

LRRK2 is reduced in Parkinson's disease gut

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Recent observations, which showed that Parkinson's disease (PD) and Crohn's disease (CD) are epidemiologically and genetically linked reinforce the assumption that the gastrointestinal (GI) tract is implicated in PD pathogenesis [5]. With regard to genetics, several studies identified the Leucine-rich repeat kinase 2 (*LRRK2*) gene, which is the gene most commonly associated with both familial and sporadic PD, as a major susceptibility gene for CD [5]. These observations suggest that LRRK2 may sit at the crossroads of GI inflammation, PD and CD.

LRRK2 is an ubiquitously expressed multi-domain kinase, which is phosphorylated at multiple sites to affect its physiological and pathological functions [1]. It has been shown that LRRK2 mRNA is upregulated in immune cells in the lamina propria in CD intestinal tissue [3], but no data are available about the expression and phosphorylation levels of LRRK2 in the GI tract in PD. Here, we first examined the expression levels of LRRK2 in the adult human colonic enteric nervous system (ENS) (Supplementary file 1, online resource), then we studied its expression, kinase activity and phosphorylation profile in sigmoid colon biopsy specimens from PD patients and compared it to samples from controls and CD patients (Supplementary table 1, 2 and file 1, online resource). Additional experiments were performed in mutant human alpha-synuclein transgenic mice and in mice with chronic DSS-induced colitis (Supplementary file 1, online resource).

Western blot and immunohistochemistry experiments showed that LRRK2 is expressed in both human colonic submucosal and myenteric plexuses (Fig. 1a). When sigmoid colon biopsies were analysed, a \approx 50% decrease in the expression levels of LRRK2 protein was observed in PD samples relative to controls (Fig. 1b and supplementary Fig.1,

online resource) with LRRK2 protein and mRNA levels showing significant positive correlation (Supplementary Fig.2a, online resource). There were no differences in age, biopsy storage time or levels of IL-1 β and IFN- γ between PD patients and controls, and no correlation between lifetime dose of levodopa and LRRK2 levels (Supplementary table 1 and supplementary Fig.2b, online resource). In contrast, a marked increase in LRRK2 was observed in CD colonic biopsies (Supplementary Fig. 3a and b, online resource); this was associated with an upregulation of IL-1 β and IFN- γ (Supplementary Fig. 4, online resource). LRRK2 phosphorylation of Rab10 at Thr73 is commonly used to monitor its kinase activity [1]. When biopsies were analysed with a Rab10 phosphospecific antibody, the amount of Rab10 phosphorylated at Thr73 was significantly lower in PD compared to controls (Fig. 1b). The phosphorylation of LRRK2 at Ser935 plays a key role in its cellular localisation [2] but no difference was observed between PD and controls when this site was analysed (Fig. 1c). The results obtained in two animal models corroborated the findings we obtained with human samples. LRRK2 expression was significantly lower in the colon of aged alpha-synuclein transgenic mice when compared to one-month old mice, while mice challenged with DSS had more colonic LRRK2 than untreated animals (Supplementary Fig. 5, online resource).

Our research is the first to study LRRK2 in the PD gut and we show that both LRRK2 expression and kinase activity are significantly reduced in the colon of PD patients. These results are in sharp contrast with those obtained from CD gut, in which LRRK2 is upregulated in inflamed areas (current study and [3]). In addition, and in keeping with previous findings obtained in mice [4], we also show that the distribution of LRRK2 in the human GI tract is not limited to the immune cells [3] but also extends to the two main plexuses of the ENS. The discrepancy observed in enteric LRRK2 expression between PD and CD suggests that these two disorders, despite their genetic links, differ mechanistically; this might explain why

epidemiological associations between CD and PD are minor [5]. Future research should be conducted to investigate the transcriptional regulation of LRRK2 and to elucidate the possible role of LRRK2 downregulation in the ENS in PD.

Figure legend

Figure 1. Expression levels, activity and phosphorylation status of LRRK2 in the ENS in PD. **(a)** Lysates from dissected human colon from 2 control subjects, which contains the submucosal and myenteric plexuses, were analysed by Western blot using a LRRK2 antibody. Colonic tissues from 8 additional control subjects were stained to examine the localisation of LRRK2 in the myenteric and submucosal ganglia (supplementary file 1, online resource). Representative photomicrographs are shown. Scale bar is 50 μ m. **(b and c)** Sigmoid biopsy lysates from 12 PD patients and 11 controls (C) (supplementary table 1, online resource) were subjected to immunoblot analysis using antibodies against LRRK2, LRRK2 phosphorylated at Ser935 (pS935 LRRK2), Rab10 and Rab10 phosphorylated at Thr73 (pT73 Rab10). Raw immunoblots are provided in supplementary Fig.1 (online resource). Quantification was performed as described in supplementary file 1 (online resource). All data correspond to mean \pm SEM, * $p < 0.05$ and *** $p < 0.001$.

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Conflict of interest

The authors declare no actual or potential conflict of interest.

Author's contribution

ADGDL, JV, TO, AP and TD performed the experiments. TO and MRD managed tissue sampling and biobanking. MN, MRD, TB and PD supervised the study. CP provided critical feedback and helped shape the research. PD and ADGDL wrote the first draft and the final version of the manuscript.

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