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CORRESPONDENCE

Enteric alpha-synuclein expression is increased in Crohn's disease

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An accumulating body of evidence has emerged in the last decade to suggest that gastrointestinal (GI) inflammation might be involved in the development of Parkinson's disease (PD). Elevated levels of proinflammatory cytokines [3] and fecal markers of inflammation have been reported in PD patients [5]. Data from genetic studies further support the close relationship between PD and GI inflammation as PD and Crohn's disease (CD) share common causative and susceptibility genes such as leucine-rich repeat kinase 2 (LRRK2) and nucleotide-binding oligomerization domain-containing protein 2 (NOD2) (reviewed in [2]). Two recent large epidemiological studies, by showing an increased risk of subsequent PD in patients with inflammatory bowel disease (IBD) when compared with non-IBD subjects, tie even closer together these two disorders [4, 7].

The mechanisms by which gastrointestinal inflammation might influence PD development or progression are still unclear. A recent report showed that viral-induced gastrointestinal inflammation is associated with alpha-synuclein upregulation in the upper gastrointestinal tract [6]. In the context of PD, this observation suggests that a sustained low-grade inflammation might increase alpha-synuclein expression and aggregation in the submucosal neurons whose terminal axons are only micrometers away from the gut lumen, thereby initiating or worsening the pathological process. The recent findings demonstrating both epidemiological and genetic links between PD and IBD logically led us to study the expression levels and aggregation of alpha-synuclein in the GI tract of IBD patients with either CD or ulcerative colitis (UC).

For biochemical analysis, a total of 34 subjects participated in this study: 10 CD and 12 UC patients with an active disease, as well as 12 healthy controls who had a normal colonoscopy for colorectal cancer screening (see suppl. Table 1 [Online Resource 1] for subjects' demographics and treatments). IBD patients had 4 biopsies (2 in an inflamed and 2 in a non-inflamed area) taken during the course of a colonoscopy. Biopsies were analyzed by Western-Blot and filter-trap assay (see Online Resource 2 for further details on the methods). For immunohistochemical analysis, full-thickness segments of colon containing the myenteric plexus were obtained from 8 IBD patients (4 CD and 4 UC) and from 4 controls who underwent colonic resection for disease management and non-obstructive colorectal carcinoma, respectively (Online Resource 2).

A significant 2.07- and 2.35-fold increase in the expression levels of alpha-synuclein protein was observed in the non-inflamed and inflamed area of CD patients when compared to controls, respectively (Fig. 1a and suppl. Fig. 1 [Online Resource 3]). By contrast, no significant differences were observed between UC patients and controls (Fig. 1b and suppl. Fig. 1 [Online Resource 3]). Filter-trap assays were performed in samples from 6 controls, 6 CD and 6 UC patients to detect alpha-synuclein aggregates. Although our assay efficiently detected alpha-synuclein aggregates in a brain sample from a patient with dementia with Lewy bodies, it did not show any appreciable difference in the immunoreactivity for alpha-synuclein or phosphorylated alpha-synuclein between colonic samples from CD, UC and controls (Fig. 1c and suppl. Fig. 2 [Online Resource 4]). Additional immunohistochemistry experiments showed that alpha-synuclein immunoreactivity was increased in

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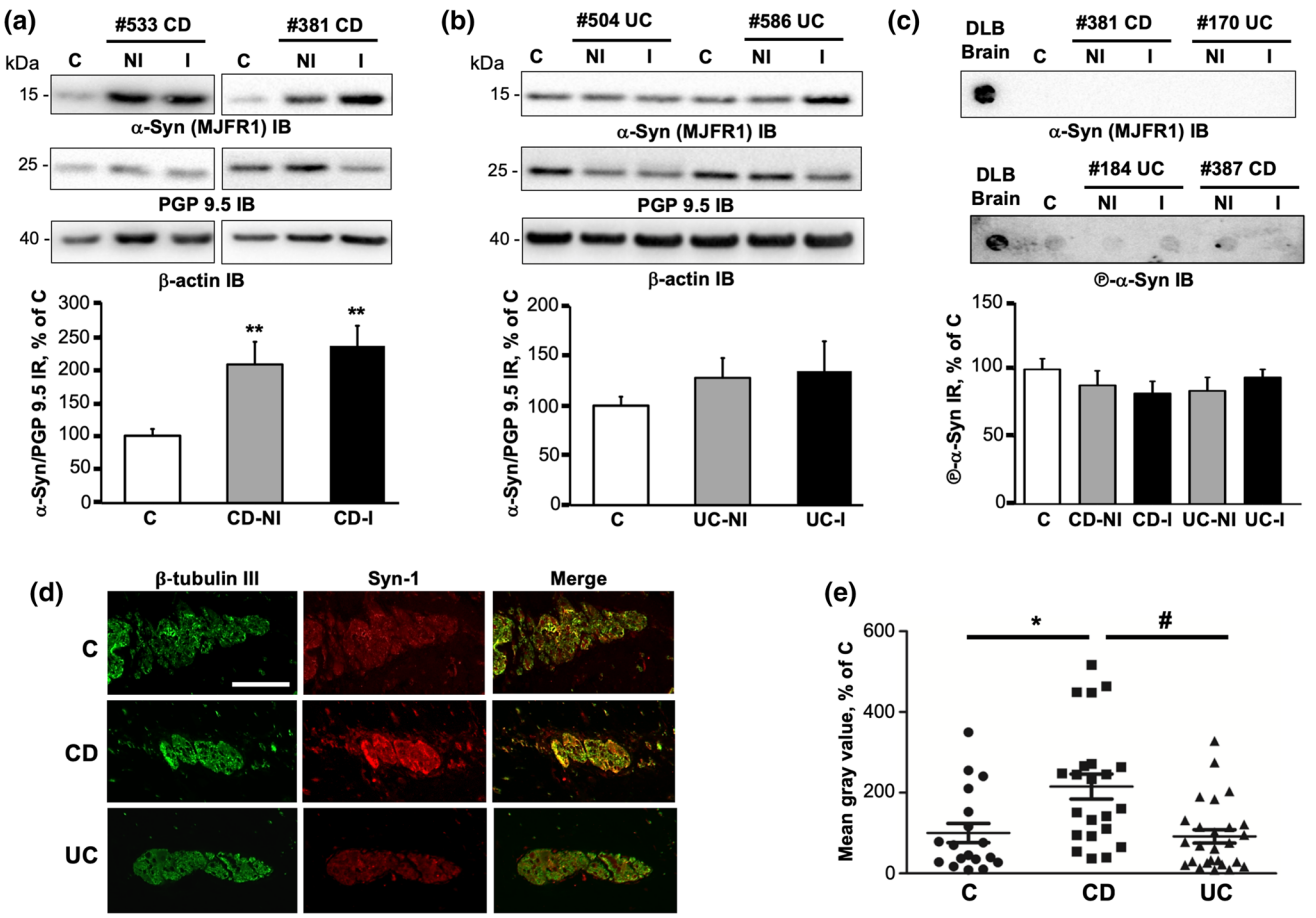

Fig. 1 Expression levels and aggregation of alpha-synuclein in colonic biopsies from patients with Crohn's disease (CD) and ulcerative colitis (UC). **a**, **b** Colonic biopsies lysates from 10 CD patients, 12 UC patients and 12 controls (C) (see suppl. Table 1 [Online Resource 1] for subjects' demographics and treatments) were subjected to immunoblot analysis using antibodies against total alpha-synuclein (α-Syn MJFR1 IB) and PGP 9.5 (PGP 9.5 IB). For UC and CD patients, biopsies taken in non-inflammatory (NI, CD-NI and UC-NI) and inflammatory area (I, CD-I and UC-I) were analyzed separately. For one UC subject (#283), no biopsies were taken in the non-inflamed area. To ensure equal protein loading, membranes were probed with anti-β-actin antibody. Illustrative images are shown; raw immunoblots are provided in suppl. Fig. 1 [Online Resource 3]. Alpha-synuclein immunoreactive bands were measured, normalized to the optical densities of PGP 9.5 and expressed as percentage of controls. Data correspond to mean ± SEM of 12 samples for control subjects (C), 12 samples for UC patients in the inflamed area, 11 samples for UC patients in the non-inflamed area and 10 samples for CD patients in both inflamed and non-inflamed area, $^{**}p < 0.005$. **c** Colonic biopsies lysates from 6 UC and CD patients and 6 controls (C) were subjected to filter-trap assay with cellulose acetate membrane using antibodies against total alpha-synuclein (α-Syn MJFR1 IB) and phosphorylated alpha-synuclein (Ⓟ-α-Syn IB). Illustrative images are shown, raw results are provided in suppl. Fig. 2 [Online Resource 4]. Phosphorylated alpha-synuclein immunoreactive spots were measured and expressed as percentage of controls. **d** Anti-alpha-synuclein Syn-1 and anti-β-tubulin III antibodies were used to detect alpha-synuclein in the myenteric ganglia in CD and UC samples and in control subjects. Scale bar is 50 µm. **e** Quantitative analysis of alpha-synuclein immunofluorescence in the myenteric plexus of CD, UC patients and controls. Data are shown as corrected mean gray value normalized to controls and presented as mean ± SEM, $n = 18$, 22 and 26 myenteric ganglia for control, CD and UC group, respectively. $^{*}p < 0.05$, CD vs C, $\#p < 0.05$, CD vs UC

myenteric neurons of CD patients when compared to UC patients and control subjects (Fig. 1d, e).

Our results show that alpha-synuclein protein levels are increased in the colon of CD, but not of UC patients and that this upregulation is not associated with more protein aggregation. These findings further reinforce the possible link between IBD and PD and the causal role of gastrointestinal inflammation in the development of PD. They also outline the fact that despite being grouped under the term IBD and sharing overlapping symptoms and complications, UC and CD are two different disorders from a pathophysiological point of view. While CD is primarily associated with T-helper type 1 immune responses, UC predominantly shows a characteristic atypical T-helper type 2 cytokine pattern [1]. It is, therefore, tempting to speculate that some of the proinflammatory cytokines and/or para-inflammatory responses that are activated in CD but not in UC are involved in the regulation of alpha-synuclein expression. Further in vitro

studies are definitely needed to identify these signaling pathways and to study if they are shared between the central and enteric nervous system. Such an approach should help us better understand the role of systemic inflammation in PD.

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Author's contribution AP, AL, ED, GC and MRD performed the experiments. AB, ED, MN and MRD were involved in tissue sampling and biobanking. MRD and PD supervised the study, wrote the first draft and the final version of the manuscript.

Compliance with ethical standards

Conflict of interest The authors declare no actual or potential conflict of interest.

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