# A potential new diagnostic test for Parkinson's disease: real-time QuiC of alpha-synuclein

ERIG, SRMS, Edinburgh

14th January 2017

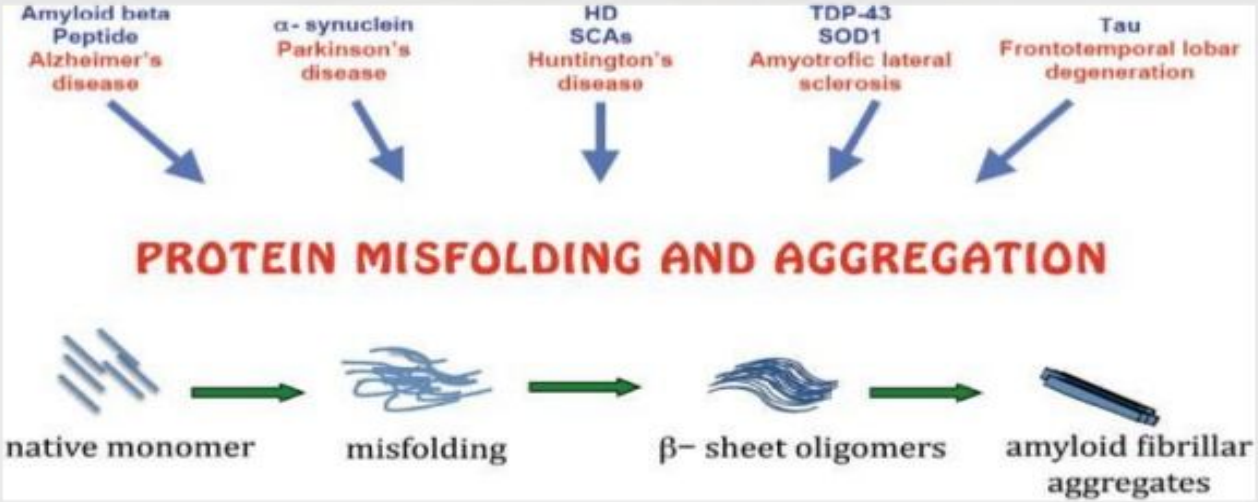
Alison JE Green

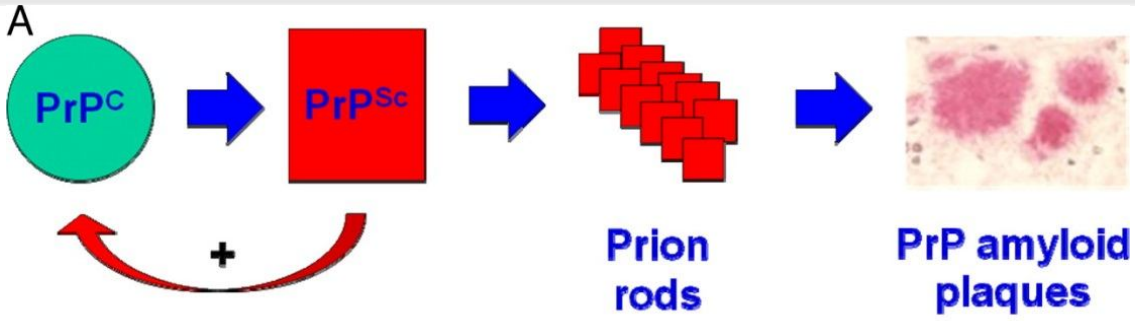
The National CJD Research & Surveillance Unit, University of Edinburgh, United Kingdom

# Neurological diseases associated with protein misfolding

| Neurological disorder | Protein involved | Length of protein | Nature of misfolding |
|---|---|---|---|
| **Alzheimer's disease** | Amyloid-β peptide | 37–43 | Intrinsically disordered |
| **Spongiform encephalopathies** | Prion protein or its fragments | 230 | Intrinsically disordered and α-helical |
| **Parkinson's disease** | α-synuclein | 140 | Intrinsically disordered |
| **Amyotrophic lateral sclerosis** | Superoxide dismutase 1 | 153 | β-sheet and Ig-like |
| **Huntington's disease** | Huntingtin fragments | Variable | Mostly intrinsically disordered |
| **Familial amyloidotic polyneuropathy** | Transthyretin mutants | 127 | β-sheet |

# Protein-misfolding diseases



# Real-Time QuiC Publications

OPEN ACCESS Freely available online

PLoS PATHOGENS

## Rapid End-Point Quantitation of Prion Seeding Activity with Sensitivity Comparable to Bioassays

**Jason M. Wilham[1], Christina D. Orrú[1,2], Richard A. Bessen[3], Ryuichiro Atarashi[4], Kazunori Sano[4], Brent Race[1], Kimberly D. Meade-White[1], Lara M. Taubner[1], Andrew Timmes[1], Byron Caughey[1]***

**1** Laboratory of Persistent Viral Diseases, Rocky Mountain Laboratories, National Institute of Allergy and Infectious Disease, Hamilton, Montana, United States of America, **2** Department of Biomedical Sciences and Technologies, University of Cagliari, Monserrato, Italy, **3** Veterinary Molecular Biology, Montana State University, Bozeman, Montana, United States of America, **4** Department of Molecular Microbiology and Immunology, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Kyushu, Japan

**Received** May 4, 2010; **Accepted** November 3, 2010; **Published** December 2, 2010

nature medicine

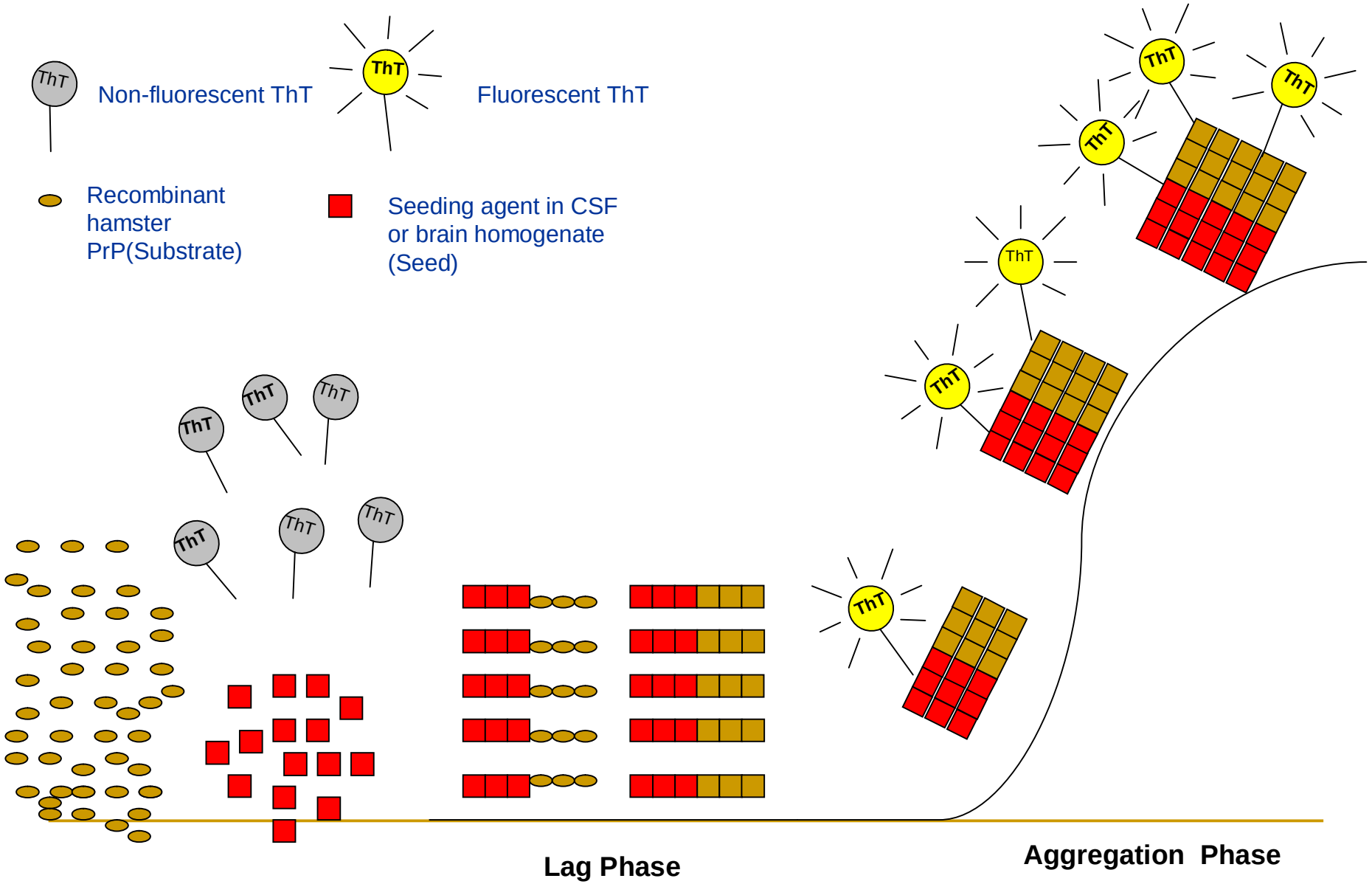
BRIEF COMMUNICATIONS

## Ultrasensitive human prion detection in cerebrospinal fluid by real-time quaking-induced conversion

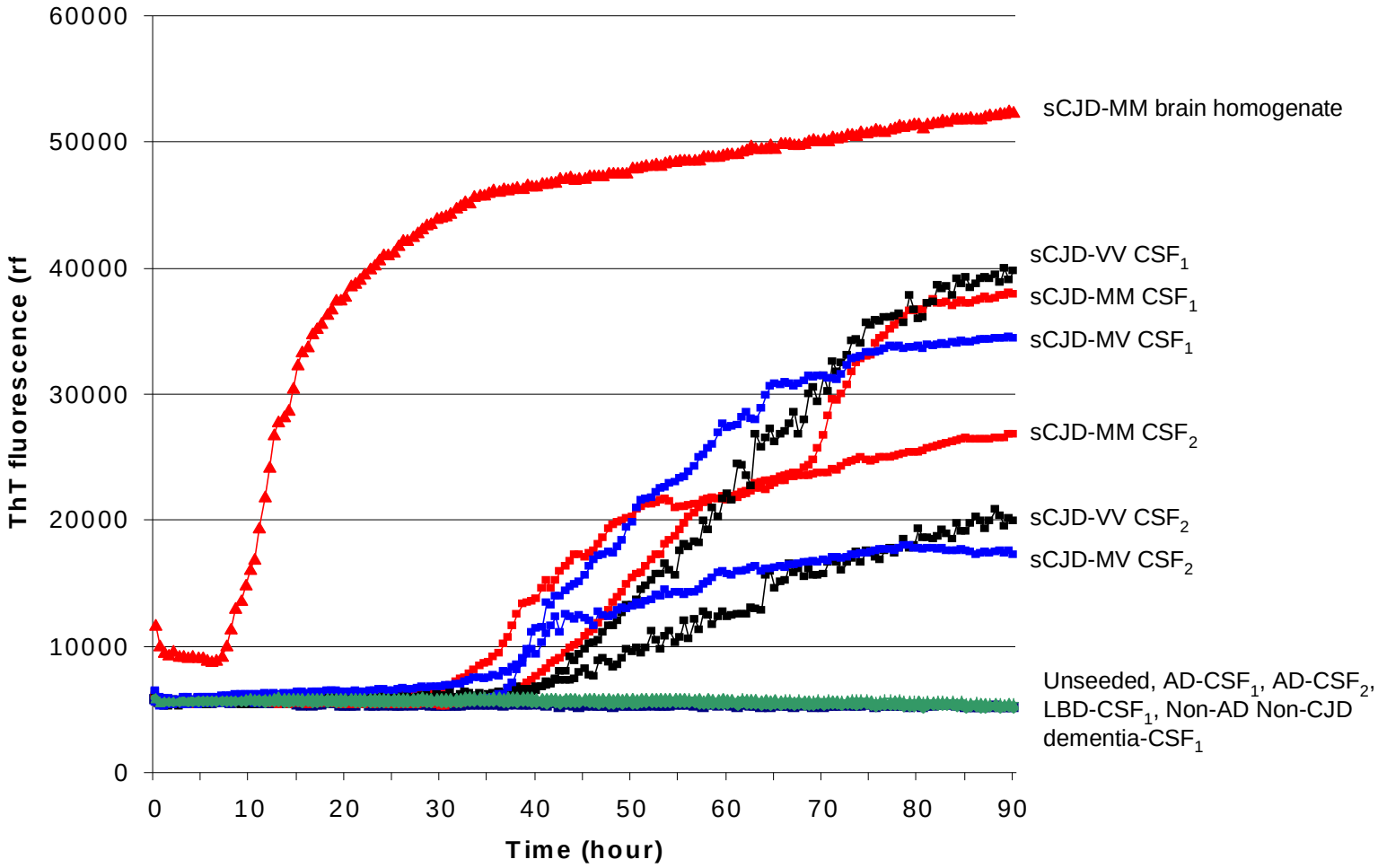
Ryuichiro Atarashi[1,2], Katsuya Satoh[1], Kazunori Sano[1,3], Takayuki Fuse[1], Naohiro Yamaguchi[1], Daisuke Ishibashi[1], Takehiro Matsubara[1], Takehiro Nakagaki[1], Hitoki Yamanaka[4], Susumu Shirabe[5], Masahito Yamada[6], Hidehiro Mizusawa[7], Tetsuyuki Kitamoto[8], Genevieve Klug[9], Amelia McGlade[9], Steven J Collins[9] & Noriyuki Nishida[1,3]

Received 2 July 2010; accepted 16 December 2010; published online 30 January 2011; doi:10.1038/nm.2294

# Diagrammatic scheme of RT-QuIC reaction



# Preliminary experiments: FL Ham rPrP seeded with CSF



# Blinded Retrospective Study

- **227 CSFs tested:**
  - 124 neuropathologically confirmed cases of sCJD.
  - 103 non-CJD control patients; initially suspected of sCJD but given an alternative clinical or pathological diagnosis.

## Patient Demographics

| Diagnosis | Number | Age (Mean ± SD) | F:M | Codon 129 | | |
|---|---|---|---|---|---|---|
| | | | | MM | MV | VV |
| sCJD | 124 | 65.2 ± 11.5 | 63:61 | 60 | 25 | 20 |
| Non-CJD Controls | 103 | 65.6 ± 11.1 | 52:51 | - | - | - |

# Comparison of RT-QuIC results with 14-3-3 and tau protein

| | RT-QuIC | 14-3-3 | tau protein >1260pg/ml |
|---|---|---|---|
| **sCJD** | 110/124 | 118/124 | 60/67 |
| **non-CJD controls** | 0/103 | 39/103 | 19/98 |
| **Sensitivity** | 89% | 95% | 90% |
| **Specificity** | 100% | 64% | 87% |

# Real Time Quaking-Induced Conversion Analysis of Cerebrospinal Fluid in Sporadic Creutzfeldt–Jakob Disease

Lynne I. McGuire, PhD,[1] Alexander H. Peden, PhD,[1] Christina D. Orrú, PhD,[2] Jason M. Wilham, PhD,[2] Nigel E. Appleford, Cbiol,[3] Gary Mallinson, PhD,[3] Mary Andrews, BSc,[1] Mark W. Head, PhD,[1] Byron Caughey, PhD,[2] Robert G. Will, FRCP,[1] Richard S. G. Knight, FRCP,[1] and Alison J. E. Green, PhD[1]

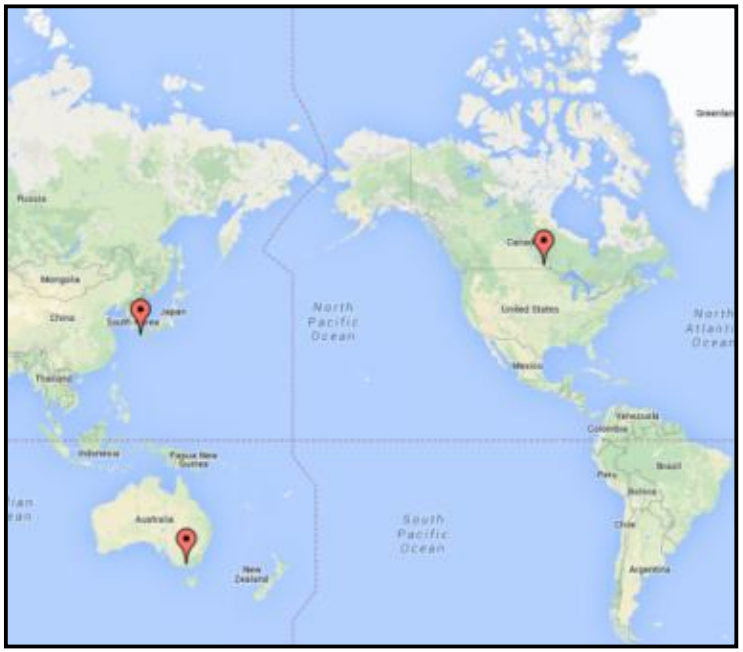
**Objective:** Current cerebrospinal fluid (CSF) tests for sporadic Creutzfeldt–Jakob disease (sCJD) are based on the detection of surrogate markers of neuronal damage such as CSF 14-3-3, which are not specific for sCJD. A number of prion protein conversion assays have been developed, including real time quaking-induced conversion (RT-QuIC). The objective of this study is to investigate whether CSF RT-QuIC analysis could be used as a diagnostic test in sCJD.
**Methods:** An exploratory study was undertaken that analyzed 108 CSF samples from patients with neuropathologically confirmed sCJD or from control patients. Of the 108 CSF samples, 56 were from sCJD patients (30 female, 26 male; aged 31–84 years; mean age, 62.3 ± 13.5 years), and 52 were from control patients (26 female, 26 male; aged 43–84 years; mean age, 67.8 ± 10.4 years). A confirmatory group of 118 patients was subsequently examined that consisted of 67 cases of neuropathologically confirmed sCJD (33 female, 34 male; aged 39–82 years; mean age, 67.5 ± 9.0 years) and 51 control cases (26 female, 25 male; aged 36–87 years; mean age, 63.5 ± 11.6 years).
**Results:** The exploratory study showed that RT-QuIC analysis had a sensitivity of 91% and a specificity of 98% for the diagnosis of sCJD. These results were confirmed in the confirmatory study, which showed that CSF RT-QuIC analysis had a sensitivity and specificity of 87% and 100%, respectively.
**Interpretation:** This study shows that CSF RT-QuIC analysis has the potential to be a more specific diagnostic test for sCJD than current CSF tests.

ANN NEUROL 2012;72:278–285

# Prospective Audit CSF RT-QuIC of 1512 CSF samples

| | CSF RT-QuIC | CSF 14-3-3 |
|---|---|---|
| Definite sCJD | 252/283 | 234/275 |
| Probable sCJD | 184/195 | 154/191 |
| Possible sCJD | 13/21 (62%) | 0/20 |
| Iatrogenic CJD (GH) | 2/4 | 1/4 |
| Genetic CJD (2 insert, 1 E200K) | 3/3 | 3/3 |
| Unknown | 5/18 | 4/17 |
| NOT CJD | 0/988 | 67/977 |
| Sensitivity | 89% (94%) | 85% (81%) |
| Specificity | 100% | 93% |
| Efficiency | 98% | 91% |

# Transferance of RT-QuIC technology

**Cerebrospinal fluid real-time quaking-induced conversion is a robust and reliable test for sporadic Creutzfeldt-Jakob disease: An international study.**

McGuire LI[1], Poleggi A[2], Poggiolini I[3], Suardi S[4], Grznarova K[5,6], Shi S[7], de Vil B[8], Sarros S[9], Satoh K[10], Cheng K[11], Cramm M[12], Fairfoul G[1], Schmitz M[12], Zerr I[12], Cras P[8], Equestre M[2], Tagliavini F[4], Atarashi R[10], Knox D[11], Collins S[13], Haïk S[5,6,14], Parchi P[3,15], Pocchiari M[2], Green A[1].

Ann Neurol. 2016 Jul;80(1):160-5

## Diagnostic criteria for surveillance of sporadic CJD from 1 January 2017

1.1 **DEFINITE:**

Progressive neurological syndrome **AND**

Neuropathologically **or** immunohistochemically **or** biochemically confirmed

1.2 **PROBABLE:**

1.2.1 I + two of II and typical EEG*

**OR** 1.2.2 I + two of II and typical MRI brain scan**

**OR** 1.2.3 I + two of II and positive CSF 14-3-3

**OR** 1.2.4 Progressive neurological syndrome and positive RT-QuIC in CSF or other tissues

1.3 **POSSIBLE:**

I + two of II + duration < 2 years

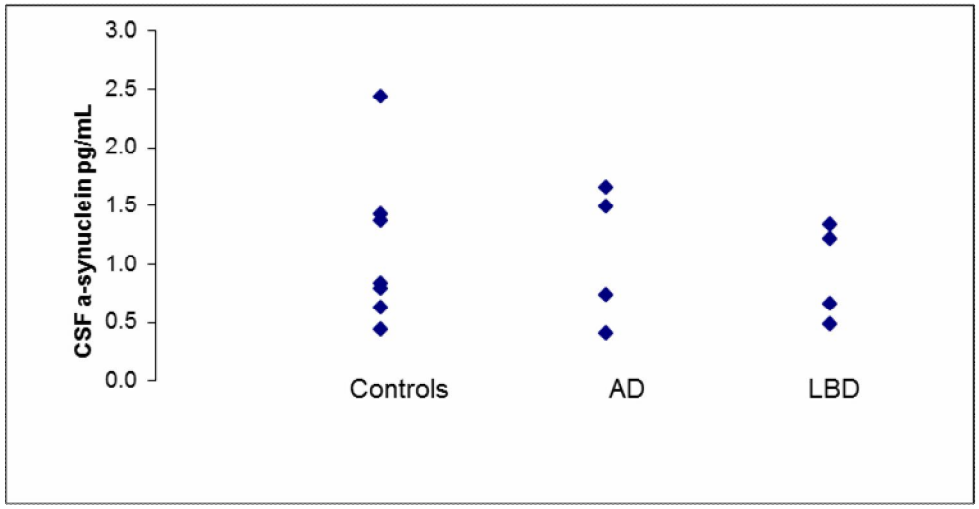
I Rapidly progressive cognitive impairment

II
- A Myoclonus
- B Visual or cerebellar problems
- C Pyramidal or extrapyramidal features
- D Akinetic mutism

*Generalized periodic complexes

**High signal in caudate/putamen on MRI brain scan or at least two cortical regions (temporal, parietal, occipital) either on DWI or FLAIR

# CSF Alpha-synuclein in Lewy body dementia




| | Mean | SD |
|---|---|---|
| 1 (Control) | 1.12 | 0.68 |
| 2 (AD) | 1.06 | 0.60 |
| 3 (LBD) | 0.92 | 0.42 |
| | | |
| T-Test (Cont vs LBD/PD) | 0.55 | |
| T-Test (AD vs LBD/PD) | 0.71 | |

Contents lists available at SciVerse ScienceDirect

Parkinsonism and Related Disorders

journal homepage: www.elsevier.com/locate/parkreldis

Review

The diagnostic utility of cerebrospinal fluid alpha-synuclein analysis in dementia with Lewy bodies – A systematic review and meta-analysis

Xuxin Lim [a], Jing Ming Yeo [a], Alison Green [b], Suvankar Pal [c,d,e,*]

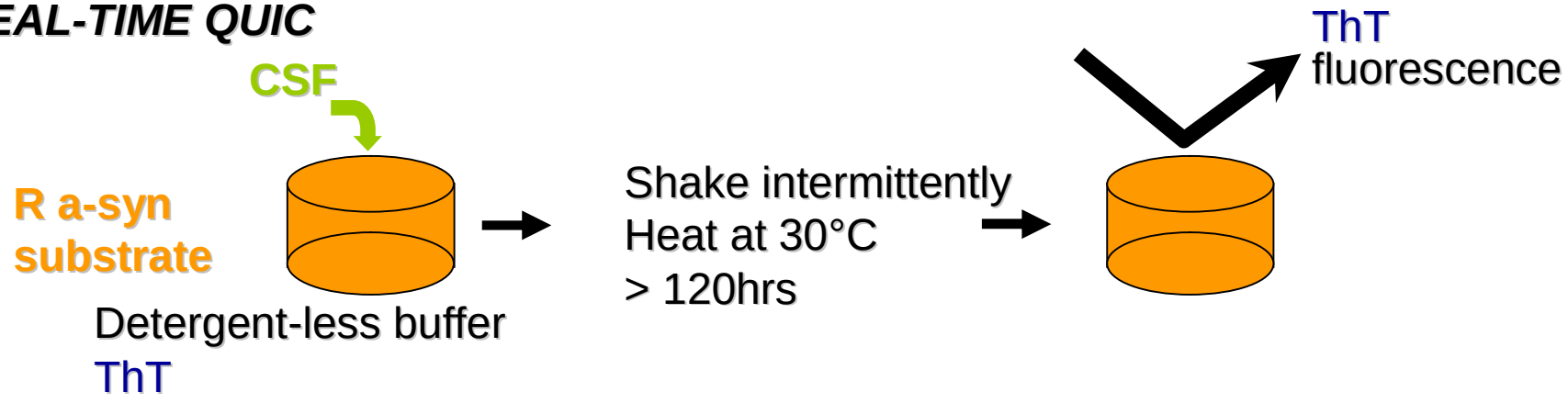
[a] College of Medicine and Veterinary Medicine, University of Edinburgh, UK
[b] CJD Research & Surveillance Unit, Western General Hospital, Edinburgh, UK
[c] Department of Neurology, Forth Valley Royal Hospital, NHS Forth Valley, UK
[d] Division of Clinical Neurosciences, Western General Hospital, Edinburgh, UK
[e] Anne Rowling Regenerative Neurology Clinic, University of Edinburgh, UK

## Conclusions

- 13 studies: 2728 patients
- CSF $\alpha$-synuclein lower in LBD vs AD, p=0.02
- Caution:
  - Heterogeneity of assays
  - Lack of consistent control groups
  - Limited PM verification

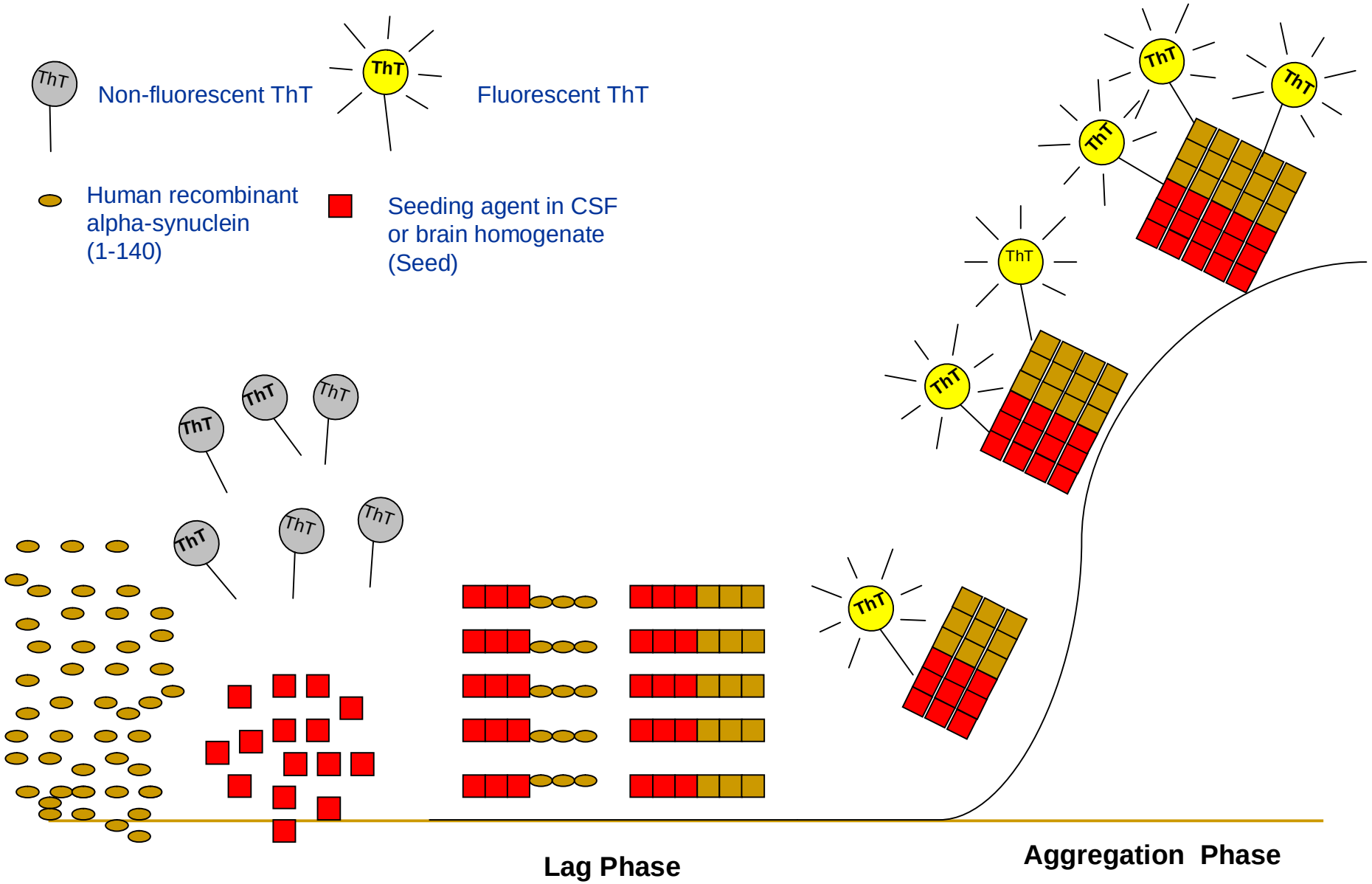
# Alpha-synuclein RT-QuIC Protocol

***REAL-TIME QUIC***

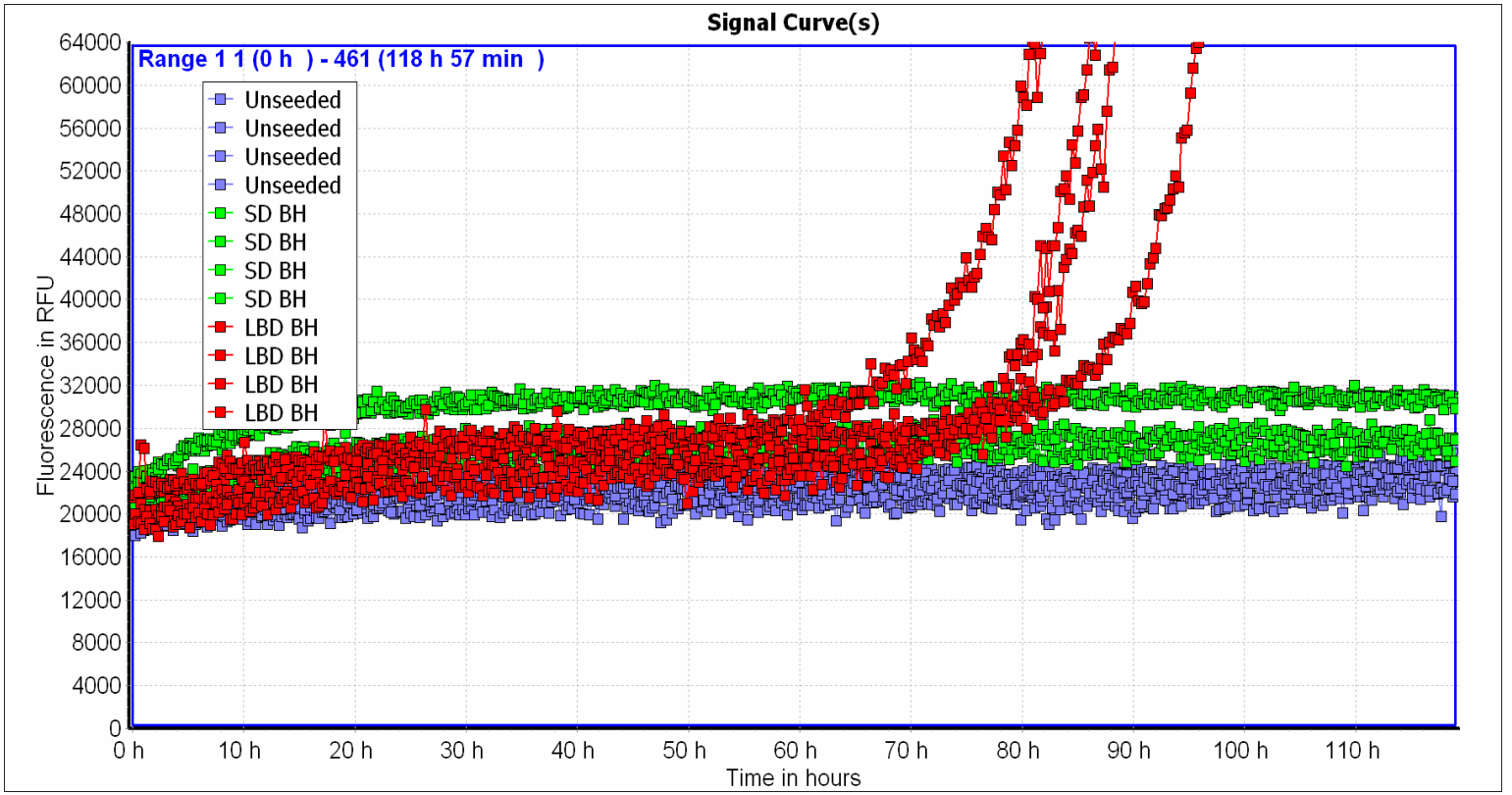



- Instrument: BMG Optima, 200rpm, 1 min shake, 14 min rest
- Substrate: 0.1mg/mL full-length human recombinant a-syn (1-140)
- Seeds: 2 µl of diluted of LBD brain homogenate
  2 µl of diluted brain from AD or SD homogenate (controls)
  15µl of undiluted CSF
- Buffer: 10 mM PBS, 200 mM NaCl, 10 µM ThT, 10 µM EDTA

# Diagrammatic scheme of RT-QuIC reaction

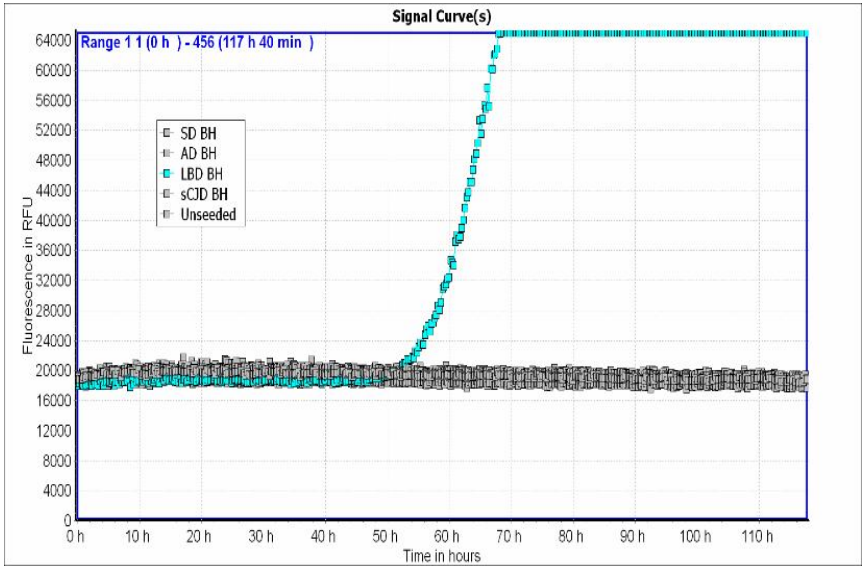


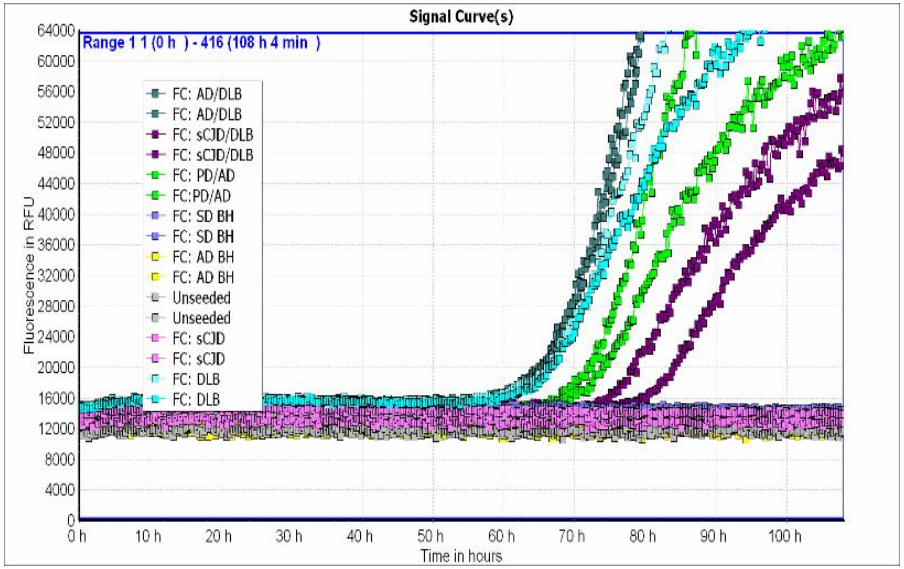
# RT-QuIC of α-synuclein




**PBS buffer, pH 8.2, 200rpm 30°C, 1min shake, 14 min off**

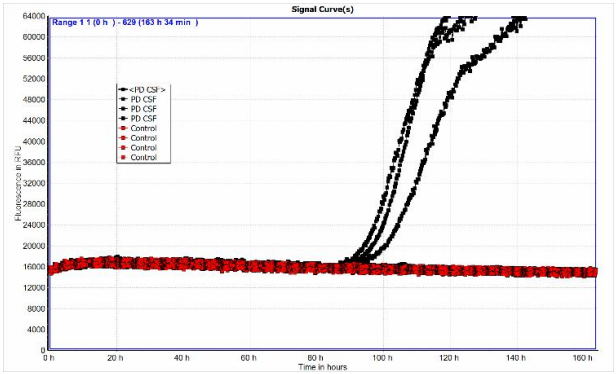
# RT-QuIC for α-synuclein (brain homogenates)



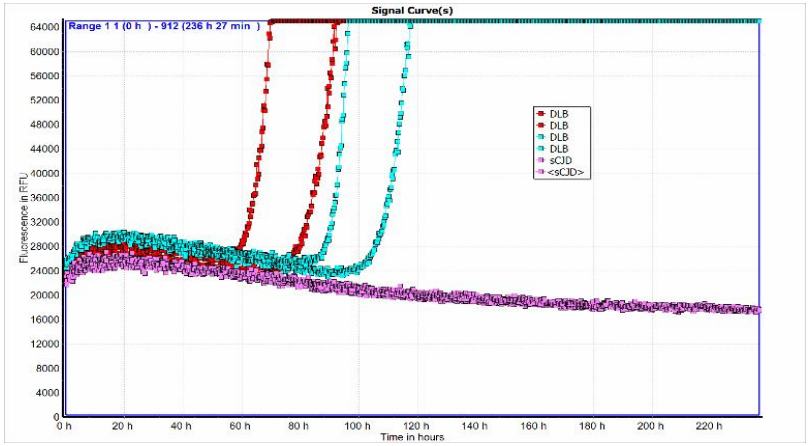




Commercially available recombinant human 1-140aa a-synuclein
Phosphate buffer, pH8.2
Temperature: 30°C
Shake speed: 200rpm
Shake cycle; 1 min on; 14 min off

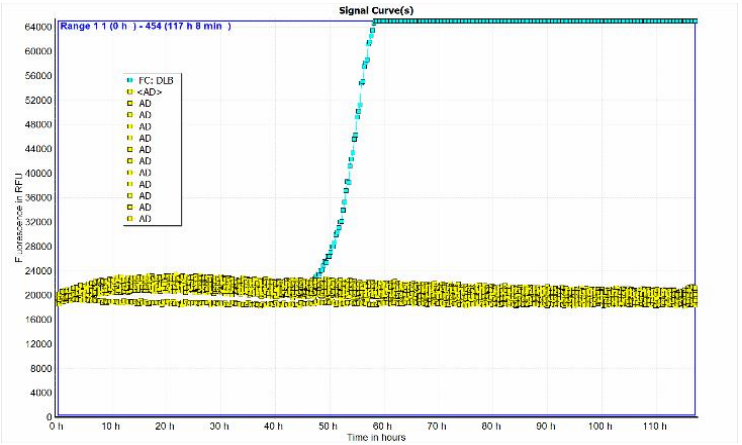
# RT-QuIC for α-synuclein (CSF samples)




CSF Parkinson's Disease




CSF LBD samples




CSF AD samples

# Retrospective analysis of α-synuclein RT-QuIC

- Collaboration with Dr Laura Parkkinen, Dept of Neuropathology, University of Oxford
- 99 CSF samples from the OPTIMA Study (Exploratory group)
  - Prospective longitudinal study of dementia and aging – CSF taken at regular time points. Established in 1988
- 38 CSF OPDC Discovery cohort (Confirmatory group)
- Each CSF sample analysed blind

# Patient Demographics

| OPTIMA patients (n) | Age at death<br>Mean ± SD (range) | F/M |
|---|---|---|
| Pure LBD (12) | 80.8 ± 6.5 (71-92) | 4/8 |
| Parkinson's disease (2) | 77.5 ± 7.8 (72-83) | 0/2 |
| Mixed LBD/AD (17) | 80.1 ± 6.4 (69-90) | 10/7 |
| AD with incidental LB (13) | 79.8 ± 7.8 (67-91) | 9/4 |
| Pure AD (30) | 77.7 ± 8.6 (61-93) | 17/13 |
| Progressive supranuclear palsy (PSP) (2) | 69.5 ± 3.5 (67-72) | 2/0 |
| Corticobasal degeneration (CBD) (3) | 64.0 ± 10.6 (52-72) | 1/2 |
| Controls (20) | 82.9 ± 6.9 (68-93) | 10/10 |
| Discovery patients (n) | | |
| Parkinson's disease (20) | 65.1 ± 9.1 (42-80) | 6/14 |
| At-risk RBD patients (3) | 67.6 ± 7.7 (59-74) | 0/3 |
| Controls (15) | 65.8 ± 7.4 (55-83) | 8/7 |

# Retrospective Study

| Exploratory Patient Group (n) | Positive RT-QuIC (%) |
|---|---|
| Pure DLB (12) | 11 (92%) |
| Mixed DLB/AD (17) | 11 (65%) |
| AD with incidental LB (13) | 2 (15%) |
| Parkinson's disease (2) | 2 (100%) |
| Progressive Supranuclear Palsy (2) | 0 (0%) |
| Corticobasal degeneration (3) | 0 (0%) |
| Pure AD (30) | 0 (0%) |
| Healthy Controls (20) | 0 (0%) |
| Sensitivity (DLB) | 92% |
| Specificity (vs controls) | 100% |
| Specificity (vs AD) | 100% |
| Specificity (vs Controls + AD) | 100% |
| | |
| Confirmatory Patient Group (n) | Positive RT-QuIC (%) |
| Parkinson disease (20) | 19 (95%) |
| At-risk RBD patients (3) | 3 (100%) |
| Healthy Controls (15) | 0 (0%) |
| Sensitivity (PD) | 95% |
| Specificity | 100% |

ANNALS of Clinical and Translational Neurology
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BRIEF COMMUNICATION

# Alpha-synuclein RT-QuIC in the CSF of patients with alpha-synucleinopathies

Graham Fairfoul[1], Lynne I. McGuire[1], Suvankar Pal[1,2], James W. Ironside[1], Juliane Neumann[3], Sharon Christie[4], Catherine Joachim[4], Margaret Esiri[4], Samuel G. Evetts[3], Michal Rolinski[3], Fahd Baig[3], Claudio Ruffmann[3], Richard Wade-Martins[5], Michele T. M. Hu[3], Laura Parkkinen[3] & Alison J. E. Green[1]

[1]The National CJD Research & Surveillance Unit, Western General Hospital, University of Edinburgh, Edinburgh EH4 2XU, United Kingdom
[2]Centre for Clinical Brain Sciences, Western General Hospital, Edinburgh EH4 2XU, United Kingdom
[3]Oxford Parkinson's Disease Centre, Nuffield Department of Clinical Neurosciences, University of Oxford, John Radcliffe Hospital, Level 6 West Wing, Oxford OX3 9DU, United Kingdom
[4]Oxford Project to Investigate Memory and Ageing (OPTIMA), Nuffield Department of Clinical Neurosciences, University of Oxford, John Radcliffe Hospital, Level 6 West Wing, Oxford OX3 9DU, United Kingdom
[5]Oxford Parkinson's Disease Centre, Department of Physiology, Anatomy and Genetics, University of Oxford, Le Gros Clark Building, South Parks Road, Oxford OX1 3QX, United Kingdom

# Second blinded retrospective study

| Patients | Number positive for RT-QuIC/total investigated |
|---|---|
| Idiopathic PD (56) | 51/56 (91%) |
| REM Sleep behavioural disorder (2) | 1/2 (50%) |
| Controls (2) | 0/2 (0%) |

# Cumulative data

| Patient Group (n) | Positive RT-QuIC (%) |
|---|---|
| Pure LBD (12) | 11 (92%) |
| Parkinson's disease (78) | 72 (95%) |
| At risk RBD (5) | 4 (80%) |
| Healthy controls (37) | 0 (0%) |
| Progressive Supranuclear Palsy (2) | 0 (0%) |
| Corticobasal degeneration (3) | 0 (0%) |
| Pure AD (30) | 0 (0%) |
| | |
| Sensitivity (DLB) | 92% |
| Sensitivity (PD) | 95% |
| Specificity | 100% |

# Further work

- Atypical PD
- Non-PD movement disorders
- Assess relations of a-syn RT-QuIC with:
  - Disease duration
  - Disease severity
  - Gender / Age at onset
- Other tissues: olfactory mucosa, blood
- Adaptation of technique

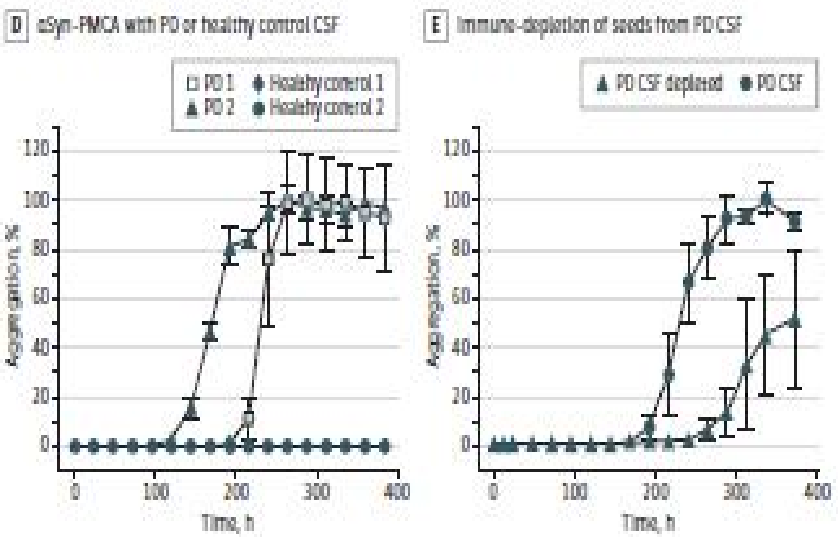
# Further studies

- Prof Bas Bloem and Dr Marcel Verbeek, University Radboud, Nijmegen, Netherlands
  - 110 CSF samples from atypical PD and appropriate controls
- Dr Guinluigi Zanusso, University of Verona, Italy
  - Olfactory mucosa samples
- bioFIND, MJFF
  - 112 CSF samples from PD (varying disease duration and severity)
- Graham Fairfoul, NCJDRSU
  - Developing a-syn RT-QuIC for blood

JAMA Neurology | Original Investigation

# Development of a Biochemical Diagnosis of Parkinson Disease by Detection of α-Synuclein Misfolded Aggregates in Cerebrospinal Fluid

Mohammad Shahnawaz, PhD; Takahiko Tokuda, MD; Masaaki Waragai, MD; Nicolas Mendez, BSc; Ryotaro Ishii, MD; Claudia Trenkwalder, MD; Brit Mollenhauer, MD; Claudio Soto, PhD

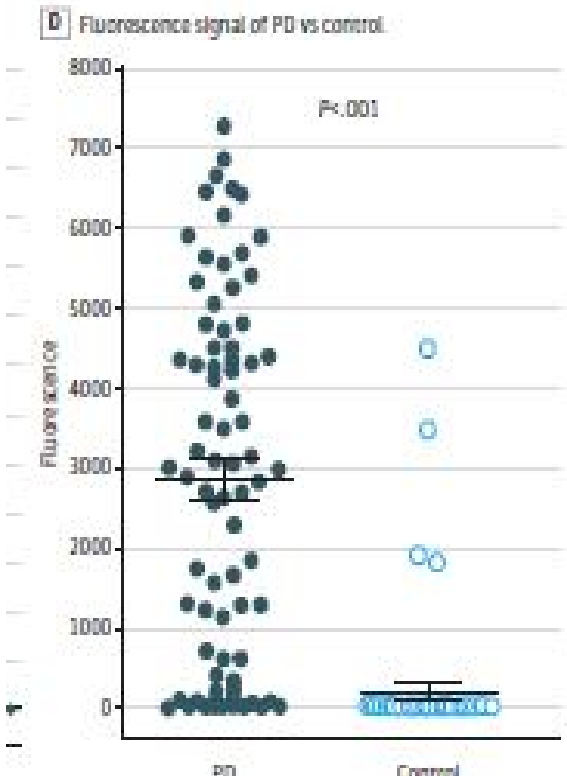


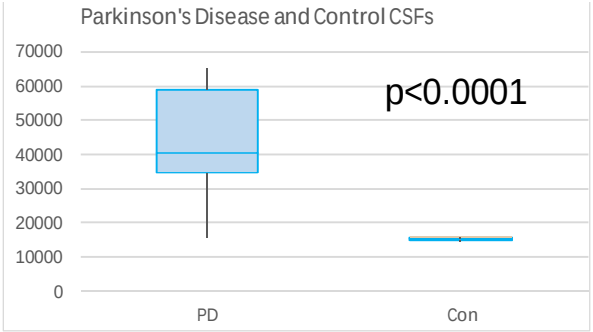

| Patient group | Number of CSF samples positive for a-syn aggregates |
|---|---|
| Parkinson's Disease (76) | 88.5% |
| Lewy body dementia (10) | 100% |
| Multi-system atrophy (10) | 80% |
| Neurological controls (97) | 4.1% |
| Sensitivity for PD | 88.5% |
| Specificity for PD | 95.9% |

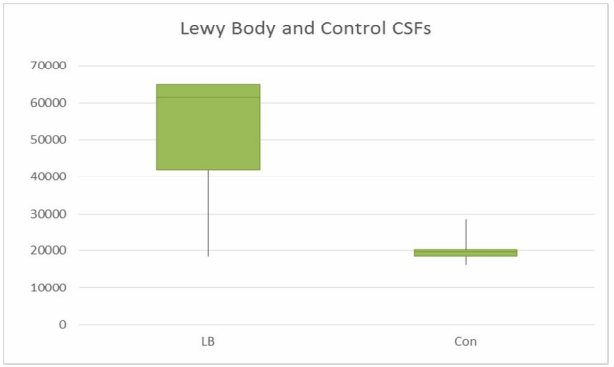
JAMA Neurology | Original Investigation

# Development of a Biochemical Diagnosis of Parkinson Disease by Detection of α-Synuclein Misfolded Aggregates in Cerebrospinal Fluid

Mohammad Shahnawaz, PhD; Takahiko Tokuda, MD; Masaaki Waragai, MD; Nicolas Mendez, BSc; Ryotaro Ishii, MD; Claudia Trenkwalder, MD; Brit Mollenhauer, MD; Claudio Soto, PhD



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Dr Diane Ritchie
Mrs Mags Le Grice
Research Registrars

**Rocky Mountain Laboratories**

Dr Byron Caughey
Dr Jason Wilham
Dr Christina Orru
Mr Andy Hughson

**Bristol Institute of Transfusion Science**

Dr Gary Mallinson
Dr Nigel Appleford
Dr Dave Jackson

**Department of Neuropathology, University of Oxford**

Dr Laura Parkkinen
Dr Ilaria Poggiolini