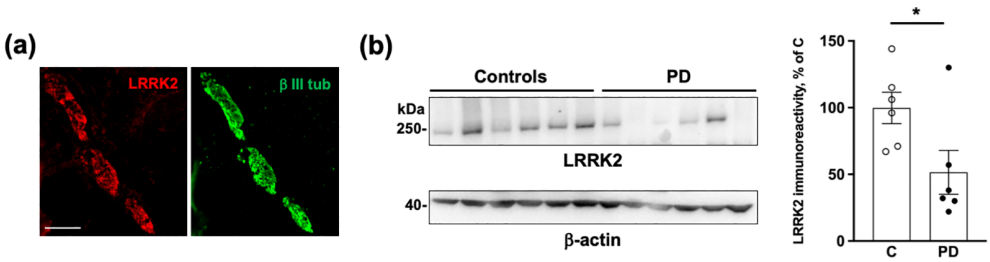

FIG. 1. Expression levels of LRRK2 in the enteric nervous system under physiological condition and in Parkinson's disease (PD). (**a**) Anti-LRRK2 antibody (1:100; MJFF2, c41-2; Abcam, Paris, France) was used to detect LRRK2 in the myenteric ganglia in a control subject; beta III-tubulin (βIII-tub, 1:250; T8660; Sigma, Saint Quentin Fallavier, France) antibody was used to specifically label neurons. Scale bar: 50 μm. (**b**) Sigmoid biopsies lysates from six patients with PD and six age-matched controls (C) were subjected to immunoblot analysis using antibodies against LRRK2 (rabbit monoclonal anti-LRRK2, 1:1000; MJFF2, c41-2; Abcam); membranes were probed with anti-β-actin antibody (1:10,000; A5441; Sigma), to ensure equal protein loading. LRRK2 immunoreactive bands were measured using ImageJ (version 1.51), normalized to the optical densities of β-actin and expressed as percentage of controls. GraphPad Prism software version 9.1.1 (GraphPad Software Inc., La Jolla, CA, USA) was used for statistical analysis. For statistical comparison between groups, a Mann–Whitney test was performed. All data shown are mean ± standard error of the mean; *$p < 0.05$. The sampling of human colon was approved by the Fédération des biothèques of the University Hospital of Nantes. Regarding sigmoid biopsies sampling, the study protocol was approved by the local Committee on Ethics and Human Research (Comité de Protection des Personnes Ouest VI) and registered on ClinicalTrials.gov (ColoBioParker, identifier NCT01353183). [Color figure can be viewed at wileyonlinelibrary.com]

2. Maekawa T, Shimayama H, Tsushima H, et al. LRRK2: an emerging new molecule in the enteric neuronal system that quantitatively regulates neuronal peptides and IgA in the gut. Dig Dis Sci 2017;62:903–912.

3. de Guilhem de Lataillade A, Verchere J, Oullier T, et al. LRRK2 is reduced in Parkinson's disease gut. Acta Neuropathol 2021;142:601–603.

Reply to: Mild Chronic Colitis Triggers Parkinsonism in LRRK2 Mutant Mice through Activating TNF-α Pathway

We are pleased to know that *LRRK2* expression is not limited to immune cells in the lamina propria but is also observed in enteric neurons of the submucosal and myenteric plexus of gastrointestinal tracts, as described by Maekawa et al.[1] In our recently published study,[2] human colonic tissues were sampled by routine biopsy, which was usually shallow to the level of the submucosal layer, and rarely to the muscular level. Therefore, the *LRRK2* expression observed in our routine colonic biopsies was mainly in the lamina propria and did not cover either the submucosal or myenteric plexus. Notably, in our study, most of the colonic biopsies were performed in the transverse or descending colon as part of the follow-up for colonic polyps in a limited patient sample.[2] We did not have sigmoid colonic biopsy samples, as Derkinderen et al showed in their letter to the editor and their recent study.[3] Since the innervation system differs between the descending colon and sigmoid,[4] we hypothesize that *LRRK2* expression may not be evenly distributed throughout the gastrointestinal tract. Further studies, enrolling more participants with multiple biopsies from different regions of the gastrointestinal tract, are needed to elucidate the distribution of LRRK2 in the gut. We agree with Derkinderen and colleagues that additional investigations are needed to delineate the role of LRRK2 in the enteric nervous system—including studies using multiplex immunohistochemistry/immunofluorescence staining to determine the specific localization of LRRK2 in individual cell types, such as ChAT-positive, VIP-secreting neurons, sympathetic TH-positive neurons, and enteric glia. Furthermore, as we have addressed,[2] *LRRK2 G2385R* is a polymorphism that may cause a partial loss-of-function mutation instead of increasing LRRK2 kinase activity.[5] Our findings from transgenic *LRRK2 G2019S* mice should be verified by a large cohort study enrolling patients with the *LRRK2 G2019S* mutation. Finally, we agree with Derkinderen and colleagues that it is insufficient to measure total *LRRK2* expression level, and necessary to also examine the levels of phospho-LRRK2 and its substrates, such as phospho-Rab10. However, phosphorylated proteins are liable to lose their antigenicity in paraffin-embedded tissue samples.[6] One study demonstrates that several phosphorylated proteins, including phospho-AKT, phosphor-ERK1/2, and phospho-tyrosine, lost their antigenicity within 2 hours of cold ischemic time during tissue preparation for the paraffin-embedding process.[6] Therefore, the expression of phospho-LRRK2 in paraffin-embedded biopsied colonic tissues should be cautiously interpreted. Future prospective studies, in which fresh biopsied gut tissues are collected in a follow-up study design, are needed to elucidate *LRRK2* expression and activity in the gastrointestinal tract during the Parkinson's disease process. ■

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References

1. Maekawa T, Shimayama H, Tsushima H, et al. LRRK2: an emerging new molecule in the enteric neuronal system that quantitatively regulates neuronal peptides and IgA in the gut. Dig Dis Sci 2017;62:903–912.
2. Lin CH, Lin HY, Ho EP, et al., Mild chronic colitis triggers parkinsonism in LRRK2 mutant mice through activating TNF-α pathway Mov Disord 2021. https://doi.org/10.1002/mds.28890. Online ahead of print.
3. de Lataillade AG, Verchere J, Oullier T, et al. LRRK2 is reduced in Parkinson's disease gut. Acta Neuropathol 2021;142:601–603.
4. Vermeulen W, De Man JG, Pelckmans PA, De Winter BY. Neuroanatomy of lower gastrointestinal pain disorders. World J Gastroenterol 2014;20:1005–1020.
5. Carrion MDP, Marsicano S, Daniele F, et al. The LRRK2 G2385R variant is a partial loss-of-function mutation that affects synaptic vesicle trafficking through altered protein interactions. Sci Rep 2017;7:5377
6. Vassilakopoulou M, Parisi F, Siddiqui S, et al. Preanalytical variables and phosphoepitope expression in FFPE tissue: quantitative epitope assessment after variable cold ischemic time. Lab Invest 2015;95:334–341.

Single-Trajectory Multiple Target for Parkinsonian Mobility and Cognition? An Inadvisable Idea

Sanskriti Sasikumar et al reported a study to target both the nucleus basalis of Meynert (NBM) and globus pallidus pars interna (GPi) with a single trajectory.[1] However, to our knowledge, this is an inadvisable idea.

The effective area of GPi for parkinsonian movement disorder is in its posterior part. However, cholinergic neurons of NBM are located anterior and ventral to the GPi.[2] Therefore, it is difficult to target both NBM and the effective part of GPi with a single trajectory (as top anterior and bottom posterior used by the authors). Therefore, when targeting NBM, motor effects of GPi will be sacrificed, as the authors demonstrated in their study. Besides, not all parts of the NBM are effective on cognition; only the areas with dense cholinergic neurons are effective. Even reserving fewer motor effects, the targeted location of the NBM is not its most effective part[3]; therefore, the cognitive effects cannot be guaranteed.

Therefore, it is inadvisable to target both NBM and GPi with a single trajectory in future trials.

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References

1. Sasikumar S, Cohn M, Harmsen IE, et al. Single-trajectory multiple-target deep brain stimulation for Parkinsonian mobility and cognition. Mov Disord 2022;37(3):635–640.
2. Vitek JL, Bakay RA, Hashimoto T, et al. Microelectrode-guided pallidotomy: technical approach and its application in medically intractable Parkinson's disease. J Neurosurg 1998;88(6):1027–1043.
3. Liu W, Yu DY. Bilateral nucleus basalis of Meynert deep brain stimulation for dementia with Lewy bodies: a randomised clinical trial. Brain Stimul 2020;13(6):1612–1613.

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