPS brain bank).

Five patients with LRRK2-PD harbored the G2019S variant and one the R1441G variant. Genetic studies were performed, as previously described,[24] either by extracting DNA from in vivo collected peripheral blood or from brain tissue. Genotyping of the G2019S variant was performed on a StepOnePlus Real-Time PCR System (Life Technologies) using the commercial predesigned TaqMan assay C-63498123-10 single-nucleotide polymorphism rs34637584, and a TaqMan assay on demand (Life Technologies) was used for identifying the R1441G variant[25] with subsequent DNA sequencing. The histological findings of five of the LRRK2-PD cases used for this study have been published.[26,27] The local ethics committee of Hospital Clinic Barcelona approved the neuropathological and the study protocols, and donors or next of kin provided informed consent for brain donation.

Collection of Samples

Brain and ventricular CSF extraction and neuropathological examination were performed following the standardized procedures of the BTN-IDIBAPS brain bank.[28]

At the time of brain extraction, ventricular CSF was collected in 14 instances (3 LRRK2-PD, 5 iPD, and 6 non-PD control subjects) in a polypropylene tube using a 10-cc syringe and kept at 4°C until the processing. CSF samples were centrifuged for 10 minutes, at 4000*g* at 4°C in less than 2 hours from the collection, and gently mixed to avoid gradient effects. After centrifugation, CSF was immediately frozen at −80°C. Brain and CSF samples were stored in the BTN-IDIBAPS brain bank at −80°C until the time of this study.

Neuropathological Description

Three cases with LRRK2-PD, all carrying the G2019S variant, exhibited LTP with moderate nigral loss and α-syn inclusions predominantly in the midbrain and extending to the temporal neocortex (Braak stages 4–5). The other three LRRK2-PD cases (two harboring the G2019S and one the R1441G variant) showed a moderate neuronal loss in substantia nigra (SN) and locus coeruleus, without LTP. No protein inclusions were detected in the R1441G case; neuronal and glial 4R tau inclusions were present in one G2019S case, in which the pathological diagnosis of progressive supranuclear palsy was established; and tau inclusions characteristic of Alzheimer's disease (AD) were present in the other case. The six cases with a clinical diagnosis of iPD exhibited the classical LTP Braak stages 5 and 6 with variable degree of AD copathology. One case without known neurodegenerative disease during life had Lewy-type synucleinopathy with olfactory bulb and brainstem involvement (Braak stage 1) and AD-type pathology Braak stage 2.

The neuropathological diagnosis of the remaining LTP-negative cases was of AD at different stages (Braak stages 2–5) in three cases and primary age-related tauopathy in two cases, with some degree of small vessel disease in all of the cases.

α-Syn RT-QuIC in Brain Homogenates and CSF Samples

Frozen brain sections from the SN (n = 17) and anterior cingulate (AC) (n = 18) and CSF (n = 14) samples were transferred on dry ice to the National CJD Research & Surveillance Unit in Western General Hospital, University of Edinburgh, for analysis in January 2021 and stored at −80°C on arrival.

Samples were analyzed without prior knowledge of the donor's pathological diagnosis or genetic status. α-syn RT-QuIC was performed following a previously described methodology.[21] Each sample was run in duplicate. A positive response was defined as a relative fluorescence unit value of >2 standard deviations greater than the mean of the negative controls at 120 hours in both the BHs and CSF duplicates. If only one of two sample replicates responded positively, the RT-QuIC analysis was repeated in quadruplicate. A positive response in two or more of the replicates was considered positive. If only one of the replicates was positive, the RT-QuIC was considered to be negative.

Statistical Analysis

All analyses were done with RStudio. Qualitative variables are presented by absolute and relative frequencies (%) and analyzed by Fisher exact test. Quantitative variables are presented by the median and interquartile range (25th and 75th percentiles) and analyzed using the Kruskal–Wallis test. Sensitivity and specificity, with 95% confidence limits, for α-syn-RT-QuIC were calculated in all groups. A P value <0.05 was considered significant.

Results

The median age of LRRK2-PD subjects at death was 75.5 (70–83) years. Half of the LRRK2-PD patients were female. There were no significant demographic differences between LRRK2-PD, iPD, and control cases. The time between the demise and brain and CSF

collection ranged from 3 to 18.5 hours and did not differ significantly between groups.

All LRRK2 cases with neuropathologically confirmed LTP showed positive SN and AC α-syn RT-QuIC, whereas in LRRK2-PD cases without abnormal α-syn aggregates on the immunohistochemical analysis, SN α-syn RT-QUIC was negative (Table 1, Fig. 1). In one LRRK2-PD patient without LTP, in which SN was not available, the α-syn RT-QuIC of the AC was negative. One of the LRRK2-PD subjects without Lewy body disease had a positive curve in the AC.

All non-LRRK2 PD cases with LTP had a positive reaction of the α-syn RT-QuIC assay in the SN and AC (Table 1). Controls brains without LTP proved negative in α-syn RT-QuIC SN in all cases. Three control cases (LTP negative) had a positive test in the AC. Overall, SN α-syn RT-QuIC had a 100% sensitivity and 100% specificity to detect an underlying LTP synucleinopathy. RT-QuIC in the AC, however, was positive in cases with a negative result in the SN (in one LRRK2 brain and three controls).

Data on ventricular CSF were available in 14 subjects: 3 LRRK2-PD, 6 brains with LTP, and 5 control subjects. α-syn RT-QuIC results in CSF showed good agreement with nigral α-syn RT-QuIC results and the presence or absence of LTP on neuropathological examination (Table 1, Fig. 1). Only one iPD case had a negative CSF α-syn RT-QuIC reaction. Overall, CSF α-syn RT-QuIC had a 88.9% sensitivity and 100% specificity to detect underlying LTP.

Discussion

We report in this article the results of an α-syn seeding assay in postmortem brain and CSF from LRRK2-PD. RT-QuIC detected α-syn aggregation in postmortem SN and CSF of LTP-proven PD cases, either carrying a LRRK2 mutation or idiopathic, with high sensitivity and specificity. A similar high accuracy of α-syn RT-QuIC has been reported earlier in CSF of non-LRRK2 PD subjects with neuropathologically confirmed diagnosis.[22,30-32]

In a previous study, performed in 31 G2019S LRRK2 carriers, including patients with manifest PD and unaffected subjects, we found that α-syn RT-QuIC in lumbar CSF was positive in 40% of the LRRK2-PD cases and in 18% of unaffected carriers.[33] We hypothesized that the smaller percentage of positive cases among LRRK2-PD compared with the iPD cases was related to the known neuropathological variability of LRRK2-PD, with the positive cases identifying those with an underlying synucleinopahty. The lack of autopsy confirmation rendered the conclusions tentative. The results of this study showing that α-syn RT-QuIC can identify the LRRK2-PD cases with pathology-proven LTP with 100% sensitivity and 100% specificity in both SN and CSF support our initial hypothesis.

TABLE 1 *α-Synuclein real-time quaking induced conversion diagnostic value in postmortem brain tissue and ventricular CSF*

Diagnostic category	n	SN pos	Sensitivity (%)	Specificity (%)	AC pos	Sensitivity (%)	Specificity (%)	CSF pos	Sensitivity (%)	Specificity (%)
LRRK2-PD LTP^{+}	3	3/3	100	–	3/3	100	–	2/2	100	–
LTP^{+} controls[a]	7	7/7	100	–	7/7	100	–	5/6	83	–
LRRK2-PD LTP^{-}	3	0/2	–	100	1/3	–	67	0/1	–	100
LTP^{-} controls[b]	5	0/5	–	100	3/5	–	60	0/5	–	100

[a]Six idiopathic Parkinson's disease cases with classical LTP (Braak stages 5–6) and one case with LTP from a patient without parkinsonism (Braak stage 1).
[b]Frontotemporal dementia (n = 1), vascular dementia (n = 1), and control subjects without neurodegenerative disease (n = 3).
Abbreviations: CSF, cerebrospinal fluid; SN, substantia nigra; pos, positive; AC, anterior cingulate; LRRK2-PD, Parkinson's disease associated with leucine-rich repeat kinase 2 mutations; LTP, Lewy-type pathology.

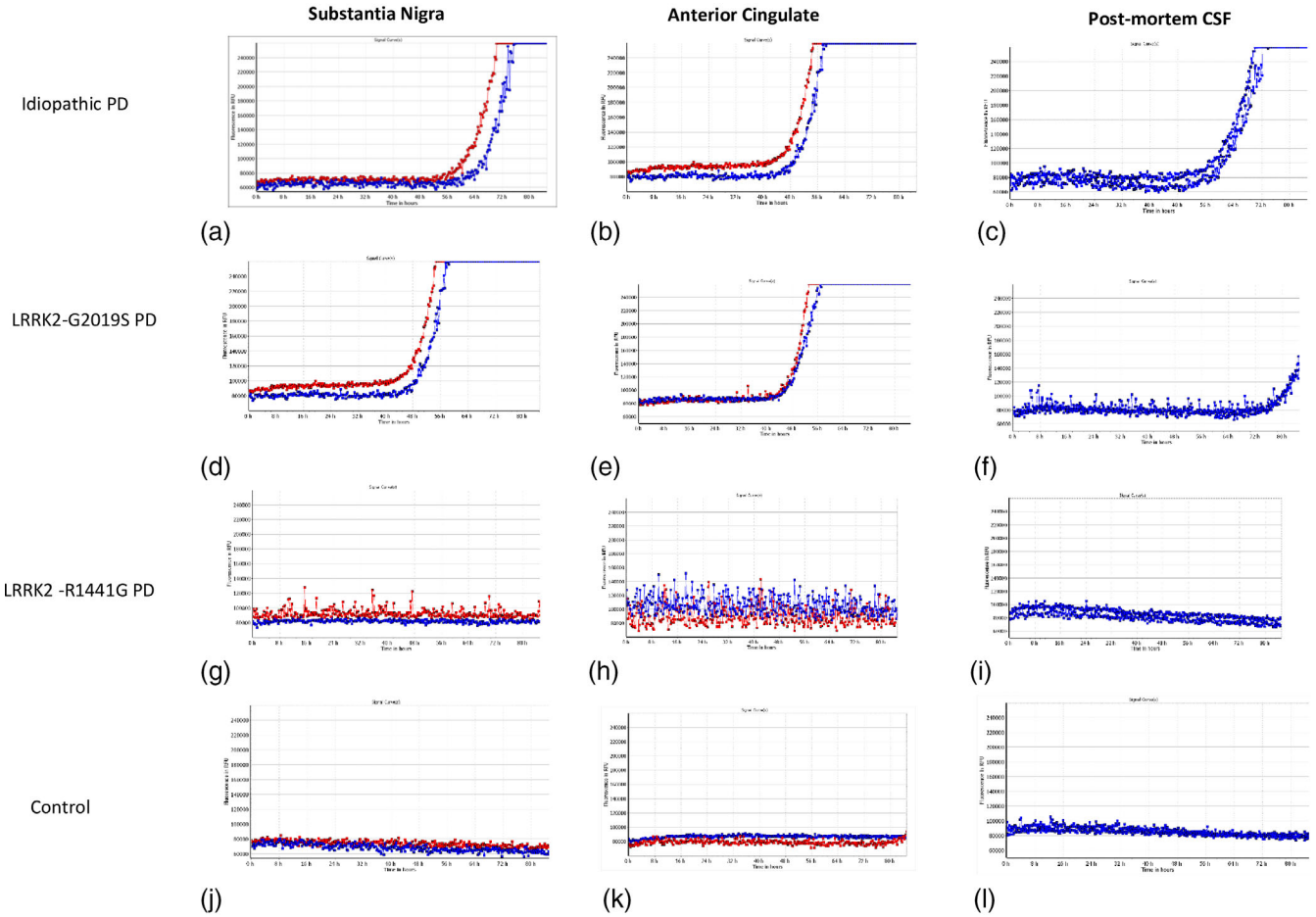

FIG. 1. α-Synuclein real-time quaking-induced conversion reaction curves of substantia nigra (**A**, **D**, **G**, **J**), anterior cingulate (**B**, **E**, **H**, **K**), and postmortem cerebrospinal fluid (**C**, **F**, **I**, **L**) from a patient with idiopathic Parkinson's disease (PD) (**A–C**), one with leucine-rich repeat kinase 2 (LRRK2)-G2019S PD (**D–F**), one with LRRK2-R1441G PD (**G–I**), and a control subject (**J–L**). The two lines shown in each figure represent replicates from the same subject. [Color figure can be viewed at wileyonlinelibrary.com]

In this study, α-syn RT-QuIC in the SN gave the highest positive and negative predictive values, in line with the known universal presence of LTP in this structure in case of LTP-associated PD. In the AC, RT-QuIC, unexpectedly, had a low specificity, being positive in some of the controls. The significance of these findings is unclear. The AC is considered a brain region very frequently involved with LTP in PD,[34,35] and we suspect that the presence of α-Syn aggregates in this brain region of the LTP-positive brains could be the reason that so many PD-LTP-positive cases were AC positive for RT-QuIC. Unexpected RT-QuIC results have been reported in CSF studies where 10% to 25% of healthy control subjects were found to be positive, and this has been attributed to an underlying subclinical synucleinopathy.[24,33] In brain tissue, a recent study[36] has demonstrated positive RT-QuIC results in brain regions without LTP, suggesting that α-Syn aggregates could remain undetected by immunohistochemical methods and still generate a positive α-Syn RT-QuIC curve. SAAs in different brain regions, with and without detectable LTP by immunochemistry, are needed to try to understand these results.

Results in ventricular CSF achieved a high level of correlation with those in the SN. Only one CSF sample of an iPD case had a negative α-syn RT-QuIC reaction. Postmortem CSF collected at the time of autopsy may provide results different from those in lumbar CSF obtained in patients in the clinic,[36] because CSF constituents' origin may vary according to the site of collection (eg, choroid plexus and brain vs. subarachnoid structures, meninges, and dorsal roots).[37] Whether lumbar CSF allows for a similar detection of α-syn aggregates as ventricular CSF has not been determined. The results in ventricular postmortem CSF reported in this article, though, mimic those in patients with and without underlying synucleinopathies,[21,22] suggesting that ventricular postmortem CSF behaves similar to in vivo lumbar CSF in regard to α-syn seeding assays.

In summary, in this α-syn aggregation study, in postmortem brain and CSF from neuropathologically documented cases, α-syn RT-QuIC correctly identified as

positive the LRRK2-PD cases with LTP, whereas it was negative in those without LTP. RT-QuIC was also positive in all control PD cases with LTP. Our results support the notion that SAA, like RT-QuIC, applied to lumbar CSF may reliably identify those cases with an underlying synucleinopathy and potentially candidates for disease modification therapies targeting α-syn. Despite the small number of cases in our study, the results suggest that α-syn RT-QuIC is a promising tool to evaluate the underlying neuropathology of LRRK2-PD in vivo. Studies comparing SAA results in postmortem brain tissue and lumbar CSF from LRRK2-PD subjects are needed to confirm the usefulness of these assays for the reliable identification of an ongoing central nervous system synucleinopathy. ■

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Data Availability Statement

The data that support 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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Author Roles

E.T., M.J.M., A. Garrido, and A. Green contributed to the design of the study. G.F. performed the experiments. A Green analyzed the RT-QuIC data. E.T., M.J.M., A. Garrido, and A. Green interpreted the results. E.T., A. Garrido, M.J.M., M.E., and A. Green wrote the manuscript. All authors reviewed and commented on the manuscript.

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