Tear fluid as a novel specimen for detection of α-synuclein seeding activity in Parkinson's Disease

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Background

Parkinson's disease (PD) is a neurodegenerative disorder whose clinical heterogeneity makes diagnosis, still based on clinical criteria, challenging[1]. Misfolded α-synuclein (αSyn) represents the molecular hallmark, and real-time quaking-induced conversion (RT-QuIC) has emerged as a promising tool to detect seeding-competent αSyn species. RT-QuIC has been applied to cerebrospinal fluid, skin and olfactory mucosa, but their collection is invasive[2]. Tear fluid (TF), an easily accessible biofluid, has not yet been tested.

Objective

We evaluated the feasibility and diagnostic performance of TF-based RT-QuIC in 44 PD patients and 32 age- and sex-matched healthy controls (HCs).

Methods

PD patients underwent standardized clinical assessment, while HCs were screened for prodromal features (**Table 1**). TF was collected using the Shirmer test and analysed by RT-QuIC under optimized conditions. Kinetic parameters were extracted. ROC-derived cutoffs were used to define replicate positivity and diagnostic performance.

Results

At the group level, PD samples exhibited significantly higher fluorescence signals (RFU) throughout the RT-QuIC amplification curve than HCs ($p < 0.0001$) (**Figure 1**). PD-derived replicates displayed significantly shorter lag phase, higher Median and MaximumRFU, and increased area under the fluorescence curve values (all $p < 0.05$) (**Figure 2**). At the subject level, a threshold of ≥3 positive replicates out of 4 (based on MedianRFU) yielded 45.5% sensitivity and 78.1% specificity. Notably, when excluding three subjects showing prodromal features, the specificity of the assay increased from 78.1% to 86.2%. RT-QuIC outcomes were unrelated to clinical variables.

Conclusions

Pathological αSyn seeding activity can be detected in PD tear fluid using RT-QuIC. Although sensitivity remains suboptimal, the method's non-invasiveness and promising specificity support further optimization of TF-based RT-QuIC as a fully non-invasive diagnostic tool for synucleinopathies.

Table 1. Demographic and clinical data of PD patients and controls

Variable	PD (n = 44)	HCs (n = 32)	p value
Age (years), mean ± SD	65.4 ± 10.3	61.6 ± 10.7	n.s
Male:Female, n	25:19	14:18	n.s
Disease duration (years), mean ± SD	6.5 ± 5.2	-	n.a
Age at onset (years), mean ± SD	58.9 ± 11.4	-	n.a
Hoehn and Yahr stage, mean ± SD	2.0 ± 0.8	-	n.a
NMSS score sum, mean ± SD	78.9 ± 61.4	-	n.a
MDS-UPDRS Part I, mean ± SD	11.4 ± 7.3	-	n.a
MDS-UPDRS Part II, mean ± SD	9.8 ± 9	-	n.a
MDS-UPDRS Part III, mean ± SD	28 ± 15.6	-	n.a
MDS-UPDRS Part IV, mean ± SD	3.3 ± 4.2	-	n.a
MoCA, mean ± SD	26.2 ± 3.8	-	n.a
MMSE, mean ± SD	28 ± 2.7	-	n.a
FAB, mean ± SD	15.7 ± 3.1	-	n.a

Figure 1. Comparison of RT-QuIC-based fluorescence kinetics between PD patients and healthy controls

All technical replicates were included in the analysis. **** denotes statistical significance ($p < 0.0001$).

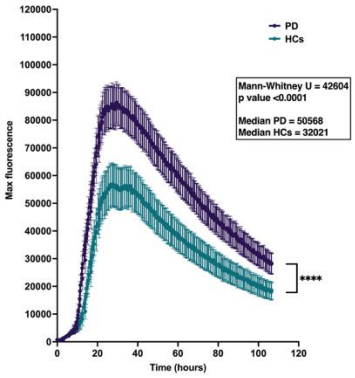
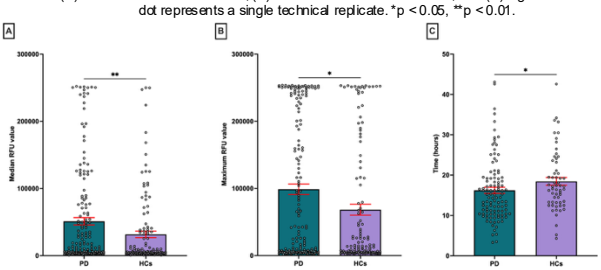


Figure 2. Comparison of RT-QuIC-derived kinetic parameters between Parkinson's disease patients and healthy controls

(A) median fluorescence values, (B) maximum fluorescence values, and (C) lag time. Each dot represents a single technical replicate. *p < 0.05, **p < 0.01.



References

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