Ultrasensitive RT-QuIC assay with high sensitivity and specificity for Lewy Body associated synucleinopathies

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Supplementary materials.

Supplementary Table 1. Diagnostic categories in the clinical control group.

| Diagnostic categories                                      | n  | %   |
|------------------------------------------------------------|----|-----|
| Chronic headache                                           | 29 | 46.8|
| Narcolepsy type 1 plus REM sleep behavior disorder         | 11 | 17.7|
| Narcolepsy type 1                                          | 4  | 6.4 |
| Peripheral polyneuropathy                                  | 3  | 4.8 |
| Periodic limb movement disorder                            | 3  | 4.8 |
| Delirium due to drug/alcohol abuse                         | 2  | 3.2 |
| Myelopathy due to vitamin B12 deficiency                   | 2  | 3.2 |
| Benign focal amyotrophy                                    | 1  | 1.6 |
| Optic atrophy                                              | 1  | 1.6 |
| Epilepsy                                                   | 1  | 1.6 |
| Subjective cognitive impairment                            | 1  | 1.6 |
| Psychiatric disorder                                       | 1  | 1.6 |
| CNS neoplasia (meningioma)                                 | 1  | 1.6 |
| Poliomyelitis                                              | 1  | 1.6 |
| Stiff limb syndrome                                        | 1  | 1.6 |
| **Total**                                                  | 62 | 100 |
**Supplementary Table 2.** Overview of MMSE results and diagnostic investigations performed in the clinical cohort.

|                  | DLB (n=34) | PD (n=71) | AD (n=43) | PSP/CBS (n=30) | MSA (n=31) | PAF (n=28) | iRBD (n=18) | Clinical ctrl (n=62) |
|------------------|-----------|-----------|-----------|----------------|------------|------------|-------------|---------------------|
| **Brain MRI (%)** | 25 (73.5) | 61 (85.9) | 36 (83.7) | 27 (90.0) | 31 (100) | 26 (92.8) | 16 (88.9) | 53 (85.5) |
| **DLB** (n=34)  |           |           |           |                |            |            |             |                     |
| **PD** (n=71)   |           |           |           |                |            |            |             |                     |
| **AD** (n=43)   |           |           |           |                |            |            |             |                     |
| **PSP/CBS** (n=30) |           |           |           |                |            |            |             |                     |
| **MSA** (n=31)  |           |           |           |                |            |            |             |                     |
| **PAF** (n=28)  |           |           |           |                |            |            |             |                     |
| **iRBD** (n=18) |           |           |           |                |            |            |             |                     |
| **Clinical ctrl** |           |           |           |                |            |            |             |                     |
| **SPECT DaTSCAN (%)** | 19 (55.9) | 55 (77.5) | -         | 15 (50.0) | 24 (77.4) | 16 (57.1) | 14 (77.8) | 4 (6.4) |
| **Cardiac 123I-MIBG scintigraphy (%)** | 15 (44.1) | 30 (42.2) | -         | 5 (16.7)  | 19 (61.3) | 19 (67.8) | -          | -         |
| **Polysomnography (%)** | 17 (50.0) | 47 (66.2) | 1 (2.3)  | 9 (30.0)  | 29 (93.5) | 22 (78.6) | 18 (100) | 20 (32.2) |
| **Autonomic tests (%)** | 24 (70.6) | 60 (84.5) | -         | 17 (56.7) | 30 (96.8) | 28 (100)  | 11 (61.1) | -          |
| **Neuropsychological assessment (%)** | 32 (94.1) | 61 (85.9) | 43 (100) | 27 (90.0) | 25 (80.6) | 14 (50.0) | 18 (100) | 7 (11.3)  |
| **MMSE (yrs from disease onset)** | 21.6±5.6 (7.9±9.9) | 27.7±3.8 (5.0±4.0) | 21.9±5.4 (3.8±2.9) | 27.1±2.5 (3.9±2.6) | 28.4±1.8 (4.5±3.4) | 28.1±1.9 (12.0±5.6) | 29.1±1.3 (6.2±5.1) | 29.2±0.8 (3.7±4.7) |

List of abbreviations: DLB, dementia with Lewy bodies; PD, Parkinson’s disease; AD, Alzheimer’s disease; PSP, progressive supranuclear palsy; CBS, corticobasal syndrome; PAF, pure autonomic failure; iRBD, isolated REM sleep behaviour disorder; ctrl, controls; MRI, magnetic resonance imaging; MMSE, Mini-Mental State Examination.

**Supplementary Table 3.** Results of CSF biomarkers (A/T/N system) across the diagnostic groups.

|                  | t-tau (pg/ml) | p-tau (pg/ml) | Aβ42 (pmol/l) | Aβ40 (pmol/l) | Aβ42/Aβ40x10 (pg/ml) |
|------------------|---------------|---------------|---------------|---------------|----------------------|
| **AD**           | 773.3±508.5 (55) | 91.7±57.9 (53) | 450.0±207.4 (53) | 8820.3±4081.8 (47) | 0.51±0.17 (47) |
| **DLB**          | 445.6±821.9 (45) | 48.1±25.1 (33) | 570.8±385.4 (34) | 6181.8±3339.9 (26) | 0.82±0.37 (26) |
| **PSP/CBS**      | 203.6±84.7 (31) | 33.3±11.5 (19) | 806.8±344.5 (21) | 5755.8±2350.3 (9) | 1.12±0.26 (9) |
| **PAF**          | 220.6±145.9 (22) | 35.2±9.0 (10) | 1034.4±558.9 (11) | 7572.2±3262.3 (9) | 1.43±0.67 (9) |
| **iRBD**         | 246.4±117.6 (16) | 30.2±10.6 (5) | 895.6±278.6 (5) | 6849.0±3248.9 (4) | 1.41±0.26 (4) |
| **PD**           | 207.9±144.9 (68) | 46.5±31.8 (17) | 656.9±206.2 (15) | -               | -                  |
| **MSA**          | 319.4±199.5 (29) | 50.6±24.5 (9) | 792.7±363.8 (9) | -               | -                  |

The number of cases analyzed for each diagnostic group and each CSF biomarker are reported in brackets. All biomarker values, except the Aβ42/Aβ40 ratio, are in pg/ml. For abbreviations see supplementary Table 1.
**Supplementary Table 4.** Percentage distribution of positive replicates across LBD groups*.

| Diagnostic categories (positive/total samples) | Number of positive wells (samples, n) | %    |
|-----------------------------------------------|--------------------------------------|------|
| Incidental NP LB syn+ (6/7)                  | 2 (3) 3 (1) 4 (2)                    | 50.0% 16.7% 33.3% |
| iRBD (18/18)                                 | 2 (2) 3 (5) 4 (11)                   | 11.1% 27.8% 61.1% |
| PAF (26/28)                                  | 2 (1) 3 (3) 4 (22)                   | 3.9% 11.5% 84.6% |
| DLB (47/48)                                  | 2 (2) 3 (6) 4 (39)                   | 4.2% 12.8% 83.0% |
| PD (67/71)                                   | 2 (6) 3 (13) 4 (48)                  | 9.0% 19.4% 71.6% |
| Total LB syn + (164/172)                     | 2 (14) 3 (28) 4 (122)                | 8.5% 17.1% 74.4% |

*Only the results of “diagnostic” positive runs are represented. For “undetermined” runs (1/4 positive wells see supplementary Table 6). For abbreviations see supplementary Table 2.

**Supplementary Table 5.** Phenoconversion from PAF to DLB, PD and MSA.

| Diagnosis at LP | Age at LP (yrs) | Time between first symptom(s) and phenotypic conversion (yrs) | Clinical diagnosis at last follow-up | α-syn RT-QuIC result |
|-----------------|-----------------|---------------------------------------------------------------|-------------------------------------|----------------------|
| PAF             | 48              | 7                                                             | MSA                                 | Negative             |
| PAF             | 69              | 7                                                             | DLB                                 | Positive             |
| PAF             | 52              | 9                                                             | PD                                  | Positive             |
| PAF, RBD        | 53              | 3                                                             | MSA                                 | Negative             |
| PAF             | 68              | 4                                                             | PD                                  | Positive             |
| PAF             | 68              | 3                                                             | PD                                  | Positive             |

LP, lumbar puncture; for the other abbreviations see supplementary Table 1.
**Supplementary Table 6.** Percentage distribution of “unclear” results across diagnostic groups

|                      | Unclear/Total runs (%) | Final result of repeated test |
|----------------------|------------------------|-------------------------------|
|                      | 0/4 (%) | ≥2/4 (%) |                      |
| **Syn –**            |          |          |                      |
| Syn- path controls   | 15*/ 193 (7.8) | 14 (93.3) | 1 (6.7) |
| Clinical controls    | 7*/ 81 (8.6) | 7 | - |
| PSP/CBS              | 5 / 62 (8.1) | 4 | 1 |
| **AD**               | 3 / 31 (9.7) | 3 | - |
| **MSA**              | 5 / 33 (15.2) | 5 | - |
| **Syn +**            |          |          |                      |
| Syn+ path controls   | 3* / 172 (1.7) | 2 (66.7) | 1 (33.3) |
| PD                   | 2* / 7 (28.6) | 1 | 1 |
| **Total**            | 28 / 439 (6.4) | 24 (85.7) | 4 (14.3) |

*One sample needed a further repetition because of a second unclear result.

For abbreviations see supplementary Table 1.

**Supplementary Table 7.** Performance of Syn RT-QuIC in positive samples repeated three times

| Diagnostic groups | 2/4 (n=7*) | First result | 4/4 (n=11§) |
|-------------------|------------|--------------|--------------|
|                   | Second result (%) | Third result (%) | Second result (%) | Third result (%) | Second result (%) | Third result (%) |
| syn + (syn+ NP, DLB, PAF, RBD, PD) |          |              |              |                      |
| 0/4               | - | - | - | - | - |
| 1/4               | 2 (28.6) | 1 (14.3) | 2 (28.6) | 1 (14.3) | - | - |
| 2/4               | 2 (28.6) | 3 (42.8) | 2 (28.6) | 3 (42.8) | 1 (9.1) | 1 (9.1) |
| 3/4               | 1 (14.2) | 1 (14.3) | 1 (20.0) | 1 (20.0) | - | - |
| 4/4               | 2 (28.6) | 2 (28.6) | 3 (60.0) | 3 (60.0) | 11 (100) | 7 (63.6) |

*Includes 4 PD, 1 iRBD, 1 DLB and 1 syn+ NP; † Includes 2 PD, 1 iRBD, and 1 PAF; § Includes 4 PD, 2 iRBD, 4 DLB and 1 PAF. For abbreviations see supplementary Table 1.
Supplementary Table 8. Intra-batch and inter-batch coefficients of variation.

| # Batch | CV I max | CV AUC | CV lag phase |
|---------|----------|--------|--------------|
| 1       | 19.82%   | 24.65% | 14.98%       |
| 2       | 7.43%    | 2.99%  | 21.79%       |
| 3       | 8.12%    | 12.26% | 16.85%       |
| 4       | 11.50%   | 16.59% | 22.97%       |
| 5       | 8.88%    | 13.00% | 18.48%       |
| 6       | 18.15%   | 24.39% | 32.25%       |
| 7       | 22.17%   | 23.88% | 25.88%       |
| 8       | 10.04%   | 5.12%  | 12.25%       |
| 9       | 16.21%   | 30.28% | 32.06%       |
| mixed   | 15.81%   | 17.16% | 20.40%       |
| Inter-batch CV | 17.65% | 21.16% | 23.48% |

| # Batch | CV I max | CV AUC | CV lag phase |
|---------|----------|--------|--------------|
| 2       | 10.58%   | 4.21%  |              |
| 3       | 7.78%    | 8.66%  |              |
| 4       | 11.56%   | 10.99% |              |
| 5       | 2.76%    | 1.32%  |              |
| 6       | 39.57%   | 28.72% |              |
| 7       | 27.67%   | 21.92% |              |
| 8       | 11.86%   | 11.28% |              |
| 9       | 16.87%   | 6.92%  |              |
| mixed   | 25.61%   | 22.83% |              |
| Inter-batch CV | 36.41% | 29.94% |        |

The intra-batch coefficient of variation (CV) of the maximum intensity of fluorescence (I max), area under the curve (AUC) and lag phase is expressed as percentage of the ratio between standard deviation and average.
Supplementary figure. Batch-to-batch and intra-batch variation of fluorescence signal induced by recombinant α-Syn aggregation in the RT-QuIC. Kinetic curves, AUC, and Lag phase of the same positive (a, c, e) and negative (b, d, f) control tested with different batches of recombinant α-Syn. Each colour depicts the performance of a different batch of α-Syn. Error bars in the kinetic curves were excluded in order to make the image easier to read. In the other graphs, error bars represent intra-batch variability, calculated for the positive control on, respectively 3 (batch #1), 2 (batch #2), 3 (batch #3), 4 (batch #4), 1 (batch #5), 2 (batch #6), 8 (batch #7), 2 (batch #8), 1 (batch #9), and 2 runs (mixed batches #2 + #6, and #8 + #9). For the negative control, error bars representing intra-batch variability, were calculated on, respectively, 1 (batch #1), 1 (batch #2), 3 (batch #3), 1 (batch #5), 2 (batch #6), 8 (batch #7), 2 (batch #8), 1 (batch #9), and 2 runs (mixed batches #2 + #6 and #8 + #9). All control samples were run in quadruplicates. Differences in the dimension (n) of the runs per batch were related to the variability in the yield of recombinant α-Syn between batch preparations.