Exercice: Investigating α-synuclein strains: GCI vs LB

LETTER

https://doi.org/10.1038/s41586-018-0104-4

Cellular milieu imparts distinct pathological α-synuclein strains in α-synucleinopathies

Chao Peng, Ronald J. Gathagan, Dustin J. Covell, Coraima Medellin, Anna Stieber, John L. Robinson, Bin Zhang, Rose M. Pitkin, Modupe F. Olufemi, Kelvin C. Luk, John Q. Trojanowski & Virginia M.-Y. Lee*

Abstract: In Lewy body diseases—including Parkinson's disease, without or with dementia, dementia with Lewy bodies, and Alzheimer's disease with Lewy body co-pathology1 —α-synuclein (α-Syn) aggregates in neurons as Lewy bodies and Lewy neurites2 . By contrast, in multiple system atrophy α-Syn accumulates mainly in oligodendrocytes as glial cytoplasmic inclusions (GCIs)3 . Here we report that pathological α-Syn in GCIs and Lewy bodies (GCI-α-Syn and LB-α-Syn, respectively) is conformationally and biologically distinct. GCI-α-Syn forms structures that are more compact and it is about 1,000-fold more potent than LB-α-Syn in seeding α-Syn aggregation, consistent with the highly aggressive nature of multiple system atrophy. GCI-α-Syn and LB-α-Syn show no cell-type preference in seeding α-Syn pathology, which raises the question of why they demonstrate different cell-type distributions in Lewy body disease versus multiple system atrophy. We found that oligodendrocytes but not neurons transform misfolded α-Syn into a GCI-like strain, highlighting the fact that distinct α-Syn strains are generated by different intracellular milieus. Moreover, GCI-α-Syn maintains its high seeding activity when propagated in neurons. Thus, α-Syn strains are determined by both misfolded seeds and intracellular environments

https://www.nature.com/articles/s41586-018-0104-4

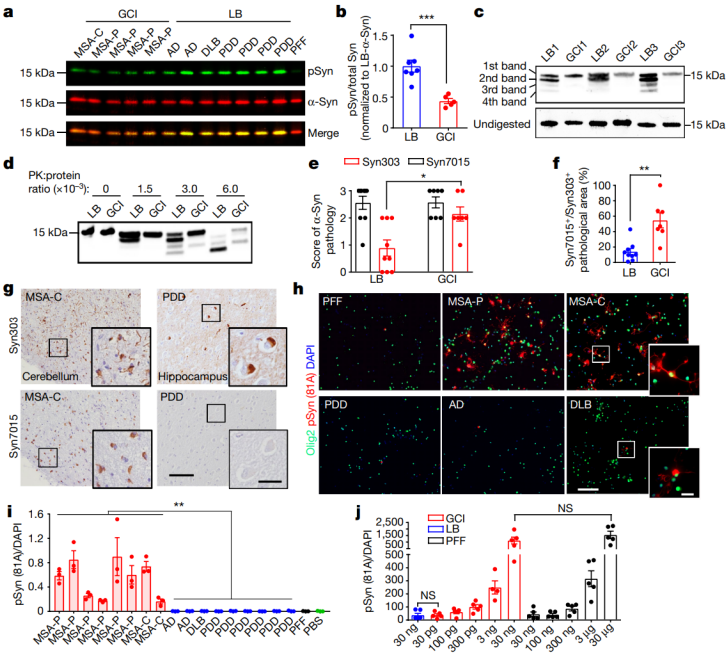
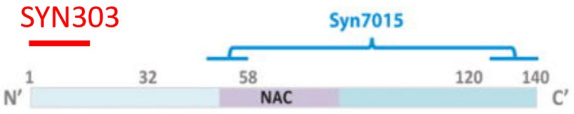


Fig. 1 | GCI-α-Syn and LB-α-Syn represent two distinct strains. **a**, GCI and Lewy body immunoblotted with antibodies against total α-Syn or pS129 α-Syn (pSyn). **b**, Quantification of pS129 α-Syn versus total α-Syn shown in **a** (GCI, $n = 5$ cases; LB, $n = 7$ cases). **c**, Proteinase K-digested LB-α-Syn and GCI-α-Syn from six cases immunoblotted with anti-α-Syn MAb (Syn211). **d**, GCI-α-Syn and LB-α-Syn incubated with increasing concentrations of proteinase K (PK) and immunoblotted with Syn211 (experiment repeated three times). **e**, Semi-quantitative scores (0–3) to quantify α-Syn pathology revealed by Syn303 or Syn7015 immunohistochemistry in adjacent brain sections of patients with MSA or Lewy body disease (LB, $n = 9$ cases; GCI, $n = 7$ cases) (statistics: Mann–Whitney *U*-test). **f**, Quantification of area occupied by Syn7015-positive (Syn7015$^+$) versus Syn303-positive (Syn303$^+$) α-Syn pathology for experiments in **e** (LB, $n = 9$ cases, GCI, $n = 7$ cases). **g**, Representative photomicrographs for experiments in **e** (repeated with seven cases). **h**, Primary oligodendrocytes expressing α-Syn-mCherry incubated with 13 ng GCI-α-Syn, LB-α-Syn or PFFs were stained with 81A (pS129 α-Syn) and anti-olig2 (experiment repeated four times). **i**, Quantification of pS129 α-Syn induced by GCI-α-Syn, LB-α-Syn and PFF in oligodendrocytes expressing α-Syn (GCI, $n = 8$ different preparations; LB, $n = 9$ different preparations) (statistics: two-tailed unpaired *t*-test using the mean value of each case). **j**, Quantification of pS129 α-Syn induced by various amounts of PFFs, GCI-α-Syn or LB-α-Syn in oligodendrocytes expressing α-Syn ($n = 6$ (LB), 4 (GCI 3 ng) or 5 (all other groups) biological replicates) (statistics: adjusted with Bonferroni correction). Results shown as mean ± s.e.m. $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; $^{****}P < 0.0001$; NS, not significant. Scale bars: 100 μm (**g**, **h**), 25 μm (**g** inset), 10 μm (**h** inset). AD, Alzheimer's disease; DLB, dementia with Lewy bodies; LB, Lewy body; PDD, Parkinson's disease with dementia. For gel source data, see Supplementary Fig. 1. See Supplementary Table 5 for statistical details.

Study Fig. 1 (panels a–j) from Peng et al. (Nature, 2018). Answer the following questions in your own words (2–5 sentences each).

1) Post-translational modification & protease resistance (panels a–d):
   What do the blots and PK digestion profiles suggest about phosphorylation (pSer129) and protease resistance in GCI-derived vs LB-derived α-syn?

2) Epitope/exposure and antibody specificity (panels e–g): Syn303 and Syn7015 antibodies detect different patterns in GCI vs LB.



   What does this imply about conformational differences / epitope masking/exposure?

3) Cell seeding and strain–cell interaction (panels h–j):
   To determine whether structural differences between GCI-α-Syn and LB-α-Syn

influence their seeding activities, we treated primary oligodendrocytes expressing α-Syn with an equal amount of GCI-α-Syn, LB-α-Syn or α-Syn preformed fibrils (PFFs=in vitro made)
Why did the authors choose to prepare oligodendrocyte and not neuronal culture? When primary cultures are exposed to different seeds (GCI, LB, PFF), p-α-syn induction differs by cell type. What does this tell us about strain potency and cell environment influence?

4) Integrative conclusion: Based on questions 1–3, why do the authors call GCI-α-syn and LB-α-syn distinct strains?

5) Linking to IP/WB methods: If you had only IP + WB available, outline how you would compare GCI vs LB α-syn in brain extracts to test:

   a) difference in phosphorylation, and

   b) difference in aggregation / oligomerization.

   Name the kind of antibodies you would use and what band/ratio patterns would support 'distinct strains.'