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Discrepancy between distribution of alpha-synuclein oligomers and Lewy-related pathology in Parkinson's disease

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Abstract

The pathological hallmarks of Parkinson's disease (PD) are α -synuclein (α SYN)-positive inclusions referred to as Lewy bodies and Lewy neurites, collectively referred to as Lewy-related pathology (LRP). LRP is thought to propagate in an ascending manner throughout the brain as the disease progresses. LRP is visible with histologic methods and is thought to represent a later stage of the disease process, while α SYN oligomers, which are not visible with routine histologic methods, are considered earlier. There is increasing evidence to suggest that α SYN oligomers may be more toxic than visible LRP. Detecting α SYN oligomers requires special techniques, and their distribution and association with clinical features are important research objectives. In this report, we describe the distribution of α SYN oligomers in multiple cortical and subcortical regions of PD using a proximity ligation assay (PLA). We observe widespread distribution of α SYN oligomers with PLA and more restricted distribution of LRP with α SYN immunohistochemistry. The distribution of α SYN oligomers differed from LRP in that α SYN oligomer burden was significantly greater in the neo-cortex, while LRP was greater in vulnerable subcortical regions, including the brainstem. We also found that cognitive impairment was associated with α SYN oligomers in the hippocampus. These results suggest that α SYN oligomers may be widely distributed in PD early in the disease process and that they may contribute to cognitive impairment in PD.

Keywords: Alpha-synuclein, Oligomers, Lewy bodies, Parkinson disease, Pathogenesis

Introduction

Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by bradykinesia, tremor, and rigidity [6, 35]. The pathological hallmark of PD is the Lewy body, a neuronal cytoplasmic inclusion found in selectively vulnerable neuronal populations [13]. The major structural component of Lewy bodies is

α -synuclein (α SYN) [3, 40]. In addition to PD, dementia with Lewy bodies [39] and multiple system atrophy [2] are the most common α -synucleinopathies [12, 19, 25]. In addition to Lewy bodies, α SYN accumulation is also observed in glia and dystrophic neurites, the latter referred to as "Lewy neurites" [44]. Lewy bodies and Lewy neurites are collectively referred to as Lewy-related pathology (LRP) [18, 23]. LRP represents a later stage of aggregation of α SYN that is visible with histologic methods, while earlier stages of α SYN aggregation are not visible and have been referred to as α SYN oligomers [1]. Recent evidence suggests that α SYN oligomers may be more toxic than LRP [10, 11, 20, 22, 33, 46]. Braak et al. examined autopsy brains from 41 PD patients and 69

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individuals without Parkinsonism and proposed a pathological staging of LRP in PD [9], based upon the hypothesis that α SYN propagation in human brains was not random, but followed a predictable pattern of selective vulnerability. While the general principles of the Braak PD staging scheme are valid, some patients do not fit the proposed staging scheme [4, 24, 34, 48]. One of the major correlates of the Braak staging hypothesis is that non-motor symptoms, such as rapid eye movement sleep behavior disorder, should precede motor symptoms due to earlier involvement of specific brainstem nuclei (e.g., pedunculopontine nucleus [17]) before the substantia nigra. On the other hand, it has been difficult to detect α SYN oligomers in pathological specimens, and the distribution of α SYN oligomers and their association with clinical features remain to be elucidated.

A novel technique to detect α SYN oligomers is with proximity ligation assay (PLA) [28, 36, 38]. This method has enabled detection of α SYN oligomers in pathological specimens and led to recognition that α SYN oligomers can be detected in brain regions previously not recognized to be vulnerable to LRP. In α SYN-PLA, two forms of oligonucleotides are attached to an epitope-blocking anti- α SYN antibody and serve as templates for the circulation of connector oligonucleotides by a ligase when α SYN oligomers exist and two molecules of α SYN are in close proximity (<40 nm). In late-stage aggregates such as LRP, due to conformational changes, the antibodies do not bind close enough together and the reaction does not proceed [36]. The circularized DNA strands remain hybridized to the proximity probes; then, after the addition of DNA polymerase, the oligonucleotide arm of one of the PLA probes acts as a primer for a rolling-circle amplification (RCA) reaction using the ligated circle as a template, generating a concatemeric product that extends from the oligonucleotide arm of the PLA probe. The oligonucleotide of the second probe has three mismatched, exonuclease-resistant 2' O-methyl RNA nucleotides at the 3' end that prevent its use as a primer for RCA. RCA produces a randomly coiled, single-stranded product of up to 1000 complements of the DNA circle. Finally, oligonucleotides labeled with horseradish peroxidase hybridize to the RCA product and a visible signal is detected as a distinct red-brown precipitate after adding substrate. In our previous study, we noticed that α SYN oligomers are also widely distributed in PD brains, although less than in multiple system atrophy (MSA) [38], but the distribution of α SYN oligomers in PD brains has not previously been explored.

In the present study, we aimed to determine the distribution of α SYN oligomers using PLA, compare it with the distribution of LRP using α -synuclein immunohistochemistry in PD, and correlate oligomers with clinical

features. We find that α SYN oligomers are more widespread than LRP and that α SYN oligomers in the hippocampus correlate with cognitive impairment.

Materials and methods

Brain samples and neuropathology

The present study included eight PD patients and five control subjects. Post-mortem brain samples from neuropathologically-confirmed cases of PD brain samples were obtained from the National Hospital Organization Hyogo-Chuo Hospital (Sanda, Hyogo, Japan), Kobe City Medical Center General Hospital (Kobe, Hyogo, Japan), and Kobe University Hospital (Kobe, Hyogo, Japan). We used five autopsy brains of subjects without parkinsonism and Lewy pathology from the National Hospital Organization Hyogo-Chuo Hospital as control. We reviewed medical records and collected clinical information of each patient. Written informed consent was obtained from the next of kin. This study was approved by the ethical committee of Kobe University Hospital.

We examined brain samples as previously reported in accordance with protocols in Research Resource Network Japan [38, 42]. Briefly, we stored portions of brain tissues were stored at -80°C and the rest of brain was fixed in 10% neutral buffered formalin. After fixation, the cerebrum was serially sliced in a coronal plane, the brainstem in an axial plane, and the cerebellum in a sagittal plane. Representative anatomical regions were embedded in paraffin. Serial 6- μm -thick sections were stained with hematoxylin and eosin (H&E). The following regions were examined: dorsal motor nucleus of the vagus, medullary raphe nuclei, locus coeruleus, substantia nigra, raphe nuclei of the midbrain, amygdala, entorhinal cortex, hippocampus, putamen, caudate, and neocortex (frontal, temporal, parietal, and occipital). Two continuous sections of each region were analyzed: one with phosphorylated- α SYN immunohistochemistry and one with α SYN-PLA.

Clinical information and diagnosis of patients

All patients included in the present study were examined by multiple board-certificated neurologists between 2006 and 2017. We reviewed the medical records of each patient and collected the following clinical information: sex, age at onset, age at death, initial symptoms, visual hallucinations, and cognitive impairment. We considered a patient to have visual hallucinations if there were documented complaints from the patient or family members. A patient was considered to have cognitive impairment if a physician had diagnosed cognitive abnormalities, such as memory disturbance, disorientation, executive dysfunction, or stereotypic behavior [15]. We confirmed the pathological diagnosis of PD based on the presence of

moderate to severe neuronal loss in the substantia nigra and the presence of LRP [14], which was assessed with H&E-stained sections and phosphorylated- α SYN immunohistochemistry of the midbrain. Control subjects were processed the same and had no neuronal loss or LRP in the substantia nigra.

Immunohistochemistry

Immunohistochemistry for phosphorylated- α SYN was performed as described previously [38]. Paraffin-embedded brain sections were dewaxed in xylene, then rehydrated in a graded series of alcohol. Antigen retrieval was performed by microwave heating of slides for 15 min in pH6 citrate buffer. After blocking in 3% bovine serum albumin in phosphate-buffered saline at room temperature for 30 min, primary antibody for phosphorylated- α SYN (1:2000; mouse monoclonal, psyn#64, FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan) was added and incubated at 4 °C overnight. Sections were washed in Tris-buffered saline (TBS) and were then incubated in hydrogen peroxide at room temperature for 30 min to inactivate endogenous peroxidase activity. After washing the sections, they were incubated with a biotin-conjugated secondary antibody (goat anti-mouse IgG) and then with avidin–biotin complex (VECTASTAIN Elite ABC Kit, Vector Laboratories, Burlingame, CA, USA). Phosphorylated- α SYN was visualized with 3,3'-diaminobenzidine-tetrahydrochloride-dihydrate and the sections were counterstained with hematoxylin. Samples were dehydrated in a graded series of alcohol and xylene before mounting with Permount mounting medium (Falma, Tokyo, Japan).

Braak neurofibrillary tangle (NFT) stage [7] and Thal amyloid phase [41] were assigned by thioflavin S fluorescent microscopy as previously described [37]. Immunohistochemistry for tau (AT8, mouse monoclonal, 1:2500, Thermo Fisher Scientific, Rockford, IL, USA) and phosphorylated transactive response DNA-binding protein of 43 kDa (pTDP-43) (pS409/410, mouse monoclonal, 1:5000, Cosmo Bio USA, Carlsbad, CA, USA) were performed on the hippocampus section of PD patients as previously described [31].

α SYN-PLA staining

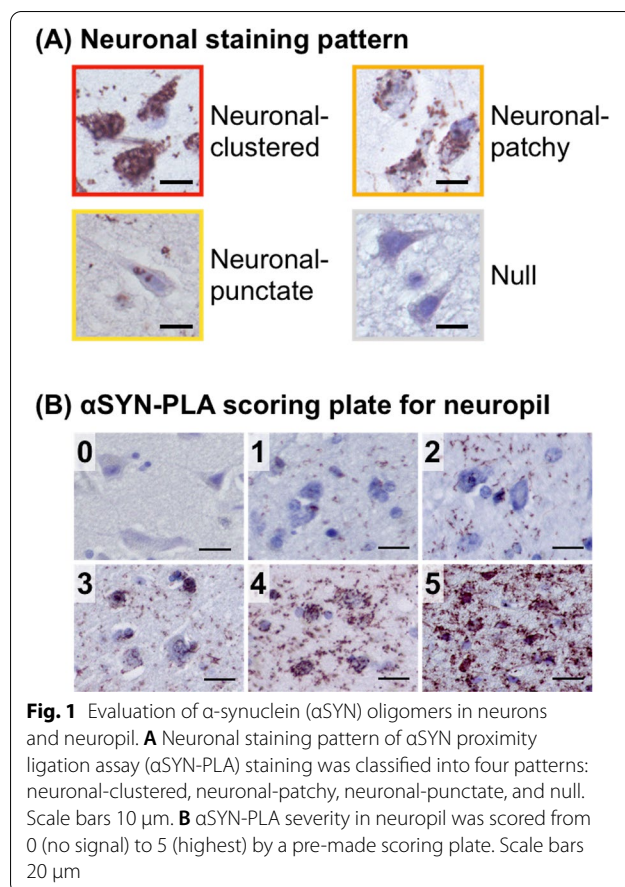
We conducted α SYN-PLA staining by using Duolink kits supplied by Sigma-Aldrich (St. Louis, MO, USA) to detect α SYN oligomers following the manufacturer instructions. We made both PLA probes with an α SYN antibody (mouse monoclonal, Syn211, Abcam, Cambridge, UK). We added 20 μ g of Syn211 antibody to 2 μ l of conjugation buffer and transferred the solution to a vial containing lyophilized oligonucleotides (plus or minus). Then, we incubated the solution at room

temperature overnight. The conjugates were incubated with 2 μ l of stop solution for 30 min at room temperature and suspended in 24 μ l of storage solution. After dewaxing and hydrating the tissue sections as above, sections were incubated with hydrogen peroxide for 1 h at room temperature and subsequently heated in a microwave for 15 min in pH6 citrate buffer. Sections were blocked in 3% bovine serum albumin in phosphate-buffered saline at 37 °C for 1 h, followed by incubation with PLA probes diluted in PLA probe diluent (1:100) at 37 °C for 1 h, and then at 4 °C overnight. Sections were washed in TBS-T (TBS + 0.05% Tween 20) and then incubated with ligation solution and ligase at 37 °C for 1 h, washed in TBS-T and then incubated with amplification reagents and polymerase at 37 °C for 2.5 h. Finally, the sections were washed in TBS-T, incubated with detection solution at room temperature for 1 h, and finally incubated with substrate solution at room temperature for 20 min. Sections were counter-stained with hematoxylin and dehydrated in a graded series of alcohol and xylene before mounting with a bright-field mounting medium.

Evaluation of LRP and α SYN oligomer burden

We assessed LRP (Lewy bodies and Lewy neurites) on phosphorylated- α SYN immunostained slides using a semi-quantitative scoring scheme at 20 $\times$ magnification. The severity of LRP was rated on a five-point scale: absent (0), slight (1+), mild (2+), moderate (3+), and severe (4+). A Braak PD stage was assigned to each case based on the distribution of LRP [8, 9].

We evaluated α SYN oligomer burden on α SYN-PLA slides as previously described [38]. Each stained slide was viewed at 20 $\times$ magnification and evaluated for the pattern and severity of neuronal and neuropil staining. Neuronal staining was classified based upon the staining pattern as follows: neuronal-clustered, neuronal-patchy, neuronal-punctate, and null (no signal detected) (Fig. 1A). Neuronal-clustered was characterized by α SYN-PLA signal throughout the neuronal perikarya; neuronal-patchy had patchy α SYN-PLA signal in the neurons; neuronal-punctate had dot-like α SYN-PLA signal. Neuropil staining was rated on a scale from zero (no signal) to five (highest signal) by using pre-made scoring plates (Fig. 1B). Because the cortical areas were large, we eliminated bias by evaluating α SYN-PLA neuropil scores in the three most affected microscopic fields and calculating the average scores in the cortex of superior frontal gyrus, superior temporal gyrus, superior parietal lobule, lingual gyrus, and parahippocampal gyrus. In other regions, such as the dorsal motor nucleus of the vagus, medullary raphe nuclei, locus coeruleus, substantia nigra, raphe nuclei of the midbrain, amygdala, hippocampus,



putamen, and caudate, the region with the most signal was scored.

To compare the severity of αSYN oligomer burden with LRP, we made a combined severity score for αSYN oligomers on a five-point scale from the neuronal staining pattern and neuropil scores. We compared the score of LRP severity and combined severity of αSYN oligomer burden in each brain region. We also compared the mean pathology scores of each patient in the brainstem and neocortex to compare regional anatomical differences in the distribution of each pathology.

In addition to the scoring described above, we quantitatively measured the immunoreactive area of αSYN-PLA staining using the software ImageJ (National Institute of Health, Bethesda, MD, USA). The cortical images were used because they were large enough to fill all areas of 1720 × 1075 pixels at 20 × magnification. Three representative images of each cortex were obtained with the Aperio AT2 Slide Scanner (Leica Biosystems, Deer Park, IL, USA) at 20 × magnification. Quantification of immunostaining was performed using the IHC Image Analysis Toolbox plugin. After the training procedure with Nova Red color, the stained images were analyzed with color

detection and converted to a 16-bit format (Additional file 1: Fig. S1A). Then, we measured the area of threshold pixels.

Statistical analysis

We conducted all statistical analysis with GraphPad Prism (version 9.1.2, GraphPad Software, La Jolla, CA, USA). Unpaired t test was used to compare differences in age at death between PD and controls. We compared the LRP scores and combined αSYN oligomer score in each brain region by Wilcoxon matched-pairs signed-rank test. The combined αSYN oligomer scores and pathological burden scores in each brain region of PD and controls were compared with respect to clinical symptoms with unpaired t test (Welch's correction was performed when the variance of the two groups was not equal). We calculated Spearman's rank correlation coefficient and examined whether there is a correlation between the duration of disease and the respective pathology scores. Statistical significance was defined by a p value < 0.05.

Results

Demographics and clinical characteristics

We summarize the demographic and clinical characteristics of each patient and control subject in Table 1. There was no significant difference in age at death between PD and control subjects (74 ± 10 years in PD patients and 64 ± 10 years in control subjects, respectively, $P = 0.089$). The median disease duration of PD patients was 15 ± 7 years. The initial symptoms of PD patients were tremor in four patients, gait difficulty in three patients, and writing disturbance in one patient. None of the control subjects had parkinsonism. Among eight PD patients, six had visual hallucinations and four developed cognitive impairment during their disease course.

Pathologic features

The pathologic features of all cases are summarized in Table 2. Among PD patients, two cases were Braak NFT stage 0, three were stage I, and three were stage III. Thal amyloid phase was 0 in four patients, 1 in one patient, 2 in two patients, and 3 in one patient. One patient had TDP-43 pathology in the hippocampus.

Distribution of αSYN oligomers and evaluation of αSYN oligomer severity

We examined the distribution and pathological burden of αSYN oligomers in neurons and neuropil on αSYN-PLA stained slides (Fig. 1). We illustrate representative αSYN-PLA staining in Fig. 2A. In the neocortex of PD2, αSYN oligomers were more prominent in neuropil than neuronal perikarya. A similar trend was observed in the neocortex of PD4, but we observed abundant αSYN

Table 1 Demographic and clinical characteristics

ID	Sex	Age at Death (Y)	Disease duration (Y)	Initial symptom	Cognitive impairment	Visual hallucinations
PD1	M	79	9.9	Hand tremor	—	+
PD2	M	76	17.0	Hand tremor	—	+
PD3	M	88	7.9	Hand tremor	—	—
PD4	M	64	27.9	Writing difficulty	+	—
PD5	M	74	19.8	Gait difficulty	+	+
PD6	M	59	13.9	Hand tremor	+	+
PD7	F	68	11.8	Gait difficulty	—	+
PD8	F	85	7.8	Gait difficulty	+	+
C1	F	55	NA	NA	—	—
C2	F	60	NA	NA	—	—
C3	M	60	NA	NA	—	—
C4	M	63	NA	NA	—	—
C5	F	80	NA	NA	—	—

Y, years; ND, no data; NA, not assessed

Table 2 Pathological characteristics

ID	PMI (hr)	Braak NFT stage	Thal amyloid phase	Braak PD stage	TDP-43 in hippocampus
PD1	1.9	III	0	3	—
PD2	2.0	0	0	4	—
PD3	3.4	I	1	4	—
PD4	2.5	I	0	4	—
PD5	16.0	III	0	5	+
PD6	4.2	I	3	5	—
PD7	3.3	0	2	6	—
PD8	10.2	III	2	6	—
C1	1.5	NA	NA	NA	NA
C2	3.5	NA	NA	NA	NA
C3	7.7	NA	NA	NA	NA
C4	5.5	NA	NA	NA	NA
C5	ND	NA	NA	NA	NA

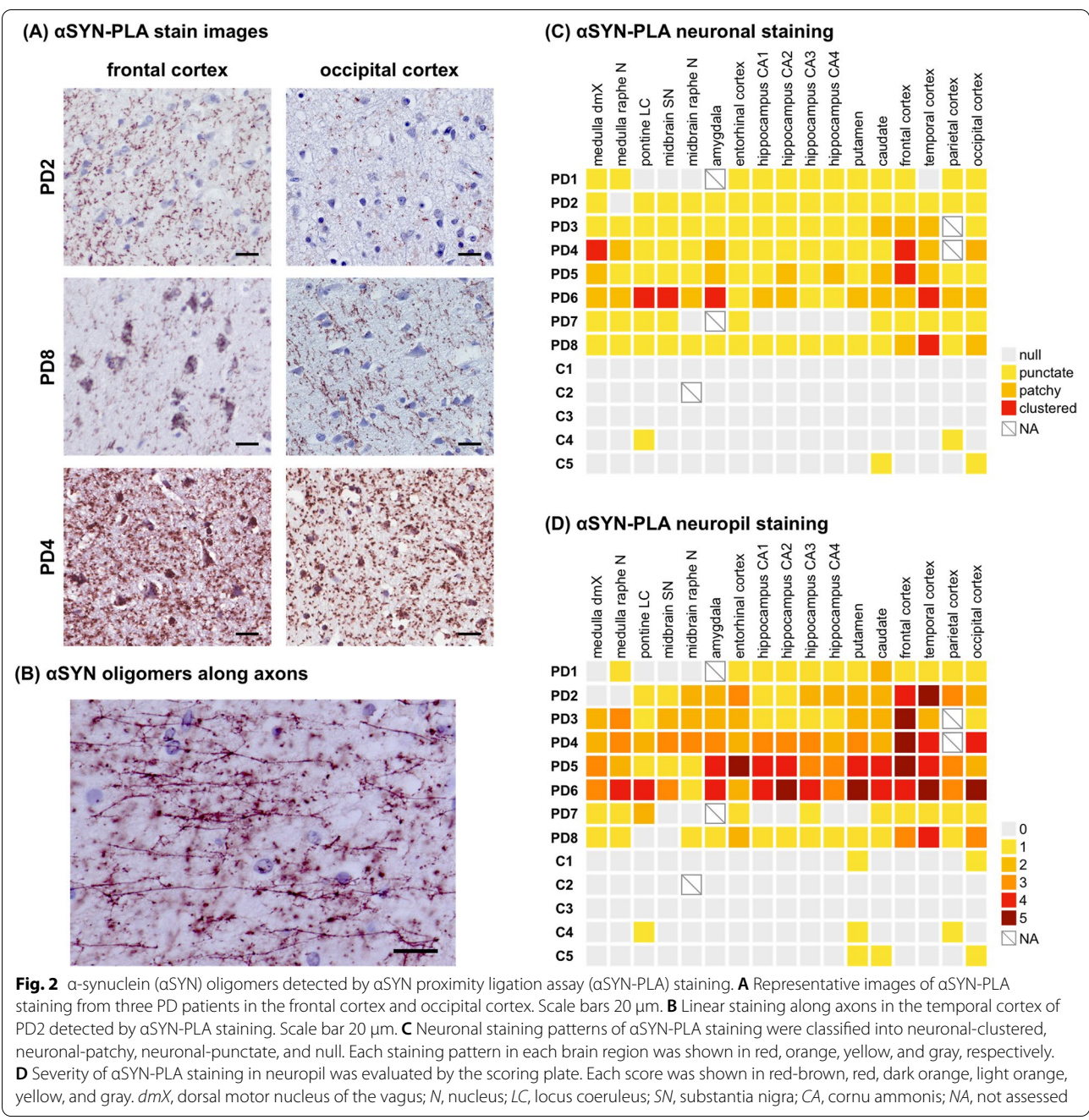
PMI, post-mortal interval; hr, hours; NFT, neurofibrillary tangle; PD, Parkinson's disease; TDP-43, transactive response DNA binding protein of 43 kDa; ND, no data; NA, not assessed

oligomers in both neurons and neuropil. PD8 showed an intermediate degree of staining in neurons and neuropil between PD2 and PD4 with more prominent signals in neuropil. A linear staining pattern of α SYN-PLA, consistent with accumulation of synuclein oligomers in axons, was noted in some PD patients (Fig. 2B).

We assessed the pattern of α SYN oligomers in neurons. Neuronal staining was evaluated as neuronal-clustered, neuronal-patchy, neuronal-punctate, or null (Fig. 1A). The results are summarized in Fig. 2C. The neuronal-clustered pattern was observed in the brainstem of two

patients (PD4 and PD6). The neuronal-clustered pattern was found in the neocortex of four patients (PD4, PD5, PD6, and PD8). The amount of α SYN-PLA neuropil staining was rated on a 0 to 5 scale by comparing images to pre-made scoring plates (Figs. 1B, 2D). We confirmed the correlation between the neuropil staining score and the stained area of α SYN-PLA staining ($r=0.8222$, $P<0.0001$, Additional file 1: Fig. S1B). There was a strong correlation between the severity of neuropil staining and the severity of neuronal staining in each brain region ($r=0.7010$, $P<0.0001$). The severity of neuropil staining tended to be higher in brain regions with the most neuronal staining. When we compared the severity of neuronal and neuropil staining in each region, neuropil staining was greater than neuronal staining in many regions. The severity of neuronal staining was greater than neuropil staining in only 3 of 132 regions examined (17 regions in 8 PD cases, excluding 4 missing).

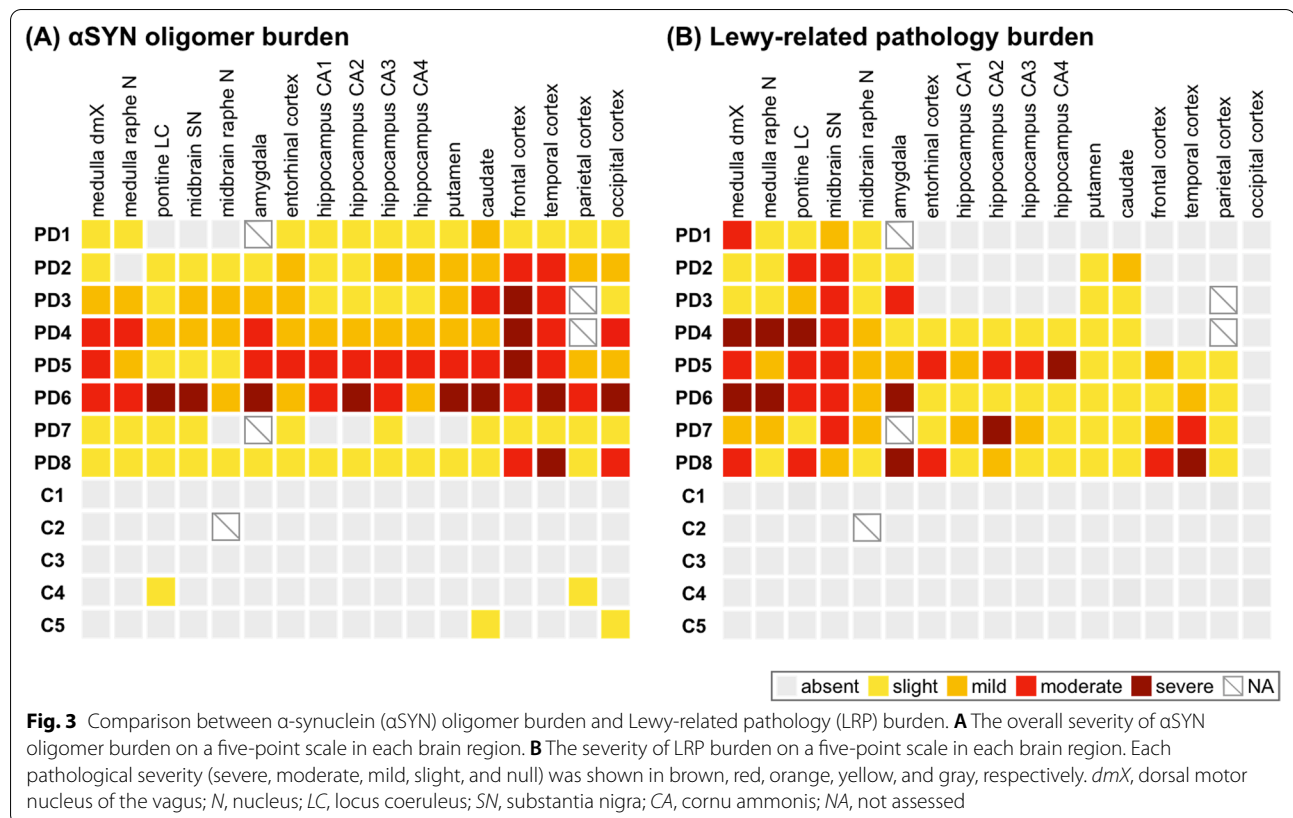
We assessed the overall burden of α SYN oligomers on a five-point scale by combining the staining of neurons and neuropil. The results are shown in Fig. 3A. α SYN oligomers were more prominent in the neocortex than in the brainstem. In the limbic system, one patient had severe, one had moderate, two had mild, and four had mild burden of α SYN oligomers in the hippocampus. Six out of eight patients had moderate to severe burden of α SYN oligomers in the neocortex. We compared the severity of α SYN oligomers in each brain region between PD patients and control subjects. The α SYN oligomer severity score and stained area of PD patients were significantly greater than those of controls, respectively (Additional file 1: Figs. S2 and S3).



Severity of LRP

We analyzed the severity of LRP (Lewy bodies and Lewy neurites) in each region with phosphorylated- α SYN immunohistochemistry and evaluated the severity on a five-point scale (0—no staining, 1—slight, 2—mild, 3—moderate, and 4—severe). We assessed Braak PD staging based on the distribution of LRP [8, 9]. There was one patient with Braak stage 3, three patients with Braak stage 4, two patients with Braak stage 5, and two patients with Braak stage 6. All PD patients had LRP in the brainstem,

including the dorsal motor nucleus of the vagus, locus coeruleus, and substantia nigra. No PD patient had LRP in the occipital cortex. The severity of LRP in each brain region is summarized in Fig. 3B. Most of the cases had moderate to severe LRP in the brainstem. With respect to LRP in the limbic system, two patients had severe pathology, one patient had moderate pathology, one patient had mild pathology, and two patients had minimal pathology in the amygdala. Four out of eight patients had no LRP in the neocortex.



Comparison of LRP and αSYN oligomer burden

We compared the severity of LRP and αSYN oligomers in the respective brain regions of PD patients. The representative comparative images of phosphorylated-αSYN immunostaining and αSYN-PLA staining are shown in Fig. 4. Lewy bodies were present in the substantia nigra, locus coeruleus, and frontal cortex, and αSYN oligomers were observed in the same regions. We observed αSYN oligomers at the periphery of some Lewy bodies in the substantia nigra and locus coeruleus (Fig. 4A, B, and Additional file 1: Fig. S4). αSYN oligomers in neuropil were more prominent than Lewy neurites (Fig. 4C). Although no LRP was detected, abundant αSYN oligomers were detected in the occipital cortex (Fig. 4D).

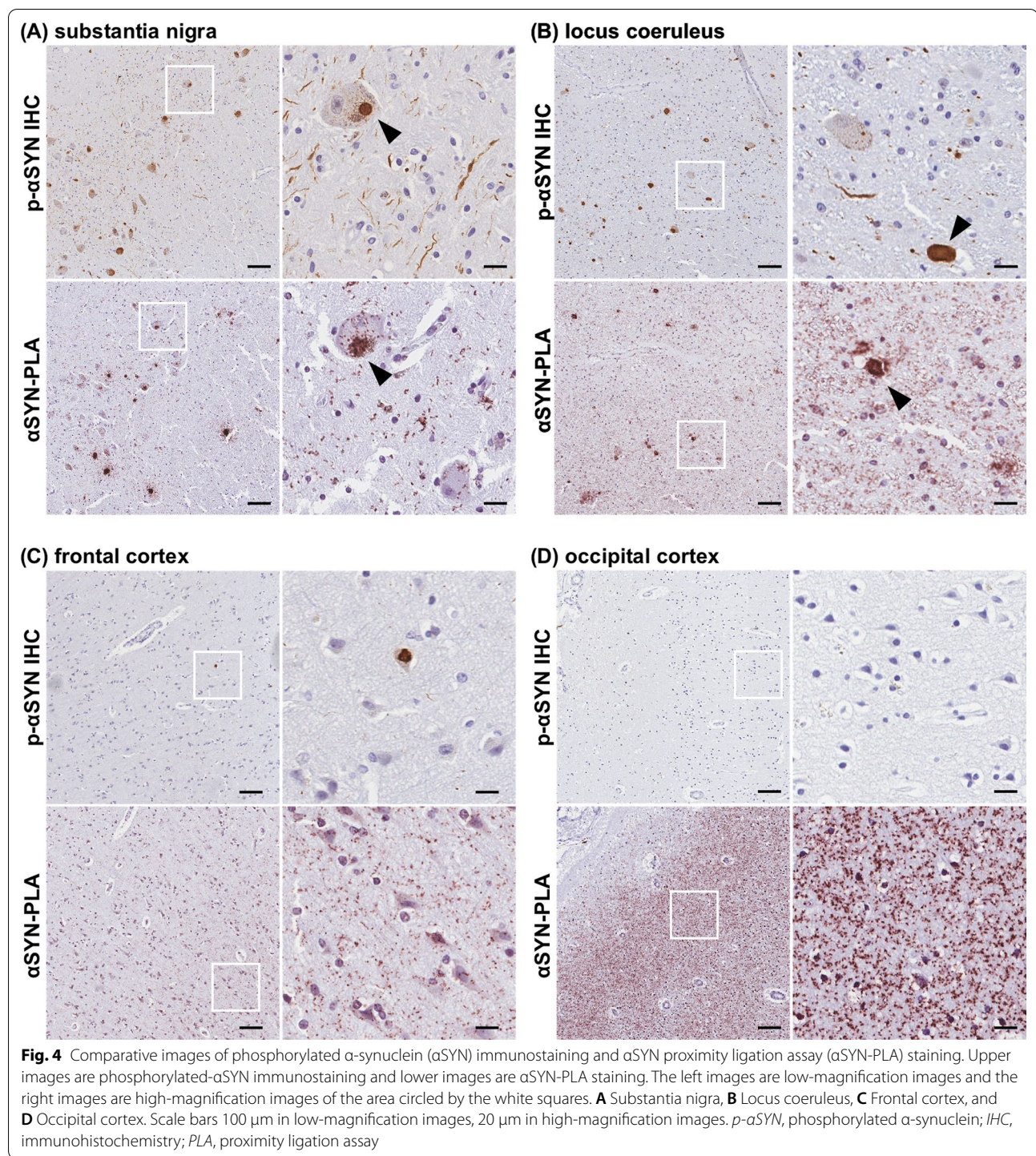
We analyzed differences in the distribution of αSYN oligomers and LRP graphically (Fig. 5). LRP was significantly higher than αSYN oligomer score in the brainstem ($P=0.031$), whereas the αSYN oligomer score was significantly higher than LRP in the neocortex ($P=0.016$). Comparison of pathological severity in each brain revealed that αSYN oligomer score was significantly higher than the LRP in the frontal cortex ($P=0.047$) and occipital cortex ($P=0.0078$). The LRP score was significantly higher than αSYN oligomer score in the substantia nigra ($P=0.039$).

Clinical characteristics and each pathological severity

Finally, we examined correlations between the severity of LRP and αSYN oligomers with clinical features. PD patients who had cognitive impairment had significantly higher αSYN oligomer scores in CA1 and CA2 of the hippocampus than those who did not have cognitive impairment ($P=0.032$ and $P=0.045$; Fig. 6). On the other hand, there was no significant difference in the severity of LRP score in the hippocampus between those with and without cognitive impairment. Braak NFT stage and Thal amyloid phase did not differ significantly between PD patients with and without cognitive impairment ($P=0.32$ and $P=0.59$). We found no significant correlation between the presence of visual hallucinations and either LRP or αSYN oligomer in the amygdala and occipital cortex. There was no significant correlation between disease duration and brainstem LRP severity ($r=0.65$, $P=0.089$), neocortical LRP score ($r=-0.28$, $P=0.50$), brainstem αSYN oligomer severity score ($r=0.31$, $P=0.45$) or neocortical αSYN oligomer score ($r=0.45$, $P=0.27$).

Discussion

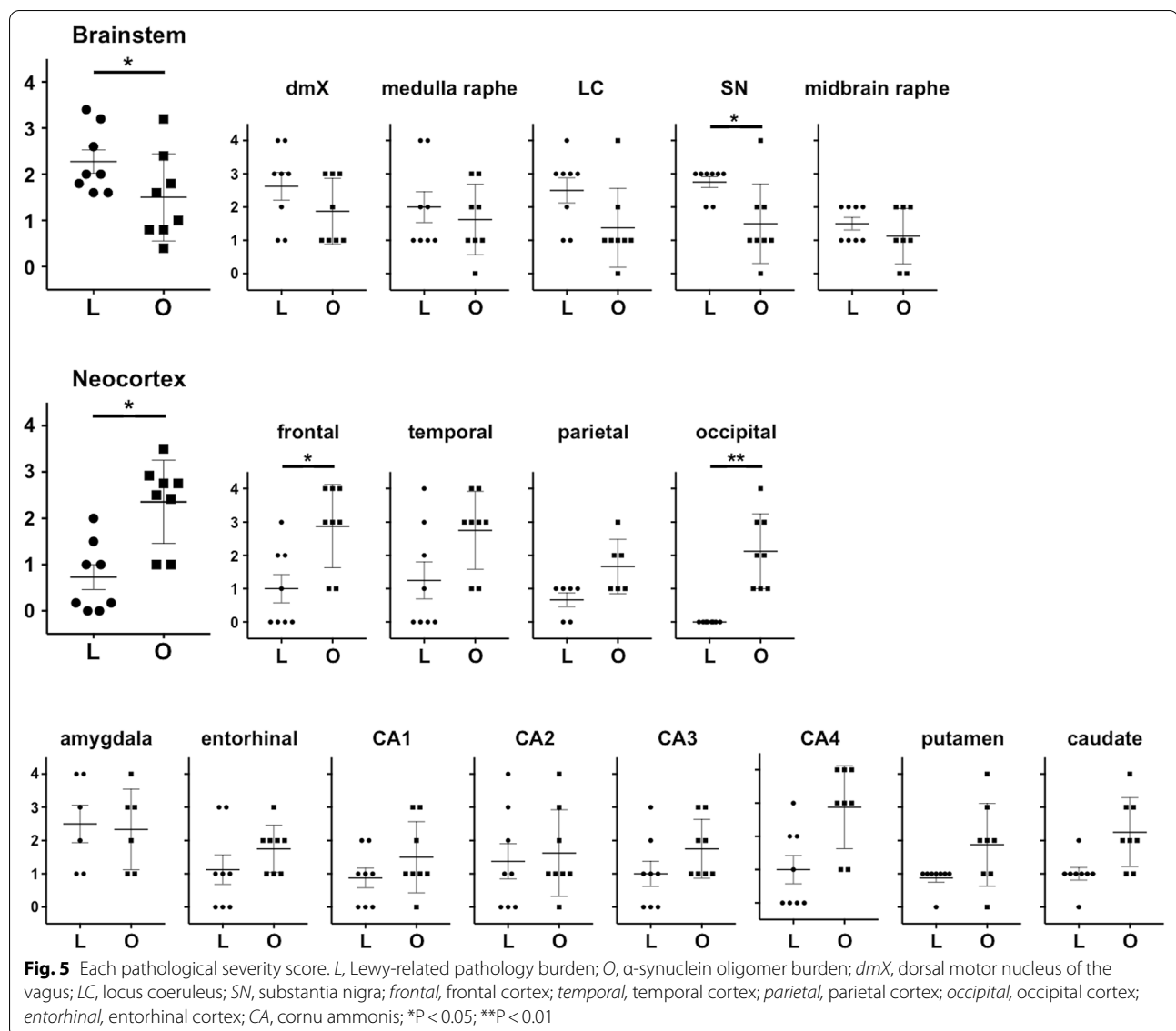
Here we report a distribution of α-synuclein oligomers in PD using a PLA. In the present study, we found a widespread distribution of αSYN oligomers in PD brains and



discordance between the distribution of αSYN oligomers and LRP. We also found that PD patients with cognitive impairment had more severe αSYN oligomers in the hippocampus.

Our results demonstrate that the distribution of αSYN oligomers was more widespread than that of LRP. Half of

the patients in the present study had no LRP in the neocortex, but at least some αSYN oligomer was detected in the neocortex of all patients. Therefore, αSYN oligomers may be widespread even early in the disease stage. Accumulation of αSYN oligomers is rarely if ever observed in control subjects, therefore, αSYN oligomers detected

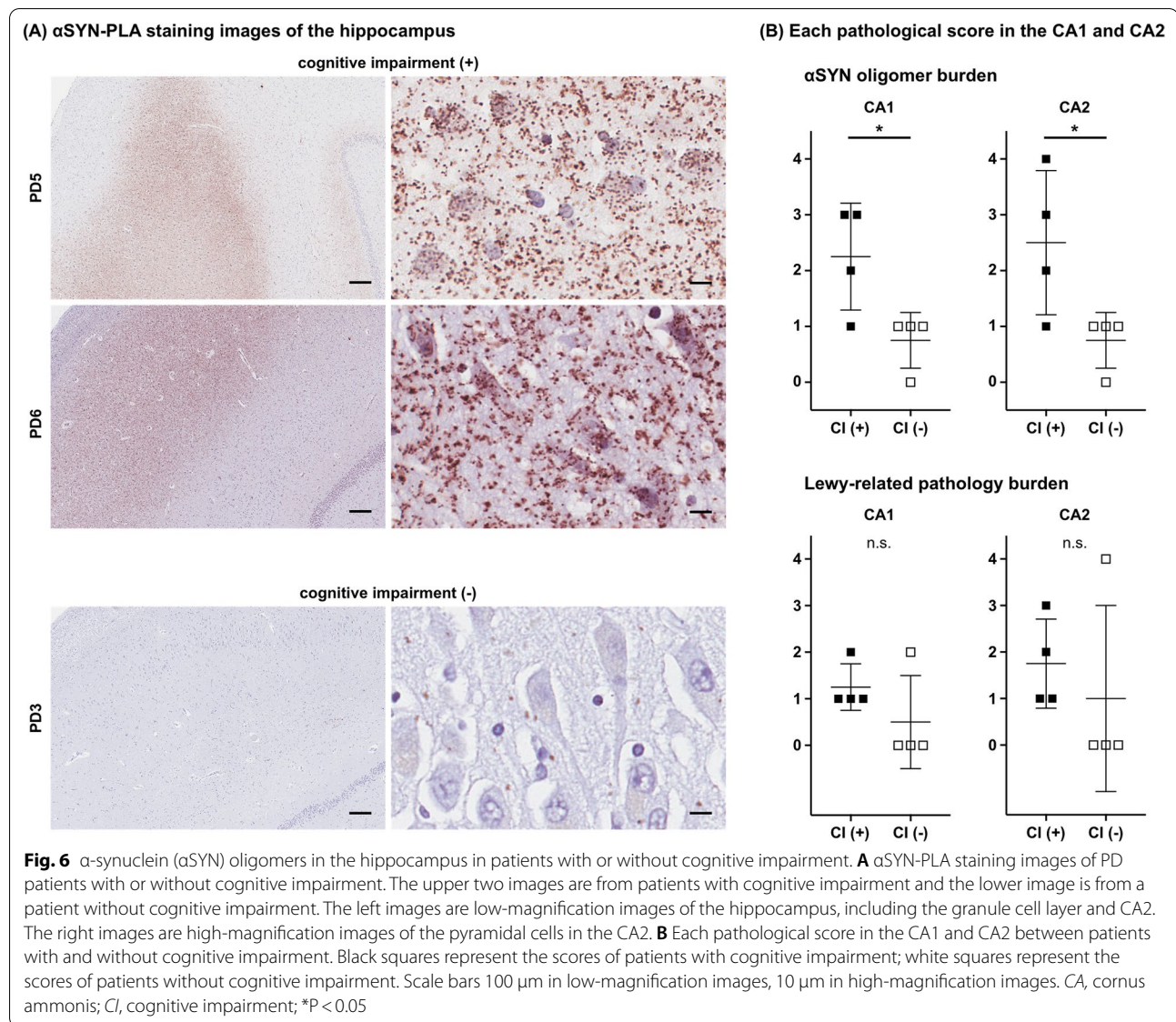


with α SYN-PLA reflects genuine pathological findings related to PD. This is supported by previous in vitro and in vivo studies. α SYN-PLA staining has detected α SYN oligomers in two different methods (bimolecular fluorescence complication and FK506 binding protein-FK506 rapamycin binding-rapamycin model) [36]. Another study successfully detected α SYN oligomers in transgenic mice (Thy1-h- α SYN*A30P) with α SYN-PLA similar to those employed in the present study. Of note, no signal was observed in the non-transgenic control mice [5].

Regarding the association between clinical features and α -synuclein pathology, in the present study, more α SYN oligomers were found in the hippocampus of patients with cognitive impairment. This association was not observed with respect to LRP. There were also no differences in Braak NFT stage or Thal amyloid phase between

patients with and without cognitive impairment. One patient with cognitive impairment had TDP-43 pathology in the hippocampus, which might have contributed to cognitive impairment [32, 43]. Mounting evidence suggests that α SYN oligomers are more toxic than LRP [11, 22, 46]. A recent study also reported MSA patients with cognitive impairment had more α SYN oligomers [30]. Therefore, cognitive impairment observed in the present study may be associated with α SYN oligomers.

We also observed differences in the distribution between α SYN oligomers and LRP, with prominent α SYN oligomer burden in the neocortex and prominent LRP in the brainstem. The reason for this distribution discrepancy could be attributed to regional differences in neuronal aggregation of α SYN. In this study, all patients had neuropathologic and clinical diagnosis of PD, with

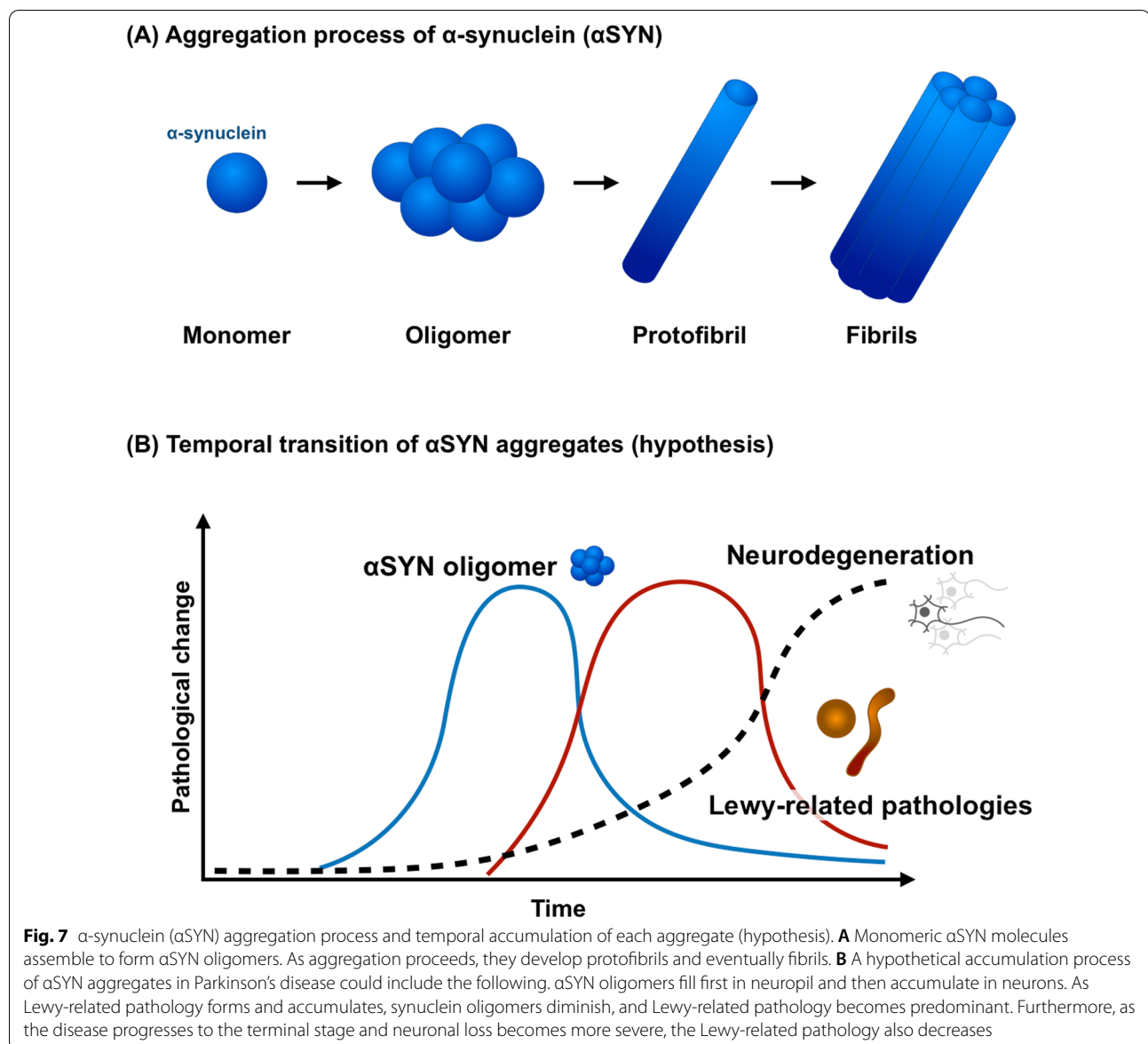


motor symptoms prior to onset of any cognitive deficits. Accordingly, α SYN pathology is likely to have first appeared in the brainstem rather than the cortex. Based upon studies of α SYN aggregation, it is likely that α SYN oligomers precede formation of LRP [47]. We speculate that the reason for differences in distribution between α SYN oligomers and LRP is that α SYN oligomers are the substrate for LRP and that as LRP increases there is a shift in the pool of oligomers to fully formed fibrils in LRP. Since the neocortex is affected later in the disease of PD, relatively more α SYN oligomers than LRP are observed in the neocortex (Fig. 7).

The present study suggests that α SYN oligomers may propagate through axons and accumulate in neuropil before they accumulate in perikarya of neurons. We observed more α SYN oligomers in the neuropil than in

neurons in most brain regions. These results suggest that α SYN oligomers appear first in neuronal processes in the neuropil, before that mature into LRP in perikarya. Moreover, we found a linear staining pattern of α SYN oligomers in the neocortex of PD patients that appeared to be in axons. This suggests that α SYN oligomers may propagate through axons.

Our results may be useful in considering the suitable targets for anti- α SYN therapy. In the Braak hypothesis, it has been thought that neurodegeneration progresses through the propagation of Lewy pathology [9]. This propagation concept was supported by the presence of Lewy bodies in neurons of fetal graft has suggested that pathological α SYN propagates [26, 27, 29]. A treatment strategy to stop the propagation of pathological synuclein has been considered [45]; however, anti- α SYN therapy



has failed to demonstrate therapeutic efficacy [16]. The reason for the failure could be the widespread distribution of α SYN oligomers in earlier pathological stages of the disease. The successful treatment of PD patients with the anti- α SYN approach requires including very early PD or even those in a preclinical phase of the disease [21].

We recognize the relatively small sample size as a limitation. The implications of α SYN oligomers in the hippocampus on cognitive impairment warrant further research. Another potential limitation of the current study lies in the inherent nature of retrospective autopsy studies. Patients did not undergo the prospective clinical evaluations. Therefore, it remains possible that some of the clinical features may be underestimated.

Nevertheless, the present study sheds new light on the neuropathology of PD from the perspective of α SYN oligomers.

Conclusion

We examined the distribution of α SYN oligomers in formalin-fixed paraffin-embedded brain samples from patients with PD. The distribution of α SYN oligomers was more widespread than that of LRP, suggesting that α SYN oligomers may be found throughout the brain earlier in the disease course than can be observed with immunohistochemistry for LRP. Of note, we observed more α SYN oligomers in the hippocampus in patients with cognitive impairment. Given the toxicity of α SYN

oligomers, clinicopathological studies focusing on α SYN oligomers may provide insight into PD pathogenesis.

Abbreviations

α SYN: Alpha-synuclein; H&E: Hematoxylin and eosin; LRP: Lewy-related pathology; NFT: Neurofibrillary tangle; PD: Parkinson's disease; PLA: Proximity ligation assay; RCA: Rolling circle amplification; TBS: Tris-buffered saline; TDP-43: Transactive response DNA-binding protein of 43 kDa.

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s40478-022-01440-6>.

Additional file 1. Fig. S1. (A) Example of image conversion for stained area measurement by the software ImageJ. (B) Correlation between stained area and neuropil score; **Fig. S2.** Score of α SYN oligomer burden; **Fig. S3** Stained area of α SYN-PLA staining; **Fig. S4** Comparative images of phosphorylated α SYN immunostaining and α SYN-PLA staining.

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Author contributions

HS, SK, DWD, HK, and TT contributed to the study conception and design. Material preparation and data collection were performed by HS, AT, YH, MT, KN, FN, MK, and NK. HS, AT, YH, MT, and SK contributed to data analysis. HS wrote the first draft of the manuscript and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate

All brain autopsies were performed with the consent of the legal next-of-kin or an individual with legal authority to grant permission for autopsy. The study was approved by Kobe University Ethical Committee (2010-073).

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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