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Original Article

Relation of Enteric α-Synuclein to Gastrointestinal Dysfunction in Patients With Parkinson's Disease and in Neurologically Intact Subjects

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Background/Aims
α-Synucleinopathy in the brain is the neuropathological hallmark of Parkinson's disease (PD). However, the functional impact of α-synucleinopathy in the enteric nervous system remains unknown. We aim to evaluate the association between gastrointestinal (GI) dysfunction and α-synuclein (αSYN) pathology in the stomach and colon of PD patients and controls, as well as to investigate the association between the αSYN pathology in GI tract and future PD risk.

Methods
A total of 35 PD patients and 52 neurologically intact subjects were enrolled in this study. Endoscopic biopsies were performed, and then immunohistochemical staining for αSYN was performed. All subjects completed the validated Rome III questionnaire for the assessment of GI symptoms. The association between GI symptoms and the αSYN pathology in GI mucosa was evaluated. Incident PD cases were assessed during a median follow-up of 46 months.

Results
The proportion of self-reported constipation and functional constipation through the Rome III questionnaire was significantly higher in PD patients than in controls ($P < 0.001$ and $P = 0.015$). However, no significant association was found between the αSYN pathology in the stomach and colon mucosa and constipation, as well as other GI symptoms including dyspepsia symptoms and abdominal discomfort or pain, regardless of the presence or absence of clinical PD ($P > 0.05$). No incident PD cases were diagnosed during study period.

Conclusions
Our present study suggests that the deposition of αSYN in the mucosal enteric nervous system may not be reflected by functional impairment of the affected segment of the gut.
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Key Words
α-Synuclein; Enteric nervous system; Gastrointestinal symptoms; Parkinson disease

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Introduction

Gastrointestinal (GI) symptoms are the most prominent and disabling non-motor manifestations of Parkinson's disease (PD).[1] Patients with PD patients develop various GI symptoms, including dry mouth, drooling, dysphagia, gastroparesis, and constipation.[1,2] Although the clinical understanding of GI dysfunction in PD patients has increased substantially, the pathophysiological mechanism of GI involvement in PD is still unclear.

α-Synuclein (αSYN) is the main component of Lewy bodies and Lewy neurites, which are the main neuropathological hallmarks of PD.[3] The abnormal deposition of aggregated αSYN initially occurs in the olfactory bulb and dorsal motor nucleus of the vagus nerve (DMV) in the medulla; thereafter, it spreads through less vulnerable nuclear gray and cortical areas in an ascending course.[4] Researchers have also found αSYN aggregation in the enteric nervous system (ENS), occurring at the earliest stage of PD, or even preceding the onset of PD.[5-7] GI symptoms also occur from the earliest stage of PD, and constipation is known to precede the motor symptoms of the disease.[1,8] In addition, the extent of GI dysfunction corresponds with widespread ENS synucleinopathy, suggesting that the abnormal deposition of αSYN in the ENS could be the main cause of GI dysfunction in PD.[9] However, no studies have shown a causative link between this pathological abnormality and the corresponding GI deficit in PD.[2]

Several recently published studies have reported that αSYN is also present in the colonic mucosa or submucosa of persons without neurodegenerative diseases.[10-12] Our group also reported that there was no difference in the αSYN immunoreactivity of gastric and colonic mucosal tissues obtained through routine endoscopy among PD patients and controls.[13] However, the functional impact of αSYN pathology in the GI tract of neurologically intact persons remains unknown. The role of ENS synucleinopathy in neurologically intact persons in predicting future PD risk has also not been evaluated.

Therefore, in the present study, we aim to evaluate the association between the αSYN pathology in the human GI tract, and GI dysfunction in PD patients and neurologically intact persons, as well as to investigate the association between the αSYN pathology in the GI tract and the subsequent risk of PD in neurologically intact subjects.

Materials and Methods

Study Population

Our group's preceding study was a prospective study comparing αSYN immunoreactivity between PD and controls (neurologically intact subjects).[13] In this study, the sample population comprised of 87 subjects who completed the validated Korean Rome III questionnaire (Rome III-K) among the participants of the preceding study.[13,14] We enrolled PD patients and controls between August 2013 and February 2015. The PD patients were enrolled from the clinical practice of the Department of Neurology at Asan Medical Center, a tertiary university hospital in Seoul, South Korea, and the diagnosis of PD was made by experienced movement disorder specialists by using the clinical diagnostic criteria of the United Kingdom PD Society Brain Bank.[15] Control subjects older than 45 years, without a history of neurological disorders, and who visited the gastroenterology outpatient clinic at the Asan Medical Center were prospectively enrolled in this research. We screened all of the controls for PD by using the "Nine-symptom screening tool,"[16] and the subjects were examined by neurologists (S.J.C., J.K., H.S.R., and M.J.K.) to exclude neurological disorders. The control subjects had no psychological diseases, no tremors, and no impairment in cognitive function or the activities of daily living. Subjects were excluded if they had chronic GI diseases, such as Crohn's disease or ulcerative colitis, or if they showed active GI diseases in their esophagogastroduodenoscopy (EGD) or colonoscopy, such as a gastric ulcer, gastritis, colitis, or cancer.

Endoscopic Biopsy

The enrolled subjects underwent an EGD, colonoscopy, or both, as recommended by the gastroenterologist in routine clinical practice. An endoscopy was performed by 3 gastroenterologists (H.J.L., K.W.J., and J.H.L.) according to the usual procedure followed by the Gastroenterology Department of Asan Medical Center. We performed gastric mucosal biopsies in the fundus and antrum by using standard biopsy forceps (FB-25K-1; standard fenestrated cups; Olympus, Tokyo, Japan). Colonic mucosal biopsies were also performed in the ascending colon, transverse colon, and sigmoid colon by using standard biopsy forceps (FB-24U-1; standard fenestrated cups with needle, Olympus). Two random biopsies were taken from each biopsy site, and the two biopsies obtained from the same site were considered as a single sample. After sampling, the gastric and colonic mucosal specimens were immediately

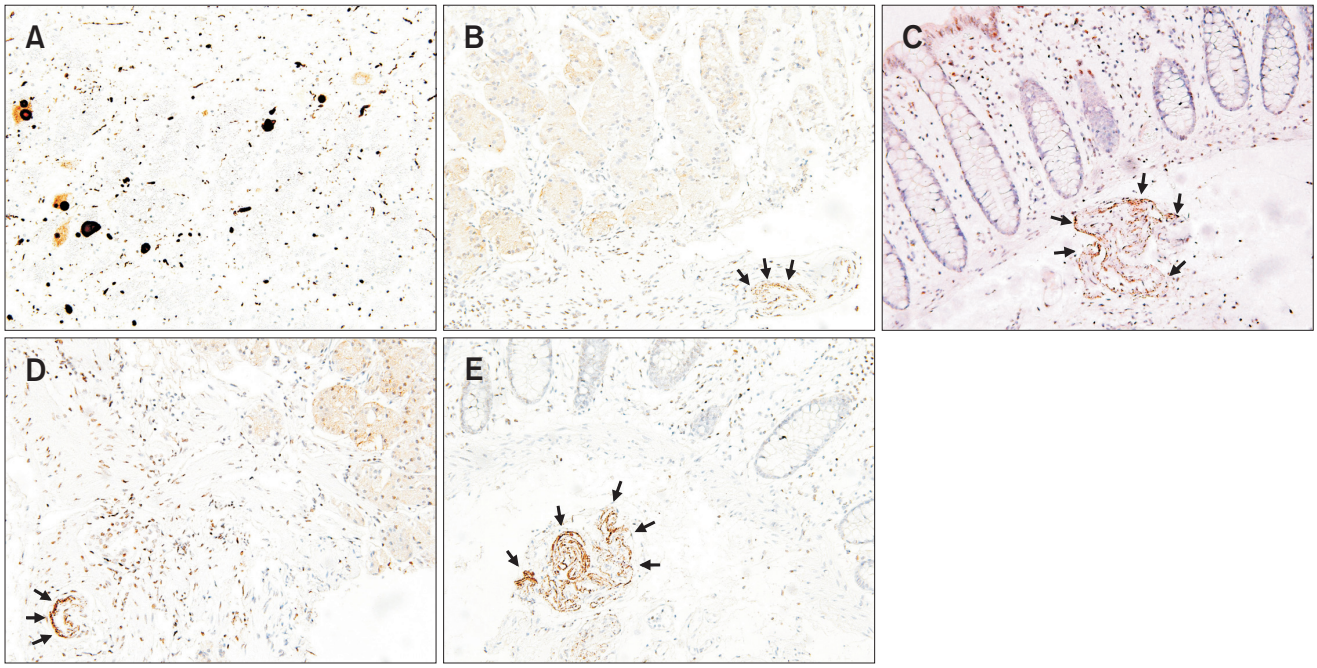

Figure. α-Synuclein (αSYN) immunostaining. (A) Positive control of αSYN immunoreactivity in this study; Lewy body-like inclusions and Lewy neurites with strong αSYN immunoreactivity in brain tissue from autopsy of a patient with Parkinson's disease. (B-E) αSYN immunostaining in gastric and colonic endoscopic biopsy tissues. (B) Gastric tissue from a 78-year-old man with an 11-year history of Parkinson's disease. (C) Colonic tissue from a 55-year-old woman with a 2-year history of Parkinson's disease (D) Gastric tissue from a 67-year-old woman as a control. (E) Colonic tissue from an 80-year-old man as a control. Note the positive αSYN immunoreactivity reveals multiple dots-like or short-linear appearance (arrows), which is located close to the muscularis mucosa (all figures, original magnification, × 200).

fixed in 10% formalin.

Our study protocol was approved by the institutional review board of Asan Medical Center (Protocol No. 2013-0175), and informed consent was obtained from all subjects.

Immunohistochemical Staining and Tissue Assessment Procedures

The primary antibodies for αSYN (1:200; EP1536Y; ABCAM, Cambridge, UK) and S-100 protein (1:200; 18-0046; Zymed, South San Francisco, CA, USA), with BenchMark autostainers (Ventana Medical Systems, Tuscan, AZ, USA), were used according to the manufacturers' protocol. Then, 4-μm tissue sections were deparaffinized, and hydrated in xylene and serially diluted ethanol, respectively. Endogenous peroxidase was blocked by incubation in 3% H_2O_2 for 10 minutes, after which heat-induced antigen retrieval was performed for 32 minutes. After primary antibody incubation for 32 minutes at room temperature, detection was carried out with an I-View detection kit (BenchMark XT, Ventana Medical Systems).

The specimens were determined to be adequate for αSYN immunostaining when sufficient numbers of sections were available for analysis, and the density of S-100-positive nerve cells was at least low to moderate, with the presence of the muscularis mucosa. A section with at least one definite αSYN immunoreactive fiber was considered to be positive when compared with the positive control of αSYN immunoreactivity (ie, Lewy body-like inclusions or Lewy neurites from the brain tissues of PD patients; Fig. A). The neuropathological findings were assessed by one neuropathologist (S.M.H.) and one experienced neurologist (J.K.) who were blinded to the clinical information of subjects.

Assessment of Gastrointestinal Symptoms

All subjects completed the validated Korean Rome III questionnaire to assess their GI symptoms (Rome III-K).[14] The questionnaires evaluated the frequency and duration of the GI symptoms, including epigastric burning, early satiation, postprandial fullness, epigastric pain, acid reflux, nausea, vomiting, abdominal pain or discomfort, constipation, bloating, and fecal incontinence, during the previous 3 months. Each self-reported GI symptom was defined as "presence" if it met the following criteria set by Rome

III[17]: epigastric burning, epigastric pain, and vomiting one or more times per week; early satiation, postprandial fullness, and nausea from 2 to 3 or more times per week; and abdominal pain or discomfort and bloating for ≥ 3 days/month. The presence of acid reflux was confirmed when the symptom occurred more than once per week, and the presence of fecal incontinence was confirmed when the symptom occurred more than once per month. The presence of self-reported constipation was confirmed when the subjects reported defecatory difficulty. Functional GI disorders (functional dyspepsia, irritable bowel syndrome, and functional constipation) were diagnosed as defined by the Rome III criteria. In the PD patients, the presence of drooling and dysphagia were also assessed by using the non-motor symptom assessment scale for PD.[18]

We evaluated the association between the presence of GI symptoms and the αSYN pathology of the GI tract in PD patients and neurologically intact subjects. We also assessed the relation between upper GI symptoms and αSYN in the gastric samples, as well as the relation between lower GI symptoms and αSYN in the colon samples. This assessment sought to evaluate the organ-specific association between the αSYN pathology and GI symptoms. Upper GI symptoms included drooling, dysphagia, epigastric burning, early satiation, postprandial fullness, epigastric pain, acid reflux, nausea, and vomiting. Lower GI symptoms included abdominal pain or discomfort, constipation, bloating, and fecal incontinence.

Ascertainment of Parkinson's Disease

The outcome of interest was defined as having a disease code for expanding benefit coverage for PD patients' (V124) registration with the Korean National Health Insurance Service.

Statistical Methods

The differences in the continuous variables between the groups were evaluated by using the Mann–Whitney U test, and the differences in the categorical variables were evaluated with the χ^2 test or Fisher's exact test. Data were analyzed with SPSS 20.0 (IBM Corp, Armonk, NY, USA), and *P*-values < 0.05 were considered to be statistically significant.

Results

Baseline Characteristics of the Study Population

A total of 35 PD patients and 52 controls were enrolled in this study, and the baseline characteristics and GI symptoms of the subjects are shown in Table 1. There were no significant differences between the two groups in terms of age, sex, and body mass index.

Among the 35 PD patients, the median disease duration was 7 years (range, 2-21 years), the mean levodopa equivalent dose was 816 mg/day, the mean Hoehn and Yahr stage was 2.4, and the mean total unified PD rating scale score was 40.9. Three patients (8.6%) underwent EGD only, 16 (45.7%) underwent colonoscopy only, and 16 (45.7%) underwent both procedures.

Among the 52 controls, 21 subjects (40.4%) were given screening endoscopies without GI symptoms. The main reasons for performing endoscopy in the remaining controls (n = 31, 59.6%) were as follows: constipation (n = 18), abdominal pain or discomfort (n = 6), acid reflux (n = 2), epigastric pain (n = 1), postprandial discomfort (n = 1), bloating (n = 1), and diarrhea (n = 2). Seven subjects (13.5%) underwent EGD only, 24 (46.2%) underwent colonoscopy only, and 21 (40.4%) underwent both procedures.

The rates of self-reported constipation and functional constipation through the Rome III questionnaire were significantly higher in PD patients than in controls ($P < 0.001$ and $P = 0.015$). The other self-reported GI symptoms and functional GI disorders did not differ between the two groups.

α-Synuclein in the Gastric or Colonic Mucosa Is Not Associated With Gastrointestinal Symptoms

Of the 87 subjects, 79 (90.8%) had one or more adequate quality samples, and were assessable for neuropathological study; αSYN was documented in 32 subjects (40.5%). αSYN immunostaining in the gastric and colonic endoscopic biopsy tissue were illustrated in Figure B-E. There was no difference in the αSYN immunoreactivity between the controls and PD patients (43.5% vs 36.4%, $P = 0.643$; Supplementary Table 1).

The GI symptoms of the subjects according to the αSYN immunostaining in the mucosal biopsies, regardless of the presence or absence of an underlying neurological disorder, are indicated in Table 2. No statistically significant differences were found in the demographic variables, self-reported GI symptoms, or functional GI disorders in Rome III between the negative αSYN group (n = 47) and the positive αSYN group (n = 32). In addition, αSYN positivity in gastric mucosal biopsy was not associated with any upper or lower GI symptoms (Supplementary Table 2), and the results according to the αSYN positivity in the colonic mucosal biopsy were the same (Supplementary Table 3). Moreover, the detailed constipation symptoms did not differ between the two groups (Supplementary Table 4).

The GI symptoms of PD patients according to αSYN immunostaining in the mucosal biopsy are indicated in Table 3. Among

Table 1. Baseline Characteristics and Gastrointestinal Symptoms of the Study Subjects

Variables	Control (n = 52)	PD (n = 35)	*P*-value
Age (median [range], yr)	63 (50-80)	67 (50-79)	0.098
Sex (male, n)	26 (50.0%)	15 (42.9%)	0.513
Body mass index (median [range], kg/m^2)	24 (18-34)	22 (17-35)	0.090
Characteristics of PD			
Duration of disease (median [range], yr)	-	7 (2-21)	
Levodopa equivalent dose (mean ± SD, mg/day)	-	816 ± 339	
Hoehn and Yahr stage (mean ± SD)	-	2.4 ± 0.6	
UPDRS of PD (mean ± SD)			
Part I	-	2.9 ± 2.3	
Part II	-	11.8 ± 5.6	
Part III	-	23.2 ± 9.5	
Part IV	-	3.1 ± 2.9	
Total	-	40.9 ± 16.8	
Self-reported GI symptoms (n)			
Epigastric burning	5 (9.6%)	2 (5.7%)	0.697
Early satiation	9 (17.3%)	3 (8.6%)	0.347
Postprandial fullness	10 (19.2%)	2 (5.7%)	0.112
Epigastric pain	6 (11.5%)	3 (8.6%)	0.735
Acid reflux	6 (11.5%)	1 (2.9%)	0.234
Nausea	1 (1.9%)	2 (5.7%)	0.562
Vomiting	0 (0.0%)	1 (2.9%)	0.402
Abdominal pain/discomfort	13 (25.0%)	3 (8.6%)	0.088
Constipation	24 (46.2%)	32 (91.4%)	< 0.001
Bloating	14 (26.9%)	6 (17.1%)	0.288
Fecal incontinence	3 (5.8%)	2 (5.7%)	1.000
Functional GI disorder (Rome III) (n)			
Functional dyspepsia	12 (23.1%)	5 (14.3%)	0.412
Irritable bowel syndrome	6 (11.5%)	1 (2.9%)	0.234
Functional constipation	25 (48.1%)	26 (74.3%)	0.015

PD, Parkinson's disease; SD, standard deviation; UPDRS, unified Parkinson's disease rating scale; GI, gastrointestinal.

the PD patients, 33 (94.3%) were assessable for neuropathological study, and αSYN was documented in 12 (36.4%) patients. More PD patients with negative αSYN had self-reported constipation than those with positive αSYN (100% vs 75%, $P = 0.040$), whereas functional constipation according to the Rome III criteria did not differ between the two groups. The other self-reported GI symptoms and functional GI disorders did not differ between the two groups.

The GI symptoms of the neurologically intact subjects, according to the αSYN immunostaining in the mucosal biopsy, are indicated in Table 4. No statistically significant differences were found in the demographic variables, self-reported GI symptoms, or functional GI disorders in Rome III between the negative αSYN group (n = 26) and the positive αSYN group (n = 20).

The Association Between α-Synuclein Pathology in the Gastrointestinal Tract and Future Parkinson's Disease Risk During Short-term Follow-up Was Not Assessed

No incident PD cases were diagnosed during a median follow up of 46 months (range 31-52) among 52 baseline neurologically intact subjects, irrespective of their GI αSYN pathology. The association between the αSYN pathology in the GI tract and future PD risk could not be evaluated.

Discussion

Our present study described the association between the αSYN pathology in the human GI mucosa, and GI dysfunction in PD patients and neurologically intact subjects. The αSYN pathol-

Table 2. Gastrointestinal Symptoms of Subjects With Positive and Negative α-Synuclein Immunostaining in the Stomach and Colon Mucosal Biopsies

Variables	Negative (n = 47)	Positive[a] (n = 32)	*P*-value[b]
Age (median [range], yr)	65 (50-77)	66 (50-80)	0.301
Sex (male, n)	25 (53.2%)	17 (53.1%)	0.995
Body mass index (median [range], kg/m^2)	23 (17-35)	24 (18-29)	0.830
Self-reported GI symptoms (n)			
Epigastric burning	1 (2.1%)	4 (12.5%)	0.152
Early satiation	5 (10.6%)	5 (15.6%)	0.515
Postprandial fullness	7 (14.9%)	4 (12.5%)	1.000
Epigastric pain	2 (4.3%)	4 (12.5%)	0.216
Acid reflux	3 (6.4%)	3 (9.4%)	0.682
Nausea	1 (2.1%)	2 (6.3%)	0.563
Vomiting	0 (0.0%)	1 (3.1%)	0.405
Abdominal pain/discomfort	6 (12.8%)	7 (21.9%)	0.358
Constipation	35 (74.5%)	17 (53.1%)	0.058
Bloating	12 (25.5%)	6 (18.8%)	0.589
Fecal incontinence	2 (4.3%)	3 (9.4%)	0.390
Functional GI disorder (Rome III) (n)			
Functional dyspepsia	8 (17.0%)	5 (15.6%)	1.000
Irritable bowel syndrome	3 (6.4%)	2 (6.3%)	1.000
Functional constipation	28 (59.6%)	20 (62.5%)	0.819

[a]Positive: α-synuclein stain (+) ≥ 1 site.
[b]Eight subjects without adequate samples were excluded from this analysis.
GI, gastrointestinal.

ogy in the stomach and colon mucosa appeared not to be associated with GI dysfunction. Therefore, our findings suggested that the deposition of αSYN in the stomach and colon mucosal nerve fiber may not be reflected by the functional impairment of the affected segment of the gut, regardless of the presence or absence of clinical PD.

GI dysfunction is the most prominent and disabling non-motor manifestation of PD, and it is significantly more prevalent in PD patients than in controls.[1] However, the current treatment strategy for GI dysfunction in PD patients has focused on symptomatic treatment.[2,19] An understanding of the pathophysiological mechanism of GI motility dysfunction in PD would help in the development of target treatments for GI symptoms. However, the pathophysiological mechanism is still poorly understood, and even the clinical-pathological correlations between the GI symptoms and PD pathology have not been well evaluated.

The normal function of the GI tract is controlled by intrinsic and extrinsic innervation; intrinsic innervation relies on the ENS, and extrinsic innervation depends on the preganglionic parasympathetic and sympathetic outputs.[20,21] The parasympathetic output originates in the DMV of the medulla and in the sacral parasympathetic nucleus of the spinal cord.[20,21] The ENS is especially thought to play a major role in GI dysfunction in PD.[9] The ENS has essential roles in controlling bowel movement and secretion, largely independently of influences from the central nervous system, and the widespread distribution of αSYN pathology in the ENS seems to be associated with various symptoms throughout the entire GI tract.[9,21] One clinical study reported a positive correlation between the amount of Lewy neurites in the submucosal plexus of the ENS and the severity of constipation in PD patients, suggesting a pathogenic role for ENS synucleinopathy in GI dysfunction in PD.[22] However, no neuronal loss in the myenteric plexus in PD was reported, and the rostrocaudal distribution of the αSYN burden in the ENS did not coincide with the characteristics of GI dysfunction in PD, in which constipation is the most frequently occurring symptom, developing in the early phase.[1,23,24] Therefore, further evaluation is required concerning the clinical-pathological correlations between ENS synucleinopathy and GI dysfunction in PD, and the possible mechanisms.

Furthermore, recent studies have reported that αSYN was detected in the ENS of neurologically intact subjects; that is, it may not be a specific finding of PD patients.[10-12,25] Our group also re-

Table 3. Gastrointestinal Symptoms of Patients With Parkinson's Disease With Positive and Negative α-Synuclein Immunostaining in the Stomach and Colon Mucosal Biopsies

Variables	Negative (n = 21)	Positive[a] (n = 12)	*P*-value[b]
Age (median [range], yr)	67 (51-77)	66 (50-79)	0.567
Sex (male, n)	12 (57.1%)	7 (58.3%)	1.000
Body mass index (median [range], kg/m^2)	22 (17-35)	22 (18-26)	0.645
Self-reported GI symptoms (n)			
Drooling	7 (35.0%)	3 (25.0%)	0.703
Dysphagia	3 (15.0%)	1 (8.3%)	1.000
Epigastric burning	0 (0.0%)	2 (16.7%)	0.125
Early satiation	2 (9.5%)	1 (8.3%)	1.000
Postprandial fullness	1 (4.8%)	1 (8.3%)	1.000
Epigastric pain	0 (0.0%)	2 (16.7%)	0.125
Acid reflux	0 (0.0%)	1 (8.3%)	0.364
Nausea	0 (0.0%)	2 (16.7%)	0.125
Vomiting	0 (0.0%)	1 (8.3%)	0.364
Abdominal pain/discomfort	2 (9.5%)	1 (8.3%)	1.000
Constipation	21 (100.0%)	9 (75.0%)	0.040
Bloating	3 (14.3%)	2 (16.7%)	1.000
Fecal incontinence	1 (4.8%)	1 (8.3%)	1.000
Functional GI disorder (Rome III) (n)			
Functional dyspepsia	2 (9.5%)	2 (16.7%)	0.610
Irritable bowel syndrome	1 (4.8%)	0 (0.0%)	1.000
Functional constipation	15 (71.4%)	9 (75.0%)	1.000

[a]Positive: α-synuclein stain (+) ≥ 1 site.
[b]Two Parkinson's disease patients without adequate samples were excluded from this analysis.
GI, gastrointestinal.

ported that the αSYN positivity in the gastric and colonic mucosal biopsied samples was comparable between 38 PD patients and 53 controls (33.3% for the stomach and 18.5% for the colon in the controls).[13] These studies have raised concerns about whether the αSYN neuropathology is the main pathophysiology of GI dysfunction in PD patients. Moreover, it is not clear what is the role of αSYN in the GI tract of neurologically intact subjects.

To our knowledge, this study is the first to describe the association between the αSYN pathology in the GI tract (upper and lower) of living humans, and comprehensive GI dysfunction in both PD and neurologically intact subjects. In this study, both controls (with or without GI symptoms) and PD patients were enrolled, and the validated and structured Korean Rome III questionnaire (Rome III-K) was used to assess the GI symptoms systematically.[14] Unfortunately, we could not find any significant association between the αSYN pathology in the GI mucosa and GI dysfunction. One unexpected finding was that PD patients without αSYN had higher rates of self-reported constipation than those with αSYN, which might be an incidental finding due to the low sample size of PD patients.

We postulate several reasons for the negative results of this study. First, the accumulation of αSYN in the ENS may not be the primary cause of the GI dysfunction in PD. The αSYN neuropathology affected not only the ENS but also the DMV, sacral parasympathetic neurons, and sympathetic preganglionic and ganglion neurons; therefore, the contribution of these factors to GI dysfunction in PD remains to be defined.[1,26,27] In particular, the DMV is almost affected in PD, and neuronal loss in this nucleus has also been reported.[4,28] The vagal innervation from the DMV is more heavily distributed in the esophagus and stomach than in the distal GI tract; therefore, the dysmotility of the esophagus and stomach is likely to be associated with the involvement of the DMV.[4,9,29] Further studies to evaluate the contribution of other affected nervous system components to GI dysfunction are required. In addition, aging, mobility, and drugs used to treat the motor symptoms of PD are also considered to be factors related to GI dysfunction in PD.[9,30] Second, pathological αSYN distinct from physiological αSYN might exist, and the immunohistochemical method used in our study may fail to distinguish between the 2 types. Böttner et al[10] reported the presence of native αSYN in surgical specimens of the

Table 4. Gastrointestinal Symptoms of Neurologically Intact Subjects With Positive and Negative α-Synuclein Immunostaining in the Stomach and Colon Mucosal Biopsies

Variables	Negative (n = 26)	Positive[a] (n = 20)	*P*-value[b]
Age (median [range], yr)	62 (50-76)	66 (51-80)	0.276
Sex (male, n)	13 (50.0%)	10 (50.0%)	1.000
Body mass index (median [range], kg/m^2)	23 (18-34)	25 (20-29)	0.485
Self-reported GI symptoms (n)			
Epigastric burning	1 (3.8%)	2 (10.0%)	0.572
Early satiation	3 (11.5%)	4 (20.0%)	0.682
Postprandial fullness	6 (23.1%)	3 (15.0%)	0.711
Epigastric pain	2 (7.7%)	2 (10.0%)	1.000
Acid reflux	3 (11.5%)	2 (10.0%)	1.000
Nausea	1 (3.8%)	0 (0.0%)	1.000
Vomiting	0 (0.0%)	0 (0.0%)	N/A
Abdominal pain/discomfort	4 (15.4%)	6 (30.0%)	0.292
Constipation	14 (53.8%)	8 (40.0%)	
Bloating	9 (34.6%)	4 (20.0%)	0.336
Fecal incontinence	1 (3.8%)	2 (10.0%)	0.572
Functional GI disorder (Rome III) (n)			
Functional dyspepsia	6 (23.1%)	3 (15.0%)	0.711
Irritable bowel syndrome	2 (7.7%)	2 (10.0%)	1.000
Functional constipation	13 (50.0%)	11 (55.0%)	0.774

[a]Positive: α-synuclein stain (+) ≥ 1 site.
[b]Six neurologically intact subjects without adequate samples were excluded from this analysis.
GI, gastrointestinal.

colon in 13 subjects without neurodegenerative diseases, and they suggested that αSYN in the ENS appears to be a normal finding and not an indicator of neurodegenerative processes. One recent interesting study demonstrated that distinct αSYN strains display differential seeding capacities, inducing strain-specific pathologies and neurotoxic phenotypes.[31] The authors reported that fibrils seem to be the major toxic strain, resulting in progressive motor impairment and cell death. Therefore, further studies are warranted to elaborate on the pathological αSYN characteristics and their functional impact on GI dysfunction. Finally, it is possible that GI dysmotility might be present even in the absence of symptoms.

Another aspect of identifying the role of αSYN pathology in the GI tract of neurologically intact patients was the possibility that GI αSYN pathology could predict PD risk prior to any motor signs of PD. Our study sought to prospectively investigate the association between the αSYN pathology in the GI tract and the subsequent risk of PD in neurologically intact subjects. However, because no incident PD cases were observed in our study period, the assessment could not be done. Although previous studies have reported that αSYN positivity in the GI tissues were obtained several years (2-20 years) prior to the onset of motor symptoms in PD patients, there have been neither large population-based nor prospective cohort studies conducted.[6,32,33] Our results were difficult to conclude because of the short follow-up period; therefore, further follow up with our cohort is required.

Our study has the following limitations. First, we did not evaluate the deep layer of the GI tract, including the submucosal and myenteric plexus of the ENS, because we obtained the GI mucosal layer by using endoscopic biopsy forceps. This sampling method was intended to obtain enteric tissues in the living human efficiently, and its usefulness has been reported in the detection of enteric αSYN in PD patients.[34-36] The low quality of the samples obtained with endoscopic biopsy is also one of the limitations of this study, and this may have affected the results of the neuropathological analysis. Future studies to evaluate the αSYN pathology in the submucosal and myenteric plexus in living humans, by using novel neuropathological methods, are needed. Second, the number of subjects with certain GI symptoms was low, and the severity of symptoms was not assessed.

In conclusion, our present study suggests that the deposition of αSYN in the mucosal ENS may not be reflected by functional impairment of the affected segment of the gut. These data provide a background for further analyses of the αSYN neuropathology and GI dysfunction. Further investigations are warranted to elucidate

the pathological αSYN strains in the ENS that are distinguishable from the physiological αSYN strains, as well as their clinical implications, and the exact mechanism of GI motility dysfunction.

Supplementary Material

Note: To access the supplementary table mentioned in this article, visit the online version of *Journal of Neurogastroenterology and Motility* at http://www.jnmjournal.org/, and at https://doi.org/10.5056/jnm17141.

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Conflicts of interest: None.

Author contributions: Hyo Jeong Lee performed the procedures, analyzed the data, and wrote the manuscript; Kee Wook Jung and Jeong Hoon Lee performed the procedures and collected the data; Seung-Mo Hong performed the pathological analysis; Sun Ju Chung designed the study; Juyeon Kim collected the data, performed neurologic examinations, and performed the pathological analysis; Ho-Sung Ryu, Mi Jung Kim collected data and performed neurologic examinations; Sung Wook Hwang, Ho-Su Lee, Myeongsook Seo, Sang Hyoung Park, Dong-Hoon Yang, Byong Duk Ye, Jeong-Sik Byeon, Jaewon Choe, Hwoon-Yong Jung, and Suk-Kyun Yang revised the manuscript; and Seung-Jae Myung designed the study and revised the manuscript. All authors have approved the final draft submitted.

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