RESEARCH ARTICLE

Increased Accumulation of α-Synuclein in Inflamed Appendices of Parkinson's Disease Patients

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ABSTRACT: Background: The accumulation of α-synuclein (α-Syn) aggregates that leads to the onset of Parkinson's disease (PD) has been postulated to begin in the gastrointestinal tract. The normal human appendix contains pathogenic forms of α-Syn, and appendectomy has been reported to affect the incidence of PD.
Objective: This study investigated appendix abnormality in patients with PD.
Methods: We assessed appendix morphology in 100 patients with PD and 50 control subjects by multislice spiral computed tomography. We analyzed the clinical characteristics of patients with PD with diseased appendices, which was confirmed in seven patients by histopathological analysis.
Results: Chronic appendicitis-like lesions were detected in 53% of patients with PD, but these were not associated with the duration of motor symptoms. Appendicitis-like lesions, impaired olfaction, and rapid eye movement sleep behavior disorder were risk factors for PD. The following clinical symptoms could be used to identify patients with PD with appendicitis-like lesions: first motor symptoms were bradykinesia/rigidity, onset of motor symptoms in the central axis or left limb, prodromal constipation, high ratio of Unified Parkinson's Disease Rating Scale Part III score to symptom duration, low Montreal Cognitive Assessment score, and high Epworth Sleepiness Scale score. The seven patients with PD who were diagnosed with chronic appendicitis underwent appendectomy, and histopathological analysis revealed structural changes associated with chronic appendicitis and α-Syn aggregation.
Conclusions: These results indicate an association between chronic appendicitis-like lesions and PD, and suggest that α-Syn accumulation in the diseased appendix occurs in PD. © 2021 International Parkinson and Movement Disorder Society

Key Words: Parkinson's disease; appendix; appendicitis; diagnosis; α-synuclein

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The etiology of Parkinson's disease (PD) is not known, and the clinical manifestations are heterogeneous.[1-3] PD has long been considered to be a brain disease, but there is increasing evidence that, in some cases, PD begins in the intestine.[4] Constipation is the earliest prodrome of PD and can occur 10–20 years before emergence of motor symptoms.[5,6] In addition, abnormal α-synuclein (α-Syn) protein, a pathogenic marker of PD, has been detected in the colon.[7] Abnormal α-Syn is similar to prion protein in that it can spread between cells and from the gastrointestinal muscle layer through sympathetic nerves to the brainstem and midbrain substantia nigra, causing the appearance of PD motor symptoms.[8,9]

The production and release of abnormal α-Syn may be related to the appendix, which is a tissue enriched in immune cells that is also involved in the regulation of intestinal microbes.[10] Evidence from several studies indicates a link between gut microbes and intestinal inflammation in PD,[11,12] and some epidemiological studies have reported an association between removal of the appendix and PD risk, although this is controversial.[13-16] However, α-Syn is enriched in the healthy appendix, and normal α-Syn has been shown to aggregate when cultured with appendix tissue lysates.[13] Both the appendix and gastrointestinal tract are innervated by the vagus nerve; thus, the appendix may provide clues regarding the molecular pathogenesis of PD, as well as the mechanisms by which α-Syn aggregates are transferred from the gut to the brain.

In this study, we investigated the relationship between appendix abnormality and PD by examining the accumulation of α-Syn in the appendix and analyzing the clinical characteristics of patients with PD with appendix lesions.

Subjects and Methods

Ethics Approval of the Study Protocol

Ethics approval for the study was obtained from the Research Ethics Board of the First Affiliated Hospital of University of Science and Technology of China (Hefei, China). The study protocol was in accordance with the Declaration of Helsinki. All patients provided written informed consent to participate. This study has been registered in the Chinese Clinical Trial Registry (registration number: ChiCTR1900026156).

Participants

Patients with PD were recruited at the Department of Neurology of the First Affiliated Hospital according to Movement Disorder Society PD criteria.[17] Inclusion criteria were as follows: age ≥40 to <80 years; duration of motor symptoms <5 years; and without family history of PD but with history of appendectomy, abdominal pain, or tenderness at the McBurney point. The 50 control subjects were from age-matched healthy volunteers. Also, six normal appendix tissue samples came from the discarded appendices of the patients undergoing right colon surgery.

Baseline and Clinical Assessments

Motor and nonmotor symptoms were assessed in patients without antiparkinsonian medicine treatment history or 24 hours after antiparkinsonian medication had been stopped in patients who were receiving it. Evaluations were performed by a specialist in movement disorders who was blinded to the results of multislice spiral computed tomography (MSSCT) and three-dimensional (3D) reconstruction of images of the appendix.

Variables

Demographic variables included in the analysis were age, sex, education level, age at PD onset, symptom duration, and symptoms at PD onset. Hoehn and Yahr stage and Parkinson's Disease Questionnaire-39 score were also obtained.[18]

Motor manifestations were assessed using the International Parkinson's Disease and Movement Disorder Society Unified Parkinson's Disease Rating Scale (MDS-UPDRS) Parts II and III.[17]

Nonmotor manifestations were also documented. Constipation was assessed using the Knowles–Eccersley–Scott Symptom questionnaire.[19] Olfactory dysfunction was determined based on the age/sex-adjusted University of Pennsylvania Smell Identification Test score.[20] Sleep behavior was assessed with the Rapid Eye Movement Sleep Behavior Disorder Questionnaire—Hong Kong (RBDQ-HK).[21] Cognitive impairment was evaluated with the Montreal Cognitive Assessment (MoCA) Scale.[22] Sleep disturbance was assessed using the Epworth Sleepiness Score (ESS) and Pittsburgh Sleep Quality Index (PSQI).[23,24] Anxiety and depression were evaluated with the Hamilton Anxiety Scale and Hamilton Depression Scale, respectively.[25,26] Fatigue was quantified with the Fatigue Severity Scale.[27] Autonomic dysfunction was determined based on total scores for the Scales for Outcomes in Parkinson's Disease Autonomic and its subsections (orofacial, constipation, urinary, cardiovascular, thermoregulatory, pupillomotor, and sexual).[28] To evaluate other nonmotor features without specific instruments for measurement (hallucinations, apathy, pain, and fatigue), we used single items from MDS-UPDRS Part I.

PD progression is expressed as MDS-UPDRS total score/duration of motor symptoms. Constipation, rapid eye movement sleep behavior disorder (RBD), and olfactory dysfunction before the appearance of motor symptoms were considered to be prodromes.

MSSCT of the Appendix and Colon

MSSCT was performed using a 64-slice SCT scanner (Brilliance 64 VCT; Philips, Amsterdam, the Netherlands). The scanning parameters were as follows: voltage = 120 kV; current = 200 mA; volume acquisition = 1 mm × 16, pitch = 1.0; scan time = 0.5 second per turn; layer thickness = 1.0 mm, pitch = 0.5 mm, and matrix = 256 × 256.

With the patient lying supine on a table, having taken orally meglumine diatrizoate (40 mL; Shanghai Xudong Haipu Pharmaceutical Co., Ltd., Shanghai, China) 3–4 hours ago, images were acquired from the iliac crest to the pubic symphysis. The thin-layer recombination image was transferred to a Vitrea2 workstation (Siemens, Munich, Germany). Coronal, sagittal, and surface images were reorganized along the appendix and colon. Radiologists evaluated the images and interpreted them at the workstation. The diagnostic criteria for chronic appendicitis were appendix enlargement >6 mm, thickening of the appendix wall >3 mm, inflammatory mass in the tissue surrounding the appendix, visible calculus in the appendix cavity, blurred frontal contour of the psoas muscle, fluid in or thickening of the right abdominal fascia, and localized thickening of the wall of the cecum.[29]

Surgical Consultation

After chronic appendicitis-like lesions were detected by MSSCT, patients were informed that they may have chronic appendicitis and were advised to visit the department of general surgery; each consulted patient underwent the palpation of the appendix during physical examination. Neither the patients nor the surgeons who treated them were told that the chronic appendix disease was related to PD. According to the Chinese version of "*Surgery*" (2020, Chen Xiaoping, Wang Jianping. People's Medical Publishing House, Ninth Edition, page 377) and "*Clinical Diagnosis and Treatment Guidelines – Surgery Division*" (2006, Chinese Medical Association, People's Medical Publishing House, pages 253–254), patients with chronic appendicitis diagnosed by MSSCT were advised to have appendectomy by the surgeon. Among 53 patients with PD and four control subjects with imaging evidence for appendicitis, only 7 patients with PD consented for surgery.

Hematoxylin and Eosin Staining

The resected appendix was fixed in 4% formalin for 24 hours, embedded in paraffin, and cut into sections at a thickness of 4 μm. The sections were deparaffinized in xylene and rehydrated in ethanol, then stained with hematoxylin solution for 3 to 5 minutes and rinsed with water. The sections were immersed in ammonia solution, followed by 85% alcohol solution for 5 minutes, 95% alcohol solution for 5 minutes, and eosin for 5 minutes. The sections were dehydrated and mounted with resin.

Fluorescence Immunohistochemistry

The appendix tissue was embedded in paraffin and cut into sections. The deparaffinized sections were subjected to antigen retrieval and incubated with an autofluorescence quencher (G1221, Servicebio, Wuhan, China), then rinsed for 10 minutes. After adding bovine serum albumin dropwise to the sections and incubating for 30 minutes to block nonspecific antibody binding, the sections were incubated overnight at 4°C with antibodies against neurofilament-200 (NF200, 1:100, GB13141, Servicebio, Wuhan, China) or α-Syn (1:200, ab6162, Abcam, Cambridge, UK), followed by Cy3-conjugated goat anti-mouse IgG (1:300, GB21301, Servicebio, Wuhan, China) or fluorescein isothiocyanate-conjugated donkey anti-goat IgG (1:300,GB22404, Wuhan, China) at room temperature for 50 minutes. The sections were then stained with 4′,6-diamidino-2-phenylindole (G1012, Servicebio, Wuhan, China) for 10 minutes at room temperature in the dark to label nuclei and then sealed with antifluorescence quenching mounting tablets. The sections were observed under a fluorescence microscope at the following excitation/emission wavelengths: 4′,6-diamidino-2-phenylindole, 330 to 380/420 nm; fluorescein isothiocyanate, 465 to 495/515 to 555 nm; and Cy3, 510 to 560/590 nm.

Statistical Analyses

Statistical analyses were carried out using SPSS v17 (IBM, Armonk, NY, USA). Quantitative data were shown as mean ± standard deviation, and qualitative data were presented as numbers and percentages. Quantitative data with a normal distribution were analyzed with the independent two-sample test, and quantitative data (PSQI, RBDQ-HK, Hamilton Anxiety Scale, and Hamilton Depression Scale scores) that did not follow a normal distribution were analyzed using nonparametric tests. Qualitative data were analyzed with the χ^2 test. A P value <0.05 was considered significant. Multivariate analysis was performed with a logistic regression model, with 0.05 and 0.10 as the significance cutoffs for selected and excluded variables, respectively.

Results

Chronic Appendicitis-like Lesions are Imaging Markers in Patients with PD

All 100 patients with PD and 50 control subjects underwent MSSCT, with 3D reconstruction of the appendix and colon. Complete appendix images were available for 147 patients; the appendix was not

visualized in three patients, but data were available after barium enema. In 53 patients with PD and four control subjects, the appendix was swollen, with a diameter >6 mm and thickening of the appendix wall to >3 mm, and the lumen was narrow or had fecal stones. These patients met diagnostic criteria for chronic appendicitis. Representative images of normal and abnormal appendices from patients with PD are shown in Figure 1. The colonic mucosa in these patients was smooth, and there were no abnormal findings, such as ulcers or stenosis of the lumen. Thus, chronic appendicitis-like lesions in these patients with PD were independent of abnormalities of the colon.

Chronic Appendicitis-like Lesions are Not Associated with PD Duration

To determine whether chronic appendicitis in patients with PD was affected by PD duration, we counted the number of patients with PD with versus without appendicitis-like lesions who had PD for 6, 12, 18, 24, 30, 36, 42, 48, 52, or 60 months and found that the incidence of appendicitis-like lesions did not increase with longer disease duration (χ^2_{trend} = 1.571, P = 0.210) (Supporting Information Fig. S1).

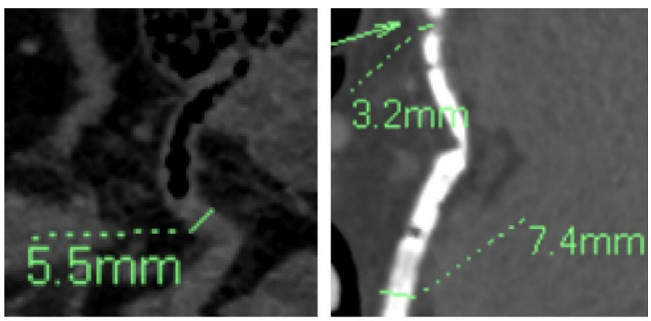

FIG. 1. Representative computed tomography images of appendices of patients with Parkinson's disease (PD) with chronic appendicitis-like lesions and non-PD patients with normal appendices (healthy control [HC]). The measured values represent the diameter of the appendix and were calculated by the imaging software. [Color figure can be viewed at wileyonlinelibrary.com]

Chronic Appendicitis-like Lesions are Risk Factors for PD

We investigated risk factors related to PD onset in 100 patients and 50 healthy subjects without PD. Ten risk factors were evaluated: age, sex, years of education, physical exercise, negative life events, constipation, olfactory disorder, exposure to an agricultural environment, RBD, and chronic appendicitis-like lesions. The univariate analysis showed that age, University of Pennsylvania Smell Identification Test score, constipation, appendicitis-like lesions, RBD, and exposure to an agricultural environment were significantly associated with development of PD ($P < 0.05$). However, in the multivariate logistic regression model, only chronic appendicitis-like lesions (odds ratio [OR] = 46.786, P = 0.026), olfactory disorder (OR = 0.673, P = 0.003), and RBD (OR = 292.098, P = 0.004) were risk factors for PD (Table 1).

Clinical Features of Patients with PD with Chronic Appendicitis-like Lesions

Demographics, prodromes, motor symptoms, and nonmotor symptoms were evaluated in 100 patients with PD. To exclude associations between variables, we analyzed nonmotor symptoms as a separate set of variables. Multivariate regression analysis was performed to predict clinical features and nonmotor symptoms in patients with PD that could lead to chronic appendicitis-like lesions.

Clinical symptoms of patients with PD with chronic appendicitis-like lesions included bradykinesia/rigidity as initial motor symptoms (OR = 0.012, $P < 0.001$), initial onset of PD in the central axis (OR = 67.422, $P < 0.001$) or left upper limb (OR = 12.520, $P < 0.001$),

TABLE 1. Single-factor and multiple-factor analyses of the risk factors for PD

			Univariate analysis		Multivariate logistic regression analysis			
Characteristics	Non-PD (n = 50)	PD (n = 100)	χ^2/t	P	B	OR	OR (95% CI)	P
Sex (men), n, (%)	29 (58.0%)	50 (50.0%)	0.856	0.355	–	–	–	–
Education history, years	7.78 ± 3.6	6.22 ± 6.83	1.837	0.068	–	–	–	–
Age, years	58.28 ± 7.43	62.07 ± 8.26	−2.736	0.007	−0.089	0.915	0.788–1.062	0.244
UPSIT (% expected)	97.64 ± 3.88	45.57 ± 27.35	18.667	<0.001	−0.396	0.673	0.519–0.872	0.003
APP (n, %)	4 (8.0%)	53 (53.0%)	28.650	<0.001	3.846	46.786	1.579–1386.160	0.026
Constipation (n, %)	6 (12.0%)	56 (56.0%)	26.066	<0.001	−0.717	0.488	0.022–10.988	0.652
RBD (n, %)	1 (2.0%)	37 (37.0%)	21.587	<0.001	5.677	292.098	5.831–14,632.865	0.004
Physical exercise, n (%)	19 (38.0%)	23 (23.0%)	3.720	0.054	–	–	–	–
Negative life event (n, %)	3 (6.0%)	13 (13.0%)	1.714	0.190	–	–	–	–
Agricultural environmental exposure (n, %)	3 (6.0%)	45 (45.0%)	23.300	<0.001	1.207	3.344	0.034–329.287	0.606

PD, Parkinson's disease; OR, odds ratio; APP, appendicitis-like lesions; CI, confidence interval; UPSIT, University of Pennsylvania Smell Identification Test; RBD, rapid eye movement sleep behavior disorder.

TABLE 2. Clinical features of patients with PD with chronic appendicitis-like lesions

Characteristics	PD no APP (n = 47)	PD with APP (n = 53)	Univariate analysis		Multivariate logistic regression analysis			
			χ^2/t	*P*	B	OR	OR (95% CI)	*P*
Sex (men), n (%)	20 (42.6%)	30 (56.6%)	1.967	0.161	–	–	–	–
Education history, years	6.64 ± 8.92	5.85 ± 4.27	0.575	0.567	–	–	–	–
Age at onset, years	59.11 ± 7.88	60.06 ± 8.70	−0.570	0.570	–	–	–	–
Initial motor symptom at onset			36.761	<0.001				
Bradykinesia/rigidity, n (%)	15 (31.9%)	48 (90.6%)				1.000		
Tremor, n (%)	32 (68.1%)	5 (9.4%)			−4.410	0.012	0.002–0.085	<0.001
Part onset			40.67	0.001				
Right limb	32 (68.1%)	5 (9.4%)				1.000		
Left limb	12 (25.5%)	22 (41.5%)			2.527	12.520	2.805–55.880	0.001
Central axis	3 (6.4%)	26 (49.1%)			4.211	67.422	8.937–508.619	<0.001
MDS-UPDRS-motor Part II / symptoms duration	0.45 ± 0.25	0.91 ± 1.27	−2.561	0.013	−4.018	0.018	0.000–1.568	0.078
MDS-UPDRS-motor Part III / symptoms duration	1.07 ± 0.47	2.43 ± 4.50	−2.178	0.034	3.126	22.793	1.897–273.901	0.014
Olfactory decline before motor symptoms (n, %)	17 (36.2%)	18 (34.0%)	0.053	0.817	–	–	–	–
RBD before motor symptoms (n, %)	15 (31.9%)	8 (15.1%)	3.980	0.046	−1.317	0.268	0.060–1.198	0.085
Constipation before motor symptoms (n, %)	5 (10.6%)	28 (52.8%)	20.056	<0.001	1.817	6.154	1.481–25.563	0.012

PD, Parkinson's disease; APP, appendicitis-like lesions; OR, odds ratio; CI, confidence interval; MDS-UPDRS, Movement Disorder Society Unified Parkinson's Disease Rating Scale; RBD, rapid eye movement sleep behavior disorder.

TABLE 3. Non-motor symptoms of patients with PD with chronic appendicitis-like lesions

Characteristics	PD no APP (n = 47)	PD with APP (n = 53)	Univariate analysis		Multivariate logistic regression analysis			
			χ^2/t	*P*	B	OR	OR (95% CI)	*P*
Pain, n (%)	6 (12.8%)	17 (32.1%)	5.244	0.022	0.561	1.752	0.448–6.847	0.420
Constipation, n (%)	19 (40.4%)	37 (69.8%)	8.730	0.003	1.963	7.121	2.092–24.242	0.002
Motor / symptoms duration	32.62 ± 18.31	27.43 ± 18.15	−1.419	0.159	−0.067	0.935	0.899–0.973	0.001
MoCA score ($\bar{x}$± SD)	21.94 ± 5.79	17.91 ± 6.08	3.384	0.001	−0.141	0.869	0.794–0.950	0.002
ESS score ($\bar{x}$± SD)	5.45 ± 4.39	9.13 ± 7.03	−3.181	0.002	0.164	1.178	1.052–1.319	0.004
PSQI score ($\bar{x}$± SD)	5.68 ± 5.66	8.64 ± 8.85	−2.015	0.047	0.042	1.043	0.958–1.134	0.332
HAMA score ($\bar{x}$± SD)	9.81 ± 10.27	11.55 ± 8.35	−0.933	0.353	–	–	–	–
HAMD score ($\bar{x}$± SD)	10.53 ± 11.39	12.15 ± 10.08	−0.754	0.453	–	–	–	–
FSS score ($\bar{x}$± SD)	2.84 ± 1.64	3.47 ± 1.86	−1.771	0.080	–	–	–	–
RBD score ($\bar{x}$± SD)	17.13 ± 19.55	12.55 ± 18.34	1.208	0.230	–	–	–	–
UPSIT ($\bar{x}$± SD)	44.45 ± 27.29	46.57 ± 27.62	−0.385	0.701	–	–	–	–

PD, Parkinson's disease; APP, appendicitis-like lesions; OR, odds ratio; CI, confidence interval; MoCA, Montreal Cognitive Assessment; SD, standard deviation; ESS, Epworth Sleepiness Score; PSQI, Pittsburgh Sleep Quality Index; HAMA, Hamilton Anxiety Scale; HAMD, Hamilton Depression Scale; FSS, Fatigue Severity Scal; RBD, rapid eye movement sleep behavior disorder; UPSIT, University of Pennsylvania Smell Identification Test.

constipation as a prodrome (OR = 6.154, P = 0.012), and a high ratio of UPDRS III score to symptom duration (OR = 22.793, P = 0.014) (Table 2). Nonmotor symptoms were constipation (OR = 7.121, P = 0.002), low MoCA score (OR = 0.927, P = 0.044), high ESS score (OR = 1.146, P = 0.003), and high PSQI score (OR = 1.080, P = 0.026) (Table 3). These results suggest that PD with chronic appendicitis-like lesions is a distinct clinical entity.

Pathological Manifestations of Chronic Appendicitis-like Lesions in Patients with PD

The hematoxylin and eosin staining was used to compare the structure of diseased appendix specimens from seven patients with PD and six normal appendix specimens. The analysis revealed intact mucosal (Fig. 2A, red arrow), submucosa (Fig. 2A, blue arrow), and serosal layers in the control. The appendix cavity in the patients with PD was obviously smaller, the mucosal epithelium was damaged with fewer mucosal glands (Fig. 2A, red arrow), the mucosa and submucosa were ruptured, the lamina propria (Fig. 2A, blue arrow) was exposed, and there was an increased number of submucosal lymphoid follicles, all of which led to an incomplete muscle layer and eosinophilic infiltration (PD patient 6 in Supporting Information Table S1). In a more severe case (patient 4 in Supporting Information Table S1), the mucosa and submucosa have disappeared, but there is extensive adipose (Fig. 2A, blue arrow) and connective tissue (Fig. 2A, red arrow) that obstruct the lumen.

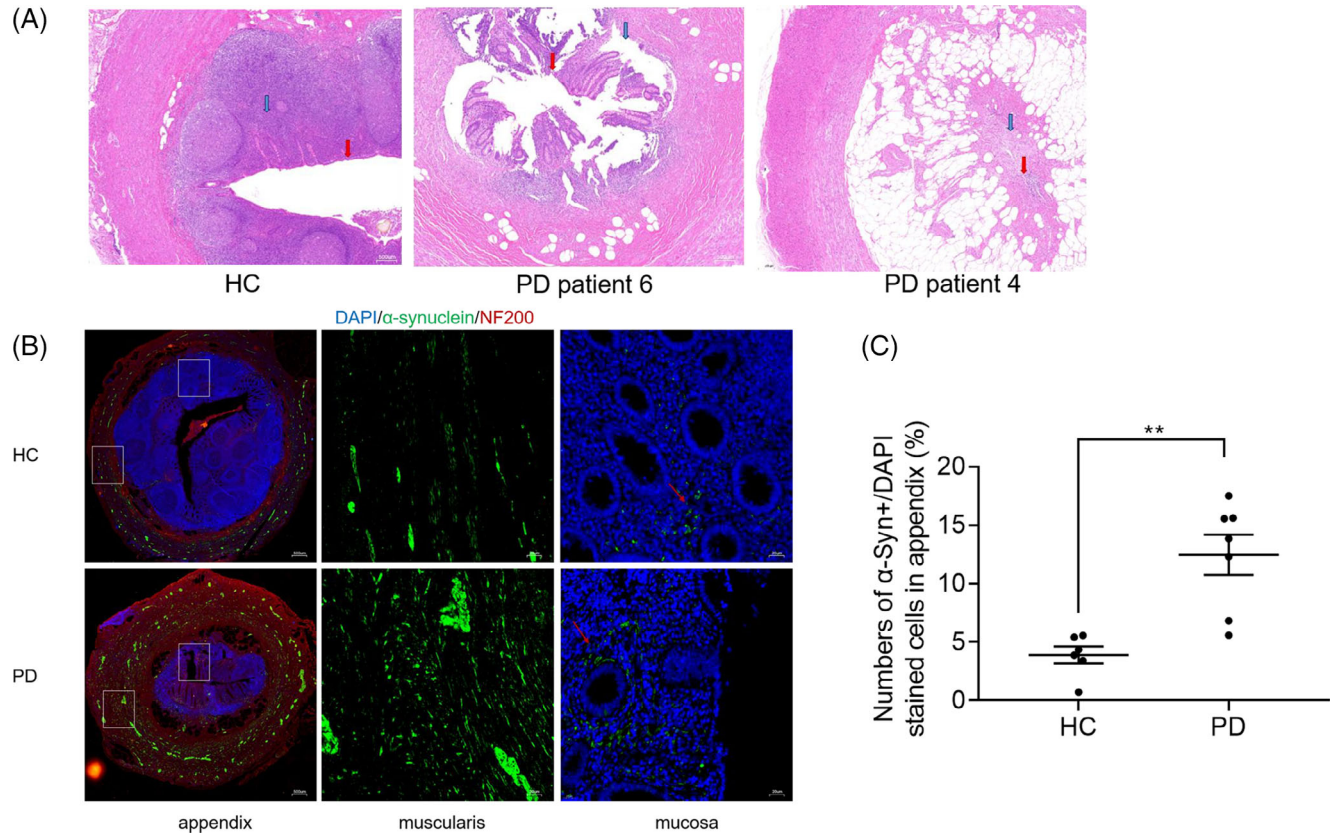

FIG. 2. The pathological changes of appendices in two representative patients with PD with chronic appendicitis-like lesions and the non-PD patient with a normal appendix (healthy control [HC]). (**A**) Hematoxylin and eosin staining of the appendices. Scale bars, 500 μm. Red and blue arrows indicate the mucosa and submucosa of the appendix. (**B**) Representative pictures of α-Syn and NF200 staining in two specimens. HC, normal appendix specimen; PD, diseased appendix specimen of patients with PD. $^{**}P < 0.05$. Scale bars, 20 μm. (**C**) Quantitative analysis of α-Syn immunoreactivity in PD (n = 7) group and control group (n = 6). DAPI, 4′,6-diamidino-2-phenylindole. [Color figure can be viewed at wileyonlinelibrary.com]

Increased Accumulation of α-Syn in the Appendices of Patients with PD with Chronic Appendicitis-like Lesions

To further investigate the link between chronic appendicitis-like lesions and PD, we performed double immunolabeling of NF200 (a neurofilament protein in neurons and nerve fibers, which is costained with synuclein to detect the expression of synuclein in neurons and nerve fibers) and α-Syn in the diseased appendices of patients with PD (n = 7) and the normal control appendix. As expected, the normal appendix was enriched in α-Syn, which colocalized with NF200; the proteins were evenly distributed in the muscle layer, mucosa, and submucosal nerves and ganglia (Fig. 2B). In contrast, in the appendix of patients with PD, α-Syn was present in larger amounts and had a disordered distribution, forming clusters that were not all colocalized with NF200 (Fig. 2C). The mean values and upper and lower limits for the PD and healthy control groups are 12.46 and 3.897, 17.5 and 5.57, 5.59 and 0.72, respectively ($P = 0.0013$).

Discussion

The results of this study provide evidence for an association between appendix pathology and PD. Half of the patients with PD in our cohort had chronic appendicitis-like lesions, which were a risk factor for PD and distinguished this subset of patients from patients with PD without such lesions. Moreover, the lesions were associated with increased accumulation of α-Syn in the appendices of these seven patients These results indicate that chronic appendicitis-like lesions may play an important role in PD pathology and possibly its pathogenesis.

The appendix is an immune organ located at the beginning of the colon that is similar to the thymus and is associated with development of the immune system and disease states.[30] The appendix is enriched in α-Syn, which is deposited in the substantia nigra and induces the degeneration of dopaminergic neurons.[13] Abnormal α-Syn is also present in the intestine of patients with PD and can be transmitted from the intestine to the brain via the vagus nerve, possibly leading to the onset of PD.[8,9] Based on these findings,

along with epidemiological data suggesting that appendectomy can influence the incidence of PD,[13-16] we speculate that patients with PD have lesions in their appendices, which produce and release abnormal α-Syn through as yet unknown mechanisms that eventually lead to the onset of PD.

MSSCT and 3D reconstruction of images of the appendix are the most accurate methods for diagnosing appendicitis. The recently published Cochrane systematic review of the use of CT for diagnosis of appendicitis in adults identified 64 studies, including 71 separate study populations; the overall sensitivity was 0.95, the specificity was 0.94, and the sensitivity of CT with oral contrast enhancement was 0.96.[31] To eliminate the influence of late complications and long-term medication use in our analysis, we selected patients with PD with a disease duration of <5 years. We found that 53 of 100 patients with PD, but only 4 of 50 non-PD patients had chronic appendicitis-like lesions in the appendix, which were not associated with PD duration. A multivariate regression analysis showed that the chronic appendicitis-like lesions together with olfactory disorder and RBD were risk factors for PD. The latter two factors are common prodromes in PD.[32,33] Moreover, regression analysis using chronic appendicitis-like lesions as the dependent variable and clinical symptoms as the independent variable demonstrated that patients with PD whose first motor symptoms were bradykinesia/rigidity and who had motor symptom onset in the central axis or left upper limb, constipation as a prodrome, a high ratio of MDS-UPDRS III score/symptom duration, low MoCA score, and high ESS score were more likely to have chronic appendicitis-like lesions. Interestingly, these clinical symptoms are similar to those of postural instability/gait difficulty PD or diffuse-malignant subtypes,[34,35] further supporting the possibility that patients with chronic appendicitis-like lesions are more prone to be related to these two clinical subtypes.

The patients with PD with chronic appendix-like lesions detected by imaging did not have fever or abdominal pain, but the stiff appendix could be palpated during physical examination, unlike for a normal appendix. Although the reason for this phenomenon is unclear, the diagnosis of chronic appendicitis in these patients with PD was well founded. According to the Chinese medical textbooks "*Surgery*" and "*Clinical Diagnosis and Treatment Guidelines – Surgery Division*", chronic appendicitis may not manifest with abdominal pain but instead with digestive symptoms, such as constipation and indigestion; the diagnosis can be based on symptoms and signs or imaging findings, and the final diagnosis depends on the pathology. The preferred treatment for elderly or infirm patients diagnosed with chronic appendicitis is appendectomy.

Seven patients with PD in our cohort underwent appendectomy, and the histopathological analysis confirmed that inflammation mainly affected the mucosa and submucosa, which were different from typical appendicitis in the extent of inflammation involved. In detail, the inflammation of the appendix of patients with PD mainly involved the mucosa and submucosa, while the inflammation of the muscle layer is relatively mild, which might explain why these patients with PD have imaging manifestations of appendicitis but without abdominal pain. Besides, unlike in the normal appendix, α-Syn in these samples formed clumps and accumulated in the mucosa and submucosa. Many studies have demonstrated the relationship of intestinal microbes to PD,[4,8,13,36] and it has been shown that in normal appendix tissue lysate, normal nonaccumulating α-Syn could be transformed into an abnormal aggregating form.[10] However, the mechanistic basis for increased accumulation of α-Syn in the appendices of patients with PD and its significance for the pathogenesis of PD have yet to be determined.

Six patients with PD with chronic appendicitis-like lesions in our study were followed up after 6 months. They did not change their medications after appendectomy (Supporting Information Table S1). Three patients showed improvement: one had complete alleviation of motor symptoms and stopped medical treatment 6 months after the surgery, and the other two had complete improvement of constipation and a 5-point decrease in MDS-UPDRS III score. However, two patients showed no significant changes in PD symptoms, and one patient had newly developed dysuria, increased frequency of urination at night and residual urine, and increased gait instability. The latter patient had a disease course of 55 weeks, and pathological changes in his appendix were also more serious: the mucosa and submucosa of the appendix had disappeared and were filled with adipose tissue. The drug treatment regimen of these patients with PD did not change during the postoperative follow-up. It should be noted that because the number of cases was small and the follow-up duration was short, these findings are completely anecdotal.

Our study had several limitations. First, the sample size was small. Second, investigation of the onset time of prodromes was based on the reports of patients and their families. In addition, we did not explore the causal relationship between chronic appendicitis-like lesions and PD. Larger studies are necessary to validate our findings and clarify the relationship between abnormal α-Syn accumulation in the appendix and the onset of PD.

The major strength of our study is that we demonstrated about half of patients with PD have appendix disease by imaging examination and confirmed the association between appendix abnormality and PD, suggesting a possible etiologic role for the appendix in PD. ●

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References

1. Espay AJ, Brundin P, Lang AE. Precision medicine for disease modification in Parkinson disease. Nat Rev Neurol 2017;13:119–126.
2. Espay AJ, Schwarzschild MA, Tanner CM, et al. Biomarker-driven phenotyping in Parkinson's disease: a translational missing link in disease-modifying clinical trials. Mov Disord 2017;32:319–324.
3. Johnson ME, Stecher B, Labrie V, Brundin L, Brundin P. Triggers, facilitators, and aggravators: redefining Parkinson's disease pathogenesis. Trends Neurosci 2019;42:4–13.
4. Horsager J, Andersen KB, Knudsen K, et al. Brain-first versus body-first Parkinson's disease: a multimodal imaging case-control study. Brain 2020;143:3077–3088.
5. Kaye J, Gage H, Kimber A, Storey L, Trend P. Excess burden of constipation in Parkinson's disease: a pilot study. Mov Disord 2006;21:1270–1273.
6. Savica R, Carlin JM, Grossardt BR, et al. Medical records documentation of constipation preceding Parkinson disease: a case-control study. Neurology 2009;73:1752–1758.
7. Lebouvier T, Chaumette T, Damier P, et al. Pathological lesions in colonic biopsies during Parkinson's disease. Gut 2008;57:1741–1743.
8. Kim S, Kwon SH, Kam TI, et al. Transneuronal propagation of pathologic α-synuclein from the gut to the brain models Parkinson's disease. Neuron 2019;103:627–641.e627.
9. Peng C, Gathagan RJ, Covell DJ, et al. Cellular milieu imparts distinct pathological α-synuclein strains in α-synucleinopathies. Nature 2018;557:558–563.
10. Guinane CM, Tadrous A, Fouhy F, et al. Microbial composition of human appendices from patients following appendectomy. mBio 2013;4:e00366–12.
11. Maini Rekdal V, Bess EN, Bisanz JE, Turnbaugh PJ, Balskus EP. Discovery and inhibition of an interspecies gut bacterial pathway for levodopa metabolism. Science 2019;364:eaau6323.
12. Sampson TR, Debelius JW, Thron T, et al. Gut microbiota regulate motor deficits and neuroinflammation in a model of Parkinson's disease. Cell 2016;167:1469–1480.e1412.
13. Killinger BA, Madaj Z, Sikora JW, et al. The vermiform appendix impacts the risk of developing Parkinson's disease. Sci Transl Med 2018;10:eaar5280.
14. Marras C, Lang AE, Austin PC, Lau C, Urbach DR. Appendectomy in mid and later life and risk of Parkinson's disease: a population-based study. Mov Disord 2016;31:1243–1247.
15. Svensson E, Horváth-Puhó E, Stokholm MG, Sørensen HT, Henderson VW, Borghammer P. Appendectomy and risk of Parkinson's disease: a nationwide cohort study with more than 10 years of follow-up. Mov Disord 2016;31:1918–1922.
16. Yilmaz R, Bayram E, Ulukan Ç, Altınok MK, Akbostancı MC. Appendectomy history is not related to Parkinson's disease. J Parkinsons Dis 2017;7:347–352.
17. Marsili L, Rizzo G, Colosimo C. Diagnostic criteria for Parkinson's disease: from James Parkinson to the concept of prodromal disease. Front Neurol 2018;9:156.
18. Jenkinson C, Fitzpatrick R, Peto V, Greenhall R, Hyman N. The Parkinson's disease questionnaire (PDQ-39): development and validation of a Parkinson's disease summary index score. Age Ageing 1997;26:353–357.
19. Knowles CH, Eccersley AJ, Scott SM, Walker SM, Reeves B, Lunniss PJ. Linear discriminant analysis of symptoms in patients with chronic constipation: validation of a new scoring system (KESS). Dis Colon Rect 2000;43:1419–1426.
20. Doty RL, Shaman P, Kimmelman CP, Dann MS. University of Pennsylvania smell identification test: a rapid quantitative olfactory function test for the clinic. Laryngoscope 1984;94:176–178.
21. Stiasny-Kolster K, Mayer G, Schäfer S, Möller JC, Heinzel-Gutenbrunner M, Oertel WH. The REM sleep behavior disorder screening questionnaire–a new diagnostic instrument. Mov Disord 2007;22:2386–2393.
22. Nasreddine ZS, Phillips NA, Bédirian V, et al. The Montreal cognitive assessment, MoCA: a brief screening tool for mild cognitive impairment. J Am Geriatr Soc 2005;53:695–699.
23. Buysse DJ, Reynolds CF 3rd, Monk TH, Berman SR, Kupfer DJ. The Pittsburgh sleep quality index: a new instrument for psychiatric practice and research. Psychiatry Res 1989;28:193–213.
24. Johns MW. A new method for measuring daytime sleepiness: the Epworth sleepiness scale. Sleep 1991;14:540–545.
25. Hamilton M. A rating scale for depression. J Neurol Neurosurg Psychiatry 1960;23:56–62.
26. Maier W, Buller R, Philipp M, Heuser I. The Hamilton anxiety scale: reliability, validity and sensitivity to change in anxiety and depressive disorders. J Affect Disord 1988;14:61–68.
27. Krupp LB, LaRocca NG, Muir-Nash J, Steinberg AD. The fatigue severity scale. Application to patients with multiple sclerosis and systemic lupus erythematosus. Arch Neurol 1989;46:1121–1123.
28. Visser M, Marinus J, Stiggelbout AM, Van Hilten JJ. Assessment of autonomic dysfunction in Parkinson's disease: the SCOPA-AUT. Mov Disord 2004;19:1306–1312.
29. Rao PM, Rhea JT, Novelline RA. Sensitivity and specificity of the individual CT signs of appendicitis: experience with 200 helical appendiceal CT examinations. J Comput Assist Tomogr 1997;21:686–692.
30. Rankin LC, Girard-Madoux MJ, Seillet C, et al. Complementarity and redundancy of IL-22-producing innate lymphoid cells. Nat Immunol 2016;17:179–186.
31. Di Saverio S, Podda M, De Simone B, et al. Diagnosis and treatment of acute appendicitis: 2020 update of the WSES Jerusalem guidelines. World J Emerg Surg 2020;15:27.
32. Postuma RB, Gagnon JF, Vendette M, Montplaisir JY. Markers of neurodegeneration in idiopathic rapid eye movement sleep behaviour disorder and Parkinson's disease. Brain 2009;132:3298–3307.
33. Zapiec B, Dieriks BV, Tan S, Faull RLM, Mombaerts P, Curtis MA. A ventral glomerular deficit in Parkinson's disease revealed by whole olfactory bulb reconstruction. Brain 2017;140:2722–2736.
34. Fereshtehnejad SM, Zeighami Y, Dagher A, Postuma RB. Clinical criteria for subtyping Parkinson's disease: biomarkers and longitudinal progression. Brain 2017;140:1959–1976.
35. Stebbins GT, Goetz CG, Burn DJ, Jankovic J, Khoo TK, Tilley BC. How to identify tremor dominant and postural instability/gait difficulty groups with the movement disorder society unified Parkinson's disease rating scale: comparison with the unified Parkinson's disease rating scale. Mov Disord 2013;28:668–670.
36. Matheoud D, Cannon T, Voisin A, et al. Intestinal infection triggers Parkinson's disease-like symptoms in Pink1(−/−) mice. Nature 2019;571:565–569.

Supporting Data

Additional Supporting Information may be found in the online version of this article at the publisher's web-site.