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Detection of alpha-synuclein aggregates in gastrointestinal biopsies by protein misfolding cyclic amplification

Alexis Fenyi[a], Laurène Leclair-Visonneau[b,c], Thomas Clairembault[b], Emmanuel Coron[b,c], Michel Neunlist[b,c], Ronald Melki[a], Pascal Derkinderen[b,c,d,*], Luc Bousset[a,*]

[a] Institut Fancois Jacob (MIRCen), CEA and Laboratory of Neurodegenerative Diseases, CNRS, 18 Route du Panorama, 92265 Fontenay-Aux-Roses cedex, France
[b] Inserm, U1235, Faculté de Médecine, 1 rue Gaston Veil, F-44035 Nantes, France
[c] University Nantes, Faculté de Médecine, 1 rue Gaston Veil, F-44035 Nantes, France
[d] CHU Nantes, Department of Neurology, Nantes, Bd Jacques Monod, F-44093 Nantes, France

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ABSTRACT

Lewy bodies and neurites, the pathological signatures found in the central nervous system of Parkinson's disease (PD) patients, are primarily composed of aggregated alpha-synuclein (aSyn). The observation that aSyn aggregates are also found in the enteric nervous system has prompted several studies aimed at developing a diagnostic procedure based on the detection of pathological aSyn in gastrointestinal (GI) biopsies. The existing studies, which have all used immunohistochemistry for the detection of pathological aSyn, have had conflicting results. In the current survey, we analyzed the seeding propensity of aSyn aggregates from GI biopsies.

A total of 29 subjects participated to this study, 18 PD patients and 11 controls. For each patient, 2 to 4 GI biopsies were taken from the same site (antrum, sigmoid colon or rectum) and used to seed the aggregation of recombinant aSyn in an assay inspired from the protein misfolding cyclic amplification (PMCA) method. In a subset of patients and controls (14 and 3, respectively), one or two additional biopsies were analyzed by immunohistochemistry for the presence of phosphorylated aSyn histopathology (PASH) using antibodies against phosphorylated aSyn and PGP 9.5.

Except for one subject, none of the control samples seeded aSyn aggregation in PMCA reaction. GI biopsies from patients with PD seeded aSyn aggregation in 10 out of 18 cases (7 from the sigmoid colon, 2 from the antrum and one from the rectum). There was good agreement between PMCA and immunohistochemistry results as, except for two cases, all PMCA-positive PD patients were also PASH-positive.

Our findings show that the PMCA method we implemented is capable of detecting aSyn aggregates in routine GI biopsies. They also suggest that rectum biopsies do not contain sufficient amounts of aggregated aSyn to detect seeded assembly by PMCA. While encouraging, our findings indicate that further studies are needed to establish the diagnostic potential of the PMCA method we implemented to detect aSyn aggregates in upper GI biopsies.

1. Introduction

The *postmortem* detection by immunohistochemistry of aggregated alpha-synuclein (aSyn) in the brain along with neuronal loss in the *substantia nigra* remain the gold standard for the definite diagnosis of Parkinson's disease (PD). Over the last 12 years, several studies have shown that aSyn aggregates can also be detected outside the central nervous system, particularly in the enteric nervous system (ENS) (Braak et al., 2006; Beach et al., 2010; Bloch et al., 2006; Gelpi et al., 2014). The ENS is a neuronal network embedded in the lining of the

Abbreviations: aSyn, alpha-synuclein; CNS, central nervous system; CSF, cerebrospinal fluid; ENS, enteric nervous system; FFPE, Formalin-fixed, paraffin-embedded; GI, gastrointestinal; PASH, phosphorylated aSyn histopathology; PD, Parkinson's disease; PGP9.5, Protein Gene Product 9.5; PMCA, protein misfolding cyclic amplification; ThT, Thioflavine T

* Corresponding authors at: Pascal Derkinderen, Inserm U1235, 1 rue Gaston Veil 44035 Nantes, France; Luc Bousset, Institut François Jacob, CEA and Laboratory of Neurodegenerative Diseases, CNRS, 18 Route du Panorama, 92265 Fontenay-Aux-Roses cedex, France.

E-mail addresses: alexis.fenyi@cnrs.fr (A. Fenyi), laurene.leclair@chu-nantes.fr (L. Leclair-Visonneau), emmanuel.coron@chu-nantes.fr (E. Coron), michel.neunlist@univ-nantes.fr (M. Neunlist), ronald.melki@cnrs.fr (R. Melki), pascal.derkinderen@chu-nantes.fr, derkinderenp@yahoo.fr (P. Derkinderen), luc.bousset@cnrs.fr (L. Bousset).

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gastrointestinal (GI) tract from the lower third of the esophagus to the rectum, whose distinguishing feature is its accessibility and suitability for routine GI biopsies (Lebouvier et al., 2010a). These findings logically prompted a substantial amount of research to determine if the immunohistochemical detection of pathological aSyn in routine GI biopsies could be used for pathological diagnosis of PD within patients lifespan (reviewed in (Corbillé et al., 2016a; Schneider et al., 2016)).

In our seminal study, using microdissection and whole-mount preparation of colonic biopsies, we detected aSyn pathology in 21 out of 29 PD patients and in none of the controls (Lebouvier et al., 2010b). Although the microdissection technique we have developed provides outstanding information on the morphology of enteric neurons, it nonetheless has several limitations as it needs to be performed immediately after the endoscopic procedure and requires technical expertise and training (Lebouvier et al., 2010a). Most subsequent studies on GI biopsies in PD have therefore been performed on formalin-fixed, paraffin-embedded (FFPE) tissue, which has the main advantages of being readily accessible to most hospital-based laboratories and to allow retrospective analyses. Nevertheless, the diagnostic utility of FFPE GI biopsies in PD remains unclear, as these studies yielded conflicting results regarding the sensitivity and specificity of GI biopsies in detecting pathological aSyn (Shannon et al., 2012; Chung et al., 2016; Stokholm et al., 2017; Antunes et al., 2016; Barrenschee et al., 2017; Visanji et al., 2015; Hilton et al., 2014; Sánchez-Ferro et al., 2015; Corbillé et al., 2016b).

One possible explanation for the observed discrepancies between existing studies on immunohistochemical detection of aSyn pathology in the ENS is the very low amount of aSyn aggregates usually found in GI samples. Protein Misfolding Cyclic Amplification (PMCA) enables ultrasensitive detection of the pathogenic prion protein PrP in Creutzfeldt-Jakob disease through amplification of its misfolding and aggregation *in vitro* under different experimental conditions (Saborio et al., 2001). This technique has been recently used to detect aggregated aSyn in the CSF of PD patients (Shahnawaz et al., 2017) and in formaldehyde-fixed samples from multiple system atrophy patients (Becker et al., 2018). We implemented this method to assess the presence of pathogenic aSyn in GI biopsies.

2. Methods

2.1. Subjects, GI biopsies and study design

A total of 29 subjects participated to this study, 18 patients with PD and 11 controls. PD patients who were diagnosed according to the United Kingdom PD Society Brain Bank criteria were recruited from the movement disorder clinic at Nantes University Hospital, France. 16 out of 18 PD patients had a rectosigmodoscopy or a colonoscopy, while the remaining 2 had upper GI endoscopy for placement of an administration tube for continuous levodopa enteral infusion. As controls, we used GI biopsies from 2 groups: (1) 7 subjects who had colonoscopy for colorectal cancer screening, (2) 4 subjects with Crohn's disease who underwent either rectosimoidoscopy or colonoscopy for disease management and surveillance (Table 1). All control subjects had a detailed neurological examination to rule out PD symptoms and cognitive deficiency. Two to 4 biopsies per subject were snap frozen in liquid nitrogen at the time of collection and stored at −80 °C. 14 PD cases and 3 control cases had additional biopsies (taken from the same site, either in the sigmoid colon [2 biopsies] or in the rectum [1 biopsy]) that were analyzed by immunohistochemistry (see below). The study protocol was approved by the local Committee on Ethics and Human Research (*Comité de Protection des Personnes Ouest IV and VI*), conformed to the Code of Ethics of the World Medical Association (Declaration of Helsinki) and was registered on ClinicalTrials.gov (identifier NCT01353183 and NCT01748409). Written informed consent was obtained from each patient and from each control.

2.2. Reagents and chemicals

Unless otherwise stated all reagents were purchased from Sigma (Saint-Quentin-Fallavier, France).

2.3. Microdissection and immunohistochemistry

Microdissection was performed as previously described in one biopsy (when taken in the rectum) or two biopsies (when taken in the sigmoid colon) (Corbillé et al., 2016a). Each whole-mount preparation of submucosa obtained from a single biopsy was permeabilized for 3 h in phosphate buffered saline (PBS)/NaN_3 containing 1% (v/v) Triton X-100 and 10% (v/v) horse serum and then incubated with antibodies against phosphorylated aSyn (1:5000, WAKO, Osaka, Japan) and PGP9.5 (1:10000; Ultraclone Limited, UK). A patient was noted as positive (phosphorylated aSyn histopathology, PASH+) when at least one structure immunoreactive for both phosphorylated aSyn and PGP9.5 was observed.

2.4. Biopsies treatment for amplification

2 to 4 Biopsies were weighted in a Safelock 2 mL Biopur tube (Eppendorf, Montesson, France). The biopsies were resuspended 5% (w/v) in buffer (150 mM KCl, 50 mM Tris-HCl pH 7.5) and disrupted by sonication using a Branson SFX 150 Cell Disruptor sonicator equipped with a 3.17 mm microtip probe (Emerson, Bron, France). Sonication was applied for 10 s at 70% of the maximum amplitude. The resulting homogenate was aliquoted and flash frozen in liquid nitrogen before storage at −80 °C.

2.5. PMCA assay

Biopsies homogenates were diluted in 150 mM KCl, 50 mM Tris-HCl pH 7.5 to a final proportion of 2% (w/v). Monomeric aSyn concentration was adjusted to 100 μM in a total volume of 300 μL. The solution was split in 2 replicates in a PCR strips (BIOplastics, Landgraaf, The Netherlands) tubes (150 μL in each). PMCA amplification was performed on 2 replicates for each patient using the Q700 generator and a 431MPX horn (both from Qsonica, Fisher scientific, Illkirch, France). The power of the horn was set to 30% of the maximum amplitude. The sonication protocol consisted of 15 s of sonication and 315 s of rest at 31 °C. Every hour, 5 μL from each tube were collected and diluted into 300 μL Thioflavine T (ThT, 10 μM). The amplification was monitored by measuring ThT fluorescence increase using a Cary Eclipse Fluorescence Spectrophotometer (Agilent, les Ulis, France) with fixed excitation and emission wavelength at 440 and 480 nm, respectively. The sensitivity of the method was assessed using recombinant preformed fibrils. The detection threshold was determined to be in the range of 10 pM of aggregated aSyn (Fig. 1). PMCA efficacy was derived from the lag phase length and was considered high when the lag phase was shorter or equal to 300 min and was considered medium to low when it lied within the time frame 300 to 425 min. Statistical analysis were perfomed using ANOVA, on the duration of the lag phase or the ThT fluorecence at 480 min (Fig. 2 C and D).

3. Results

A total of 29 subjects participated to this study, 18 PD patients (12 males, mean age ± standard deviation: 63.7 ± 7.7 years) and 11 controls (7 males, 52.4 ± 15.3 years). Age and sex did not differ significantly between patients and controls (Table 1).

Two to 4 biopsies were obtained from each PD cases and control cases. For each subject multiple biopsies were pooled and homogenized as follow: 2 pooled biopsies for 6 PD cases, 3 pooled biopsies for 3 PD cases and 3 controls, 4 pooled biopsies for 9 PD cases and 8 controls (Table 1). Homogenates were blindly analyzed by means of PMCA

Table 1
Histology and amplification summary.

	Age, sex	Diagnosis	Biopsy			PMCA results			
			Site	Number	PASH	Amplification	Lag time	ThT increase at 480 min for each duplicate	
1	68/M	Control	Rectum	4	(0/1)	+	420	4,4	< 1
2	58/F			4	(0/1)	−	> 540	< 1	< 1
3	38/F			4	NA	−	540	< 1	< 1
4	43/M			4	NA	−	> 540	< 1	< 1
5	62/M		Sigmoid	3	NA	−	> 540	< 1	< 1
6	66/M			3	NA	−	540	< 1	< 1
7	61/F			3	NA	−	> 540	< 1	< 1
8	72/M			4	NA	−	540	< 1	< 1
9	56/F			4	NA	−	> 540	< 1	< 1
10	53/M			4	(0/2)	−	540	< 1	< 1
11	33/M			4	NA	−	> 540	< 1	< 1
12	67/M	Parkinson's disease	Rectum	4	(0/1)	−	> 540	< 1	< 1
13	66/M			4	(0/1)	−	> 540	< 1	< 1
14	55/M			4	(0/1)	−	> 540	< 1	< 1
15	68/F			4	(0/1)	+	420	1,4	1,2
16	70/M		Sigmoid	4	(1/2)	+ +	210	12,6	9,1
17	70/M			4	(0/2)	−	540	< 1	< 1
18	55/M			4	NA	+	360	2,6	2,0
19	47/F			3	NA	+	300	8,9	1,5
20	53/M			2	(0/2)	−	> 540	< 1	< 1
21	67/M			3	(0/2)	−	540	< 1	< 1
22	58/M			3	(2/2)	+ +	300	16,1	5,8
23	69/M			2	(2/2)	−	> 540	< 1	< 1
24	72/F			2	(0/2)	−	> 540	< 1	< 1
25	59/M			2	(0/2)	+	390	3,8	1,8
26	58/F			2	(2/2)	+	360	2,1	1,5
27	70/M			2	(2/2)	+ +	360	14,6	5,4
28	72/F		Antrum	4	NA	+ +	180	19,0	18,8
29	70/F			4	NA	+ +	240	10,5	9,3

Age and sex: age of patients at the time of the biopsies and gender; Diagnosis: differentiates controls from parkinsonian patients; Biopsy: site of the biopsy (Site), number of biopsies used in the PMCA assay (Number), result of the histological analysis (PASH; NA = not assessed; (X/Y) = number of positive biopsy[ies]); PMCA results: amplification efficacy ranked base on lag phase duration and ThT increase (Amplification; + +: high amplification; +: low amplification; −: no amplification detected), lag phase duration in minutes (Lag Time), ThT increase calculated between time point 60 min and 480 min (ThT increase at 480 min for each duplicate).

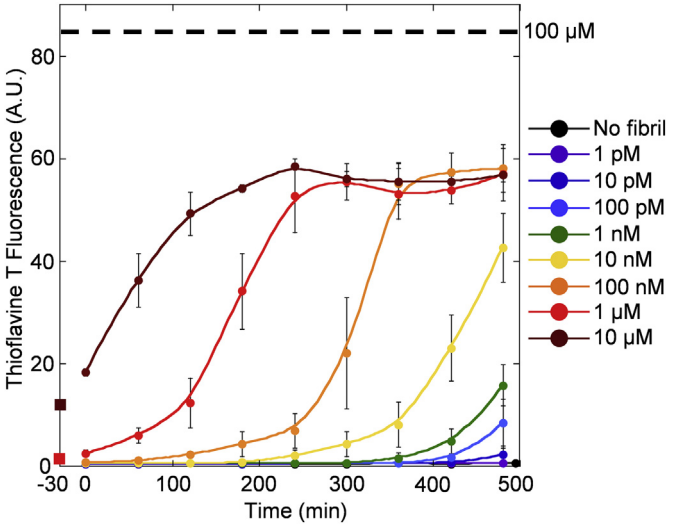

Fig. 1. PMCA of preformed recombinant aSyn fibrils. Increasing concentration of recombinant seeds were added in the reaction to assess the sensitivity of the PMCA method we implemented. The fluorescence due to the seeds is indicated at time − 30 min corresponding to the mixing of seeds and monomeric substrate for 1 µM, 10 µM and, as a dashed line, for 100 µM of aSyn fibrils. Time zero corresponds to the time where PMCA amplification is initiated. The concentrations of recombinants fibrils are expressed as initial monomeric concentration. The curves represent the average of 4 replicates +/− SD.

(Fig. 2, Supplementary Fig. 1). Except for subject #1, none of the GI biopsies homogenates from the control group, regardless of the biopsy site (sigmoid colon or rectum), seeded the aggregation of monomeric aSyn. Subject #28 was initially enrolled in 2008 as a control and as such had no signs or symptoms suggestive of PD. She underwent upper GI endoscopy for the investigation of anemia and 4 antrum biopsies were taken and stored as part of our research protocol. Because of the positive PMCA result, we decided to clinically reevaluate this patient 10 years after GI biopsies collection. Neurological examination showed that she had developed in the meantime typical signs and symtoms of PD, including right bradykinesia and mild bilateral rest tremor.

GI biopsies homogenates from patients with PD seeded monomeric aSyn aggregation in 10 out of 18 cases (Table 1). Among these PMCA+ biopsies, 2 sets of biopsies were taken from the antrum, 7 sets of biopsies were taken in the sigmoid colon while the remaining one was from the rectum. Of note rectal biopsies from 3 out of 4 PD patients yielded negative results with PMCA. Identical results were observed for the rectal biopsies from controls (Table 1).

In 14 patients with PD and 3 controls, one or two additional biopsies were immunohistochemically assessed for the presence of PASH (Fig. 3). All 3 controls as well as the 4 PD patients who had rectal biopsies were PASH- (Table 1). When sigmoid biopsies were analyzed, 5 out of 10 PD patients were PASH+ and among these 5 cases, 4 were also PMCA+ (Table 1).

4. Discussion

Here, we have used GI biopsies from PD patients to show that aSyn aggregates from peripheral autonomic nervous system can be amplified through PMCA. Only one control biopsy induced aSyn aggregation

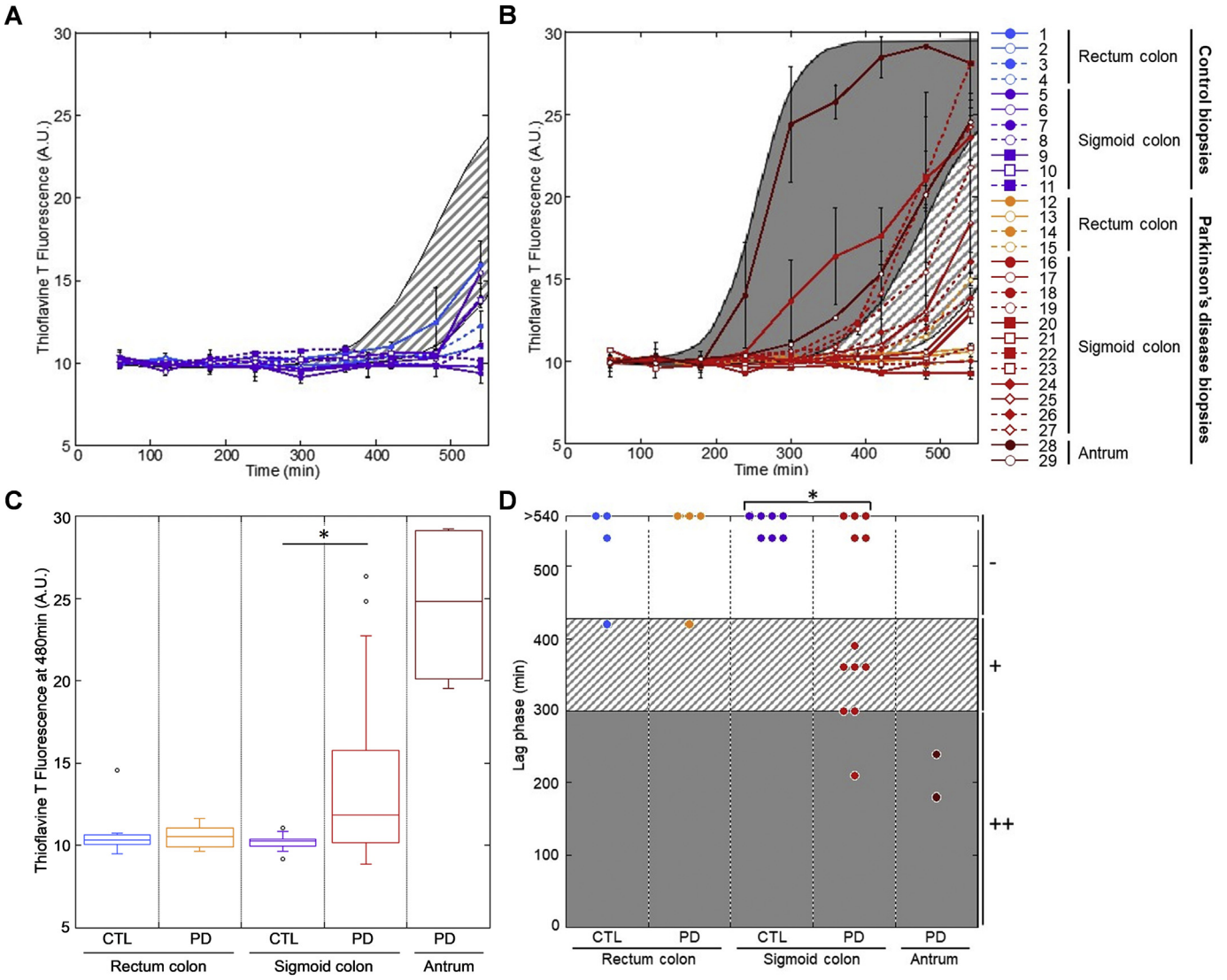

Fig. 2. PMCA assay in controls (CTL) and PD. PMCA was performed using gastrointestinal biopsies homogenates from controls (A) or PD patients (B). aSyn seeded assembly reactions obtained in the presence of homogenates from rectum biopsies are coloured in blue (controls) and orange (PD patients). Those obtained in the presence of sigmoid colon biopsies homogenates are coloured in purple (controls) and red (PD patients). Those obtained in the presence of antrum biopsies homogenates are coloured in brown (PD patients). PMCA efficacy was considered high when the assembly kinetics lied within the grey surface area. PMCA efficacy was considered medium or low when the assembly kinetics lied within the hatched grey surface area. The curves represent an average of 2 replicates, +/− SD. (C) Box plot representation of the distribution of ThT fluorescence at time 480 min, (D) Distribution of the lag phase duration. One-way analysis of variance (ANOVA) test was used for statistical analysis; *: $P < .05$. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

suggesting that the seeding method we implemented has a high specificity. Regarding PD, 2/2 upper GI biopsies, 7/12 sigmoid biopsies and only 1/4 rectal biopsies were PMCA+. Although our sample size is too small to draw firm conclusions, our findings are in line with previous reports that showed a rostro-caudal distribution of aSyn pathology in the gut, with the lower esophagus and stomach having the highest frequency of PASH, followed by small bowel regions, colon and rectum (Beach et al., 2010; Pouclet et al., 2012; Wakabayashi et al., 1988). They also suggest that rectal biopsies are not suited for PMCA-based PD diagnosis. There was significant agreement between PMCA and immunohistochemistry as 4 out of 5 PASH+ PD patients were also PMCA+. Samples from the remaining PMCA+ patients were either not assessed by immunohistochemistry (4 cases) or were PMCA+ but PASH- (2 cases). All PMCA+ and PASH+ samples originated from the sigmoid colon (4 cases). Limited agreement between PMCA and immunohistochemistry was observed for rectal samples as none was PASH + while 1 out of 4 was PMCA+. The same figure was observed for control samples. Altogether, our results suggest that rectal biopsies are not suited for PMCA-based PD diagnosis.

An interesting observation is the positive detection of aSyn aggregates in GI samples initially collected as controls from one subject who subsequently developed clinical symptoms of PD (subject #28). In a recent report that used aSyn PMCA in CSF, Shahnawaz et al. identified two subjects originally enrolled as controls who were subsequently diagnosed as having PD 1 and 4 years after sample collection (Shahnawaz et al., 2017). These findings suggest that PMCA might be used as a preclinical or premotor diagnostic method for PD.

In conclusion, our preliminary study supports the idea that PMCA could be used to detect aSyn aggregates in GI biopsies from PD patients. Further studies are needed to establish the diagnostic potential of PMCA to detect aSyn aggregates in GI biopsies especially in the upper GI tract.

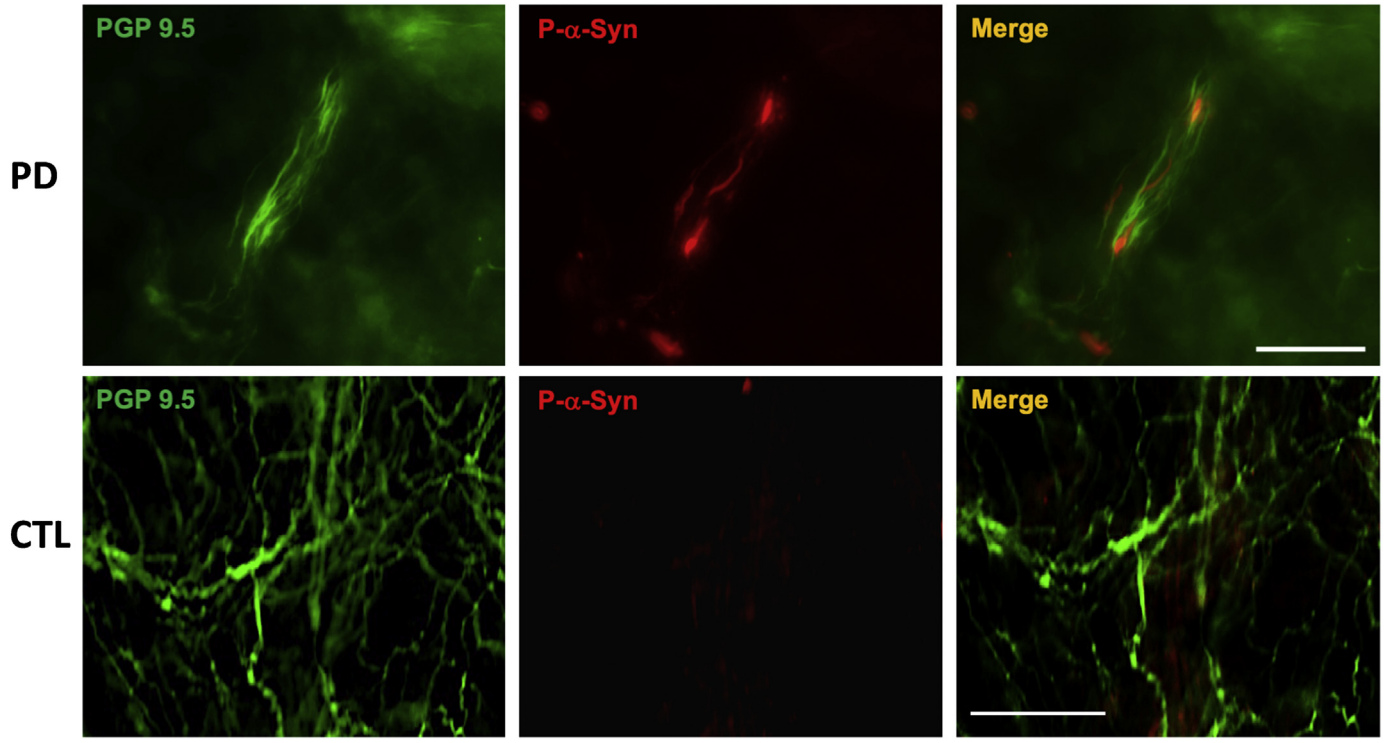

Fig. 3. Representative photomicrographs of phosphorylated alpha-synuclein and PGP 9.5 staining in the submucosa of PD patients and controls. Sigmoid biopsies from one PD patient (PD, #22; upper panels) and one control (CTL, #10; lower panels) were microdissected and analyzed by immunohistochemistry with antibodies against PGP 9.5 and phosphorylated alpha-synuclein (P-aSyn). The representative photomicrographs from PD patient show multiple PASH in whole-mount of submucosa immunoreactive for PGP9.5 (green) that were also positive for P-aSyn (red) in PD. By contrast, no immunoreactivity for P-aSyn was observed in control, with the exception of some faint nonspecific background labeling. Scale bar: 75 µm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Ethics approval and consent to participate

Regarding sigmoid biopsies sampling, the study protocol was approved by the local Committee on Ethics and Human Research (*Comité de Protection des Personnes Ouest IV and VI*) and was registered on ClinicalTrials.gov (identifier NCT01353183 and NCT01748409). Written informed consent was obtained from each patient and from each control.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analyzed during the current study available from the corresponding author on reasonable request.

Competing interests

None.

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Author contributions

AF, RM, TC, PD and LB performed the experiments and analyzed the data. LLV and MN managed the biobanking and dissected the colonic samples. EC performed the endoscopy. AF, RM, PD and LB designed the research and wrote the manuscript.

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References

Antunes, L., Frasquilho, S., Ostaszewski, M., Weber, J., Longhino, L., Antony, P., et al., 2016. Similar α-Synuclein staining in the colon mucosa in patients with Parkinson's disease and controls. Mov. Disord. 31, 1567–1570.

Barrenschee, M., Zorenkov, D., Böttner, M., Lange, C., Cossais, F., Scharf, A.B., et al., 2017. Distinct pattern of enteric phospho-alpha-synuclein aggregates and gene expression profiles in patients with Parkinson's disease. Acta Neuropathol. Commun. 5 (1).

Beach, T.G., Adler, C.H., Sue, L.I., Vedders, L., Lue, L., White Iii, C.L., et al., 2010. Multi-organ distribution of phosphorylated alpha-synuclein histopathology in subjects with Lewy body disorders. Acta Neuropathol. 119, 689–702.

Becker, K., Wang, X., Vander Stel, K., Chu, Y., Kordower, J., Ma, J., 2018. Detecting alpha Synuclein seeding activity in formaldehyde-fixed MSA patient tissue by PMCA. Mol. Neurobiol. 55, 8728–8737.

Bloch, A., Probst, A., Bissig, H., Adams, H., Tolnay, M., 2006. Alpha-synuclein pathology of the spinal and peripheral autonomic nervous system in neurologically unimpaired elderly subjects. Neuropathol. Appl. Neurobiol. 32, 284–295.

Braak, H., de Vos, R.A.I., Bohl, J., Del Tredici, K., 2006. Gastric alpha-synuclein immunoreactive inclusions in Meissner's and Auerbach's plexuses in cases staged for Parkinson's disease-related brain pathology. Neurosci. Lett. 396, 67–72.

Chung, S.J., Kim, J., Lee, H.J., Ryu, H.-S., Kim, K., Lee, J.H., et al., 2016. Alpha-synuclein in gastric and colonic mucosa in Parkinson's disease: limited role as a biomarker. Mov. Disord. 31, 241–249.

Corbillé, A.-G., Clairembault, T., Coron, E., Leclair-Visonneau, L., Preterre, C., Neunlist, M., et al., 2016a. What a gastrointestinal biopsy can tell us about Parkinson's disease? Neurogastroenterol. Motil. 28, 966–974.
Corbillé, A.-G., Letournel, F., Kordower, J.H., Lee, J., Shanes, E., Neunlist, M., et al., 2016b. Evaluation of alpha-synuclein immunohistochemical methods for the detection of Lewy-type synucleinopathy in gastrointestinal biopsies. Acta Neuropathol. Commun. 4, 35.
Gelpi, E., Navarro-Otano, J., Tolosa, E., Gaig, C., Compta, Y., Rey, M.J., et al., 2014. Multiple organ involvement by alpha-synuclein pathology in Lewy body disorders. Mov. Disord. 29, 1010–1018.
Hilton, D., Stephens, M., Kirk, L., Edwards, P., Potter, R., Zajicek, J., et al., 2014. Accumulation of α-synuclein in the bowel of patients in the pre-clinical phase of Parkinson's disease. Acta Neuropathol. 127, 235–241.
Lebouvier, T., Coron, E., Chaumette, T., Paillusson, S., Bruley des Varannes, S., Neunlist, M., et al., 2010a. Routine colonic biopsies as a new tool to study the enteric nervous system in living patients. Neurogastroenterol. Motil. 22, e11–e14.
Lebouvier, T., Neunlist, M., Bruley des Varannes, S., Coron, E., Drouard, A., N'Guyen, J.-M., et al., 2010b. Colonic biopsies to assess the neuropathology of Parkinson's disease and its relationship with symptoms. PLoS One 5, e12728.
Pouclet, H., Lebouvier, T., Coron, E., des Varannes, S.B., Rouaud, T., Roy, M., et al., 2012. A comparison between rectal and colonic biopsies to detect Lewy pathology in Parkinson's disease. Neurobiol. Dis. 45, 305–309.
Saborio, G.P., Permanne, B., Soto, C., 2001. Sensitive detection of pathological prion protein by cyclic amplification of protein misfolding. Nature 411, 810–813.
Sánchez-Ferro, Á., Rábano, A., Catalán, M.J., Rodríguez-Valcárcel, F.C., Fernández Díez, S., Herreros-Rodríguez, J., et al., 2015. In vivo gastric detection of α-synuclein inclusions in Parkinson's disease. Mov. Disord. 30, 517–524.
Schneider, S.A., Boettner, M., Alexoudi, A., Zorenkov, D., Deuschl, G., Wedel, T., 2016. Can we use peripheral tissue biopsies to diagnose Parkinson's disease? A review of the literature. Eur. J. Neurol. 23, 247–261.
Shahnawaz, M., Tokuda, T., Waragai, M., Mendez, N., Ishii, R., Trenkwalder, C., et al., 2017. Development of a biochemical diagnosis of Parkinson disease by detection of α-Synuclein Misfolded aggregates in cerebrospinal fluid. JAMA Neurol. 74, 163–172.
Shannon, K.M., Keshavarzian, A., Mutlu, E., Dodiya, H.B., Daian, D., Jaglin, J.A., et al., 2012. Alpha-synuclein in colonic submucosa in early untreated Parkinson's disease. Mov. Disord. 27, 709–715.
Stokholm, M.G., Iranzo, A., Østergaard, K., Serradell, M., Otto, M., Svendsen, K.B., et al., 2017. Assessment of neuroinflammation in patients with idiopathic rapid-eye-movement sleep behaviour disorder: a case-control study. Lancet Neurol. 16, 789–796.
Visanji, N.P., Marras, C., Kern, D.S., Al Dakheel, A., Gao, A., Liu, L.W.C., et al., 2015. Colonic mucosal a-synuclein lacks specificity as a biomarker for Parkinson disease. Neurology 84, 609–616.
Wakabayashi, K., Takahashi, H., Takeda, S., Ohama, E., Ikuta, F., 1988. Parkinson's disease: the presence of Lewy bodies in Auerbach's and Meissner's plexuses. Acta Neuropathol. 76, 217–221.