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Examination of Abnormal Alpha-synuclein Aggregates in the Enteric Neural Plexus in Patients with Ulcerative Colitis

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ABSTRACT

Background & Aims: Parkinson's disease (PD) is the second most neurodegenerative disease after Alzheimer's disease. Accumulating knowledge points to the notion that abnormal aggregation of alpha-synuclein (αSyn) starts in the gut and ascends to the substantia nigra via the vagus nerve in about a half of PD patients. Epidemiological studies revealed that ulcerative colitis (UC) increased the a risk for PD 1.3 to 1.8-folds. However, it remains unknown whether αSyn is abnormally aggregated in the enteric neurons in UC patients.
Methods: We first inspected and optimized the immunostaining protocols with an anti-phosphorylated αSyn antibody, pSyn#64, using the brain and the gut of eight autopsied cases (five with PD and three without PD). Then, we examined abnormal αSyn aggregation in the enteric neurons in 23 and 18 colectomized patients with and without UC, respectively. Five or more sections were stained for αSyn in each of 87 and 25 paraffin-embedded blocks in patients with and without UC, respectively.
Results: Ten different protocols of epitope exposure appropriately stained aggregated αSyn in the brain, but only a complete lack of epitope exposure stained aggregated αSyn in the colon with low background. Abnormal αSyn aggregates, which was confirmed by co-localization of p62, in the enteric neurons were detected in a single patient with UC but in no patient without UC.
Conclusions: Omission of epitope exposure enabled us to immunostain aggregated αSyn in the colon by pSyn#64 with low nonspecific staining, but the number of 23 UC patients was not high enough to discern whether abnormal αSyn aggregation in the colonic neural plexus was increased in UC or not.

Key words: Parkinson's disease – α-synuclein – p62 – ulcerative colitis – enteric neural plexus.

Abbreviations: ENS: enteric nervous system; IBD: inflammatory bowel disease; IHC: immunohistochemistry; LPS: lipopolysaccharides; PD: Parkinson disease; UC: ulcerative colitis; αSyn: α-synuclein.

INTRODUCTION

Parkinson's disease (PD) is the second most common neurodegenerative disease presenting not only motor symptoms but also non-motor symptoms [1]. Parkinson's disease is caused by the abnormal accumulation of α-synuclein (αSyn) aggregates called Lewy bodies in dopaminergic neurons in the substantia nigra, as well as in the autonomic nervous system [2], the lower brainstem [3], the cerebral cortex, the olfactory bulb [4], and non-neuronal tissues including the skin [5], the salivary glands [6], and the intestine [6-9]. The following lines of evidence point to the notion that aggregated αSyn starts in the gastrointestinal tract and is transmitted to the lower brain stem via the vagus nerve. First, Braak et al. [8, 10] reported in autopsies that Lewy bodies ascend from the dorsal nucleus of the vagus nerve to the substantia nigra in the midbrain. Second, constipation, rapid eye movement sleep behavior disorder, and depression are sometimes observed about 20, 10, and 5 years, respectively, before the onset of motor symptoms in PD [11], which is in accordance with the ascending pathology of Lewy bodies from the vagal nucleus to the locus ceruleus. Third, colonic biopsies frequently show abnormal accumulation of αSyn fibrils in the submucosal neural plexus in PD [12]. Fourth, total vagotomy for treating duodenal ulcer in more than 30 years ago lowers the incidence of PD to approximately 50% in Denmark and Sweden [13, 14].

In addition, three observations in animal models support an intestinal origin for PD. First, αSyn fibrils have the same feature

as prions [15, 16]. Second, intraperitoneal [17] and intragastric [18] injection of αSyn fibrils leads to abnormal αSyn aggregates in the central nervous system. In addition, the vagal nerve has a direct synaptic pathway to the substantia nigra and also to the striatum, which could serve as a route for propagation of αSyn aggregates [19]. Third, vagotomy ameliorates orally administered rotenone-induced accumulation of αSyn fibrils in the dorsal vagal nucleus [20].

Ulcerative colitis (UC) is a chronic inflammatory disease of the colon that causes erosions and ulcers in the colonic mucosa [21, 22]. It is characterized by bloody diarrhea and frequent abdominal pain, and the lesion usually involves the rectum and extends proximally in a continuous pattern [22]. Nationwide cohort studies in USA [23], Denmark [24], South-Korea [25], and Sweden [26] have shown that UC increases a risk of developing PD 1.3- to 1.8-folds compared to individuals without inflammatory bowel disease (IBD) (Table I). This notion, however, was not supported in other studies in USA [27, 28], which showed no or inverse correlation between PD and IBD.

Functional and morphological alterations of the intestinal epithelial barrier, which should also occur in UC, have been demonstrated in PD. First, we and others reported that in patients with PD intestinal permeability was increased and the levels of serum lipopolysaccharide (LPS)-binding protein were decreased [29, 30]. Second, expression of the tight junction protein, occludin, was decreased in PD [31]. Third, intraperitoneal administration of LPS increased intestinal permeability and caused the accumulation of phosphorylated αSyn in the intestinal mucosa and the dorsal vagal nucleus [32].

The epidemiological studies [23-26] prompted us to hypothesize that increased intestinal permeability in patients with UC may facilitate the formation of αSyn aggregates in the enteric nervous system (ENS) of the colon. The presence of aggregated αSyn in the ENS of chronically inflamed colon would support the notion that αSyn aggregates start from the colon and ascend via the vagal nerve into the dorsal vagal nucleus, which is subsequently linked to the dopaminergic neurons in the substantia nigra [19]. Although the immunohistochemistry (IHC) to detect aggregated αSyn in the brain has been well established [33, 34], various immunostaining protocols have been reported with or without success to detect aggregated αSyn in the intestine [35-38]. Thus, we first inspected and optimized the IHC protocols to stain aggregated αSyn in the colon in PD patients. We then stained aggregated αSyn in the ENS in the colon in both UC patients and non-IBD individuals.

METHODS

Subjects

The study was approved by the Ethics Review Committee of the Nagoya University Hospital (NUH) (approval number 2018-292). As the Auerbach's plexus is not usually included in endoscopic biopsy specimens, surgical specimens were analyzed in this study. While Crohn's disease is generally treated with restricted intestinal resection to preserve intact intestinal segments as much as possible, UC is frequently treated with total colectomy to prevent the development of neoplasm and the recurrence of inflammation. We therefore analyzed surgically resected colons in 23 patients with UC and 18 patients without IBD who had undergone colectomy at NUH from 2011 to 2020 and in 2018, respectively.

Specimens from 5 PD patients and 3 non-PD patients who underwent autopsy at NUH from 2008 to 2017 were used to inspect and optimize the IHC protocols for αSyn in the ENS. We stained αSyn aggregates in the ENS of the colons with UC and without IBD. Five or more slices of 4 μm sections of the colon were prepared from formalin-fixed paraffin-embedded tissue block in each patient. Clinicopathological data were retrieved from the medical records of the NUH. The clinical activity and the endoscopic severity of UC before colectomy were assessed by the Mayo score [39] and the UC endoscopic index of severity [40], respectively. Additionally, the histologic activity of collected colonic specimens was scored by the Geboes histopathology score [41].

Immunohistochemistry

Sections of the midbrain and the colon were deparaffinized using standard procedures. Briefly, paraffin was melted at 60°C for 15 minutes and removed from the tissue by rinsing the sections in Lemosol® (Fujifilm Wako Chemicals). The sections were rehydrated by decreasing concentrations of ethanol.

As stated in the results, we inspected and optimized the immunostaining conditions for αSyn aggregates. Our final protocols were as follows. The sections were incubated in 0.3% hydrogen peroxide in methanol at room temperature for 15 minutes to block endogenous peroxidase activity. After blocking with 1.5% horse serum in phosphate buffered saline (PBS), the sections were added with a 1:5000 dilution of the monoclonal antibody, pSyn#64 (Fujifilm Wako Chemicals) followed by incubation at 4°C overnight. The section was rinsed in PBS, and treated with biotinylated anti-mouse IgG diluted in 1.5% horse serum in PBS for 30 minutes at 37°C. The section was rinsed again in PBS and overlaid with Avidin-Peroxidase-Complex (ABC) solution (Vector Laboratories).

Table I. Four retrospective cohort studies showing that ulcerative colitis increases the risk of Parkinson's disease

Study	Country	Study period	Effect estimates	[a]Adjusted risk
Peter et al., 2018 [23]	USA	2000-2016	Rate ratio	1.31 (1.14-1.51)
Villumsen et al., 2019 [24]	Denmark	1977-2014	Hazard ratio	1.35 (1.20-1.52)
Park et al., 2019 [25]	Korea	2010-2013	Hazard ratio	1.85 (1.38-2.48)
Weimers et al., 2019 [26]	Sweden	2002-2014	Hazard ratio	[b]1.3 (1.0-1.7)

[a]95% confidence interval is indicated in parentheses; [b]Adjusted risk was reported in two significant digits.

The section was rinsed again in PBS, and then incubated with 3,3'-diaminobenzidine (DAB) and hydrogen peroxide for 3 minutes, followed by counterstaining with hematoxylin.

For co-immunofluorescent staining for aggregated αSyn and p62 of the Auerbach's neural plexus, a section serial to that for DAB staining stated above was obtained. After serial deparaffinization, the slice was blocked with 5% BSA overnight at 4°C. The section was treated with a 1:1000 dilution of the anti-mouse monoclonal pSyn#64 antibody, and anti-guinea pig polyclonal p62/SQSTM1 (C-terminus) antibody (PROGEN Biotechnik) followed by incubation at 4°C overnight. The section was rinsed in PBS and treated with a 1:1000 dilution of Alexa Fluor 488-conjugated anti-mouse IgG and Alexa Fluor 594-conjugated anti-guinea pig IgG for 1 hour at room temperature.

Observations

Sections immunostained for aggregated αSyn were examined with an upright microscope (BX53, Olympus), an inverted microscope (IX73, Olympus), and a virtual slide system (VS120-S5-J, Olympus). In the brain and colon sections, αSyn aggregation was scrutinized in the substantia nigra and the Meissner's or Auerbach's neural plexuses, respectively. Positively stained sections were anonymously mixed with negatively stained sections and were inspected by a blinded observer.

Sections co-immunostained for aggregated αSyn and p62 were observed under confocal microscopy (SpinSR10, Olympus).

RESULTS

Inspection and Optimization of IHC Method for Aggregated αSyn

We firstly stained the substantia nigra in five autopsied PD patients for aggregated αSyn using anti-phosphorylated αSyn antibody, pSyn#64. The epitope exposure by autoclaving at 105°C for 10 min followed by 99% formic acid treatment at room temperature for 10 min positively stained aggregated αSyn in the substantia nigra in five PD patients (left panel of Fig. 1I). This condition, however, nonspecifically stained the colonic muscular layer in PD patients (right panel of Fig. 1I). We thus inspected protocols flor epitope exposure with nine additional combinations of autoclaving and formic acid treatment using sections containing the substantia nigra and the colonic Auerbach's neural plexus in three to five PD patients. Although all the tried conditions appropriately stained aggregated αSyn in the substantia nigra, all the conditions failed to stain aggregated αSyn in the Auerbach's neural plexus except for that without autoclaving or formic acid treatment (Fig. 1). The failure was either due to nonspecific nuclear (Fig. 1CDE) or diffuse (Fig. 1BFG) staining of the neural plexus, or strong background staining (Fig. 1HIJ). To our surprise, complete elimination of the epitope exposure procedures enabled us to appropriately stain aggregated αSyn in the substantia nigra as well as in the colon (Fig. 1A and Fig. S1). Our final protocol is indicated in the materials and methods.

Inspection of Aggregated αSyn in the Colon in Patients with UC

Demographic and clinical features of patients with UC and control subjects without IBD are shown in Table II. There was no significant difference in age and gender between the two groups. The disease durations of the 23 UC patients was widely variable from 3 to 444 months with the mean of 153 months. A total of 87 colonic samples in 23 patients with UC and 25 colonic samples in 18 patients without IBD were analyzed (Tables III and IV). We analyzed all available non-cancerous specimens for each patient, but the number of specimens in each colonic segment was variable from patient to patient because of the heterogeneity in the purpose of colectomy, localization of the tumor, if any, and the surgeon's preferences. Five or more sections of each colon sample were immunostained for αSyn aggregates. Aggregated αSyn was observed in a single sample in a UC patient, but not in any of the control samples (Fig. 2). Co-immunostaining for pSyn#64 and p62 revealedt heir co-localization in the Auerbach's neural plexus (Fig. 3). Thus, one of the 87 samples with UC and none of the 25 samples without IBD were positive for Syn aggregates, which gave rise to no statistical significance.

DISCUSSION

We observed that variable protocols of epitope exposure appropriately stained aggregated αSyn in the substantia nigra with pSyn#64 antibody, an anti-phosphorylated αSyn monoclonal antibody [42]. Most of these protocols, however, failed to specifically stain aggregated αSyn in the colon (Fig. 1). In a multi-center study, four IHC methods from four expert laboratories to stain aggregated αSyn in colonic biopsies were evaluated by four expert pathologists [43]. The study gave rise to low specificity and low sensitivity, and none of the four methods appropriately immunostained aggregated αSyn in colonic biopsies. A systematic review similarly concluded that IHC for aggregated αSyn in the gastrointestinal tract is poorly reproducible [35]. When pSyn#64 was not used, most previous reports exposed epitopes before immunostaining aggregated αSyn in the gastrointestinal tract [43-50]. In contrast, when pSyn#64 was used, most previous reports omitted epitope exposure to immunostained aggregated αSyn in the gastrointestinal tract [51-55]. We also confirmed that lack of autoclaving and lack of formic acid treatment stained aggregated αSyn in the colon with low nonspecific staining in autopsied patients with PD (Fig. S1). Phosphorylated Ser129 recognized by pSyn#64 is located in the flexible domain close to the C terminal end of αSyn [56, 57]. We speculate that phosphorylated Ser129 in the flexible domain was not cross-linked by formalin fixation, and procedures to expose epitope rather enhanced artifacts in other molecules. Alternatively, phosphorylated Ser129 in early stages of αSyn aggregation that we observed in the colon might be vulnerable to epitope exposure procedures.

Multiple epidemiological studies have shown that UC is a risk for PD [23-26], although a lack of association [27] or an inverse association [28] has been also reported.

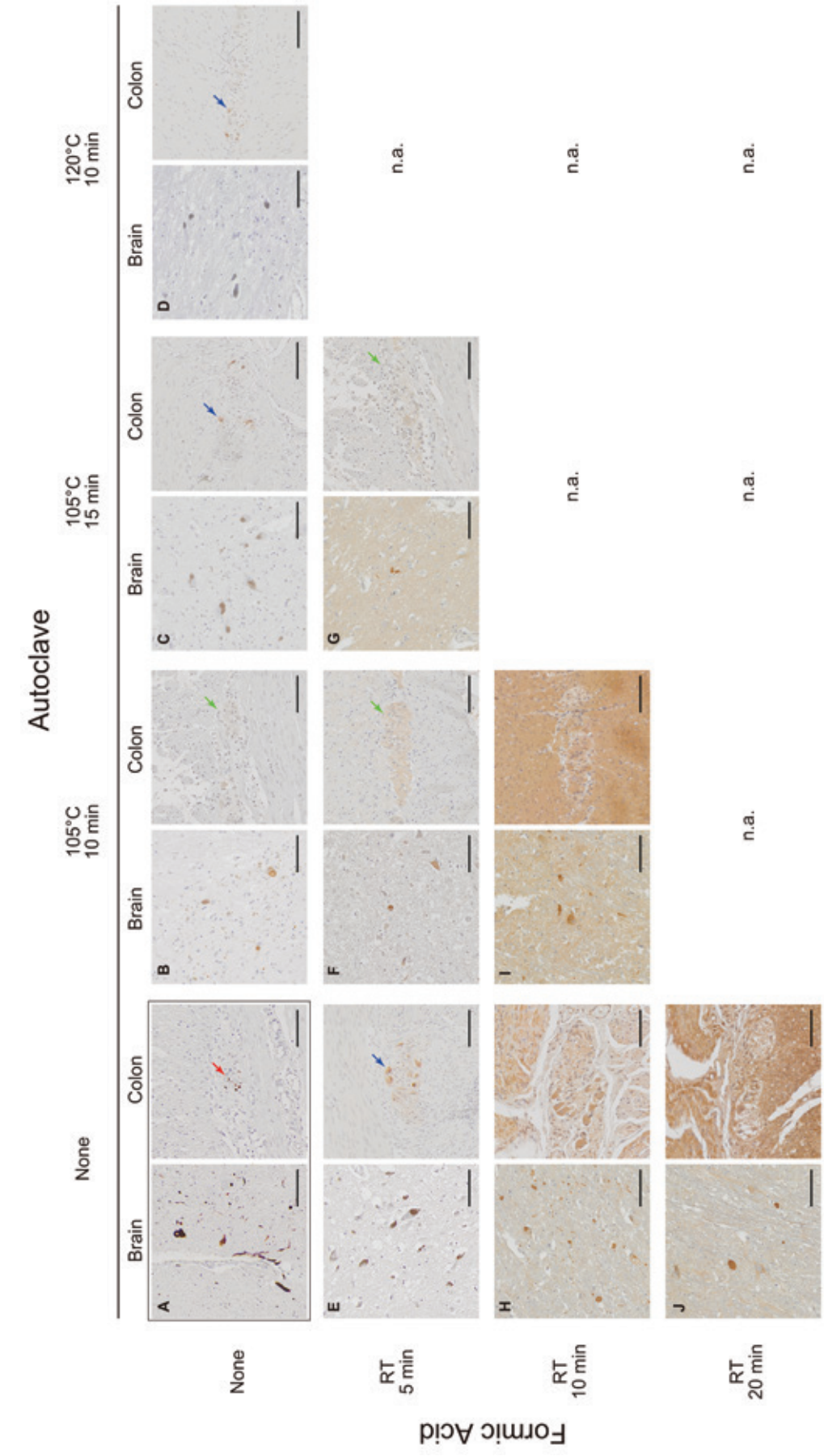

Fig. 1. Representative immunostaining images for aggregated αSyn of sections treated with different conditions of autoclaving and formic acid treatment. The substantia nigra and the Auerbach's neural plexus of the colon are immunostained for aggregated αSyn (pSyn #64) in five autopsied patients with Parkinson's disease, and representative images are indicated on the left and right panels, respectively, in a pair. The inner ring muscle is aligned to the top of each right panel. Note that αSyn aggregates in the substantia nigra are appropriately stained under all the conditions and that αSyn aggregates in the colon are appropriately stained only in A. A red arrow points to one of successfully stained aggregated αSyn. Blue and green arrows point to representative nonspecific nuclear and diffuse staining of the neural plexus, respectively. Images in A are also indicated at low magnification in Supplementary Fig. 1. Note that colon sections in H, I, and J are nonspecifically stained for the background. n.a., not available. Scale bar = 100 μm.

Table II. Demographic and clinical features of ulcerative colitis (UC) and control groups

	UC group (n = 23)	Control group (n = 18)	P value
[a]Age (range)	52.0 ± 13.4 years (33-79)	52.3 ± 14.8 years (28-80)	[b]0.950
Male/Female	15/8	11/7	[c]0.793
[a]Disease duration (range)	183 ± 127 months (3-444)	-	n.d.
Reason for colectomy	19 UC-associated neoplasia 4 Refractory to medical treatment	13 Colorectal cancer 2 Colon perforation 1 Endometriosis 1 Ovarian tumor 1 Uterine sarcoma	n.d

[a]Mean and SD are indicated; [b]Student's t-test; [c]Fisher's exact test; n.d.: not determined.

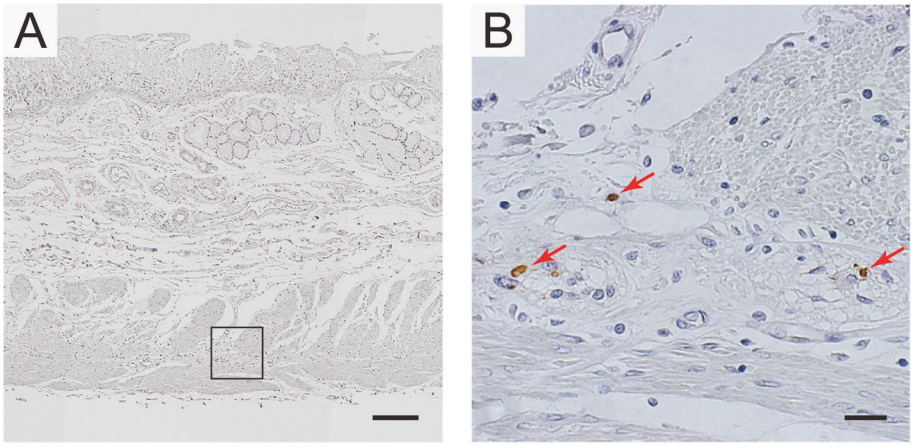

Fig. 2. Aggregated αSyn observed in the ascending colon of a patient with ulcerative colitis. Arrows point to aggregated αSyn. A rectangle in A is enlarged in B. Scale bar = 200 μm (A) and 20 μm (B).

We hypothesized that there might be aggregated αSyn in the colon in patients with UC. Contrary to our hypothesis, however, abnormal aggregation of αSyn in the colon in UC patients was not frequent compared to controls. This was consistent with a previous report showing that the expressions of αSyn were similar in 4 UC patients and 4 healthy controls [58]. Lack of significant difference between UC patients and controls was likely to be accounted for the following reasons. First, the colectomized sample positive for aggregated αSyn was from a 62-year-old man with a disease duration of UC for 16 years and 2 months. Although 15 of the 22 UC cases without colonic αSyn aggregation had a disease duration of 10 years or more, this period of time might be too short to observe aggregated αSyn in the colon. Secondly, the number of UC patients and the number of samples per patient were likely to be insufficient. Even in the colonic samples in PD patients, one or two aggregated αSyn were observed per section in the colon. Analysis of more UC patients and more samples per patient would have produced more positive samples. Thirdly, UC patients in our cohort might be well controlled compared to those that were analyzed in epidemiological studies [23-26]. Currently, there are several effective drugs available for UC, and many patients remained in remission, which potentially preclude inflammation in the colon. αSyn might not aggregate in the UC colons when chronic inflammation has been under control, even if the patients have been suffering from UC for a long term.

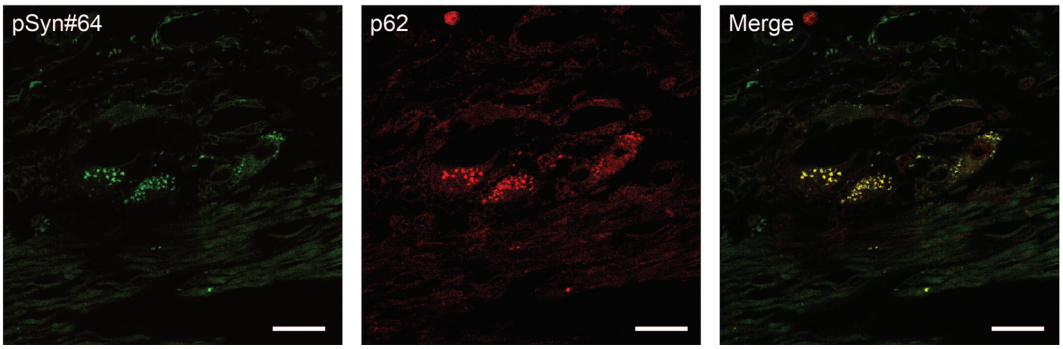

Fig. 3. Aggregated αSyn colocalized with p62 in the Auerbach's neural plexus in a patient with ulcerative colitis
A section serial to that stained in Fig. 2 was immunostained for aggregated αSyn (pSyn #64) and p62. Scale bar = 20 μm.

Table III. Details of each specimen in the ulcerative colitis group

Age	Gender	Extent of involvement	Disease duration (months)	Medication upon colectomy	Mayo Score [39]	UCEIS [40]	Reason for colectomy	Specimen site	GHS [41]	αSyn
73	M	Pancolitis	444	5-ASA	1	1-0-1	UCAN	A/C	1.1	-
								S/C		-
52	F	Pancolitis	422	5-ASA AZA Golimumab	2	1-1-2	UCAN	A/C	1.1	-
								T/C		-
								T/C		-
								D/C		-
								S/C		-
								R		-
								R		-
52	M	Pancolitis	303	5-ASA Infliximab	n.a.	n.a.	UCAN	A/C	1.1	-
								T/C		-
50	M	Pancolitis	303	5-ASA PSL	2	1-0-2	UCAN	C	1.2	-
								C		-
								C		-
								C		-
								C		-
								C		-
								A/C		-
								A/C		-
								A/C		-
								A/C		-
								A/C		-
57	F	Left-sided colitis	301	5-ASA	1	1-0-1	UCAN	R	1.2	-
								D/C		-
								C		-
49	M	Pancolitis	295	5-ASA	2	1-2-2	UCAN	D/C	3.1	-
								T/C		-
40	M	Pancolitis	278	5-ASA AZA	0	0-0-0	UCAN	C	1.1	-
								D/C		-
								S/C		-
56	M	Left-sided colitis	250	5-ASA	0	1-0-0	UCAN	S/C	2B.1	-
46	F	Pancolitis	237	5-ASA PSL	n.a.	n.a.	UCAN	A/C	4.1	-
								S/C		-
								T/C		-
33	F	Pancolitis	194	5-ASA	0	0-0-0	UCAN	C	0.1	-
								A/C		-
								T/C		-
								T/C		-
								T/C		-
								T/C		-
								T/C		-
								T/C		-
								D/C		-
								S/C		-
								S/C		-
								R		-
62	M	Pancolitis	194	5-ASA	1	1-0-0	UCAN	A/C	1.2	+

Table III (continued)

33	M	Pancolitis	153	5-ASA AZA PSL	3	1-1-1	UCAN	R C D/C	1.3	- - -
35	M	Unknown	151	5-ASA PSL	6	2-1-2	UCAN	D/C S/C	5.4	- -
39	F	Pancolitis	147	5-ASA PSL Infliximab	5	2-2-2	UCAN	A/C D/C S/C	5.4	- - -
61	F	Pancolitis	125	5-ASA PSL	3	1-0-1	UCAN	R S/C D/C A/C T/C	5.1	- - - - -
37	F	Left-sided colitis	122	5-ASA AZA Golimumab	7	2-2-2	UCAN	R R R R	5.4	- - - -
58	M	Pancolitis	99	5-ASA	1	0-0-0	UCAN	R S/C T/C D/C	1.1	- - - -
57	M	Pancolitis	91	5-ASA	1	0-0-0	UCAN	A/C	1.1	-
37	M	Pancolitis	45	5-ASA PSL Infliximab	11	2-3-3	Refractory to medical treatment	App A/C T/C S/C	5.4	- - - -
75	M	Left-sided colitis	26	5-ASA	1	0-0-0	UCAN	R R	1.1	- -
65	F	Left-sided colitis	24	5-ASA PSL Adalimumab	9	2-1-2	Refractory to medical treatment	S/C A/C C T/C D/C	4.1	- - - - -
50	M	Pancolitis	10	5-ASA Cyclosporine	8	2-1-2	Refractory to medical treatment	S/C T/C	5.3	- -
79	M	Pancolitis	3	5-ASA PSL	10	2-3-3	Refractory to medical treatment	A/C T/C S/C D/C	5.4	- - - -

5-ASA: 5-aminosalicylic acid; A/C: ascending colon; αSyn: phosphorylated α-synuclein in the intestinal neural plexus; App: appendix; AZA: azathioprine; C: cecum; GHS: D/C: descending colon; Geboes histopathology score; n.a.: not available; PSL: prednisolone; R: rectum; UCAN: ulcerative colitis-associated neoplasia; S/C: sigmoid colon; T/C: transverse colon; UCEIS: ulcerative colitis endoscopic index for severity.

As shown in Table III, 19 of the 23 patients with UC had undergone colectomy for UC-associated neoplasia (UCAN) not for aggravation of UC. In patients with UCAN, 78.9% had the Mayo score [39] of 5 or less, and 68.4% had the Geboes histopathology score [41] of grade 2 or less, indicating that the majority were in remission both clinically and pathologically. We might have detected aggregated αSyn if we would have had examined more patients who had repeated relapses and remissions over a long period of time, such as refractory patients or patients with poor drug adherence. Fourthly, a meta-analysis [59] based on five studies showed that patients aged 60 years or older at the time of IBD diagnosis had a significantly increased risk of PD, whereas patients aged less than 50 years had no association with PD. In our cohort, the only patient with positive colonic αSyn aggregates was diagnosed with UC at the age 45 years and was colectomized at the age of 62 years.

Table IV. Details of each specimen in the control group

Age	Gender	Reason for colectomy	Specimen cite	αSyn
28	F	Ovarian tumor	S/C	-
			C	-
36	F	Colorectal cancer	S/C	-
37	F	Colorectal cancer	T/C	-
39	F	Endometriosis	S/C	-
40	M	Colorectal cancer	A/C	-
43	M	Colorectal cancer	R	-
48	M	Colorectal cancer	R	-
48	M	Colorectal cancer	A/C	-
			App	-
			C	-
50	M	Colorectal cancer	S/C	-
56	M	Colorectal cancer	R	-
57	M	Colorectal cancer	S/C	-
58	F	Colorectal cancer	R	-
64	F	Colorectal cancer	A/C	-
66	F	Uterine sarcoma	A/C	-
73	M	Colon perforation	S/C	-
75	F	Colorectal cancer	S/C	-
75	M	Colorectal cancer	S/C	-
80	M	Colon perforation	S/C	-

For abbreviations see Table III.

However, 17 out of 23 patients (73.9%) in our cohort were less than 50 years of age when UC was diagnosed. Paucity of positive colonic αSyn aggregates in our cohort may be partly accounted for by younger ages at UC diagnosis. Fifth, early exposure to anti-tumor necrosis factor (anti-TNF) therapy was associated with a substantially reduced incidence of PD [60]. In our study, six patients (26.1%) were on anti-TNF therapy, and the single case with positive colonic αSyn aggregates had no history of anti-TNF therapy. Anti-TNF therapy might have reduced a chance of detecting aggregated αSyn in our cohort.

We observed that p62 was co-localized with aggregated αSyn in the Auerbach's neural plexus in a UC patient positive for aggregated αSyn in the intestine. Sequestosomal protein p62, which is a ubiquitin-binding protein, is a component protein of Lewy bodies [61]. p62 is an adaptor protein that links ubiquitous proteins to autophagosome, which serves as a defense mechanism against misfolded proteins [62]. Therefore, colocalization of p62 and pSyn#64 indicates that the pSyn#64 signals observed in the intestine in a UC patient was likely to have represented aggregated αSyn.

Gut dysbiosis has been repeatedly reported in UC patients [63-69] and PD patients [70-77]. We reported in a meta-analysis of the gut microbiota in Japan, U.S., Finland, Russia, and Germany that genus Akkermansia was increased in PD patients across countries [76]. Akkermansia degrades the intestinal mucin layer [78], which should increase permeability of the intestinal wall [29, 30], leading to αSyn aggregates in the intestinal neural plexus. Indeed, the serum LPS-binding protein was decreased in PD [29, 30]. Thus, UC [79] and PD [80] share the same feature of increased permeability of the intestinal wall, so-called leaky gut. Frequent observation of aggregated αSyn in the colon in patients with PD but not in UC, however, may indicate that another unidentified factor is required to initiate the aggregation of αSyn in the colon.

CONCLUSIONS

Omission of epitope exposure enabled us to immunostain aggregated αSyn in the colon by pSyn#64 with low nonspecific staining. However, the number of 23 UC patients, which was the highest among similar studies, was still not high enough to discern whether abnormal αSyn aggregation in the colonic neural plexus was increased in UC or not.

Conflicts of interest: None to declare.

Authors' contributions: N.G., T.H., Y.H., and K.O. conceived the study. N.G. collected the samples with the help of T.Y., M. Nakaguro., M. Nakamura., and H.K. N.G. performed the immunostaining and evaluation with the help of T.H., Y.M., M.I., M.H., and K.W. N.G., T.H., and K.O. wrote the manuscript. All authors critically revised and approved the manuscript.

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