55° Congresso AINPeNC Associazione Italiana Neuropatologia e Neurobiologia Clinica
45° Congresso AIRIC Associazione Italiana Ricerca Invecchiamento Cerebrale
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Efficient RT-QuIC seeding activity for α-Synuclein in olfactory mucosa samples of patients with Parkinson's disease and multiple system atrophy

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PARKINSON'S DISEASE AND OTHER PARKINSONISMS

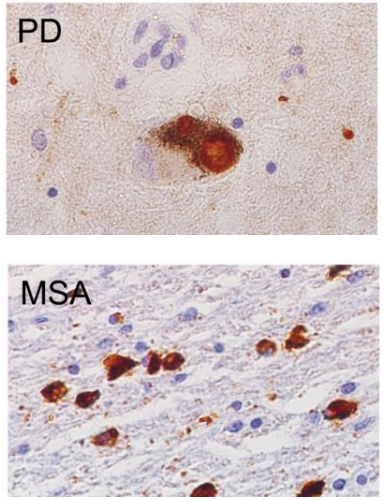
Parkinson's disease is the second most common neurodegenerative disorder after Alzheimer's disease and is characterized by bradykinesia with rigidity, tremor and postural instability

α-synucleinopathies

- Parkinson's disease (PD)
- Multiple System Atrophy (MSA)

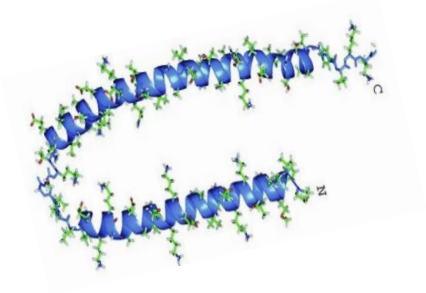
tauopathies

- Corticobasal Degeneration (CBD)
- Progressive Supranuclear Palsy (PSP)

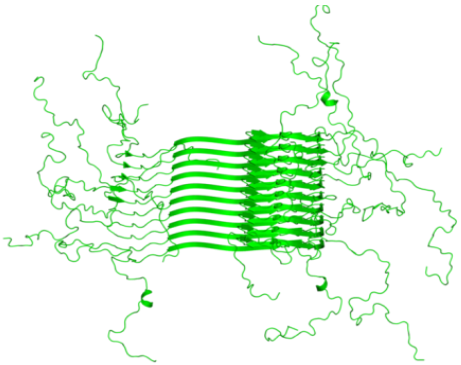


α-SYNUCLEIN PROTEIN

Normal structure



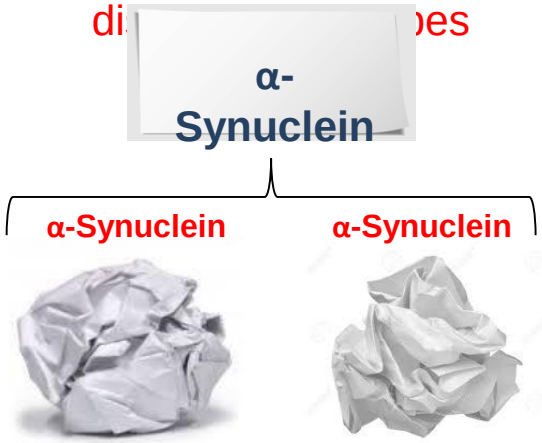
Misfolded structure



Abnormal α-synuclein proteins are rich in β-sheet structures and are prone to aggregate and form amyloid fibrils

The aggregates are partially resistant to Proteinase K (PK)

Misfolded proteins can acquire different abnormal conformations («strains») which might be associated with distinct diseases or di pes



Abnormal conformation #**1**

Abnormal conformation #**2**

ARTICLE

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Structural and functional characterization of two alpha-synuclein strains

Luc Bousset[1], Laura Pieri[1], Gemma Ruiz-Arlandis[1], Julia Gath[2], Poul Henning Jensen[3], Birgit Habenstein[4], Karine Madiona[1], Vincent Olieric[5], Anja Böckmann[4], Beat H. Meier[2] & Ronald Melki[1]

Distinct α-Synuclein strains and implications for heterogeneity among α-Synucleinopathies

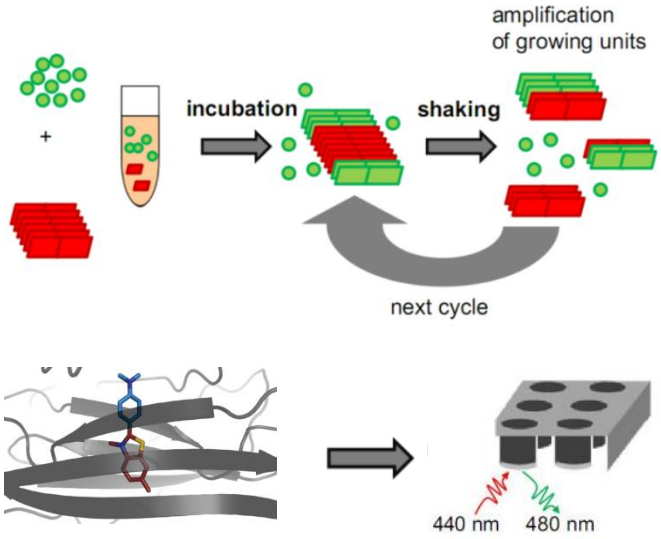
Chao Peng, Ronald J. Gathagan, Virginia M.-Y. Lee *

The Department of Pathology and Laboratory Medicine, Institute on Aging and Center for Neurodegenerative Disease Research, The Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA 19104, USA

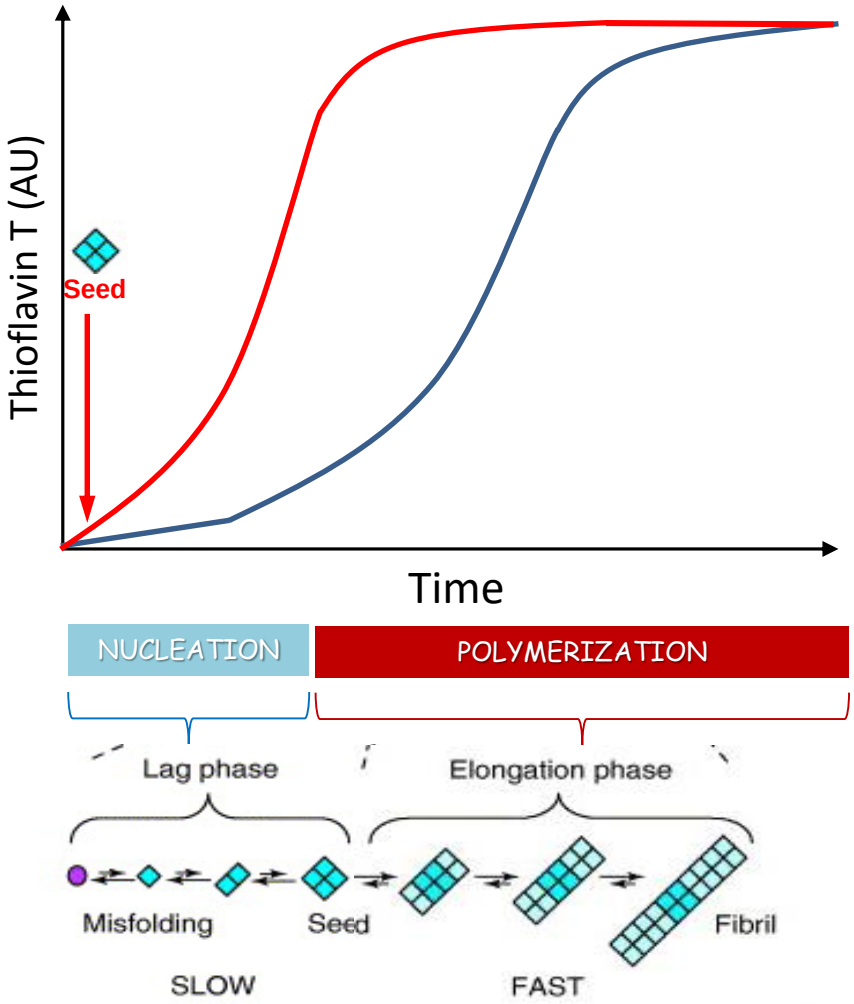
REAL-TIME QUAKING-INDUCED CONVERSION ASSAY (RT-QuIC)

Substrates: Human recombinant α-Synuclein protein

Seeds: Disease-associated misfolded proteins usually organize in β-sheet conformations that form aggregates

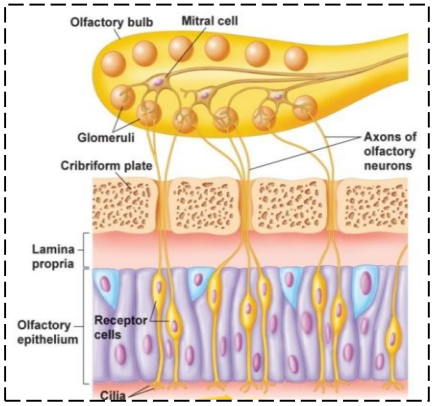


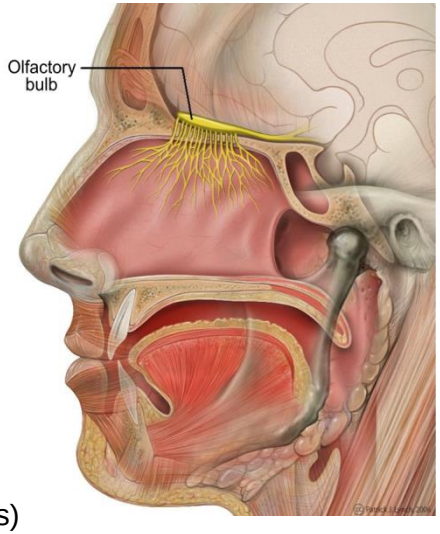
Thioflavin-T (ThT) intercalates with amyloid aggregates



The addition of pre-formed aggregates (seeds) to the reaction induces an acceleration of the kinetics of aggregation of the substrate

MISFOLDED PROTEINS IN OLFACTORY EPITHELIUM





5 milion of olfactory receptor neurons/nostril
(interspersed within supporting cells)

Olfactory receptor neurons collected post-mortem from patients with different neurodegenerative diseases contain misfolded proteins


The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

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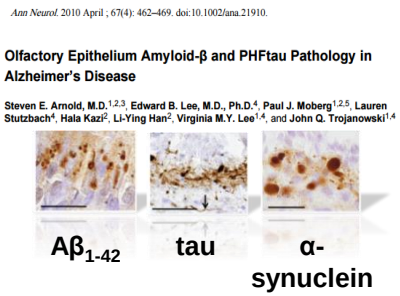
Ann Neurol. 2010 April ; 67(4): 462–469. doi:10.1002/ana.21910.

Olfactory Epithelium Amyloid-β and PHFtau Pathology in Alzheimer's Disease

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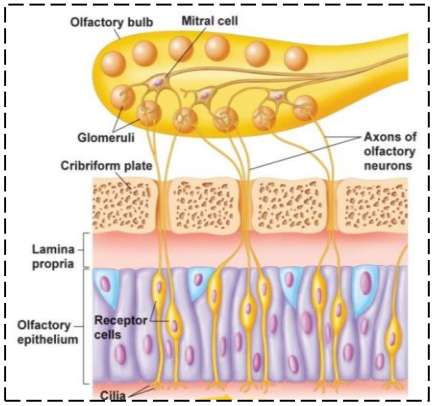
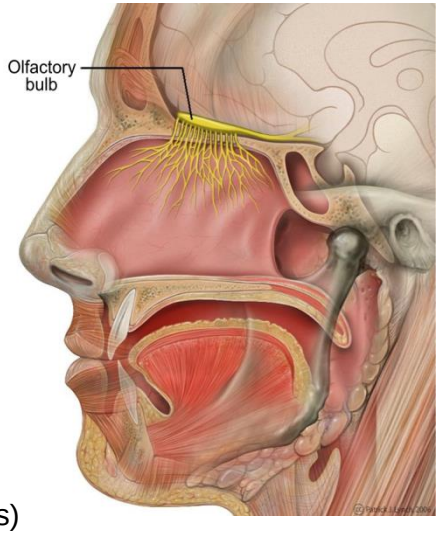
Aβ$_{1-42}$ tau α-synuclein

MISFOLDED PROTEINS IN OLFACTORY EPITHELIUM





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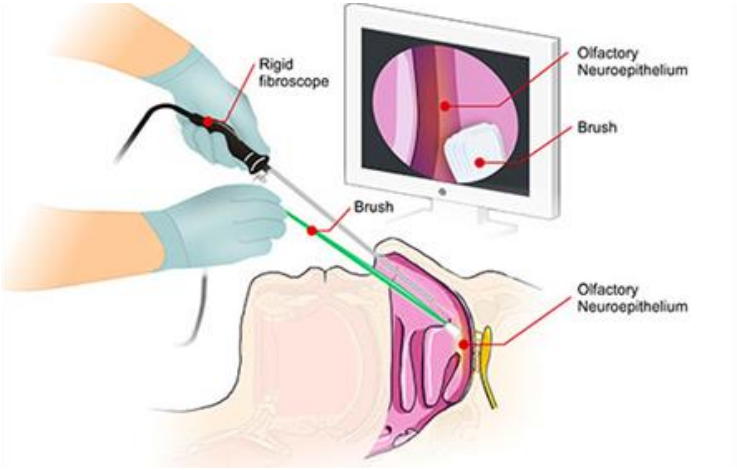

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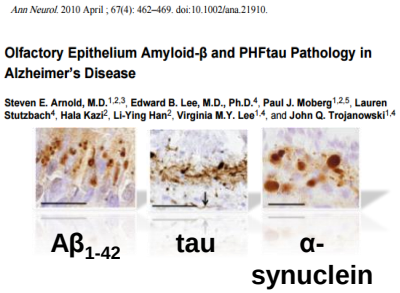
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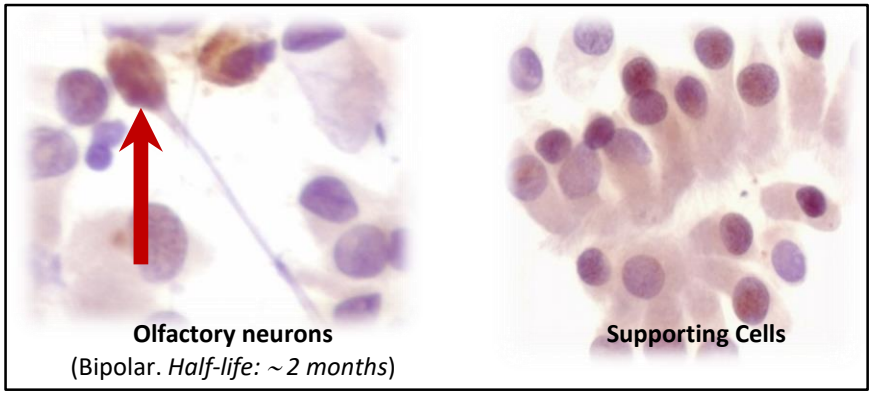


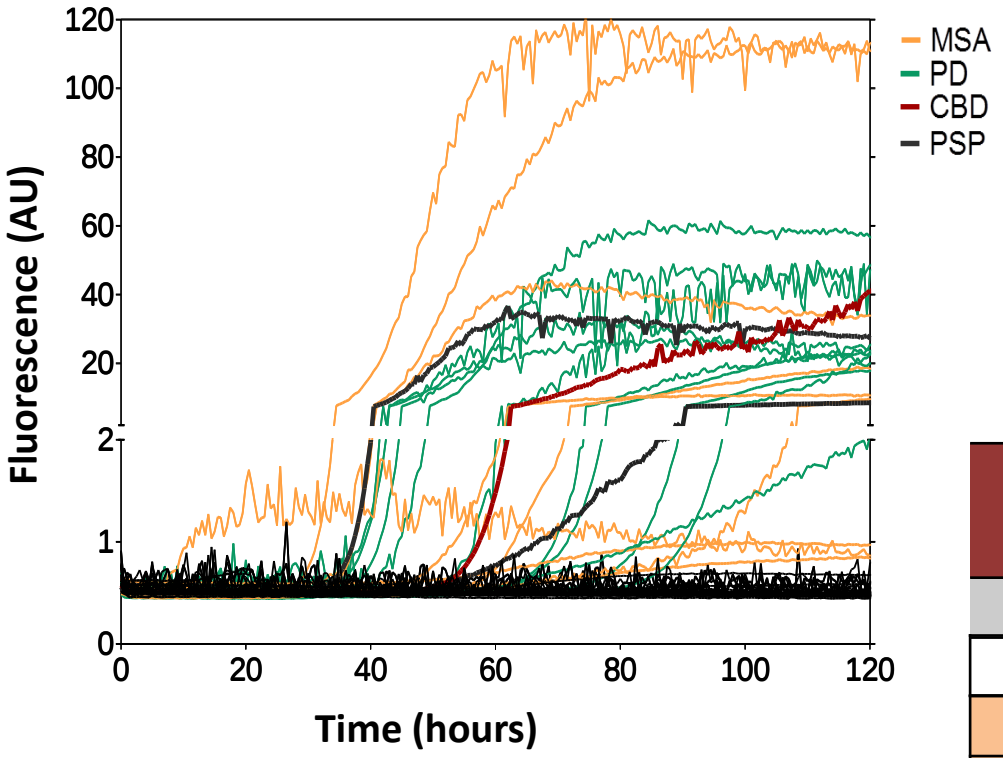
Olfactory epithelium collected from patients with different neurodegenerative diseases
contains **trace-amount** of misfolded proteins not detectable with current analytic techniques

DEMOGRAPHIC AND CLINICAL DATA

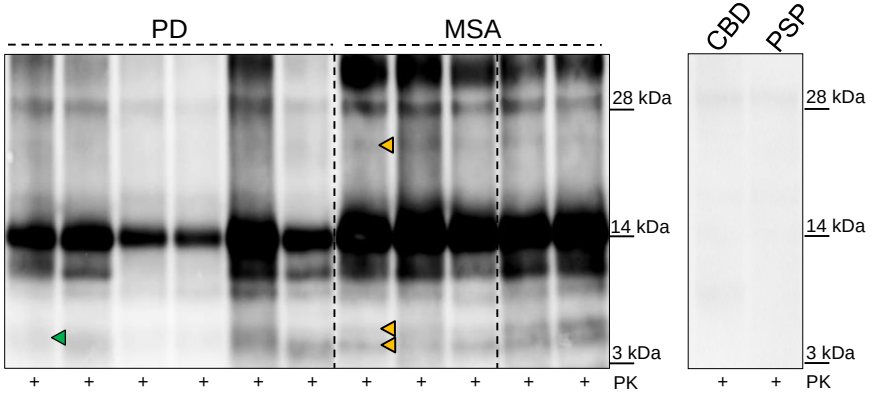
	Parkinson's Disease (PD)	Multiple System Atrophy (MSA)	Corticobasal Degeneration (CBD)	Progressive Supranuclear Palsy (PSP)
Diagnostic Criteria	Postuma R.B., Mov Dis, 2015	Gilman S., Neurology, 2008	Armstrong M.J., Neurology, 2006	Litvan I.,Neurology, 1996
Number of patients	18	11	6	12
Age at time of evaluation	64.2 ± 7.8	62.3 ± 9.2	63.3 ± 10.6	68.3 ± 7.0
Age at disease onset	52.4 ± 6.1	56.5 ± 9.5	60.2 ± 10.9	64.3 ± 8.2
Disease duration	10.1 ± 5.1	5.8 ± 3.4	3.2 ± 1.6	4.0 ± 3.6
Gender (F/M)	8/10	5/6	4/2	5/7
Frequency of symptoms (%)				
• Rigid akinetic parkinsonism	100	90.1	83.3	91.7
• Tremor	88.9	81.8	50	8.3
• Ataxia	0	90.1	50	91.7
• Apraxia	0	0	100	33.3
• Delusions	16.7	9.1	0	8.3
• Dementia	11.1	0	16.7	58.3
• Hyposmia	16.7	9.1	16.7	0
• Psychiatric disorders	33.3	45.5	33.3	50

RT-QuIC ANALYSIS OF OLFACTORY MUCOSA

α-Synuclein aggregation induced by the addition of OM



PK digestion of final RT-QuIC products



Clinical diagnosis	OM samples analyzed	α-Synuclein seeding activity (RT-QuIC)
MSA	11	9
PD	18	10
CBD	6	1
PSP	12	2

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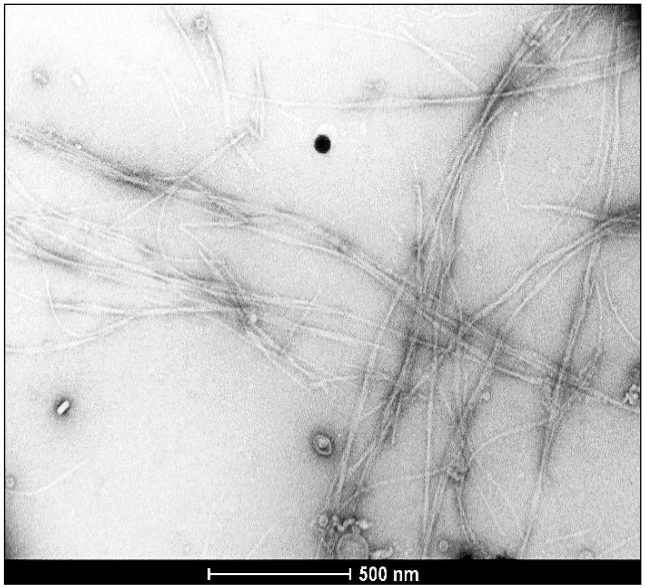
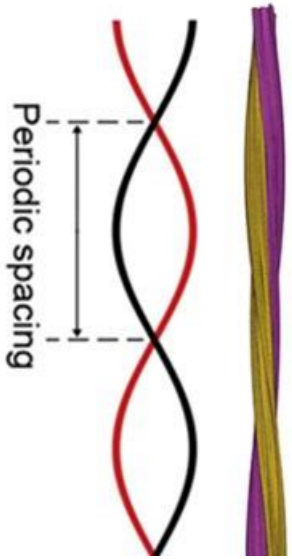
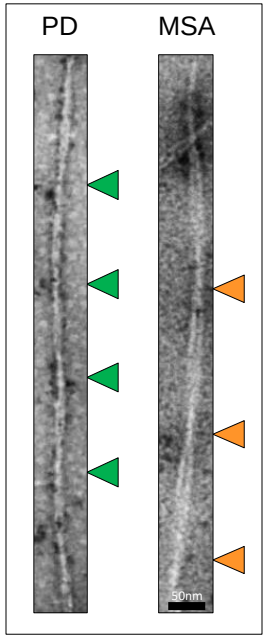
STRUCTURAL CHARACTERIZATION OF α-SYNUCLEIN AGGREGATES

Electron Microscopy Analysis of α-synuclein amyloid fibrils





Li Y et al. Cell Res. 2018



131 nm ± 1.1 **142 nm ± 1.3**

	PD	MSA
Patients included in the analysis	5	5
Number of fibrils analyzed per patient	50	50
Total fibrils	250	250
N° over-twists	613	654

Statistical analysis
Mann-Whitney
($p < 0.0001$)

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CONCLUSIONS

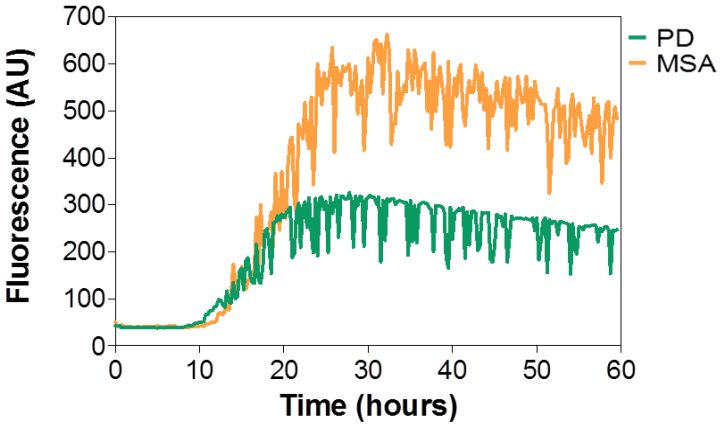
- ✓ OM samples collected from patients with PD and MSA possess seeding activity for α-synuclein;
- ✓ Final RT-QuIC products seem to acquire different biochemical and structural features when seeded with OM samples of PD or MSA patients;
- ✓ RT-QuIC analyses of OM are still in their **embryonic phases** and require further steps of standardization and harmonization before introducing them as a diagnostic procedures;
- ✓ Integrating RT-QuIC analysis of CSF and OM with other instrumental and biochemical tests might significantly improve the clinical diagnostic accuracy of PD and other neurodegenerative parkinsonisms.

Limitations of the study

- ✓ Number of samples analyzed
- ✓ Diagnosis are not neuropathologically confirmed
- ✓ Specificity and sensitivity cannot be properly estimated

INOCULATION OF FINAL RT-QuIC PRODUCTS

Inocula preparation



Animal bioassay

INOCULUM
OM RT-QuIC PD
OM RT-QuIC MSA
OM PD
OM MSA
Brain Homogenate PD
Brain Homogenate MSA

- ✓ Behavioural tests
- ✓ Biochemical analyses
- ✓ Histological analyses
- ✓ RT-QuIC assessments

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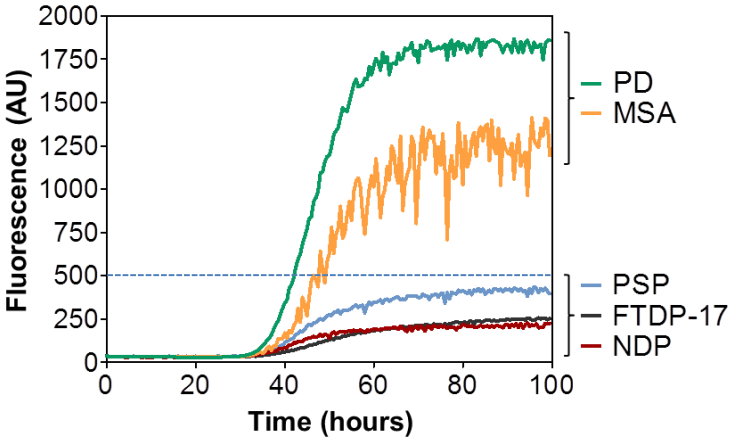
Elena De Cecco
Joanna Narkiewicz
Giulia Salzano

RT-QuIC ANALYSIS FROM BRAIN HOMOGENATES



Brain homogenates of patients with PD and MSA efficiently seeded RT-QuIC reaction (compared to those of CBD and PSP)

Serial dilutions of PD and MSA brain homogenates were able to efficiently seed rec-αSynuclein aggregation