Gut bacterial tyrosine decarboxylase associates with clinical variables in a longitudinal cohort study of Parkinson’s disease

Sebastiaan P. van Kessel, Petri Auvinen, Filip Scheperjans and Sahar El Aidy

Gut microbiota influences the clinical response of a wide variety of orally administered drugs. However, the underlying mechanisms through which drug–microbiota interactions occur are still obscure. Previously, we reported that tyrosine decarboxylating (TDC) bacteria may restrict the levels of levodopa reaching circulation in patients with Parkinson’s disease (PD). We observed a significant positive association between disease duration and the abundance of the bacterial tdc-gene. The question arises whether increased exposure to anti-PD medication could affect the abundance of bacterial TDC, to ultimately impact drug efficacy. To this end, we investigated the potential association between anti-PD drug exposure and bacterial tdc-gene abundance over a period of 2 years in a longitudinal cohort of PD patients and healthy controls. Our data reveal significant associations between tdc-gene abundance, several anti-PD medications, including entacapone, rasagiline, pramipexole, and ropinirole but not levodopa, and gastrointestinal symptoms, warranting further research on the effect of anti-PD medication on microbial changes and gastrointestinal function.

Recent studies have shown that tyrosine decarboxylating (TDC) bacteria can decarboxylate levodopa into dopamine in the periphery, thus restricting levodopa availability to the brain. Potentially, TDC-harboring bacteria could create a vicious circle, wherein peripheral dopamine production affects gut motility, favoring the colonization of (TDC)-bacteria. Additionally, non-levodopa anti-PD medications (monoaminoxidase inhibitors, COMT inhibitors, and dopamine agonists), which affect the peripheral dopaminergic balance, may lead to an altered GI function, potentially contributing to (TDC)-bacterial overgrowth and ultimately variable bioavailability of levodopa. However, levels of TDC-bacteria have not yet been measured nor previously correlated with GI symptoms in longitudinal PD cohorts.

In this study, we focused on measuring fecal tdc-gene abundance and its association with anti-PD medication exposure in a 2-year longitudinal cohort consisting of 67 PD patients and 65 healthy matched subjects, previously used in an investigation of microbiota and PD.

INTRODUCTION

In recent years, many studies have focused on the changes in microbiota composition in individuals with Parkinson’s disease (PD) compared to healthy subjects (extensively covered in several systematic reviews among others). While certain differential abundance alterations were reproduced across studies, variation of results remained considerable. One of the reasons that may explain the inconsistency among these studies are confounding factors, such as anti-PD medications, disease duration, and gastrointestinal (GI) symptoms. Indeed, studies took these factors into account with variable effort. Catechol-O-methyltransferase (COMT) inhibitors, anticholinergics, and potentially levodopa/carbidopa were found to have a significant effect on the changes in the microbiota. Apart from medication, GI dysfunction should be considered when analyzing the altered microbiota in PD patients. Indeed, PD patients usually experience more GI dysfunction symptoms compared to healthy controls (HCs) and intestinal transit time can impact microbiota composition.

Moreover, it has been shown that there is an association between anti-PD medication and GI symptoms. For example, anti-PD medications have been associated (corrected for disease duration) with the total GI Symptoms Rating Score, upper GI symptoms, and hypomotility GI functions. Furthermore, COMT inhibitor dosage was significantly higher in patients with an abnormal transit time compared to those with normal transit time. However, the statistical analysis in that study could not distinguish whether levodopa equivalent daily dose (LEDD) or disease duration was the larger contributing factor to slow colon transit. In addition, ex vivo rodent studies and in vivo dog and human studies showed an effect of dopamine agonists and/or dopamine (which can originate from levodopa in PD patients) on gut motility, as recently reviewed and citations therein. Gut microbial metabolism of unabsorbed residues of levodopa were also shown to influence ileal motility ex vivo.

RESULTS

Clinical variables

Clinical variable comparison between the longitudinal cohort of PD and HCs did not reveal any significant differences in sex, age (at stool collection), or body mass index, with no systemic antibiotics used by either group within the last month (Supplementary Table 1). The duration of motor and non-motor symptom onset in the PD cohort at baseline was ~8 years (Supplementary Table 1). Over time (between baseline and follow-up), the LEDD significantly increased by an average of 116 mg (Table 1). On average, the Unified Parkinson’s Disease Rating Scale (UPDRS) I and II scores significantly increased over time, while UPDRS III (at ON state) significantly decreased (Supplementary Table 2). The latter may be explained by the

1,2 Host-Microbe Metabolic Interactions, Groningen Biomolecular Sciences and Biotechnology Institute (GBB), University of Groningen, Groningen, the Netherlands.
3,4 Institute of Biotechnology, DNA Sequencing and Genomics Laboratory, University of Helsinki, Viikinkaari 5D, 00014 Helsinki, Finland. 5Department of Neurology, Helsinki University Hospital, and Clinicum, University of Helsinki, Haartmaninkatu 4, 00290 Helsinki, Finland. These authors jointly supervised this work: Filip Scheperjans, Sahar El Aidy. Email: Filip.Scheperjans@h us.f c; sa har.elaid y@rug.nl

Published in partnership with the Parkinson’s Foundation
Table 1. Paired tests between follow-up and baseline samples in PD and HCs (significant test results are printed in bold).

| Paried tests (follow-up–baseline) | PD | Control | PD on medication |
|-----------------------------------|-----------------|-----------|------------------|
| Paired mean difference (n) | Baseline mean ± SD | Follow-up mean ± SD | T test | Wilcoxon | McNemar | FDR | Baseline (%) | Follow-up (%) |
| tdc-gene abundance (outliers removed) | 2.2E−06 | 1.5E−6 ± 1.3E−6 (55) | 3.7E−6 ± 2.6E−6 (55) | 9.7E−07 | n.a. | 8.61E−07 | 2.2E−6 ± 2.2E−6 (54) | 3.1E−6 ± 2.7E−6 (54) | 0.167 | n.a. |
| Gastrointestinal symptoms | | | | | | | | | | |
| Wexner total score | 0.72 | 5.66 ± 4.01 (67) | 6.37 ± 4.63 (67) | 0.033 | 0.059 | −0.54 | 2.92 ± 2.61 (65) | 2.38 ± 2.58 (65) | 0.010 | 0.030 |
| Rome III (constipation and defecation) | −0.01 | 7.37 ± 5.96 (67) | 7.36 ± 5.17 (67) | 0.685 | 0.685 | −0.22 | 2.97 ± 3.60 (65) | 2.75 ± 3.60 (65) | 0.651 | 0.977 |
| Anti-PD medication exposure | | | | | | | | | | |
| LEDD (mg) | 116.40 | 444.2 ± 315.6 (66) | 560.6 ± 294.0 (66) | 1.8E−06 | 3E−05 | | | | | |
| Levodopa | | | | | | | | | | |
| Levodopa IR (mg) | 54.10 | 99.63 ± 159.46 (67) | 153.73 ± 204.92 (67) | 0.028 | 0.221 | | | | | 35.8 | 41.8 |
| Duodopa (mg) | 19.33 | 0.00 ± 0.00 (66) | 19.33 ± 157.06 (66) | 0.317 | 0.708 | | | | | 0.0 | 1.5 |
| Levodopa CR (mg) | −8.96 | 47.76 ± 157.98 (67) | 38.81 ± 115.41 (67) | 1.000 | 1.000 | | | | | 11.9 | 14.9 |
| Levodopa (with entacapone) (mg) | 19.03 | 88.06 ± 220.17 (67) | 107.09 ± 226.33 (67) | 0.513 | 0.872 | | | | | 14.9 | 22.4 |
| COMT inhibitors | | | | | | | | | | |
| Entacapone (mg) | 49.25 | 146.27 ± 357.74 (67) | 195.52 ± 385.51 (67) | 0.319 | 0.708 | | | | | 14.9 | 22.4 |
| MAO inhibitors | | | | | | | | | | |
| Selegeline (mg) | −0.51 | 4.10 ± 4.84 (67) | 3.59 ± 4.74 (67) | 0.348 | 0.708 | | | | | 43.3 | 38.8 |
| Rasagiline (mg) | 0.06 | 0.25 ± 0.44 (67) | 0.31 ± 0.47 (67) | 0.102 | 0.434 | | | | | 25.4 | 31.3 |
| Dopamine agonists | | | | | | | | | | |
| Rotigotine (mg) | 0.42 | 0.00 ± 0.00 (67) | 0.42 ± 1.62 (67) | 0.039 | 0.221 | | | | | 0.0 | 7.5 |
| Pramipexole (mg) | −0.06 | 0.62 ± 0.82 (67) | 0.56 ± 0.88 (67) | 0.613 | 0.885 | | | | | 43.3 | 38.8 |
| Ropinirole (mg) | 0.04 | 3.15 ± 5.18 (67) | 3.19 ± 6.02 (67) | 0.951 | 1.000 | | | | | 34.3 | 29.9 |
| Bromocriptine (mg) | n.a. | 0.15 ± 1.22 (67) | 0.15 ± 1.22 (67) | 1.000 | 1.000 | | | | | 1.5 | 1.5 |
| Other medication | | | | | | | | | | |
| Amantadine (mg) | 1.49 | 6.72 ± 40.73 (67) | 8.21 ± 38.53 (67) | 1.000 | 1.000 | | | | | 3.0 | 4.5 |
significantly increased over time. The Hoehn & Yahr (at ON state) score slightly increased over time (Supplementary Table 2). Gut bacterial tdc-gene abundance, GI symptoms, and medication exposure significantly increased over time in PD patients. It has recently been shown that TDC-bacteria in the GI tract interfere with the availability of levodopa medication in animal models and that longer disease duration and exposure to levodopa may further increase the abundance of TDC-bacteria in the gut. Thus, we sought to investigate the changes in the levels of gut bacterial tdc-gene abundance over time in a longitudinal PD cohort, including the differences between PD patients and matching HCs.

High prevalence of the tdc-gene was detected at baseline in 97% (n = 61/63) and 98% (n = 61/62) of the HC and PD samples, respectively. When comparing PD patients and HCs, PD patients tended to have a higher tdc-gene abundance (p = 0.057) at follow-up (Fig. 1 and Table 2). Correspondingly, the increase in tdc-gene abundance over time was significantly higher in PD patients compared to HC subjects (Wilcoxon test, p = 9.7E−07), with a mean increase of 2.6-fold (Table 1 and Fig. 1). The results indicate that, over time, tdc-gene abundance increases more rapidly in PD patients compared to HC subjects.

Because GI transit time also impacts microbial composition (including TDC bacteria), differences in GI symptoms were assessed at baseline and follow-up. At both time points, GI symptoms were significantly more severe in PD patients compared to HC subjects (Table 2). Only the Wexner scores, but
Independent tests between PD and HCs (significant test results are printed in bold).

|                  | Baseline Mean ± SD (n) | Control Mean ± SD (n) | 7 test Mann–Whitney | Fisher’s test | FDR               |
|------------------|------------------------|-----------------------|---------------------|---------------|------------------|
| tdc-gene abundance | −7.4E−07 −6 ± 1.1E−07 | 2.2E−06 −6 ± 1.2E−06 | 0.177               | n.a.          | 6.47E−07 3.8E−06  |
| Gastrointestinal symptoms | 2.73 5.66 ± 4.01 (67) | 2.92 ± 2.61 (65) | 2.4E−05 2.4E−05 3.99 | 6.37 ± 6.43 (67) | 2.38 ± 2.58 (65) 5.9E−09 1.2E−08 |
| Rome III (constipation and defecation) | 4.40 7.37 ± 5.96 (67) | 2.97 ± 3.60 (65) | 1.2E−06 1.2E−06 4.60 | 7.36 ± 5.17 (67) | 2.75 ± 3.60 (65) 1.3E−08 1.3E−08 |
| Medication | Use of anacidic (yes/no) | −2% 6/61 (67) | 0.777 0.777 | 6% 4/63 (67) | 0/65 (65) 0.119 0.238 |
| Use of anticholinergic (yes/no) | 9% 6/61 (67) | 0/65 (65) | 0.028 0.056 | 3% 3/64 (67) | 1/64 (65) 0.619 0.619 |

Unpaired T test for normally distributed unpaired data, unpaired Mann–Whitney test for non-normally distributed unpaired data, and Fisher’s test for binary distributed unpaired data. The p values were corrected for false discovery rate (FDR) per section.

n.a. not applicable.

Table 2. Independent tests between PD and HCs (significant test results are printed in bold).

Unpaired T test for normally distributed unpaired data, unpaired Mann–Whitney test for non-normally distributed unpaired data, and Fisher’s test for binary distributed unpaired data. The p values were corrected for false discovery rate (FDR) per section.

n.a. not applicable.

AntipD medication and GI symptoms associate with tdc-gene abundance independent of the changes in GI symptoms.

Using general linear models (GLMs), the contribution of the difference in antipD medication exposure to the difference in tdc-gene abundance was assessed (Table 3). The model showed that dose changes over time contributed to the difference in tdc-gene abundance independent of the changes in GI symptoms.

Although the LEDD increased significantly for any individual drug in PD patients after correction was observed for FDR, possibly due to changes in the PD and HC groups was compared at baseline, antipD medication use was significantly higher in PD patients compared to HC subjects (Table 2). In the HC group only, a significant decrease in antipD medication use was observed over time, while entacapone still contributed significantly to the tdc-gene abundance independent of the changes in GI symptoms.
abundance (Supplementary Table 3). These results indicate that the difference in drug exposure over time between slow and rapid progressing PD patients (Table 4) reflect their contribution to the tdc-gene abundance in the GLMs (Table 5 and Supplementary Table 3). In summary, these observations indicate that the change in exposure to specific anti-PD medications, like entacapone, can be a significant contributing factor to an increase in tdc-gene abundance in rapid progressing PD patients. Concomitantly, other anti-PD medications contribute to tdc-gene abundance in slow progressing PD patients.

**DISCUSSION**

In this study, we have established that gut bacterial tdc-gene abundance significantly increases over time in PD patients (Table 1), in line with previous results, where a significant correlation between disease duration and tdc-gene abundance was observed. The levels of gut bacterial tdc-gene abundance were not significantly different compared to HCs at baseline but close to significant at follow-up (Table 2). Accordingly, the increase in tdc-gene abundance was 2.6-fold higher in PD than in HCs, suggesting that the increased gene abundance occurs more rapidly in PD patients. Here, we did not find a significant correlation between levodopa dosage and tdc-gene abundance.

This discrepancy could be explained by the relatively low proportion of high levodopa dosages in this study. At baseline and follow-up, 19.4% (max 900 mg) and 26.9% (max 875 mg) of the PD patients had a dose higher than 400 mg/day, respectively, while in the previous study 60% of the PD patients received a dosage higher than 400 mg/day (max 1100 mg).

Using GLMs, we showed that several anti-PD medications other than levodopa contributed significantly to the tdc-gene abundance. Importantly, all tested medications (Table 3) affect the (peripheral) dopaminergic system; COMT inhibitors prevent methylation of levodopa, dopamine, and norepinephrine; MAOIs prevent dopamine and norepinephrine oxidation; and DA agonists act on dopamine receptors expressed in the gut. Collectively, these medications were recently shown to elicit an effect on GI symptoms. Although GI dysfunction might be caused by the degeneration of enteric neurons, as observed in PD patients with chronic constipation and reported in an MPTP mouse model for PD, additional dopaminergic medication may impact the GI function even further. Indeed, the Wexner score, which significantly increased over time in PD patients, did not change the associations between anti-PD medication and tdc-gene abundance (except for ropinirole exposure) when considered as a confounder. The potential link between changes in GI symptoms, as measured by Wexner score, and anti PD medications is in

---

**Table 3.** General linear model of the difference tdc-gene abundance overtime with anti-PD medication and Wexner score as variables (significant variable contributions to the model are printed in bold).

| All PD patients (n = 55) | Difference tdc-gene abundance 2y–0y (no outliers) | Not corrected for Wexner score | Corrected for Wexner score |
|-------------------------|---------------------------------------------|-------------------------------|---------------------------|
|                         | β                                          | p value                       | VIF                       |
| (Intercept)             | 2.1E−06                                     | 0.000                         |                           |
| Difference levodopa sum (mg) | 1.0E−09                                      | 0.671                         | 1.375                     |                           |
| Difference entacapone (mg) | 2.1E−09                                     | 0.032                         | 1.163                     |                           |
| Difference selegeline (mg) | −9.4E−08                                     | 0.258                         | 1.215                     |                           |
| Difference rasagiline (mg) | −2.7E−06                                     | 0.035                         | 1.366                     |                           |
| Difference rotigotine (mg) | 2.3E−07                                     | 0.245                         | 1.161                     |                           |
| Difference pramipexole (mg) | 2.0E−06                                     | 0.001                         | 1.415                     |                           |
| Difference ropinirole (mg) | 2.2E−07                                     | 0.028                         | 1.367                     |                           |
| Difference in Wexner total score | Not included                                |                               |                           |

VIF variance inflation factor.

**Table 4.** Independent tests (rapid–slow progressing) PD patients of exposure of anti-PD medications over time (significant test results are printed in bold).

| Difference levodopa sum (mg) | 103.5 | 208.33 ± 156.43 (12) | 104.86 ± 116.32 (36) | 0.022 | 0.051 |
| Difference entacapone (mg) | 361.1 | 366.67 ± 510.50 (12) | 5.56 ± 255.17 (36) | 0.009 | 0.051 |
| Difference selegeline (mg) | 1.5   | 0.83 ± 5.15 (12) | −0.68 ± 4.46 (36) | 0.737 | 0.983 |
| Difference rasagiline (mg) | 0.0   | 0.08 ± 0.29 (12) | 0.08 ± 0.28 (36) | 0.934 | 0.983 |
| Difference rotigotine (mg) | 0.4   | 0.83 ± 2.89 (12) | 0.39 ± 1.34 (36) | 0.983 | 0.983 |
| Difference pramipexole (mg) | −0.4 | −0.31 ± 0.65 (12) | 0.10 ± 0.58 (36) | 0.019 | 0.051 |
| Difference ropinirole (mg) | −0.3 | −0.58 ± 5.53 (12) | −0.28 ± 3.18 (36) | 0.785 | 0.983 |

Unpaired T test for normally distributed unpaired data, unpaired Mann–Whitney test for non-normally distributed unpaired data, and Fisher’s test for binary distributed unpaired data. The p values were corrected for false discovery rate (FDR).
Table 5. General linear model of the difference of tdc-gene abundance over time with anti-PD medication and Wexner score as variables in slow and rapid progressing PD patients (significant variable contributions to the model are printed in bold).

| Cohort                        | Difference tdc-gene abundance 2y-0y (no outliers) | Corrected for Wexner score | VIF |
|-------------------------------|---------------------------------------------------|-----------------------------|-----|
| Slow progressing PD patients  | β                                                 | p value                     | VIF |
| (n = 36)                      |                                                   |                             |     |
| (Intercept)                   | 2.13                                              | 0.001                       | 2.120|
| Corrected for Wexner score    | 2.4E-06                                           | 0.000                       | 2.7E-07|
| Not corrected for Wexner score| 5.4E-07                                           | 0.003                       | 7.1E-07|
| VIF                           |                                                   |                             |     |
| Rapid progressing PD patients | β                                                 | p value                     | VIF |
| (n = 38)                      |                                                   |                             |     |
| (Intercept)                   | 6.5E-07                                           | 0.080                       | 2.585|
| Corrected for Wexner score    | 4.1E-07                                           | 0.002                       | 3.538|
| Not corrected for Wexner score| 3.8E-08                                           | 0.002                       | 4.149|

Rome III (constipation and defecation) score did not change over time in PD patients, which may be explained by the fact that Rome III assesses symptoms retrospectively over a 3-month period and may reduce sensitivity to change. The difference observed between the two questionnaires confirms the need to develop more sophisticated protocols to detect and investigate GI symptoms in PD patients.

Notably, only entacapone exposure in rapid progressing PD patients contributed to fecal tdc-abundance. Enterococcus (genus consisting of species harboring TDCs) among others were found to be significantly increased only in PD patients treated with entacapone. However, in their study, Weis et al. did not report whether the tested PD patients were on medications such as MAOIs or DA agonists, other than levodopa and/or entacapone. Here we show that, in addition to entacapone, other anti-PD medications seem to affect gut bacterial tdc-gene abundance (Table 3).

The major limitation of this study is that we determined bacterial tdc-gene abundance in fecal samples, which may not be reflective of actual tdc-gene levels in the small intestine, the main absorption site of levodopa and other medications. Moreover, the presence of these genes does not necessarily reflect TDC activity.

In summary, the present study implies important associations between anti-PD medication and gut bacterial tdc-gene abundance. These associations point toward complex interactions between anti-PD medication, GI symptoms, and gut bacterial tdc-gene abundance, which warrants further research.

METHODS

Cohort

The original age and sex-matched cohort was recruited for a pilot study in 2015 investigating PD and gut microbiota. All subjects were invited to a follow-up on average 2.25 ± 0.20 years later to investigate temporal stability in the PD microbiota. The study was approved by the ethics committee of the Hospital District of Helsinki and Uusimaa. All participants gave written informed consent and the study was registered at clinicaltrials.gov (NCT01536769).

Of the total 165 subjects (77 PD, 88 HCs) recruited at baseline and follow-up, 13 subjects (6 PD, 7 HC) were excluded because they did not return for the follow-up study and 20 subjects (4 PD, 16 HCs) were excluded because of various other reasons at baseline or follow-up. In the control group, 1 subject was excluded for a sibling with PD, 3 subjects for having a common cold, 8 subjects for hyposmia (pre-motor PD symptom), 2 subjects for recent surgery, 1 subject had no matching sample, and 1 sample was missing. In the PD group, 1 subject was excluded because of recent surgery, 1 subject had a change in diagnosis, 1 subject because of a sampling handling issue, and 1 subject because of medical history. In total, 33 subjects (10 PD, 23 HCs) were excluded, resulting in 132 subjects (67 PD, 65 HCs) in this study.

The following parameters were assessed as described in the previous studies Scheperjans et al. and Aho et al: GI symptoms (Wexner constipation score), Rome III questionnaire, disease severity (UPDRS), and medication exposure.

DNA extraction

Stool sample collection and DNA isolation were performed in a previous study. Briefly, stool samples were collected by study subjects into collection tubes pre-filled with DNA stabilizer (PSP Spin Stool DNA Plus Kit, STRATEC Molecular) and stored in the refrigerator until transport (for up to 3 days). After receipt of samples, they were transferred to −80°C. DNA from both baseline and follow-up samples were extracted with the PSP Spin Stool DNA Plus Kit (STRATEC Molecular). Each extraction batch included one blank sample to assess potential contamination. (Of note, to prevent potential technical differences, DNA from baseline samples were extracted at the baseline and at follow-up, thus the baseline samples were thawed twice.)
Determination of tdc-gene abundance

DNA concentration of samples was directly estimated from 96-well plates by measuring the (pathlength corrected) absorbance at 260 and 320 nm in a multimode reader. The DNA concentration was calculated as follows: 50 μg (sample) / 100 μl = blank 260–320. Samples that were negative, very low, or very high in concentration were measured with the nanodrop to confirm. All DNA samples were diluted 20× so that the concentration would fall within the range of 2–25 ng/μl (median, 13.7 ng/μl, interquartile range, 6.9–21.8 ng/μl) and 2 μl was used for quantitative PCR (qPCR). qPCR of tdc genes was performed using primers Dec5 (5′-CGTGTGTTGTTTGTGGACACNGAR GARG-3′) and Dec3r (5′-CGCCACGCAATGGGAAARYTANCCCAT-3′), targeting a 350 bp region of the tdc gene [25]. For primers targeting 16S rRNA gene for all bacteria [26], Eub338 (5′-ACTCCTACGGGAGGCAGCAG-3′) and Eub518 (5′-ATTACCGCGGCTGCTGG-3′) were used as internal controls for sample bias and total bacterial load. All qPCR experiments were performed in a Bio-Rad CFX96 RT-PCR system (Bio-Rad Laboratories, Veenendaal, The Netherlands) with iQ SYBR Green Supermix (170–8882, Bio-Rad) in 10 μl reactions using the manufacturer’s protocol. qPCR was performed using the following parameters: 3 min at 95 °C; 15 s at 95 °C, 1 min at 58 °C, 40 cycles. A melting curve was tested using the appropriate statistical tests. The group sizes and appropriate statistical tests are indicated in the tables. GLMs were performed using the IBM SPSS Statistics version 26. The Wilk tests using the Explore function were tested using Wald Chi Square test. Additionally, the VIF was computed to check for potential collinearity between variables.

Statistics

All statistical tests were performed in IBM SPSS Statistics version 26. The p value adjustments were performed in R version 4.0.0 using padjust (p-values, “fdr”). The qPCR data were tested for outliers per group and time point using the ROUT method (Q = 0.1%) in GraphPad Prism v7 and the identified outliers were removed. Outlier removal was restricted to the qPCR data only. All variables were tested for normality using Kolmogorov–Smirnov and Shapiro–Wilk tests using the Explore function in SPSS. Based on the distribution of data, the differences were tested using the appropriate statistical tests. The group sizes and appropriate statistical tests are indicated in the tables. GLMs were performed using the Generalized Linear Models function in SPSS and the main effects were tested using Wald Chi Square test. Additionally, the VIF was computed to check for potential collinearity between variables.

Reporting summary

Further information on research design is available in the Nature Research Reporting Summary linked to this article.

DATA AVAILABILITY

Clinical data are not publicly available due to participant privacy and are available from the corresponding authors on reasonable request.

Received: 7 June 2021; Accepted: 21 November 2021; Published online: 15 December 2021

REFERENCES

1. Boertien, J. M., Pereira, P. A. B., Aho, V. T. E. & Scheperjans, F. Increasing comparability and utility of gut microbiome studies in Parkinson’s disease: a systematic review. J. Parkinsons Dis. 9, S297–S312 (2019).
2. van Kessel, S. P. & El Aidy, S. Bacterial metabolites mirror altered gut microbiota composition in patients with Parkinson’s disease. J. Parkinsons Dis. 9, 5359–5370 (2019).
3. Hill-Burns, E. M. et al. Parkinson’s disease and Parkinson’s disease medications have distinct signatures of the gut microbiome. Mov. Disord. 32, 739–749 (2017).
4. Aho, V. T. E. et al. Gut microbiota in Parkinson’s disease: temporal stability and relations to disease progression. EbioMedicine 44, 691–707 (2019).
5. Scheperjans, F. et al. Gut microbiota are related to Parkinson’s disease and clinical phenotype. Mov. Disord. 30, 350–358 (2015).
6. Weis, S. et al. Effect of Parkinson’s disease and related medications on the composition of the fecal bacterial microbiota. npj Parkinsons Dis. 5, 1–9 (2019).
7. Fasano, A., Visani, N. P., Liu, L. W. C., Lang, A. E. & Pfeiffer, R. F. Gastrointestinal dysfunction in Parkinson’s disease. Lancet Neurol. 14, 625–639 (2015).
8. Kenna, J. E. et al. Characterization of gastrointestinal symptom type and severity in Parkinson’s disease: a case–control study in an Australian cohort. Mov. Disord. Clin. Pract. 8, 245–253 (2021).
9. Falony, G. et al. Population-level analysis of gut microbiome variation. Science 352, 560–4 (2016).
10. Khoshbin, K., Hassan, A. & Camilleri, M. Cohort study in Parkinsonism: delayed transit, accelerated gastric emptying, and prodromal dystomyosity. Neurol. Clin. Pract. 11, e407–e413 (2021).
11. van Kessel, S. P. & El Aidy, S. Contributions of gut bacteria and diet to drug pharmacokinetics in the treatment of Parkinson’s disease. Front. Neurol. 10, 1087 (2019).
12. van Kessel, S. P. et al. Gut bacterial degradation of levodopa in Parkinson’s disease. Brain 143, 187 (2020).
13. Maini Rekdal, V., Bess, E. N., Bisanz, J. E., Turner-Baith, P. A. & Baluks, E. P. Discovery and inhibition of an interspecies gut bacterial pathway for levodopa metabolism. Science 364, eaau6323 (2019).
14. Tomlinson, C. L. et al. Systematic review of levodopa dose equivalency reporting in Parkinson’s disease. Mov. Disord. 25, 2649–2653 (2010).
15. Singaram, C. et al. Dopaminergic defect of enteric system in Parkinson’s disease. Gut 71, e413 (2021).
16. Anderson, G. et al. Loss of enteric dopaminergic neurons and associated changes in colon motility in an MPTP mouse model of Parkinson’s disease. Exp. Neurol. 207, 4–12 (2007).
17. Kulisevsky, J. & Pagonabarraga, J. Tolerability and safety of ropinorelin versus other dopamine agonists and levodopa in the treatment of Parkinson’s disease: meta-analysis of randomized controlled trials. Drug Saf. 33, 147–161 (2010).
18. Agachan, F., Chen, T., Pfeiffer, J., Reissman, P. & Wexner, S. D. A constipation scoring system to simplify evaluation and management of constipated patients. Dis. Colon Rectum 39, 681–685 (1996).
19. Longstreth, G. F. et al. Functional bowel disorders. Gastroenterology 130, 1480–1491 (2006).
20. Fahn, S., Elton, R. & Members of the UPDRS Development Committee. In Recent Developments in Parkinson’s Disease (eds Fahn, S., Marsden, C. D., Calne, D. B. & Goldstein, M.) 153–163 (McMillan Health Care Information, 1987).
21. Torriani, S. et al. Rapid detection and quantification of tyrosine decarboxylase gene (tdc) and its expression in gram-positive bacteria associated with fermented foods using PCR-based methods. J. Food Prot. 71, 93–101 (2008).
22. Frier, N., Jackson, J. A., Vilgalys, R. & Jackson, R. B. Assessment of soil microbial community structure by use of taxon-specific quantitative PCR assays. Appl. Environ. Microbiol. 71, 4117–4120 (2005).
23. Livak, K. J. & Schmittgen, T. D. Analysis of relative gene expression data using real-time quantitative PCR and the 2−ΔΔCT method. Methods 25, 402–408 (2001).

AUTHOR CONTRIBUTIONS

S.P.v.K. performed the qPCR and statistical analysis and wrote the original manuscript, which was reviewed and modified by P.A., F.S., and S.E.A. S.P.v.K., F.S., and S.E.A. contributed to interpreting the results and the conceptualization of the study. F.S. performed the clinical evaluations of subjects and was responsible for the original cohort data.

COMPETING INTERESTS

F.S. has patents issued (P11276718 and US10139408B2) and pending (US16/186,663 and EP3149205) that are assigned to NeuroBiome Ltd. F.S. is founder and CEO of NeuroInnovation Oy and NeuroBiome Ltd., is a member of the scientific advisory board, and has received consulting fees and stock options from Axial Biotherapeutics. F.S. has received consulting and lecture fees from Orion, Abbvie, Herantis, GE Healthcare, Merck, and Teva. S.E.A. has acquired a research grant from Weston Brain Institute, from which the research of S.P.v.K. is funded. The funders have no role in the preparation of the manuscript.

Published in partnership with the Parkinson’s Foundation
