ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

IRCCS Istituto delle Scienze Neurologiche di Bologna

# α-Synuclein SAA: A highly specific marker for the early diagnosis and stratification of patients with synucleinopathies

*"Protein aggregation seeding assays: From prions to other ADRD proteinopathies". - Bethesda, MD, November 2024*

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*DIBINEM, University of Bologna, Italy*

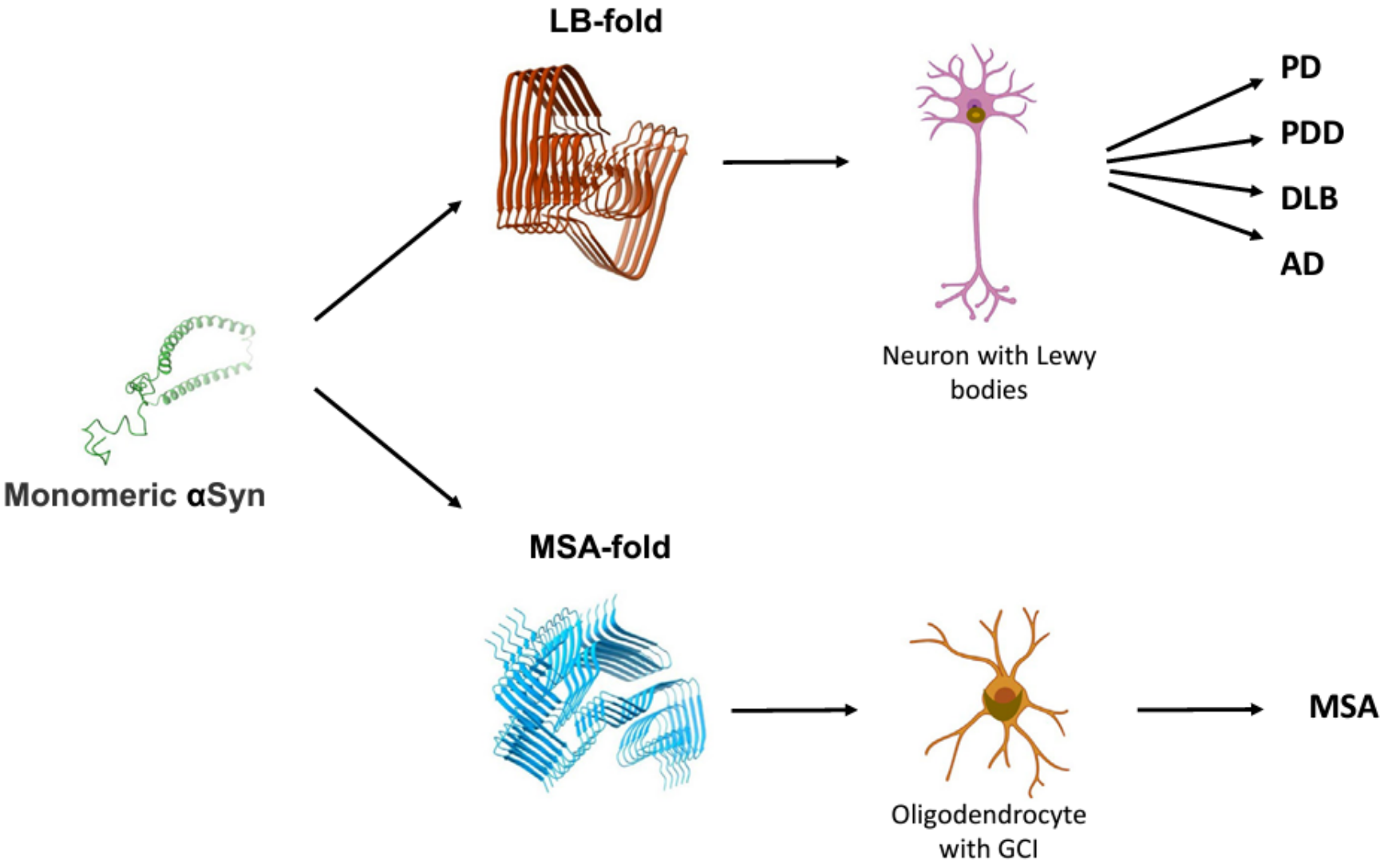
# Presentation Outline

- Summary of differences between α-Syn SAA protocols

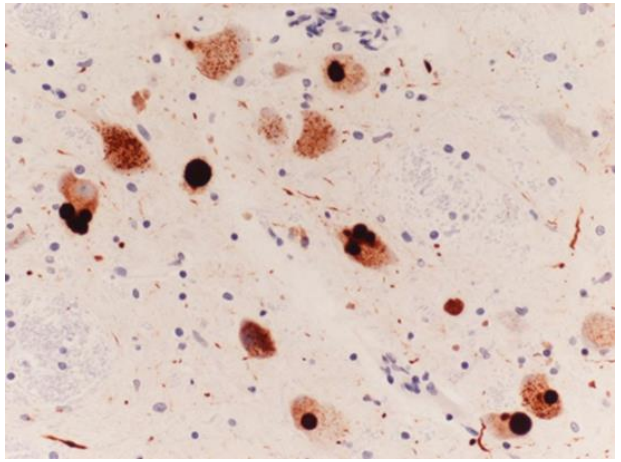
✓ **Focus on CSF results**

- Performance in cohorts with **neuropathologic assessment**
- Performance in patients with clinical parkinsonism (**PD**, MSA, PSP)
- Performance in patients with prodromal Lewy body disease (isolated **RBD**)
- Performance in patients with **cognitive decline** with or without **AD** pathology
- Performance in **"asymptomatic"** aged individuals (with no cognitive or motor dysfunction)
- Progress towards the development of a **"quantitative"** α-Syn SAA

# Two α-Synuclein(αSyn) conformational strains in synucleinopathies

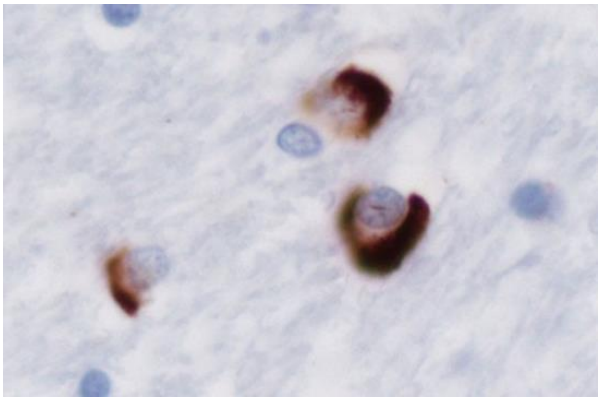



## PD-DLB



Lewy bodies

## MSA



oligodendroglial cytoplasmic inclusions

*Schweighauser et al* **Nature 2020;** *Yang Y et al* **Nature 2022,** Soto C, **TIBTEC 2024**

# α-Synuclein SAA protocols

1. **Alison Green's Lab**
   **Fairfoul et al., 2016**

Bargar *et al. acta neuropathol commun* (2021) 9:62
https://doi.org/10.1186/s40478-021-01175-w

Acta Neuropathologica Communications

METHODOLOGY ARTICLE — Open Access

Streamlined alpha-synuclein RT-QuIC assay for various biospecimens in Parkinson's disease and dementia with Lewy bodies

Connor Bargar[1], Wen Wang[1], Steven A. Gunzler[2], Alexandra LeFevre[3], Zerui Wang[1], Alan J. Lerner[2], Neena Singh[1,2], Curtis Tatsuoka[2], Brian Appleby[1,2], Xiongwei Zhu[1,2], Rong Xu[4], Vahram Haroutunian[5], Wen-Quan Zou[1,2*], Jiyan Ma[6*] and Shu G. Chen[1,2*]

- All reagents commercially available
- Rec. α-Syn from rPeptide (USA)
- CSF seeding activity detected in 30-40% of MSA cases

2. **Caludio Soto's Lab**
   **Shahnawaz et al., 2017**

**SYNTap® Biomarker Test**

The SYNTap Test helps physicians diagnose synucleinopathies such as: Parkinson's, LBD, Alzheimer's with Lewy Bodies, MCI, and MSA.

- Reagents from the Amprion Company
- Seeding activity detected in 80-90% of MSA cases with a distinctive profile (lower signal than in LBD)

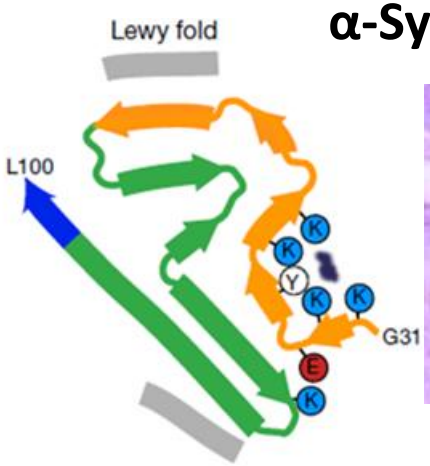
3. **Byron Caughey's Lab**
   **Groveman et al., 2018**

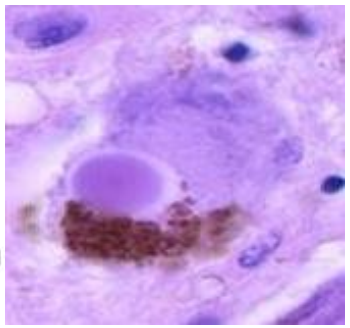
- Original plasmid for home made rec- α-Syn production made available and distributed to several laboratories

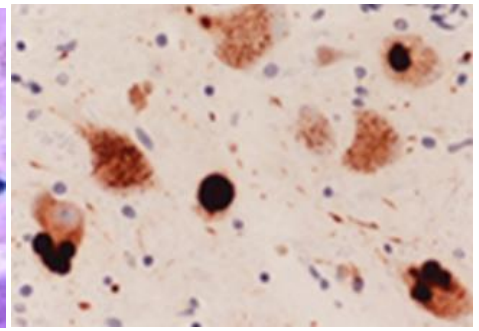
- Home made α-Syn from original Caughey's lab plasmid
- No seeding activity detected in MSA

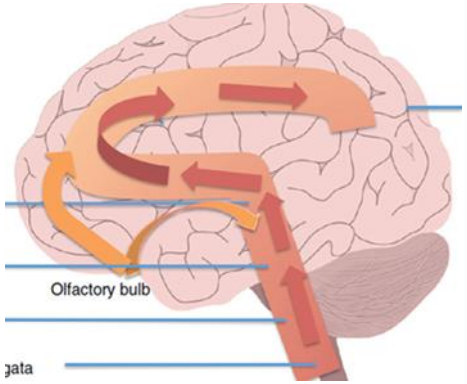
# Neuropathological staging of Lewy body disease

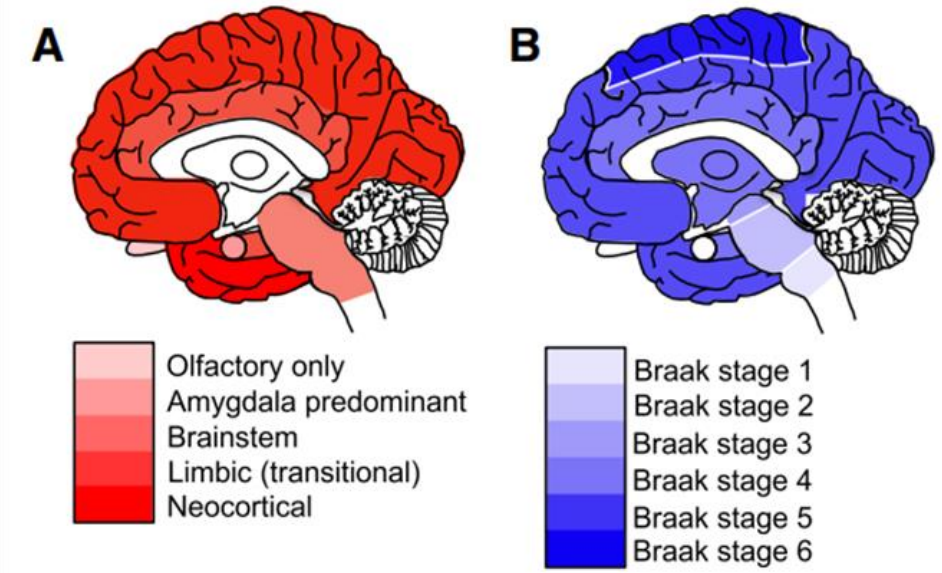
## α-Synuclein







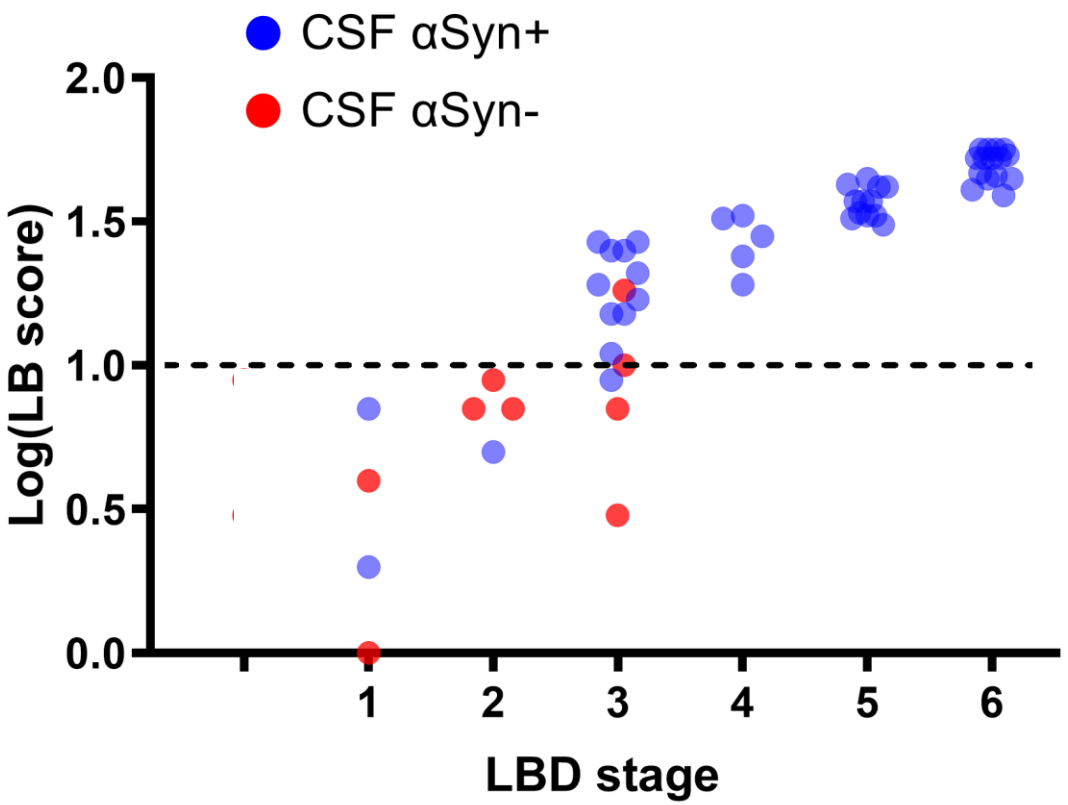




| Brainstem regions | Basal forebrain/limbic regions | Neocortical regions |
|---|---|---|
|  X LC SN |  AC nbM Trans GC |  Te Fr Pa |
| Brainstem predominant | Limbic/transitional | Diffuse neocortical |

### Staging of α-syn deposition

- Braak described six stages of LBP progression
- The Newcastle-McKeith criteria distinguishes between **brainstem**, **limbic (transitional)**, and **neocortical**.
- The most recent consensus (2017) included the "*olfactory only*", and "*amygdala predominant*" stages

# CSF α-Syn RT-QuIC performance in a large autopsy cohorts




## Studied cohort

- ✓ CSF-brain pairs from **269 patients** with **rapidly progressive dementia** (death less than 1 year after LP in most cases)
- ✓ **214** subjects lacking LB pathology (αSyn **LB-** group) and **55** with LB pathology (αSyn **LB+** group)

| | Pos/n | Sensitivity | Specificity |
|---|---|---|---|
| LB- α-Syn + | 46/55 | 83.6%* | |
| Neocortical | 15/15 | 100% | |
| Limbic | 17/17 | 100% | |
| Brainstem | 14/23 | 61% | |
| Stage III | 11/15 | 73% | |
| Stage II or I | 3/8 | 38% | |
| LB- α-Syn - | 3/214 | | 98.6% |

**High sensitivity (91.5%) of α-Syn RT-QuIC in individuals with LB Braak stage ≥ 3**

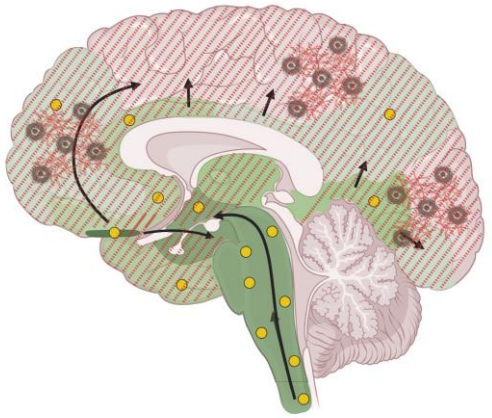
*Bentivenga et al.* ***Acta Neuropathol 2024***

# Other studies in autopsy cohorts (cognitive decline)

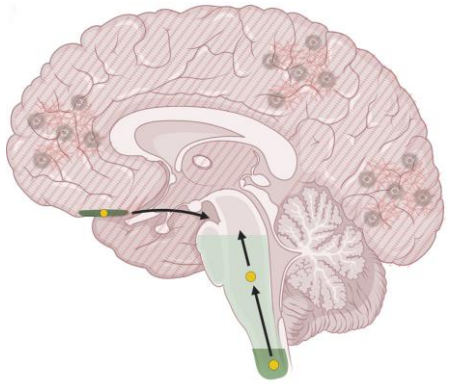
## Amprion protocol

**(Arnold - Ann Neurol 2022, Samudra - Alz Dem 2024, Tosun - Alz Dem 2024):**

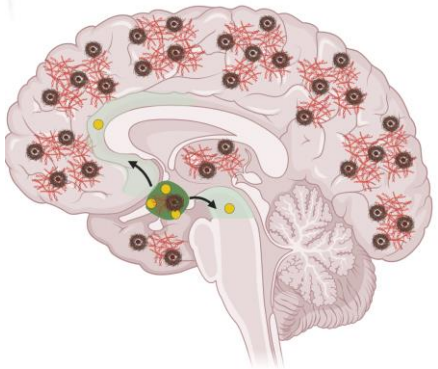
- ✓ High sensitivity of the αSyn SAA in **limbic** and **neocortical** (**n=77**) LBD stages (**92.2 %** )
- ✓ Significantly decreased sensitivity (**33.3%**) is observed in the **amygdala-predominant** **(n=45)** and **brainstem** **(n=9)** LB pathology stages
- ✓ The combined specificity was **97.5 %** **(n=119)**



**NEOCORTICAL**
Braak stages 5 and 6



**BRAINSTEM**
Braak stages 1 and 2



**AMYGDALA-PREDOMINANT VARIANT**

# CSF α-syn RT-QuIC SAA in PD and atypical Parkinsonisms

Acta Neuropathologica
https://doi.org/10.1007/s00401-020-02160-8

ORIGINAL PAPER

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

Marcello Rossi[1] · Niccolò Candelise[2] · Simone Baiardi[1,3] · Sabina Capellari[1,3] · Giulia Giannini[3] · Christina D. Orrù[4] · Elena Antelmi[3] · Angela Mammana[1] · Andrew G. Hughson[4] · Giovanna Calandra-Buonaura[1,3] · Anna Ladogana[5] · Giuseppe Plazzi[1,3] · Pietro Cortelli[1,3] · Byron Caughey[4] · Piero Parchi[1,2]

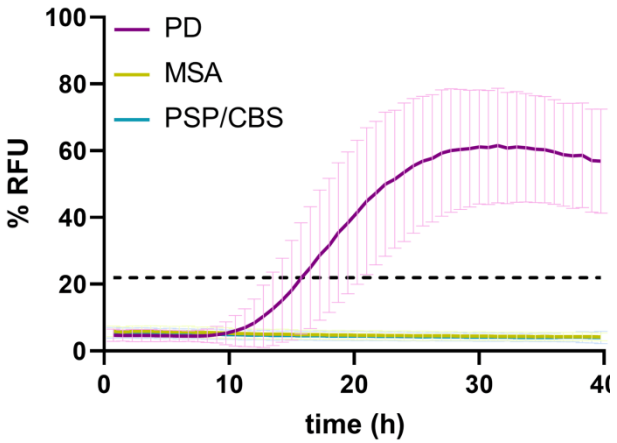
npj | Parkinson's Disease
www.nature.com/npjparkd

ARTICLE OPEN

## Neurofilament light chain and α-synuclein RT-QuIC as differential diagnostic biomarkers in parkinsonisms and related syndromes

Corinne Quadalti[1], Giovanna Calandra-Buonaura[1,2], Simone Baiardi[1,3], Andrea Mastrangelo[2], Marcello Rossi[1], Corrado Zenesini[1], Giulia Giannini[2], Niccolò Candelise[1], Luisa Sambati[2], Barbara Polischi[1], Giuseppe Plazzi[1,4], Sabina Capellari[1,2], Pietro Cortelli[1,2] and Piero Parchi[1,3]

| Diagnostic group | N° tot | N° of pos. | N° of neg. | Sensitivity | Specificity |
|---|---|---|---|---|---|
| PD (ISNB cohort) | 236 | 207 | 19 | 91.9% | |
| PD (Tuebingen cohort)* | 208 | 183 | 25 | 88.8%* | |
| PD (Biofinder cohort) | 185 | 165 | 20 | 89.2% | |
| MSA | 108 | 5 | 103 | | 95.4% |
| PSP/CBS | 88 | 2 | 86 | | 97.8% |
| Controls (Clinical) | 130 | 4 | | | 97.0% |




*Rossi et. al.* ***Acta Neuropath 2020****, Brockmann et al* ***ANC 2021****, Quadalti et al* ***NPJ Parkinson 2021*** *and unpublished data*

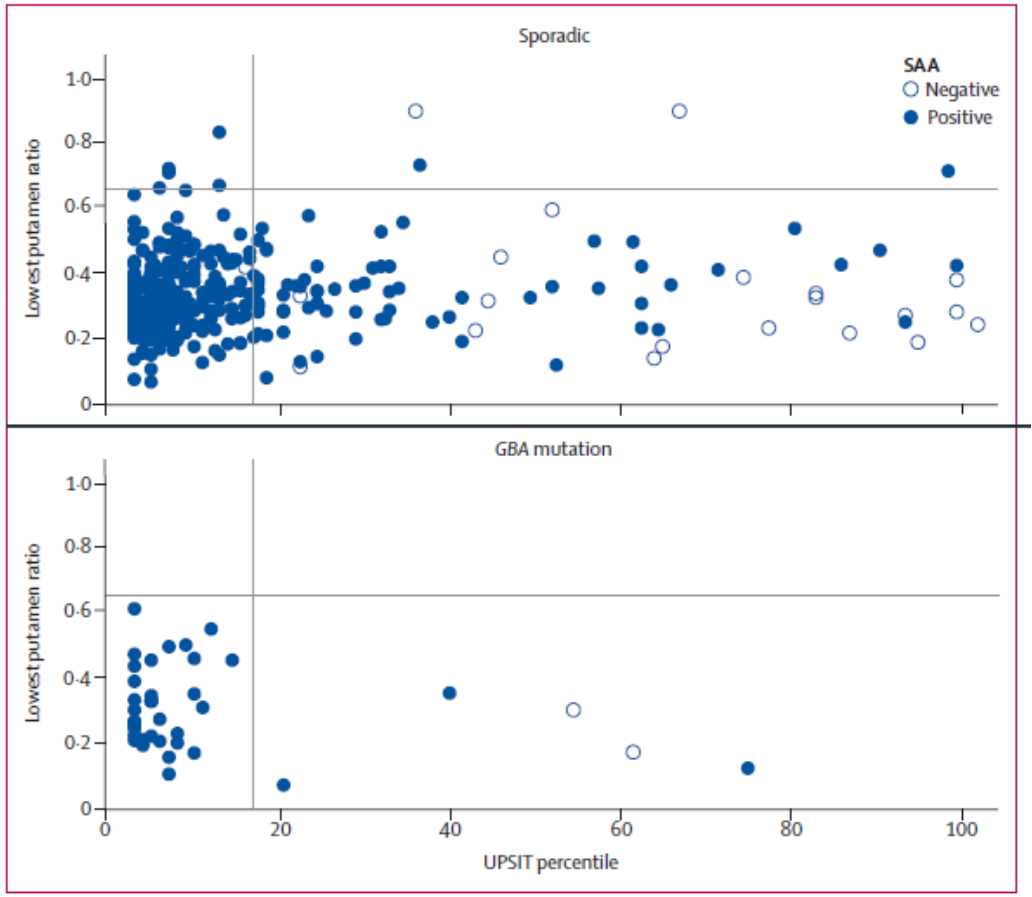
# CSF α-syn SAA in PD (Soto's Lab – Amprion assay)

*Siderowf et al LANCET NEUROL 2023 (PPMI cohort)*
*Concha et al MOV DISORD 2023 (DeNoPa cohort)*

| Diagnostic group | N° tot | N° of pos. | N° of neg. | Sens. | Spec. |
|---|---|---|---|---|---|
| PD sporadic (PPMI)* | 348 | 323 | 25 | 93.9% | |
| Olfactory deficit | 278 | 274 | 4 | 98.6% | |
| NO olfactory deficit | 69 | 57 | 12 | 78.3% | |
| PD (DeNoPa)* | 95 | 91 | 4 | 95.8% | |
| Controls (Clinical) | 163 | 7 | 156 | | 96.3% |

* **Final diagnosis** after up to 10 yrs of follow-up as gold standard

✓ **The assay identify and classify people with PD early in the course with high sensitivity and specificity and also provides information about disease heterogeneity**




**Association between dopamine transporter binding, olfaction, and α-synuclein SAA results among PD patients (PPMI cohort)**

# CSFα-Syn SAA performance in iRBD

## Detection of α-synuclein in CSF by RT-QuIC in patients with isolated rapid-eye-movement sleep behaviour disorder: a longitudinal observational study

Alex Iranzo, Graham Fairfoul, Anutra Chumbala Na Ayudhaya, Monica Serradell, Ellen Gelpi, Isabel Vilaseca, Raquel Sanchez-Valle, Carles Gaig, Joan Santamaria, Eduard Tolosa, Renata L Riha, Alison J E Green

RESEARCH ARTICLE

## Misfolded α-Synuclein Assessment in the Skin and CSF by RT-QuIC in Isolated REM Sleep Behavior Disorder

Alex Iranzo, MD, PhD,* Angela Mammana, PhD, Amaia Muñoz-Lopetegi, MD, Sofia Dellavalle, MSc, Gerard Mayà, MD, PhD, Marcello Rossi, PhD, Monica Serradell, BSc, Simone Baiardi, MD, PhD, Aurora Arqueros, MSc, Corinne Quadalti, PhD, Andres Perissinotti, MD, PhD, Edoardo Ruggeri, MSc, Joan Santamaria Cano, MD PhD, Carles Gaig, MD, PhD, and Piero Parchi, MD, PhD.*

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*Neurology*® 2023;100:e1944-e1954. doi:10.1212/WNL.0000000000207147

## Assessment of heterogeneity among participants in the Parkinson's Progression Markers Initiative cohort using α-synuclein seed amplification: a cross-sectional study

Andrew Siderowf*, Luis Concha-Marambio*, David-Erick Lafontant, Carly M Farris, Yihua Ma, Paula A Urenia, Hieu Nguyen, Roy N Alcalay, Lana M Chahine, Tatiana Foroud, Douglas Galasko, Karl Kieburtz, Kalpana Merchant, Brit Mollenhauer, Kathleen L Poston, John Seibyl, Tanya Simuni, Caroline M Tanner, Daniel Weintraub, Aleksandar Videnovic, Seung Ho Choi, Ryan Kurth, Chelsea Caspell-Garcia, Christopher S Coffey, Mark Frasier, Luis M A Oliveira, Samantha J Hutten, Todd Sherer, Kenneth Marek, Claudio Soto, on behalf of the Parkinson's Progression Markers Initiative†

https://doi.org/10.1038/s41531-024-00770-7

## CSF markers of neurodegeneration Alzheimer's and Lewy body pathology in isolated REM sleep behavior disorder

Check for updates

Amaia Muñoz-Lopetegi[1], Simone Baiardi[2,3], Mircea Balasa[4], Angela Mammana[2], Gerard Mayà[1], Marcello Rossi[2], Mónica Serradell[1], Corrado Zenesini[2], Alice Ticca[3], Joan Santamaria[1], Sofia Dellavalle[2], Carles Gaig[1], Alex Iranzo[1] ✉ & Piero Parchi[2,3] ✉

RESEARCH ARTICLE

## Accurate Detection of α-Synuclein Seeds in Cerebrospinal Fluid from Isolated Rapid Eye Movement Sleep Behavior Disorder and Patients with Parkinson's Disease in the DeNovo Parkinson (DeNoPa) Cohort

Luis Concha-Marambio, PhD,[1] Sandrina Weber, MD,[2,3] Carly M. Farris, MSc,[1] Mohammed Dakna, PhD,[2] Elisabeth Lang, BSc,[3] Tamara Wicke, MSc,[3] Yihua Ma, MSc,[1] Maritta Starke,[3] Jens Ebentheuer, MD,[3] Friederike Sixel-Döring, MD,[3,4] Maria-Lucia Muntean, MD,[3] Sebastian Schade, MD,[3] Claudia Trenkwalder, MD,[3,5] Claudio Soto, PhD,[1,6] and Brit Mollenhauer, MD[2,3*]

| Study | N° of patients | N° of pos. | Sensitivity % |
|---|---|---|---|
| Rossi et al, ANP 2020* | 18 | 18 | 100 |
| Iranzo et al Lancet* Neurol 2022 | 52 | 47 | 90.0* |
| Iranzo et al Neurology 2023 Munoz-Lopetegi et al npj Parkinson 2024 | 142 | | 75.6* |
| Siderowf et al Lancet Neurol 2023 | 33 | 28 | 84.8 |
| Concha-Marambio et al Mov Disord 2023** | 29 | 27 | 93.1 |
| TOTAL | 220 | 186 | 84.6 |

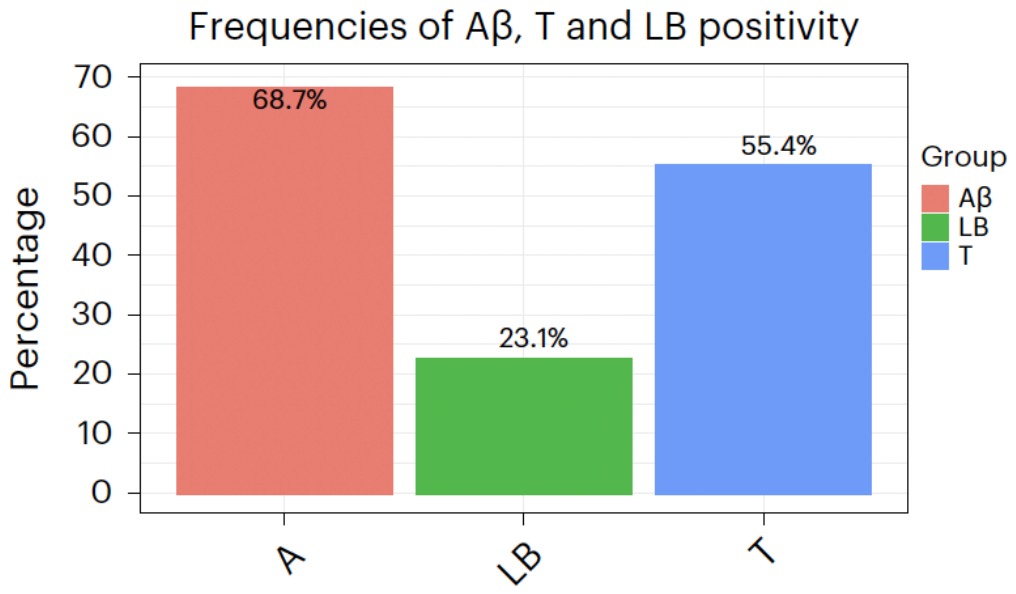
*Higher % of SAA positive patients explained by a higher frequency of MCI and other signs of prodromal PD

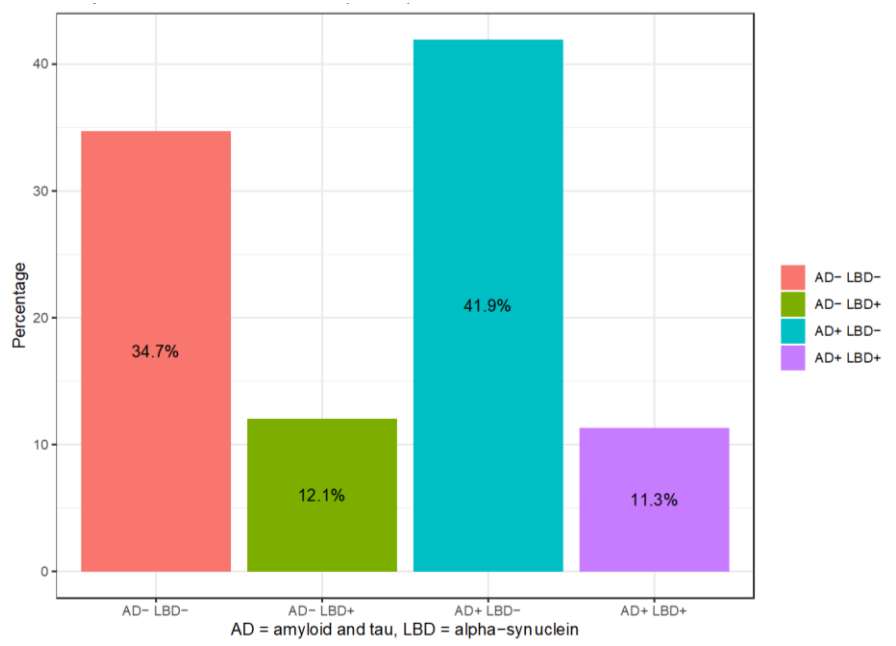
* 96.7% sensitivity in 45 patients who convereted to PD or DLB

** Multiple samples analysed at baseline and follow-up

# Prevalence of LB and AD pathology in **cognitively impaired** patients

α-Syn SAA analysed using CSF in 883 cognitively impaired BioFINDER partcipants




✓**48%** of **LB+** patients had AD pathology

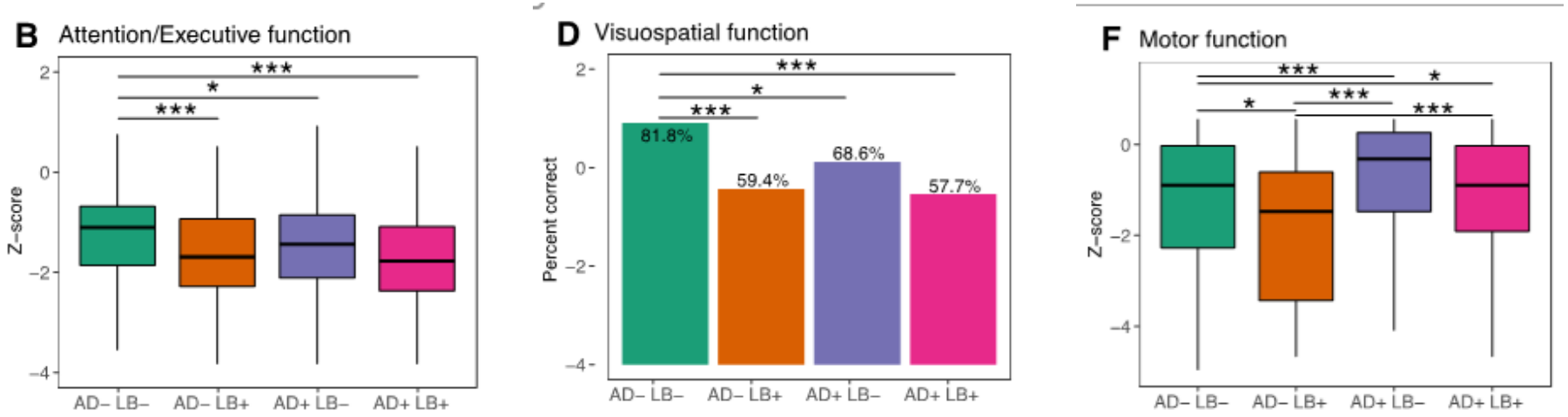
✓Among patients <u>with AD pathology</u>, **17% of MCI** and **24%** of patients with **dementia were LB+**

**Only 1 of 5 (21%)** of **LB+** patients fulfilled clinical criteria of PD or DLB at baseline

***Quadalti et al, Nature Medicine, 2023***

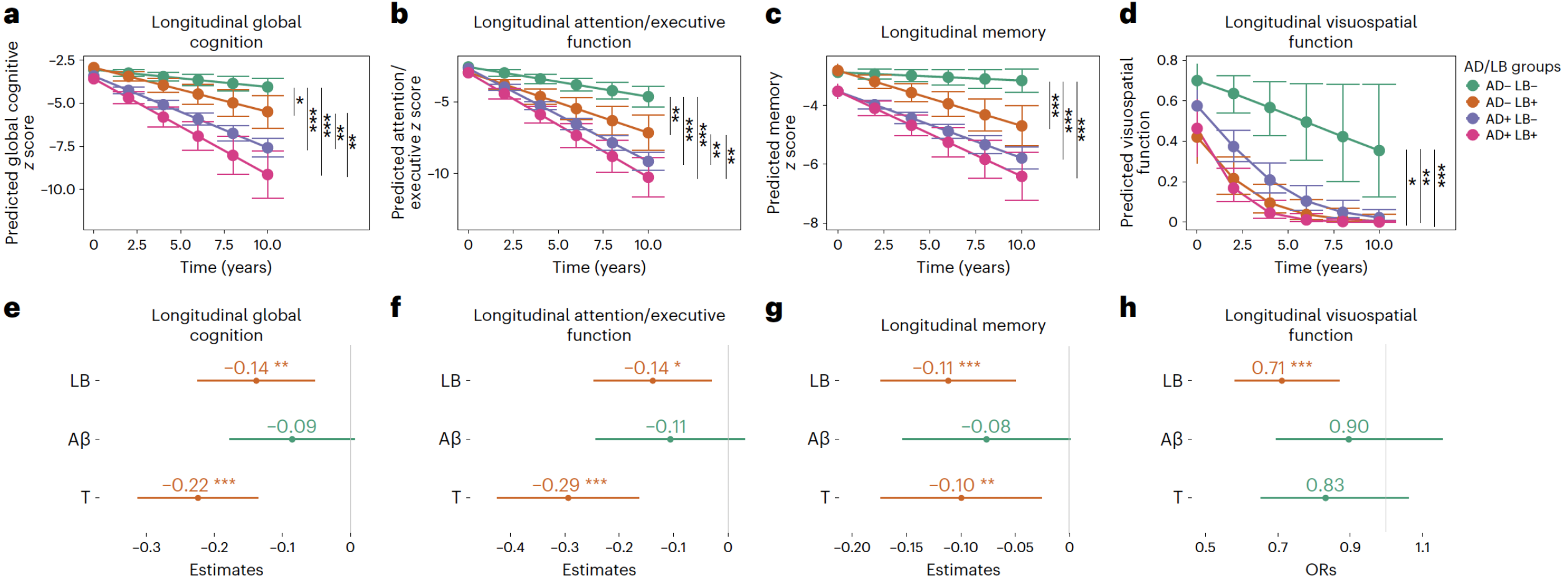
# Cross-sectional effects of LB pathology in cognitively impaired patients

- LB pathology had AD-independent effects on:
  - Attention/executive function
  - Visuospatial function
  - Motor dysfunction (e.g. bradykinisia)




*Quadalti et al, Nature Medicine, 2023*

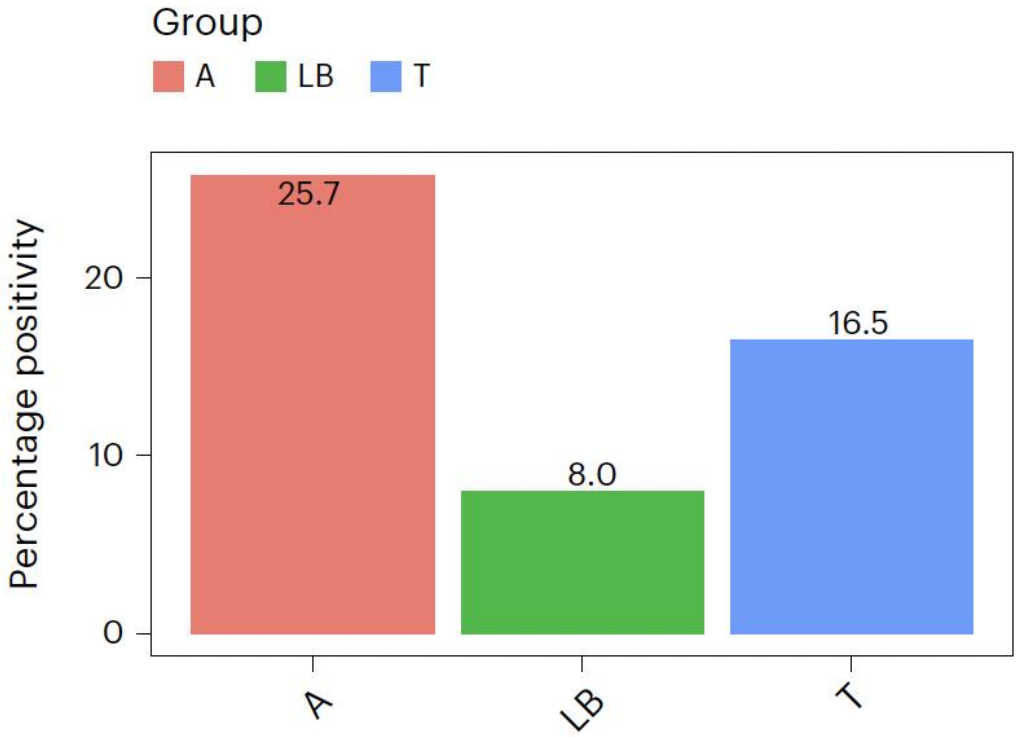
# Longitudinal effects of LB pathology in clinically impaired individuals

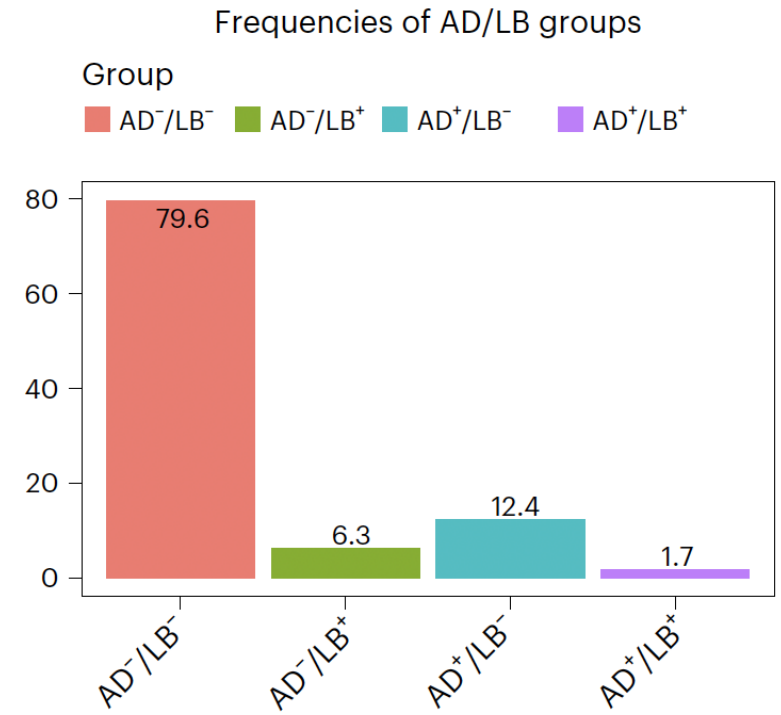



*Quadalti et al, Nature Medicine, 2023*

# Prevalence of LB and AD pathology in cognitively and neurologically **unimpaired** individuals

α-Syn SAA analysed using CSF in 1182 clinically unimpaired BioFINDER partcipants

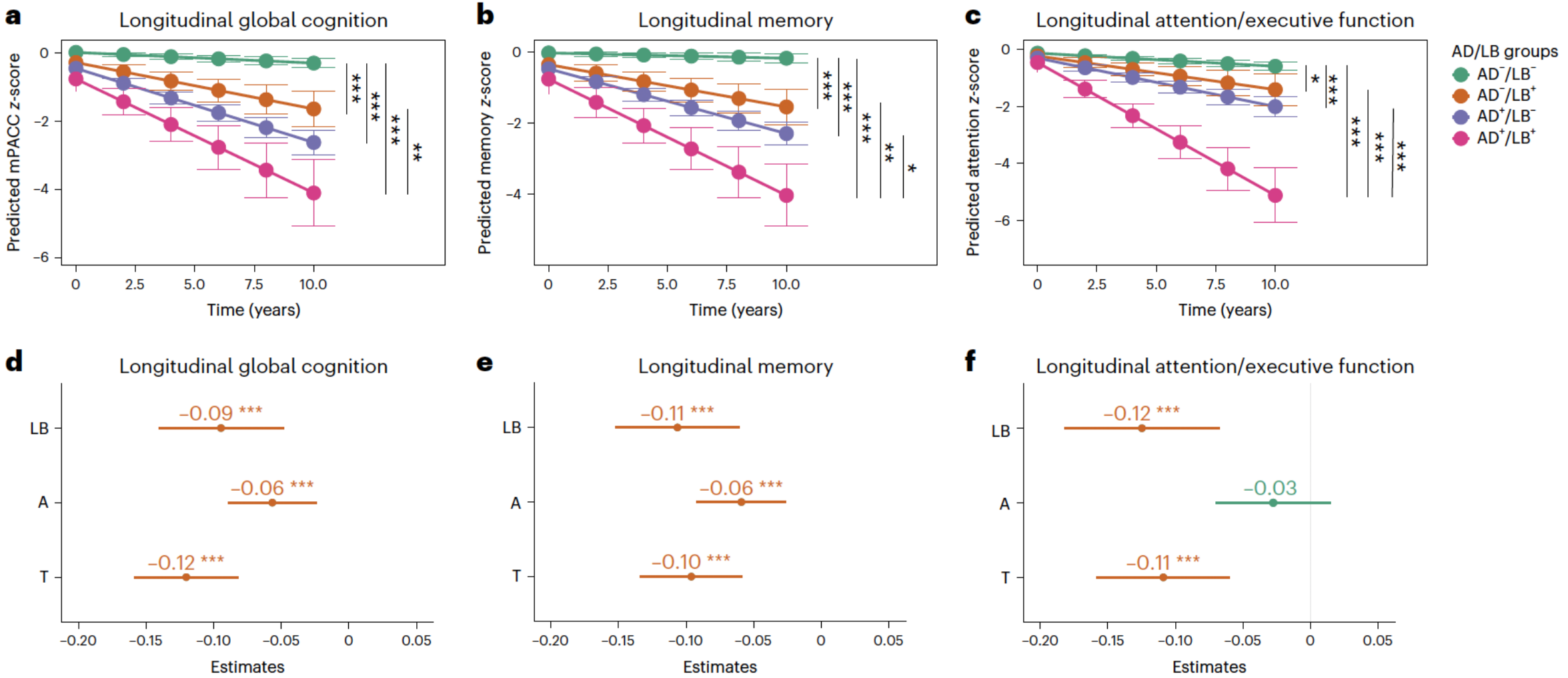



LB positivity was significantly more likely to occur in the presence of Aβ positivity (OR 1.72) but not tau positivity

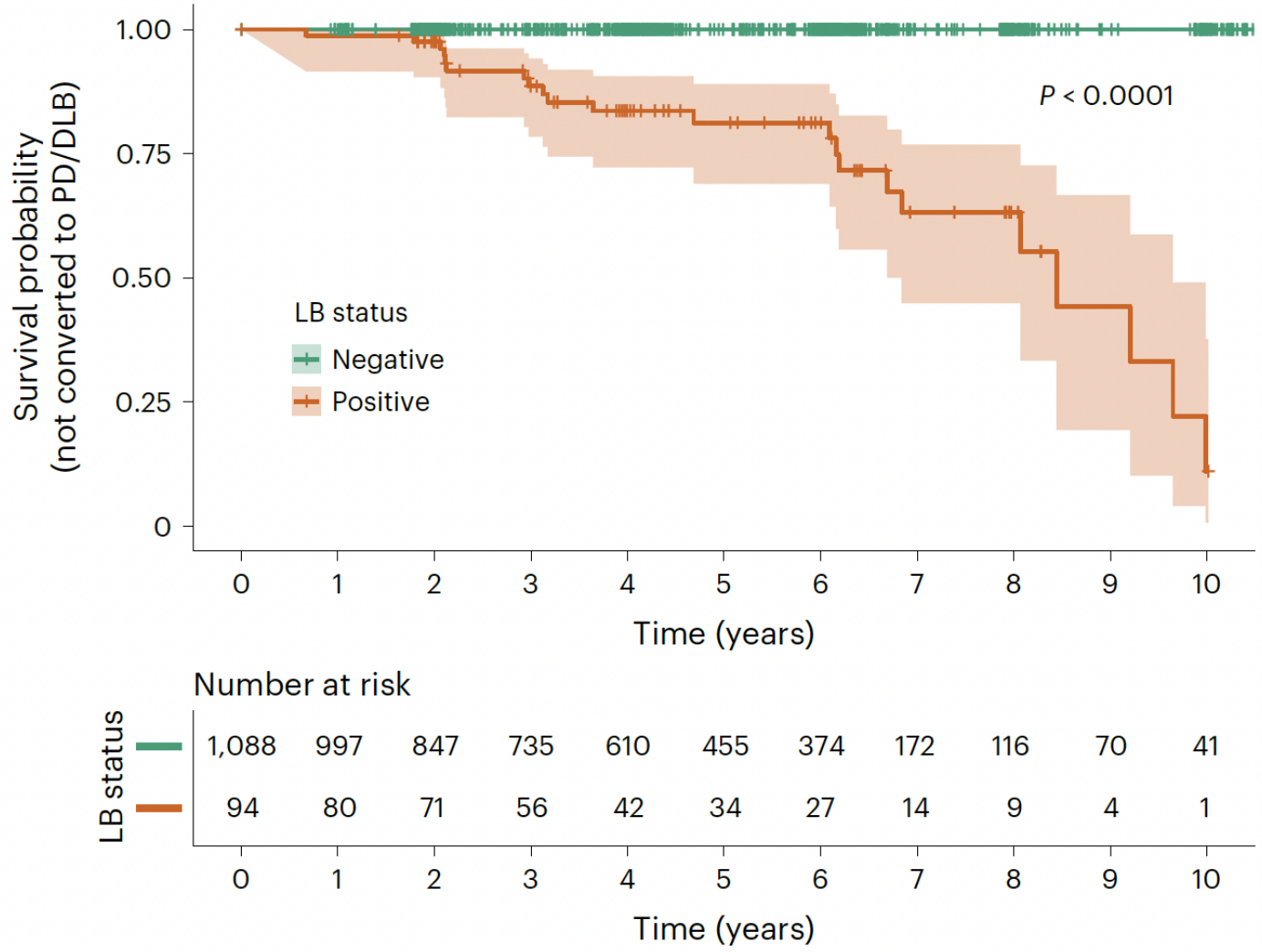
***Palmqvist et al, Nature Medicine, 2023***

# Longitudinal effects of LB pathology in clinically unimpaired individuals




*Palmqvist et al, Nature Medicine, 2023*

# Development of clinical LBD (PD or DLB)




- ✓ Lines show point **estimates** of survival curves and shaded areas 95% CI
- ✓ **No** participants who were **LB–** at baseline **progressed** to PD/DLB
- ✓ Progression to PD or DLB was observed in 24 of 94 **LB+ after a mean follow-up of 4.46 years**
- ✓ Other 14 patients developed LB pathology-related signs without fulfilling the clinical criteria for DLB or PD

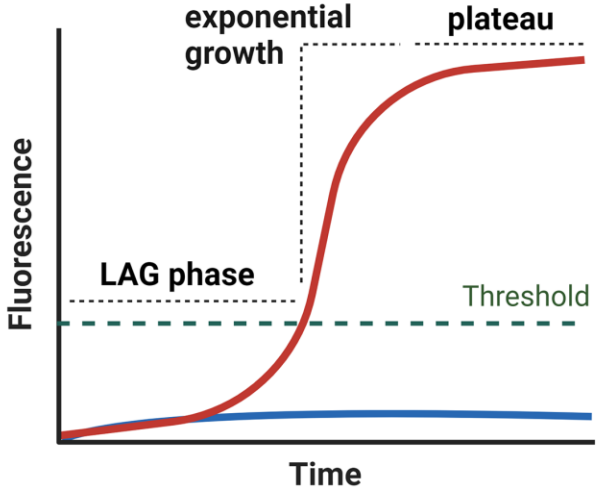
***Palmqvist et al, Nature Medicine, 2023***

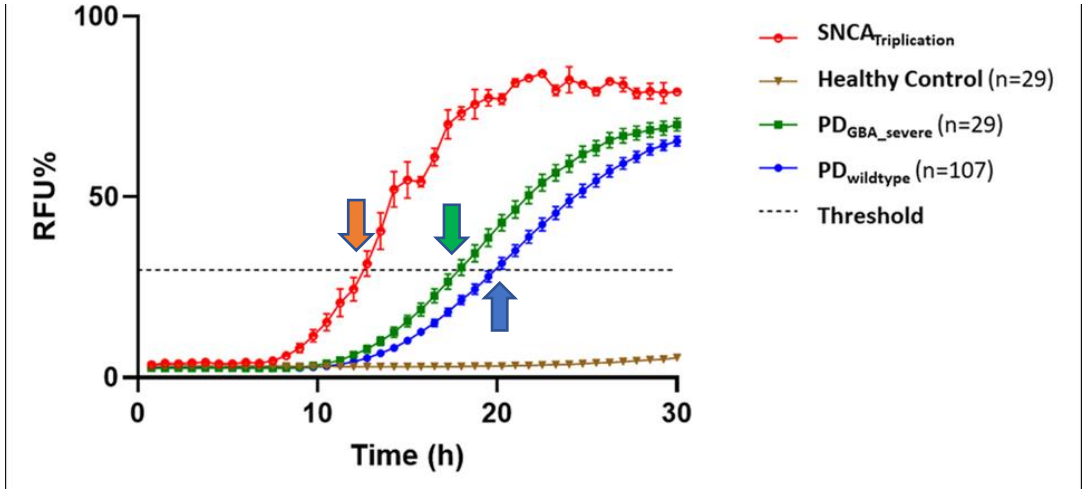
# Summary of α-syn CSF SAA results in patients with LBD

- α-syn SAAs **accurately differentiate** people with PD from healthy controls and patients with atypical Parkinsonism with high sensitivity and specificity
- The assay accurately identifies patients with LB pathology in the **prodromal** (and even **asymptomatic**) **stages** establishing biomarker-defined at-risk cohorts
- α-syn SAA identify patients with **mixed AD/LB pathology** and provides a **better precision medicine approach to predict clinical trajectories** independent of AD biomarkers and clinical characterization

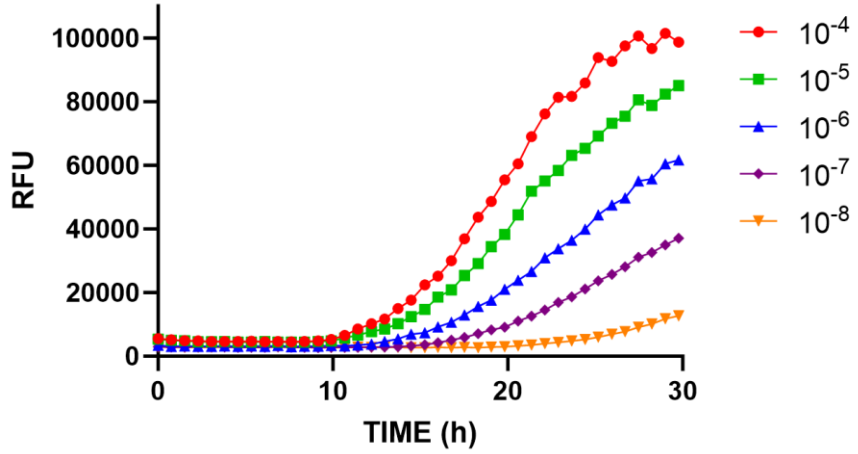
# Can α-Syn RT-QuIC provide a quantitative readout of seeding activity?

## Analysis of SAA kinetic parameters: The LAG time




*Wurster et al.* ***NPJ Parkinson 2024***




*Bentivenga et al.* ***Acta Neuropathol 2024***

**α-Syn seeding activity is relatively low in** CSF collected from living patients making **lag phases more erratic and quantitation more challenging**

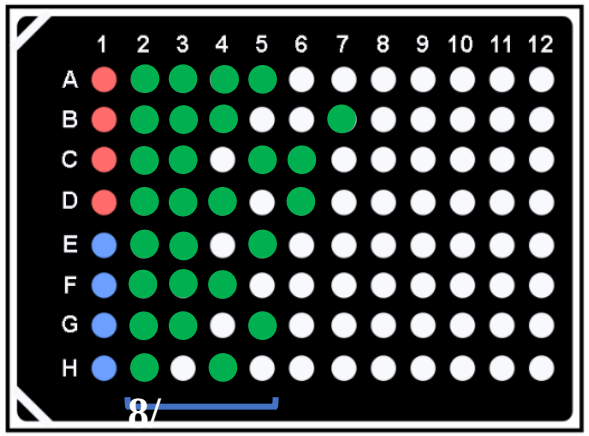
Lag times can also be influenced by **differences in matrix composition** or **other factors in addition to the initial seed concentration** that could vary between individual patients' biospecimens

*Srivastava A, et al.* ***PLoS Pathog 2024***

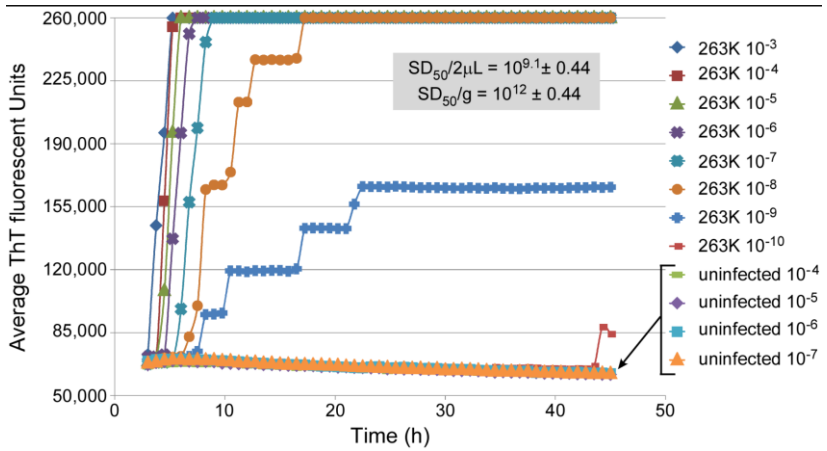
# End-point Dilution (ED) RT-QuIC

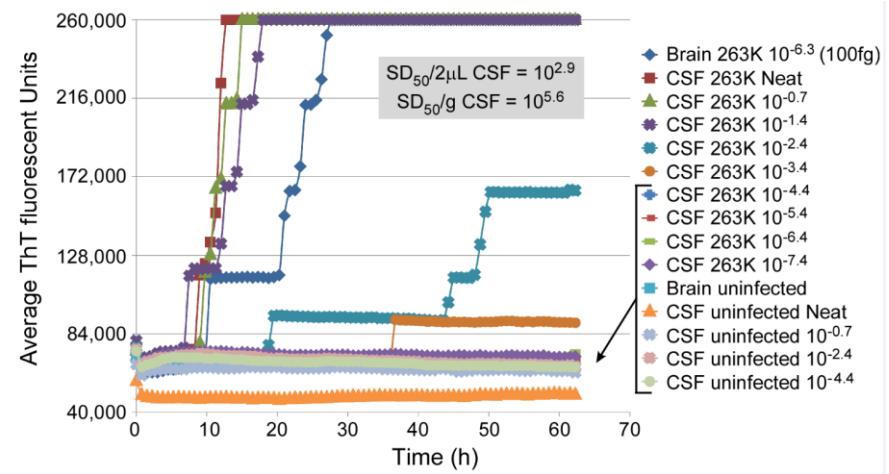
**ED RT-QuIC** is an alternative (or complementary) approach to the analysis of kinetics parameters like the LAG time that **depends primarily on how seeds dilute out**

96 well plate




- Negative control
- Positive control
- Positive sample replicates




✓ The **duration** of the **lag phase increases** and the **number of positive replicates decreases** with higher dilutions of 263K brain and CSF

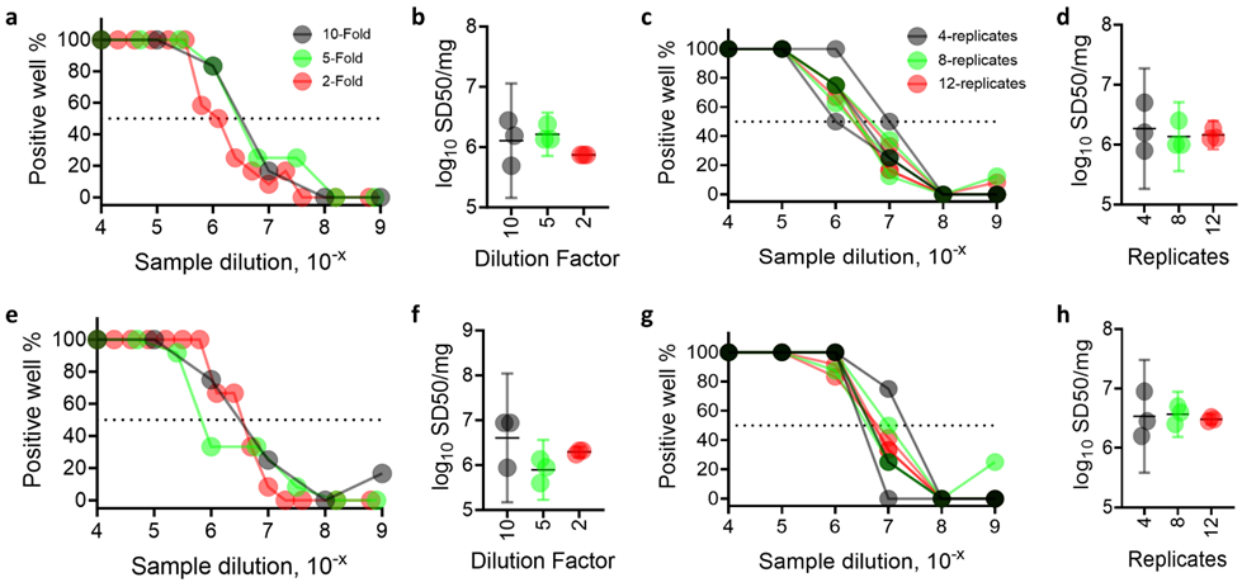
✓ Analogous to end-point dilution animal bioassays, this approach involves testing of serial dilutions of samples and statistically **estimating the seeding dose** (SD) giving **positive responses in 50%** of replicate reactions (SD50)

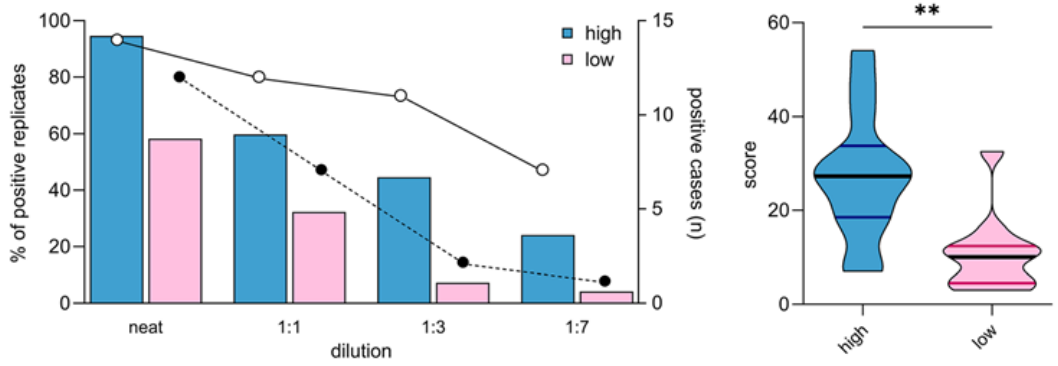
Wilham JM, et al. **PLoS Pathog 2010**

# ED α-Syn RT-QuIC:
## Influence of Dilution Factor and Replicate Number

**10F vs 5F vs 2F**

**4R vs 8R vs 12R**



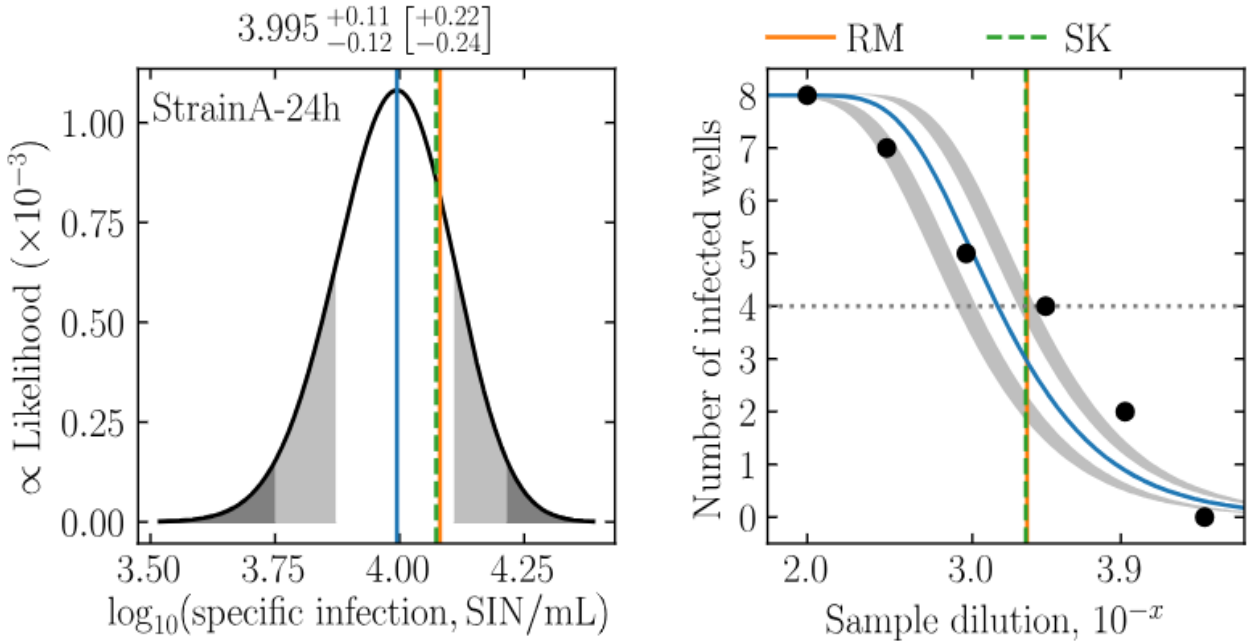

- ✓ **Consistencies of** $\log_{10}$ SD50/mg **estimates improve** by decreasing **dilution factor**
- ✓ **Consistencies of** $\log_{10}$ SD50/mg **estimates improve** by increasing **replicate numbers**

- ➢ The assay accuracy (slightly) improves by **increasing replicates** from 4 to 8 or 12
- ➢ Most significantly, the distinction among patients with **different α-syn seeding** activity improved by **increasing replicate numbers**

*Srivastava A, et al.* ***PLoS Pathog 2024***

*Mammana et al.* ***Clin Chem Lab Med, 2024***

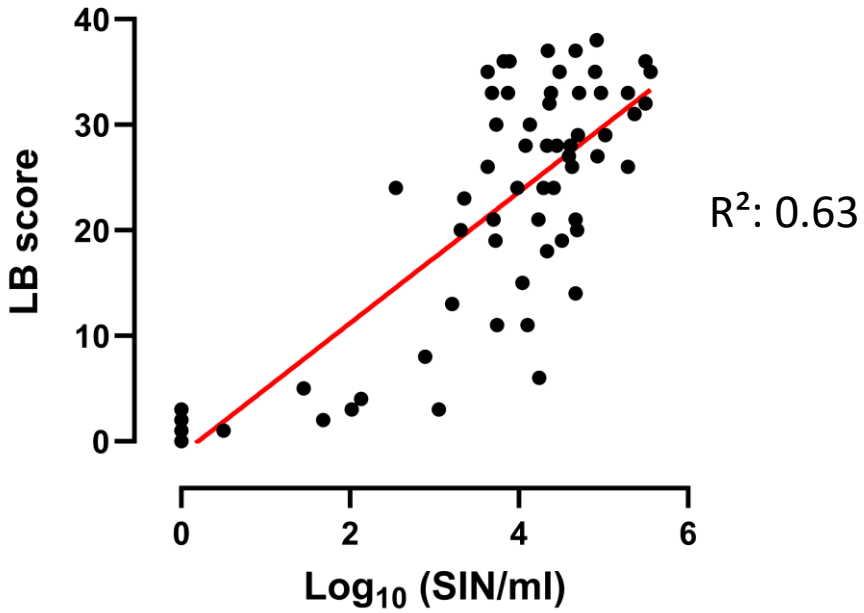
# ED α-Syn RT-QuIC SAA:
# Influence of data processing (used algorithm)



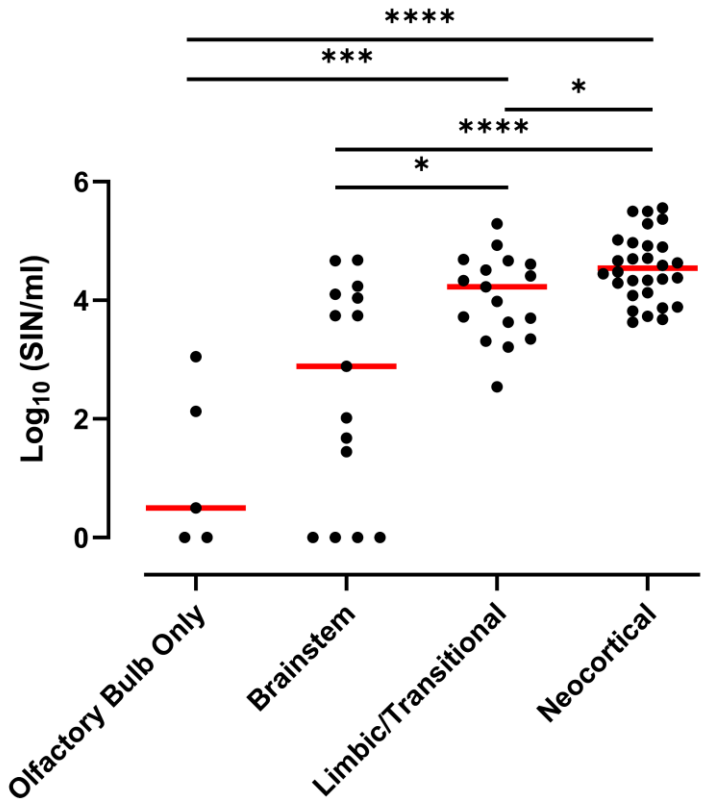

- **Consistencies** of $\log_{10}$ SD50/mg **estimates improve** by analysing the data using Poisson and (especially) midSIN algorithms compared to the (previously used) Spearman-Kaerber (SK) method

*Srivastava A, et al.* ***PLoS Pathog 2024***

# Results of CSF α-Syn ED RT-QuIC SAA in patients with LBD




$R^2$: 0.63




## Sample cohort

✓ 70 ***postmortem*** CSF collected at autopsy from LBD patients (Arizona Study of Aging and Neurodegenerative Disorders)

## Results

✓ We found a significant positive association between $Log_{10}$SIN values, the LB score and the LBD stage

***Unpublished results***

# Results of CSF α-Syn ED RT-QuIC SAA in patients with PD

| Dependent variable | N patients | Association with SIN50 | |
|---|---|---|---|
| | | P value | Rho |
| Age of onset, years | 164 | 0.0003 | 0.28 |
| Age at visit, years | 164 | <0.0001 | 0.28 |
| Disease duration, years | 164 | 0.15 | 0.11 |
| Hoehn&Yahr scale | 163 | 0.006 | 0.21 |
| MDS-UPDRS III scale | 151 | 0.0006 | 0.27 |
| MoCA scale | 151 | 0.01 | -0.20 |
| PD-NMS scale | 72 | 0.08 | 0.21 |
| BDI-2 scale | 114 | 0.29 | 0.10 |
| Sniff Sticks, % | 56 | 0.05 | -0.27 |
| UMSARS – Orthostatic symptoms | 63 | 0.004 | 0.35 |
| UMSARS – Urge | 62 | 0.38 | 0.11 |
| UMSARS – Erectile dysfunction | 62 | 0.007 | 0.34 |
| UMSARS – Constipation | 63 | 0.010 | 0.32 |
| CSF t-tau | 150 | 0.004 | 0.24 |
| CSF p-tau181 | 145 | 0.02 | 0.20 |
| CSF NfL | 143 | 0.0001 | 0.31 |
| RT-QuIC Nrep (/8) | 164 | <0.0001 | 0.79 |

## Sample cohort

- ✓ CSF collected *in vivo by LP* from **164 PD** participants (University Hospital of Tuebingen, Germany)

## Results

- ✓ $Log_{10}$ SIN50/ml values in the CSF of PD patients with a positive α-Syn SAA ranged from **0.3 to 2.0** (from **0.3 to 5.3** in *post-mortem* CSF)
- ✓ Most clinical variables of PD severity showed significant association with $log_{10}$ SIN50/ml values

- Cognitive function
- Motor function
- Olfactory function
- Autonomic function
- CSF biomarkers

***Unpublished results***

# Summary of results of α-syn ED RT-QuIC

- ED α-syn RT-QuIC SAA provides **"quantitative" data** that correlate with the LB pathology "load"/stage and clinical scores of motor, cognitive, and autonomic dysfunction

- The **degree relative levels of α-syn seeding activity** in CSF could serve as an **index of disease severity and progression**

- These (preliminary) data support the use of ED RT-QuIC SAA as surrogate markers of response to **experimental drugs in clinical trials or as a prognostic marker in clinical practice**

# ACKNOWLEDGEMENTS

## NDG Program at ISNB

**Clinical assessment:**

Sabina Capellari MD

Simone Baiardi MD PhD

Andrea Mastrangelo MD

Giuseppe Bentivenga MD

**Histopathology:**

Simone Baiardi MD PhD

**Molecular genetics:**

Sabina Capellari MD

**RT-QuIC and Biomarkers:**

Marcello Rossi PhD

Angela Mammana PhD

Alice Ticca MSc

Franco Magliocchetti MSc

Serena Caldera MSc

Claudia Vargiu MSc

## Collaborators

### Clinical Neurologists at ISNB

Pietro Cortelli MD PhD

Giovanna Calandra-Buonaura MD PhD

Luisa Sambati MD PhD

Giulia Giannini MD PhD

### External (RT-QuIC support)

**Rocky Mountain Laboratories, NIAID, NIH, Hamilton, MT, USA**

Byron Caughey PhD

### External (sample cohorts)

**Department of Clinical Sciences, Malmö, Lund University, Sweden**

Oskar Hansson MD, PhD

**Sleep Centre, Hospital Clínic Barcelona, Universitat de Barcelona**

Alex Iranzo MD

**German Center for Neurodegenerative Diseases, University of Tuebingen, Germany, DE**

Kathrin Brockmann MD