Colonic mucosal α -synuclein lacks specificity as a biomarker for Parkinson disease

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ABSTRACT

Objective: To determine the utility of detecting α -synuclein (α Syn) in colonic mucosal biopsy tissue as a potential diagnostic biomarker for Parkinson disease (PD).

Methods: We used the paraffin-embedded tissue (PET) blot, which degrades physiologic nonaggregated α Syn using proteinase K and enhances antigen retrieval allowing sensitive and selective detection of remaining protein aggregates, to detect α Syn in colonic mucosal biopsies from 15 patients with early PD (<3 years), 7 patients with later PD (>5 years), and 11 individuals without PD. α Syn and serine 129-phosphorylated α Syn (Ser129p- α Syn) were assessed by PET blot and conventional immunohistochemistry.

Results: PET blot-resistant aggregated α Syn and Ser129p- α Syn was present in 12 of 15 individuals with early PD, 7 of 7 individuals with later PD, and 11 of 11 control subjects. The number of biopsies positive by PET blot relative to conventional immunohistochemistry was significantly lower in both PD groups compared with the control group for both α Syn and Ser129p- α Syn, whereas routine immunohistochemistry was positive more often in PD, but was positive in as many as 9 of 11 control individuals.

Conclusion: Strong evidence of the presence of aggregated hyperphosphorylated α Syn in individuals with and without PD, using such a sensitive and specific method as the PET blot, suggests that colonic deposition of α Syn is not a useful diagnostic test for PD. The utility of detecting α Syn in the colon as a biomarker in combination with other assessments remains to be determined.

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GLOSSARY

α Syn = α -synuclein; ENS = enteric nervous system; PD = Parkinson disease; PET = paraffin-embedded tissue; Ser129p- α Syn = serine 129-phosphorylated α -synuclein; UPDRS-III = Unified Parkinson's Disease Rating Scale, Part III.

An accurate diagnostic test for early or prodromal Parkinson disease (PD) is a significant unmet need. Studies suggesting that abnormal deposition of α -synuclein (α Syn) may originate in the peripheral autonomic nervous system,^{1,2} coupled with evidence of a prion-like spread of synucleinopathy,³ have generated much interest in evaluating enteric nervous system (ENS) α Syn to assess early PD neuropathology before disease spread and long before markers reliant on advanced disease are affected.⁴

Several recent studies of α Syn staining in colonic tissue from living patients with PD report sensitivities ranging from 72% to a remarkable 100%.^{5–7} However, the description of some degree of α Syn labeling in controls in these studies and reports of α Syn in colonic tissue from individuals without PD have raised concerns regarding specificity.^{8,9}

The aim of the present study was to optimize the immunohistochemical detection of α Syn in colonic biopsies by adapting the paraffin-embedded tissue (PET) blot, a method developed to increase sensitivity and specificity of detecting aggregated proteins in a related proteinopathy, Creutzfeldt–Jakob disease. The PET blot degrades nonaggregated proteins allowing the selective detection of remaining, presumably pathologic, protein aggregates.¹⁰ Using this innovative

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method, we show that detection of α Syn in colonic mucosal biopsies lacks specificity as a potential diagnostic biomarker for PD.

METHODS Subjects. Patients undergoing routine colon cancer screening, as recommended by the Canadian Association of Gastroenterology,¹¹ were recruited from the Toronto Western Hospital. Individuals meeting the UK Parkinson's Disease Society Brain Bank criteria for PD¹² without known primary gastrointestinal illness were eligible as PD subjects. All subjects with PD were assessed using the Unified Parkinson's Disease Rating Scale, Part III (UPDRS-III) in the "on" medication state during the subjects' regular clinic visit. Control subjects were asymptomatic individuals with no evidence of a movement disorder upon examination by a movement disorder specialist (8/11 controls were examined using the UPDRS-III).

Colonic mucosal biopsy. Colonoscopy was performed according to standard procedures of the University Health Network Division of Gastroenterology. Briefly, after bowel preparation and under conscious sedation, mucosal biopsies were taken using standard biopsy forceps (Radial Jaw 4, 2.8 mm; Boston Scientific, Marlborough, MA) in the sigmoid colon (20 cm from the anal verge, 4 biopsies) and the rectum (5 cm from the anal verge, 4 biopsies). Tissue was immediately fixed in 10% formalin before paraffin embedding to maintain anatomy.

Immunohistochemistry and PET blot. Details of the immunohistochemistry and PET blot methods are provided in e-Methods on the *Neurology*[®] Web site at Neurology.org.

Immunohistochemistry and PET blot control studies. Control and PD brain tissue were used to optimize the PET blot for detection of pathologic α Syn, and representative images are presented in the supplementary material (figures e-1 and e-2). These studies demonstrated that the PET blot increased both sensitivity and specificity of detecting pathologic α Syn in PD brain. In addition, control studies using blocking peptides specific for the α Syn and serine 129-phosphorylated α Syn (Ser129p- α Syn) antibodies, or with the primary antibodies omitted, were conducted to confirm the specificity of the PET blot for the detection of α Syn and Ser129p- α Syn in colon (figure e-3).

Data analysis. Immunostained sections were scanned at 40 $\times$ using a ScanScope digital slide scanner (Aperio Technologies, Inc., Vista, CA). Digital images were viewed using ImageScope software (Aperio Technologies, Inc.). For each participant, the presence or absence of α Syn and Ser129p- α Syn, as revealed by both PET blot and immunohistochemistry, was assessed in 4 colon biopsy samples at each of the 2 sites (5 and 20 cm). The number of positive samples per site was used to create a semi-quantitative 0 to 4 rating of overall consistency of staining.⁶ All immunohistochemical analyses were performed by a neuropathologist blinded to diagnosis. Statistical analysis was performed using GraphPad Prism v5.00 for Windows (GraphPad, La Jolla, CA, www.graphpad.com).

Standard protocol approvals, registrations, and patient consents. All participants provided written informed consent and all procedures were conducted in accordance with a protocol approved by the University Health Network Research Ethics Board.

RESULTS Participant demographics. Fifteen patients with early PD (<3 years), 7 with later PD (>5 years), and 11 healthy individuals consented during the

study recruitment period and participated. Patient demographics are in table e-1. There was no significant difference in age or sex among the 3 groups. Disease duration was significantly longer in the later PD group compared with the early PD group. UPDRS-III scores were significantly higher in both the early and later PD groups compared with the control group. Levodopa equivalent daily dose¹³ was significantly higher in the later PD group compared with the early PD group.

α Syn and Ser129p- α Syn are present in colonic mucosal biopsies in individuals without PD. Figure 1 illustrates α Syn staining by immunohistochemistry and PET blot at 5 cm (figure 1, A and B, respectively) and 20 cm (figure 1, E and F, respectively) from the anal verge in a control. Both regions display strong staining using either method in adjacent sections. The PET blot sections have little background staining, indicating increased specificity over immunohistochemistry. Table 1 shows a summary of the results of staining in each group using both methods. Complete results of all biopsies, using both PET blot and immunohistochemistry, can be found in table e-2. Overall, in control subjects, PET blot detected α Syn in the colonic mucosa of 10 of 11 participants at 5 cm and 10 of 11 participants at 20 cm. In adjacent sections, immunohistochemistry detected α Syn in 5 of 11 and 6 of 11 participants at 5 and 20 cm, respectively. Thus, in subjects without PD, the PET blot detected α Syn in more samples than immunohistochemistry.

A similar pattern was observed for Ser129p- α Syn in control subjects. The PET blot detected Ser129p- α Syn in 10 of 11 participants at both 5 and 20 cm from the anal verge, whereas immunohistochemistry in adjacent sections detected Ser129p- α Syn in 8 of 11 and 5 of 11 participants at 5 and 20 cm, respectively (table e-2). Figure 1 illustrates representative staining for Ser129p- α Syn by immunohistochemistry and PET blot at 5 cm (figure 1, C and D, respectively) and 20 cm (figure 1, G and H, respectively) in a control case. Both regions display strong staining for Ser129p- α Syn using either method. Of interest, we note that, using PET blot, every section that was positive for α Syn was also positive for Ser129p- α Syn. In contrast, detection of both α Syn and Ser129p- α Syn by immunohistochemistry in the same sample was inconsistent (table e-2).

α Syn is present in colonic mucosal biopsies in 100% of subjects with PD. In early PD, immunohistochemistry detected α Syn in 15 of 15 subjects at both 5 and 20 cm, whereas PET blot only detected α Syn in 11 of 15 and 10 of 15 subjects, respectively (table e-2). Similar to controls, all but one section positive for α Syn by PET blot was also positive for Ser129p- α Syn by PET

Figure 1 α Syn and Ser129p- α Syn are present in colonic mucosal biopsies in individuals without PD

α Syn in colonic biopsies at 5 cm from the anal verge by (A) conventional immunohistochemistry and (B) PET blot in a corresponding section. Ser129p- α Syn in colonic biopsies at 5 cm from the anal verge by (C) conventional immunohistochemistry and (D) PET blot in a corresponding section. α Syn by (E) conventional immunohistochemistry and (F) PET blot in a corresponding section at 20 cm from the anal verge. Ser129p- α Syn by (G) conventional immunohistochemistry and (H) PET blot in a corresponding section at 20 cm from the anal verge. Scale bar represents 150 μ m in A, B, E, and F and 50 μ m in C, D, G, and H. α Syn = α -synuclein; IHC = immunohistochemistry; PD = Parkinson disease; PET = paraffin-embedded tissue; Ser129p- α Syn = serine 129-phosphorylated α -synuclein.

blot. Again, detection of α Syn and Ser129p- α Syn by immunohistochemistry in adjacent sections was inconsistent, with detection of Ser129p- α Syn being much lower.

In late PD, the pattern of both α Syn and Ser129p- α Syn detection by PET blot and immunohistochemistry was remarkably similar to early PD.

Immunohistochemistry detected α Syn in 7 of 7 subjects at both 5 and 20 cm, but PET blot was less sensitive, detecting α Syn in 6 of 7 and 5 of 7 subjects at 5 and 20 cm, respectively (table e-2). Every section positive for α Syn by PET blot was also positive for Ser129p- α Syn by PET blot. However, detection of α Syn and Ser129p- α Syn by immunohistochemistry

Table 1 α Syn in colonic mucosal biopsy tissue assessed by PET blot and immunohistochemistry

Group	PET blot α Syn		IHC α Syn		PET blot Ser129p- α Syn		IHC Ser129p- α Syn	
	5 cm	20 cm	5 cm	20 cm	5 cm	20 cm	5 cm	20 cm
Control (n = 11)	2 (0-4)	2 (0-4)	0 (0-2)	1 (0-4)	2 (0-4)	2 (0-4)	1 (0-3)	1 (0-2)
	40/88 biopsies, 11/11 controls		18/88 biopsies, 9/11 controls		40/88 biopsies, 11/11 controls		18/88 biopsies, 10/11 controls	
Early PD (<3 y from diagnosis) (n = 15)	1 (0-3)	2 (0-4)	4 (3-4)	4 (2-4)	1 (0-3)	2 (0-4)	2 (0-4)	2 (0-4)
	41/120 biopsies, 12/15 patients		107/120 biopsies, 15/15 patients		42/120 biopsies, 12/15 patients		64/120 biopsies, 15/15 patients	
Later PD (>5 y from diagnosis) (n = 7)	1 (0-3)	2 (0-3)	4 (3-4)	4 (2-4)	1 (0-3)	2 (0-3)	2 (1-4)	2 (0-3)
	20/56 biopsies, 7/7 patients		50/56 biopsies, 7/7 patients		20/56 biopsies, 7/7 patients		28/56 biopsies, 7/7 patients	

Abbreviations: α Syn = α -synuclein; IHC = immunohistochemistry; PD = Parkinson disease; PET = paraffin-embedded tissue; Ser129p- α Syn = serine 129-phosphorylated α Syn.

Data are expressed as group median (range) number of positive biopsies at each site using each method and number of positive biopsies/total number of biopsies at 5 and 20 cm combined below. Results of PET blot and immunohistochemistry for α Syn in colonic mucosal biopsies taken at 5 and 20 cm from the anal verge in participants with early (<3 years) PD, later (>5 years) PD, and healthy individuals. α Syn was assessed by PET blot and immunohistochemistry for both α Syn and Ser129p- α Syn in 4 separate biopsy samples per region to yield a score for each individual at each site.

in the same biopsy samples was again inconsistent, with detection of Ser129p- α Syn being much lower.

Figure 2 illustrates staining of α Syn and Ser129p- α Syn in early and late PD. In early PD, there was a clear overlap in positivity by immunohistochemistry and PET blot for both α Syn (figure 2, A and B, respectively) and Ser129p- α Syn (figure 2, C and D, respectively), noted also in late PD (figure 2, E and F). The PET blot depicted networks of small fine processes around large ganglion cells (figure 2D). Conventional immunohistochemistry for Ser129p- α Syn stained the same networks of small fine processes but also showed additional staining of the soma and large nerve fibers (figure 2C). The origin of these cells (noted by asterisks in figure 2, C and D) is currently unknown and the focus of future research.

Increased PET blot-resistant α Syn in colonic mucosal biopsies from healthy individuals compared with PD. Tables 1 and e-2 illustrate that the level of consistency

in α Syn and Ser129p- α Syn staining using PET blot and immunohistochemistry appears to differ between controls and patients with PD. Thus, to compare the relative sensitivity of PET blot and immunohistochemistry to detect α Syn and Ser129p- α Syn in each subject, we subtracted the number of biopsies positive by immunohistochemistry from the number of corresponding biopsies positive by PET blot. We then calculated the median relative sensitivity of PET blot and immunohistochemistry for both α Syn and Ser129p- α Syn at both 5 and 20 cm in each experimental group. Within a given experimental group, the relative sensitivity of PET blot and immunohistochemistry for both α Syn and Ser129p- α Syn was the same at both 5 and 20 cm and the median differences in the number of positive biopsies between groups were similar between the 2 sites. Therefore, further analyses were conducted on combined data from 5 and 20 cm to gain increased statistical power.

Figure 2 α Syn and Ser129p- α Syn are present in colonic mucosal biopsies in early and late PD

An early PD case illustrating α Syn detected by (A) conventional immunohistochemistry and (B) PET blot, and Ser129p- α Syn detected by (C) conventional immunohistochemistry and (D) PET blot. Conventional immunohistochemistry for Ser129p- α Syn stained networks of small fine processes as well as the soma of large ganglion cells (noted by asterisks in panel C). In an adjacent section, following PET blot, the networks of small fine processes remained Ser129p- α Syn positive without staining of the soma (noted by asterisks in panel D). α Syn visualized by (E) conventional immunohistochemistry and (F) by PET blot in an adjacent section in a late PD case. Scale bar represents 100 μ m in A and B, 150 μ m in C and D, and 350 μ m in E and F. α Syn = α -synuclein; PD = Parkinson disease; PET = paraffin-embedded tissue; Ser129p- α Syn = serine 129-phosphorylated α -synuclein.

Figure 3 illustrates the relative sensitivity of PET blot and immunohistochemistry for α Syn (figure 3A) and Ser129p- α Syn (figure 3B). Regarding α Syn, the number of positive biopsies by the PET blot relative to immunohistochemistry was significantly lower in both early PD (median -2, range -4 to 0) and later PD (median -2, range -4 to 1) compared with controls (median 1, range -2 to 4) (both $p < 0.001$ Kruskal-Wallis test with Dunn post hoc test for multiple comparisons). Similarly, regarding Ser129p- α Syn, the sensitivity of the PET blot relative to immunohistochemistry was significantly lower in both early PD (median -1, range -3 to 4) and later PD (median 0, range -4 to 2) compared with controls (median 1, range -1 to 4) ($p < 0.001$ and

$p < 0.05$ respectively, Kruskal-Wallis test with Dunn post hoc test for multiple comparisons; figure 3B). Thus, the PET blot appears to increase sensitivity for detecting both α Syn and Ser129p- α Syn more so in control than in PD colonic mucosa. Indeed, within the control samples, there were several biopsies devoid of signal using immunohistochemistry for which α Syn was detectable using the PET blot, but only one biopsy sample for which the reverse was true (table e-2).

This pattern of α Syn being more readily detectable by immunohistochemistry than PET blot in PD was also apparent within a single biopsy, illustrated in figure 4. Of 4 separate regions positive for Ser129p- α Syn by immunohistochemistry, only one remained positive by PET blot in an adjacent section. Close inspection of staining for Ser129p- α Syn in ganglion cells in PD also demonstrated a region that was positive using immunohistochemistry (figure 2C) but negative by PET blot (figure 2D). Thus, of note, some of the regions of Ser129p- α Syn detected by immunohistochemistry appear not to be PET blot-resistant.

DISCUSSION The primary aim of this study was to determine the utility of detecting α Syn in colonic mucosal biopsy tissue as a diagnostic biomarker for PD. We adapted the PET blot, expressly developed to increase the sensitivity and specificity of detecting misfolded/aggregated proteins, to optimize detection of abnormal α Syn and increase antigen retrieval, and hypothesized that this would be superior to conventional immunohistochemistry in detecting early pathology in the ENS. The PET blot degrades physiologic nonaggregated proteins using proteinase K¹⁰ and has been demonstrated to have superior sensitivity in detecting aggregated protein.^{14,15}

Our control studies in postmortem brain agree with previous studies showing that PET blot confers increased sensitivity for detecting α Syn in PD brain tissue, with a clear increase in the number of visible Lewy neurites compared with conventional immunohistochemistry.¹⁴ We also demonstrate increased specificity for detecting pathologic α Syn, whereby after PET blotting, no signal for α Syn is apparent in control brain. Conventional immunohistochemistry, by virtue of detecting physiologic α Syn, is less specific and therefore requires more detailed morphologic analysis to distinguish PD from controls. Thus, PET blot would seem ideal for addressing outstanding issues of specificity and sensitivity regarding α Syn as a biomarker for PD in colonic biopsy tissue.

In colonic biopsies from living individuals, we found PET blot-resistant aggregated α Syn in 12 of 15 individuals with early PD, 7 of 7 individuals with later PD, and 11 of 11 subjects with no evidence of

Figure 3 Sensitivity of PET blot vs immunohistochemistry for both α Syn and Ser129p- α Syn in individuals without PD (control), with early PD, and with later PD

For each subject, the combined number of biopsies at 5 and 20 cm from the anal verge positive by immunohistochemistry was subtracted from the number of corresponding biopsies positive by PET blot and plotted for both (A) α Syn and (B) Ser129p- α Syn. Data are presented as median and range. *Significant difference to the control group (* $p < 0.05$ and *** $p < 0.001$), Kruskal-Wallis test with Dunn post hoc test for multiple comparisons. α Syn = α -synuclein; EPD = early Parkinson disease; IHC = immunohistochemistry; LPD = later Parkinson disease; PD = Parkinson disease; PET = paraffin-embedded tissue; Ser129p- α Syn = serine 129-phosphorylated α -synuclein.

Figure 4 Increased Ser129p- α Syn detected by conventional immunohistochemistry compared with PET blot in colonic mucosal biopsies in early PD

(A) Ser129p- α Syn by conventional immunohistochemistry reveals 4 regions of positivity denoted by broken circles, illustrated in high-power microphotographs in B-E. (F) Ser129p- α Syn visualized by PET blot in an adjacent section reveals one overlapping region of positivity denoted by a broken circle and illustrated at high power in G. Scale bar in A represents 1,500 μ m in A and F, 300 μ m in B-E, and 450 μ m in G. IHC = immunohistochemistry; PD = Parkinson disease; PET = paraffin-embedded tissue; Ser129p- α Syn = serine 129-phosphorylated α -synuclein.

parkinsonism. In addition, we identified α Syn staining by conventional immunohistochemistry in both patients with PD and controls. Detailed visual analysis performed by a neuropathologist in 8 biopsy samples per subject showed no difference in either the area of α Syn deposition or the intensity of staining in patients with PD or controls. Thus, we conclude that, by the methods applied, there is no feature of colonic mucosal α Syn staining able to distinguish PD from controls. These findings are consistent with and extend 2 recent reports. One study showed the presence of α Syn in individuals without PD in recto-sigmoid segments obtained during partial colectomy in 13 of 13 subjects examined.⁸ The same report found evidence of Ser129p- α Syn in 11 of 13 subjects without PD, associated with increasing age. In our study using PET blot, where α Syn was present, we also found Ser129p- α Syn in adjacent sections. A second study reported α Syn in 52% of 77 colonic biopsy samples obtained from the general aged population without PD.⁹ Although these authors described staining of α Syn with a higher prevalence (100%)

in PD colon, the high positivity in the general aged population, far more than could be reasonably attributed to coincident detection of prodromal PD, led the authors to conclude that colonic α Syn would not make a good diagnostic biomarker for PD. Of note, these earlier studies reported findings in autopsy samples⁹ or surgical specimens⁸ in the deeper submucosal and myenteric plexuses. Mucosal biopsies obtained by colonoscopy or sigmoidoscopy are necessarily superficial for safety reasons. Obtaining full-thickness biopsies, which would include the myenteric plexus, would require more invasive surgery. Thus, our study, by virtue of using colonoscopic biopsy samples from living individuals, predominantly examined the mucosa and occasionally the submucosa. In tissue in which the submucosa was included and examined, we observed that α Syn was predominantly submucosal in controls whereas α Syn was both mucosal and submucosal in PD. A combination of findings from several regions of the upper and lower gastrointestinal tract demonstrate that in PD, α Syn is more frequently in the myenteric or

submucosal plexus, than in the mucosa.¹⁶ Further studies capturing deeper structures should investigate whether there is redistribution of α Syn to different layers of the colon wall in PD. Immunohistochemical and biochemical studies suggest that, in contrast to nonaggregated forms of the protein, the majority of aggregated α Syn within Lewy bodies in PD is phosphorylated at serine residue 129.^{17–20} Thus, our observation that PET blot-resistant α Syn is hyperphosphorylated in all but one section examined provides support that the PET blot is detecting aggregated forms of the protein. As we and others have recently discussed, a key issue in using α Syn as a peripheral tissue PD biomarker concerns what signal is to be considered “pathologic” and what is “normal.”^{5,21} Many studies reporting lack of colonic α Syn staining in healthy individuals nevertheless note some degree of positivity that is labeled “normal” or “non-specific.”^{6,7,22} Our study, along with other studies, would suggest that Ser129p- α Syn in healthy colon is not necessarily pathologic.⁸ Future studies should address the suggestion that this process is an age-related phenomenon.

Our finding that α Syn is present in control colon using conventional immunohistochemistry contrasts with others who report a lack of staining.^{7,22} Sampling frequency may explain this because we investigated 8 separate biopsies from 2 different sites within the colon in each participant. In 11 controls (88 biopsies), we noted the presence of either α Syn or Ser129p- α Syn by immunohistochemistry in only 18 of 88 biopsies. Indeed, all control subjects had at least one negative biopsy sample, although 10 of 11 had at least one positive biopsy by immunohistochemistry. The frequency of biopsy positivity was much higher in PD. As we and others have discussed, sensitivity and specificity are clearly influenced by details of sampling technique, such as the number of sections, section thickness, number of slides analyzed, and amount of tissue available.^{5,7,22,23} Future efforts involving sharing of tissues and methodologies will be critical in determining the potential of colonic α Syn as a PD biomarker.

Although we demonstrate that the colonic mucosa is not a valuable biopsy location for diagnostic purposes, there are several reports investigating the utility of assessing α Syn deposition in a variety of different tissues, including skin and submandibular gland.^{24–26} However, currently, none of these tissues have demonstrated 100% sensitivity or specificity for detecting PD. It is possible that the PET blot may be useful in increasing the sensitivity or specificity of α Syn deposition for PD in these alternate tissues.

Our demonstration that there is more PET blot-resistant colonic α Syn in control individuals than in patients with PD is quite unexpected and raises an

important hypothesis regarding PD pathogenesis. A long-standing proposal posits that initiation of PD involves a pathogen that may gain access to the brain through the gut.^{1,27} In vitro, proteinase K-resistant oligomers of α Syn can disaggregate to release cytotoxic fibrillar oligomers,²⁸ and furthermore, α Syn is capable of cell-to-cell spread and recruitment of non-pathologic α Syn in a prion-like manner (reviewed in reference 3). Our observation that there is a reduction in PET blot-resistant α Syn aggregates in PD compared with control colon tempts us to hypothesize that in PD colon, these aggregates may have disaggregated and infiltrated the ENS then CNS as fibrillar α Syn oligomers, as proposed by Braak et al.¹ This intriguing hypothesis certainly warrants further investigation.

Using a technique tailored to increase both sensitivity and specificity for detecting aggregated α Syn, we demonstrate the presence of α Syn in colonic mucosal biopsies from control individuals and patients with PD. This raises serious concern about the use of colonic biopsies as a diagnostic test for PD. It is likely that future diagnostic assessments, particularly those evaluating prodromal diagnosis for research purposes, will involve a battery of observations. In accordance with previous reports,⁷ we do, however, note that α Syn is found in a much higher proportion of biopsies from patients with PD than from controls, suggesting that colonic α Syn may reflect a pathogenic process in PD.

AUTHOR CONTRIBUTIONS

N.P.V.: collection, management, statistical analysis, and interpretation of the data, preparation of manuscript. D.S.K., L.W.C.L., A.A.D.: collection of the data. A.G.: editing of manuscript. L.W.C.L., C.M., A.E.L., L.-N.H.: design and conduct of study, review and approval of the manuscript.

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DISCLOSURE

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