Alpha-synuclein oligomers in Parkinson's disease with LRRK2 mutations

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ABSTRACT

BACKGROUND

Mutations in leucine-rich repeat kinase 2 (LRRK2) are a frequent cause of familial Parkinson's disease (PD). Lewy-related pathologies (Lewy bodies and Lewy neurites) are aggregates of alpha-synuclein (αSYN) and are considered a hallmark of PD. Although clinical presentations of LRRK2-PD patients are similar to sporadic PD, some lack Lewy-related pathologies. The toxicity of αSYN oligomers, early aggregates of αSYN, has garnered attention, but their distribution in LRRK2-PD is unknown. We aimed to examine the distribution of αSYN oligomers in LRRK2-PD brains.

METHODS

Brain samples from four LRRK2-PD patients with G2019S mutation and five with I2020T mutation were analyzed. Two G2019S and three I2020T samples lacked Lewy-related pathologies. Five control brains were also analyzed. αSYN oligomers were visualized by homotypic proximity ligation assay (PLA) and examined in the brainstem, basal ganglia, limbic, and neocortex.

RESULTS

Results showed that all LRRK2-PD patients, including those without Lewy-related pathologies, had an accumulation of αSYN oligomers in all regions. Control brains showed little αSYN-PLA signals. The αSYN oligomer burden in the neocortex, brainstem, and hippocampus was significantly higher in LRRK2-PD patients with G2019S mutation than in those with I2020T mutation. In the entorhinal cortex, the αSYN oligomer burden was higher in LRRK2-PD patients without Lewy-related pathologies than in those with Lewy-related pathologies.

CONCLUSIONS

Our findings indicate widespread accumulation of αSYN oligomers in LRRK2-PD patient brains, suggesting that they may contribute to the pathogenesis of the disease, regardless of the presence of Lewy-related pathologies.

OBJECTIVE

- To elucidate the distribution of α-synuclein (αSYN) oligomers in Parkinson's disease (PD) with leucine-rich repeat kinase 2 (LRRK2) mutations with and without Lewy-related pathologies (LRP).

METHODS

- We examined the following autopsy brains:
  - 5 I2020T LRRK2-PD (2 with LRP, 3 without LRP)
  - 4 G2019S LRRK2-PD (2 with LRP, 2 without LRP)
  - 5 control subjects
- αSYN oligomers were visualized with αSYN-PLA staining.
- We examined αSYN oligomers in the brainstem, limbic system, basal ganglia, and neocortex.
- The severity of αSYN oligomer burden in neuropil was evaluated semi-quantitatively based on the pre-made scoring plate.
- The accumulation pattern of αSYN oligomers in neurons was classified into four types: neuronal-clustered, neuronal-patchy, neuronal-punctate, and null.

TABLE Summary of Patient Characteristics

	Mutations	Age at onset	Age at Death	Disease duration	Initial symptom	Lewy-related pathologies
1	I2020T	70	80	10.8	Gait difficulty	-
2	I2020T	58	77	18.8	Bradykinesia, rigidity	+
3	I2020T	39	67	28.1	Gait difficulty	+
4	I2020T	56	81	24.3	Gait difficulty	-
5	I2020T	49	78	28.8	Rigidity	-
6	G2019S	ND	79	ND	Cognitive impairment	-
7	G2019S	69	80	10.7	Shuffling gait	+
8	G2019S	68	85	16.3	falls	+
9	G2019S	73	80	6.1	gait difficulty, falls	-

RESULTS

- We detected αSYN oligomers in all LRRK2-PD brains examined in the present study, both in brains with and without Lewy-related pathologies.
- The accumulation of αSYN oligomers was more prominent in neuropil than in neurons.
- αSYN oligomer burden was significantly greater in LRRK2-PD than in control.
- Among LRRK2-PD, αSYN oligomer burden was significantly greater in the G2019S group than in the I2020T group.

Figure 1 αSYN oligomers in the substantia nigra of LRRK2-PD patients

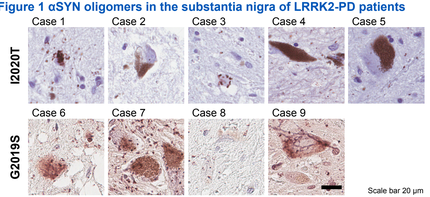


Figure 2 αSYN oligomers in the frontal cortex of LRRK2-PD patients

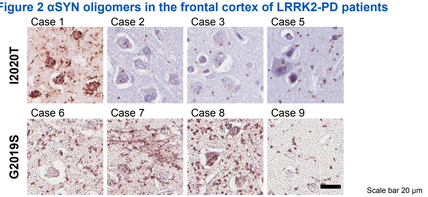
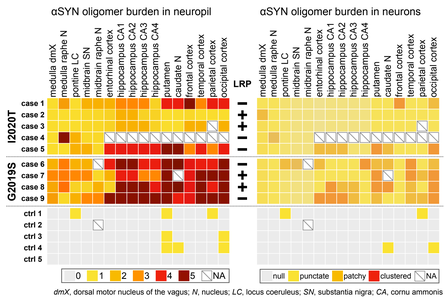
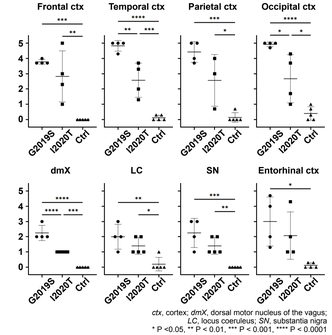


Figure 3 αSYN oligomer burden in neuropil and neurons



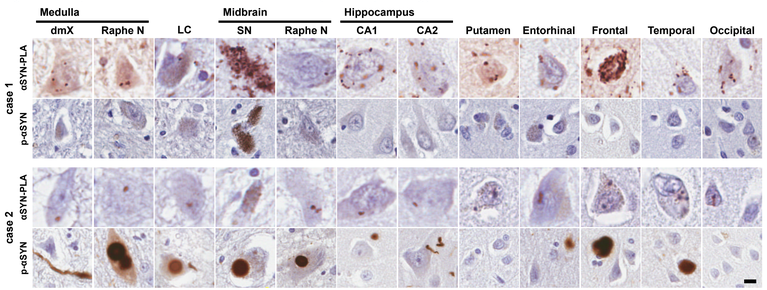
dmX, dorsal motor nucleus of the vagus; *N*, nucleus; *LC*, locus coeruleus; *SN*, substantia nigra; *CA*, cornu ammonis

Figure 5 αSYN-PLA score



ctx, cortex; *dmX*, dorsal motor nucleus of the vagus; *LC*, locus coeruleus; *SN*, substantia nigra
* P <0.05, ** P < 0.01, *** P < 0.001, **** P < 0.0001

Figure 4 Comparison between αSYN oligomer and Lewy-related pathology



dmX, dorsal motor nucleus of the vagus; *N*, nucleus; *LC*, locus coeruleus; *SN*, substantia nigra; *CA*, cornu ammonis
Scale bar 10 μm

DISCUSSION

- Mutations in LRRK2 are the most common cause of familial PD. The clinical presentation of LRRK2-PD is indistinguishable from sporadic PD; however, about half of LRRK2-PD cases show no Lewy-related pathologies[1].
- Lewy-related pathologies are late-stage αSYN aggregates, while αSYN oligomers are early-stage αSYN aggregates. Recent evidence suggests that αSYN oligomers may be more toxic than Lewy-related pathologies[2,3].
- Conventional immunostaining cannot detect αSYN oligomers, and a special staining method is required. Proximity ligation assay (PLA) has enabled detection of αSYN oligomers[4,5,6].
- We examined the distribution of αSYN oligomers in brain samples from patients with LRRK2-mutations (I2020T and G2019S).
- In the present study, αSYN oligomers were observed in LRRK2-PD brains regardless of the presence of Lewy-related pathologies.
- A previous study showed an increase of αSYN oligomer in CSF of asymptomatic LRRK2 mutation carriers[7].
- αSYN oligomers may be involved in the common disease causative mechanism of LRRK2-PD with and without Lewy-related pathologies.

CONCLUSIONS

- We observed an accumulation of α-synuclein oligomers in LRRK2-PD brains with and without Lewy-related pathologies.
- αSYN oligomers may play a common role in pathogenesis of LRRK2-PD.

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