Towards an early RT-QuIC-based diagnostic biomarker for Lewy body-related synucleinopathies

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CSF RT-QuIC in α-synucleinopathies

Synucleinopathies are neurodegenerative diseases pathologically characterized by aggregates of α-synuclein protein in selectively vulnerable neurons and/or glia.[1] These include neuronal Lewy bodies (LB) and Lewy neurites in Parkinson's disease (PD), dementia with LB (DLB), and PD dementia, and predominantly oligodendroglial cytoplasmic inclusions in multiple system atrophy (MSA).[1] Isolated REM sleep behavior disorder (iRBD) and pure autonomic failure (PAF) are often underpinned by α-synuclein pathology, sometimes revealing themselves as prodromal stages of the above-mentioned disorders in retrospect.[2, 3]

The self-propagation of misfolded proteins assembling into seeds that induce normal like proteins to misshape and aggregate was first recognized as mechanism of neurotoxicity in prion diseases. Increasing evidence supports the prion-like behaviour of other proteins in neurodegeneration, including amyloid-β, tau, and α-synuclein.[4] Therefore, detecting protein aggregates in easily accessible biospecimens represents a promising diagnostic avenue,[5] especially in synucleinopathies which can be difficult to clinically differentiate in early stages and lack specific PET radiotracers. Moreover, since the prion-like process may precede their clinical onset by years or decades, iRBD and PAF are potential targets for an early biochemical diagnosis of synucleinopathies.[2, 3, 5]

Real-Time Quaking-Induced Conversion (RT-QuIC) is a high-sensitive amplification assay to detect the seeding activity of protein aggregates in biospecimens at the expense of a substrate (i.e., the recombinant protein) under shaking conditions, which is monitored by thioflavin T fluorescence. RT-QuIC has first been validated in sporadic Creutzfeldt-Jacob disease, being now included in its diagnostic criteria,[6] and recently explored in synucleinopathies.[7]

Following an established protocol,[8] Rossi et al. performed CSF α-synuclein RT-QuIC in a large cohort of: 1) neuropathologically verified cases of LB-positive and negative dementia, atypical parkinsonism, and LB-negative controls; and 2) clinically diagnosed cases of PD, atypical parkinsonism, dementia, iRBD, and PAF.[9] The assay accurately detected α-synuclein seeding activity across LB-related synucleinopathies (i.e., PD, DLB, iRBD, and PAF), with an overall sensitivity of 95.3% (100% in definite DLB and iRBD) and an overall specificity of 98% against cases lacking LB on neuropathology.[9] Intriguingly, negative RT-QuIC results were found in almost all MSA cases, one iRBD case with subsequent phenoconversion to MSA, and one PAF case showing normal adrenergic cardiac innervation on MIBG-SPECT, as observed in MSA.[9] Finally, quantification of α-synuclein seeding activity through fluorescence analysis did not discriminate among LB-related clinical syndromes.[9]

Besides supporting the prion-like nature of LB-related synucleinopathies, this study demonstrates that α-synuclein RT-QuIC provides an accurate and reliable biomarker for these disorders in the largest cohort hitherto investigated.[9] It also proves that RT-QuIC detects α-synuclein seeding activity in prodromal syndromes, allowing early diagnosis of synucleinopathy and identification of candidates for disease-modifying trials.[9] Interpretation of negative assay results in most MSA cases is arguable. Although it might confirm that MSA and LB-related syndromes are associated with different

conformational α-synuclein strains,[10] further research needs to clarify whether different RT-QuIC set-ups may detect α-synuclein seeding activity in MSA, thus differentiating synucleinopathies.

In conclusion, these findings endorse the inclusion of an RT-QuIC-based biomarker in diagnostic criteria for LB-related synucleinopathies and promote studies to test α-synuclein RT-QuIC of other biospecimens and as progression/prognostic tool.

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Author Roles

1. Research project: A. Conception, B. Organization, C. Execution;

2. Data Analysis: A. Design, B. Execution, C. Review and Critique;

3. Manuscript Preparation: A. Writing of the first draft, B. Review and Critique.

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Ethical Compliance Statement

We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this work is consistent with those guidelines. The authors confirm that the approval of an institutional review board was not required for this work. The authors confirm that no patient consent was required for this work.

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