

Gut Microbiota Are Related to Parkinson's Disease and Clinical Phenotype

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ABSTRACT: In the course of Parkinson's disease (PD), the enteric nervous system (ENS) and parasympathetic nerves are amongst the structures earliest and most frequently affected by alpha-synuclein pathology. Accordingly, gastrointestinal dysfunction, in particular constipation, is an important non-motor symptom in PD and often precedes the onset of motor symptoms by years. Recent research has shown that intestinal microbiota interact with the autonomic and central nervous system via diverse pathways including the ENS and vagal nerve. The gut microbiome in PD has not been previously investigated. We compared the fecal microbiomes of 72 PD patients and 72 control subjects by pyrosequencing the V1–V3 regions of the bacterial 16S ribosomal RNA gene. Associations between clinical parameters and microbiota were analyzed using generalized linear models, taking into account potential confounders. On average, the abundance of Prevotellaceae in feces of PD patients was reduced by 77.6% as com-

pared with controls. Relative abundance of Prevotellaceae of 6.5% or less had 86.1% sensitivity and 38.9% specificity for PD. A logistic regression classifier based on the abundance of four bacterial families and the severity of constipation identified PD patients with 66.7% sensitivity and 90.3% specificity. The relative abundance of Enterobacteriaceae was positively associated with the severity of postural instability and gait difficulty. These findings suggest that the intestinal microbiome is altered in PD and is related to motor phenotype. Further studies are warranted to elucidate the temporal and causal relationships between gut microbiota and PD and the suitability of the microbiome as a biomarker. © 2014 International Parkinson and Movement Disorder Society

Key Words: microbiome; gastrointestinal dysfunction; biomarker; gut-brain-axis; non-motor symptoms

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Funding agencies: This study was funded by The Michael J. Fox Foundation for Parkinson's Research, The Finnish Parkinson Foundation, Helsinki University Central Hospital (T1010NL101), and Hyvinkää Hospital (M6095PEV12).

Relevant conflicts of interest/financial disclosures: F.S., V.A., P.A.B.P., K.K., L.P., and P.A. are listed as inventors on Finnish patent application 20145492. The authors report no other conflicts of interest relative to the research covered in this manuscript.

Full financial disclosures and author roles may be found in the online version of this article.

Received: 8 July 2014; **Revised:** 21 August 2014; **Accepted:** 8 October 2014

Published online 00 Month 2014 in Wiley Online Library (wileyonlinelibrary.com). DOI: 10.1002/mds.26069

In Parkinson's disease (PD), motor symptoms are mainly related to the loss of dopaminergic neurons in the substantia nigra.¹ However, neuropathological changes are much more widespread, involving the autonomic nervous system, olfactory structures, lower brainstem, and cerebral cortex.^{2–6} Extranigral pathology is related to a broad spectrum of non-motor symptoms (NMS) that have been increasingly recognized as an important feature of PD.^{7,8} Gastrointestinal dysfunction, in particular constipation, affects up to 80% of PD patients and may precede the onset of motor symptoms by years.^{5,7,9–12} Idiopathic constipation is one of the strongest risk factors for PD.¹² Prolonged intestinal transit time and constipation are associated with neurodegenerative changes in the enteric nervous system (ENS).^{5,13} These changes can

be found in earliest stages of PD, sometimes years before motor symptoms appear, and therefore have been suggested as a premotor biomarker.^{3,6}

Which factors initiate the pathophysiological cascade in PD is unknown, but an environmental factor likely plays a key role, probably against a background of genetic vulnerability.^{14,15} The early involvement of the gastrointestinal tract in PD lends support to the hypothesis that this environmental factor exerts its influences primarily via the gut.^{3,14} No conclusive evidence has been found of any specific microbe being linked to PD. However, recent studies have indicated that, instead of one microorganism, changes in the complex equilibrium of the entire microbiome may be related to human disease.^{16,17}

Next-generation DNA sequencing methods allow a relatively unbiased assessment of nearly all bacterial groups in a sample without the need for selecting specific groups of interest beforehand.¹⁸ The human body has been estimated to contain 10 times more microbial cells than human cells, and these microbes carry approximately 100 to 200 times more protein-coding genes than the human genome. Most (99%) of these genes are of bacterial origin.¹⁹ Intestinal microbiota influence the immune system and the absorption of nutrients, vitamins, medications, and toxic compounds.²⁰⁻²⁵ Furthermore, evidence is accumulating of an intense bidirectional interaction between gut microbiota and the nervous system, influencing brain activity, behavior, as well as levels of neurotransmitter receptors and neurotrophic factors.²⁶⁻³⁰ Recent studies implicate the intestinal microbiome in metabolic, neoplastic, and immunologic diseases, but hardly any research has been conducted on its relevance for neurological disorders.^{16,17,31}

Based on the early gastrointestinal involvement in PD and the vast potential of (patho-)physiological microbiome–host interactions, we speculated that intestinal microbiota may be implicated in PD. We hypothesized that the fecal microbiome of PD patients differs from that of matched control subjects in terms of bacterial diversity or taxonomic composition.

Methods

The study was approved by the ethics committee of the Hospital District of Helsinki and Uusimaa, and all participants gave informed consent. The study was registered at clinicaltrials.gov (NCT01536769).

Study Subjects

This case-control study compared patients with a diagnosis of PD according to the Queen Square Brain Bank criteria with sex- and age-matched (± 5 years) control subjects without any signs of parkinsonism or potential premotor symptoms.³² No consensus has

been reached regarding matching criteria in microbiome studies. We chose to match subjects by age and sex, because these parameters are associated with microbiome composition.^{33,34} Furthermore, exclusion criteria covered a broad range of conditions and medications that could independently affect the fecal microbiome. Seventy-two patients and 72 control subjects were included in the final analysis. Please refer to the Supplemental Data eMethods section and eTable 1 for more information about subject selection and recruitment.

Clinical Data

Parkinsonian symptoms were measured using the Unified Parkinson's Disease Rating Scale (UPDRS) and the modified Hoehn & Yahr scale (H&Y).^{35,36} Overall NMS severity was assessed using the Non-Motor Symptoms Scale (NMSS).³⁷ The degree of constipation was quantified in more detail using the Wexner constipation score (Cleveland Clinic Constipation Scoring System).³⁸ Because irritable bowel syndrome (IBS) is associated with alterations of gut microbiota, we screened our subjects for IBS symptoms using the Rome-III questionnaire to be able to account for this potential confounder in our calculations.^{39,40}

Please refer to the Supplemental Data eMethods section for more details about the clinical assessments.

Analysis of Fecal Microbiota

The subjects collected the fecal samples at home. After extraction of total DNA from the samples, we polymerase chain reaction amplified and pyrosequenced the V1–V3 regions of the bacterial 16S ribosomal RNA gene and used these sequences for taxonomic assignment. Please refer to the Supplemental Data eMethods section for further details on the laboratory protocols.

Statistical Analysis

From each subject, random subsamples of 4,500 sequences were used for statistical analysis of family-level data. Differences in bacterial communities between patients and controls and between postural instability and gait difficulty (PIGD) and tremor dominant (TD) phenotypes (a priori defined subgroup analysis) were analyzed using mothur, Metastats, and generalized linear models (GLM).^{36,41-44} Please refer to the Supplemental Data eMethods section for more information on the statistical methods used.

Results

Demographics and Clinical Data

The patient and control groups were similar with respect to most of the studied variables, but, as

TABLE 1. Selected demographic and clinical parameters of the cohort, including all parameters that showed significantly different distributions between groups.

	Patients	Controls	P-Value
Demographics			
n	72	72	
Female subjects	48.6%	50.0%	1.000
Age (years, mean \pm SD)	65.3 \pm 5.5	64.5 \pm 6.9	0.448
Body -mass -index (kg/m ² , median [IQR])	26.3 [23.8–29.3]	26.2 [23.7–28.1]	0.795
Comorbidities			
Atrial fibrillation	4.2%	18.1%	0.015
TIA or ischemic stroke	7.0%	37.5%	< 0.001
Non-motor symptoms			
Overall NMS severity (NMSS score)	40 [25.25–55.00]	8 [4.00–11.75]	< 0.001
Constipation (Wexner score)	5 [3–9]	2 [1–4]	< 0.001
Medication			
Levodopa	54.2%	0%	< 0.001
COMT inhibitor	15.3%	0%	0.001
Dopamine agonist	77.8%	0%	< 0.001
MAO inhibitor	70.8%	0%	< 0.001
Anticholinergic	8.3%	0%	0.028
Warfarin	1.4%	15.3%	0.004
Statin	20.8%	54.2%	< 0.001

SD, standard deviation; IQR, Interquartile range; TIA, transient ischemic attack; NMS, non-motor symptoms; NMSS, Non-Motor Symptoms Scale; COMT, catechol-O-methyl transferase; MAO, Monoamine oxidase.

expected, NMS were more severe in PD patients (Table 1 and eTable 2). Clinical details of the patient cohort are summarized in Supplemental Data eTable 3. All but two PD patients were using antiparkinsonian medication (Table 1). Two patients were treated by deep brain stimulation (DBS).

Microbiome

The full dataset included bacteria from 360 genera, 125 families, 60 orders, 29 classes, and 18 phyla. Most of the reads (94%) represented the phyla Firmicutes and Bacteroidetes, which are typically the dominant phyla in the gut microbiome (Supplemental Data eFig. 1).¹⁹ Although no statistically significant differences were found with respect to commonly used alpha diversity indices (Chao1, ACE, Shannon, Inverse Simpson, data not shown), comparisons of the clustering of patient and control samples in dendrograms based on beta diversity metrics (Yue & Clayton theta, Morisita-Horn index, and Bray-Curtis index, calculated with family-level data) showed a significant difference between groups (unweighted UniFrac $P < 0.02$ and weighted UniFrac $P < 0.001$ for all three indices).

The mean abundance of Prevotellaceae in the feces of PD patients was reduced by 77.6% in comparison with control subjects (Fig. 1, Supplemental Data eTable 4). The explorative analysis suggested that five families were more abundant in PD patients than in

controls, but the absolute differences between groups were smaller than for Prevotellaceae (Supplemental Data eTable 4).

To estimate effects of possible confounders (Table 1 and Supplemental Data eResults) on the observed group differences, we applied GLMs to model the distribution of bacterial abundances (Table 2). For those bacterial families that were more abundant in PD subjects, we also included Prevotellaceae abundance in the model to disentangle a group-related effect from an unspecific effect compensating low Prevotellaceae levels. Study group was the only factor that was significantly associated with Prevotellaceae abundance (Table 2). Thus, the decreased abundance of Prevotellaceae was not explained by, for example, more severe constipation in the PD group or differences in medications or comorbidities. The abundances of all of the other families except for Ruminococcaceae were also independently related to PD diagnosis. Ruminococcaceae abundance was significantly associated only with levels of Prevotellaceae, suggesting that the higher levels of Ruminococcaceae in the PD group were not related to PD itself, but rather compensating lower levels of Prevotellaceae (Table 2). The independent associations of bacterial abundances with PD diagnosis remained significant when subjects fulfilling Rome III criteria for IBS were excluded from the analysis (Supplemental Data eTable 5).

Based on a receiver operating characteristic curve analysis (Fig. 2), identification of PD patients based on low Prevotellaceae abundance had 86.1% sensitivity but only 38.9% specificity (LR+ = 1.41, LR- = 0.36). A logistic regression analysis with study group as the dependent variable and all bacterial families

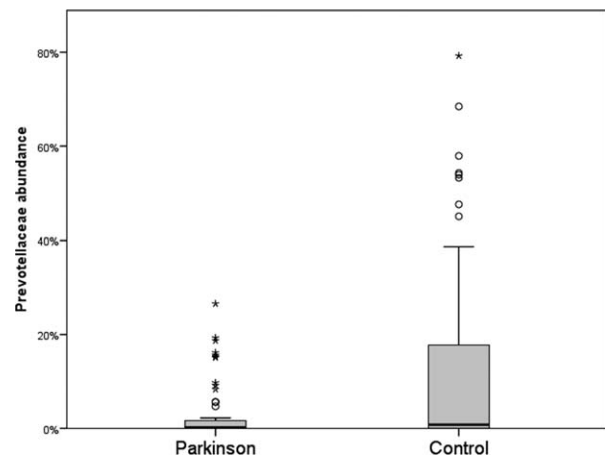


FIG. 1. Box plots showing the distributions of Prevotellaceae abundance in both study groups. Black horizontal lines indicate the median values and the boxes around them delineate the IQR. Whiskers extend to the highest value within 1.5 IQR of the upper quartile. Circles represent outliers beyond the whisker limit and asterisks represent extreme outliers beyond 3 IQR of the upper quartile. High levels of Prevotellaceae were rare in the PD group whereas low levels were found in both groups. Median [IQR]: Parkinson 0.16% [0.00%–1.66%]; Control: 0.77% [0.00%–18.18%]

TABLE 2. GLM Results (Control vs. PD)

Family	Control vs. PD	Atrial Fibrillation No vs. Yes	TIA or Ischemic Stroke No vs. Yes	Warfarin No vs. Yes	Statin No vs. Yes	Total NMSS Score	Total Wexner Score	Prevotellaceae Abundance	COMT-Inhibitor No vs. Yes
Prevotellaceae	1.490 [1.005-1.976], 36.179, <0.001	0.432 [-0.489-1.353], 0.845, 0.358	0.025 [-0.517-0.567], 0.008, 0.927	-0.145 [-1.218-0.928], 0.070, 0.791	-0.096 [-0.542-0.349], 0.180, 0.671	0.038 [-0.154-0.230], 0.152, 0.696	-0.040 [-0.270-0.191], 0.114, 0.736		
Lactobacillaceae	-5.095 [-6.190 - -4.000], 83.151, <0.001	0.017 [-1.502-1.535], 0.000, 0.983	1.754 [0.958-2.549], 18.667, <0.001	-0.258 [-1.943-1.427], 0.090, 0.764	-1.859 [-2.373 - -1.344], 50.134, <0.001	-0.199 [-0.590-0.193], 0.988, 0.320	-0.015 [-0.280-0.250], 0.012, 0.911	0.377 [0.142-0.611], 9.925, 0.002	-3.998 [-4.756 - -3.241], 107.025, <0.001
Verrucomicrobiaceae	-1.126 [-1.784 - -0.468], 11.254, 0.001	1.031 [-0.714-2.775], 1.341, 0.247	0.228 [-0.475-0.931], 0.405, 0.525	-1.768 [-3.654-0.117], 3.378, 0.066	0.081 [-0.496-0.658], 0.076, 0.783	-0.340 [-0.626 - -0.054], 5.444, 0.020	0.406 [0.141-0.672], 9.001, 0.003	-0.954 [-1.523 - -0.385], 10.801, 0.001	
Bradyrhizobiaceae	-2.368 [-3.069 - -1.666], 43.748, <0.001	-0.109 [-1.307-1.088], 0.032, 0.858	-0.440 [-1.237-0.356], 1.174, 0.279	-0.213 [-1.631-1.204], 0.087, 0.768	0.687 [0.004-1.370], 3.891, 0.049	-0.376 [-0.703 - -0.049], 5.076, 0.024	-0.595 [-0.888 - -0.301], 15.769, <0.001	-0.285 [-0.618-0.048], 2.813, 0.094	
Clostridiales Incertae Sedis IV	1.441 [0.613-2.269], 11.628, 0.001	0.173 [-0.418-0.764], 0.330, 0.565	-0.080 [-0.549-0.388], 0.113, 0.737	0.481 [-0.208-1.170], 1.871, 0.171	-0.856 [-1.303 - -0.409], 14.116, <0.001	-0.144 [-0.397-0.109], 1.239, 0.266	0.153 [-0.074-0.380], 1.742, 0.187	-0.712 [-0.938 - -0.485], 37.966, <0.001	2.237 [1.557-2.918], 41.535, <0.001
Ruminococcaceae	-52.526 [-317.204-212.151], 0.151, 0.697	-243.780 [-719.394-231.834], 1.009, 0.315	96.856 [-175.166-368.877], 0.487, 0.485	190.885 [-347.816-729.585], 0.482, 0.487	-112.421 [-339.258-114.416], 0.944, 0.331	-27.605 [-157.321-102.112], 0.174, 0.677	95.836 [-16.063-207.736], 2.818, 0.093	-212.358 [-311.798 - -112.917], 17.519, <0.001	

Results of the GLMs for bacterial abundances (sequence counts) based on the group factor and possible confounders. Normal distribution for Ruminococcaceae. Negative binomial distribution with log link for all others. COMT-inhibitor medication effect was nested within the study group effect for Lactobacillaceae and Clostridiales Incertae Sedes IV. Based on z-transformed values. Results shown as: B [95% CI], Wald chi-square, P-value.

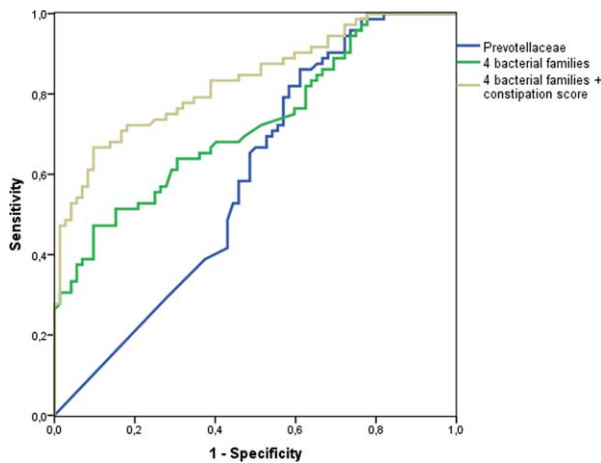


FIG. 2. ROC curves of discriminators of study group membership. The ROC curve analysis of Prevotellaceae abundance was somewhat hampered by the considerable number of samples in both groups being devoid of this family: AUC = 0.591 [95% CI 0.496-0.685], $p=0.060$; optimal cut-off for PD: $\leq 6.5\%$. Since samples devoid of Prevotellaceae were equally frequent in PD and control groups (PD: 29.2%; control: 27.8%; $p=1.000$), we performed a second ROC curve analysis only including subjects positive for Prevotellaceae: $n=103$; AUC = 0.664 [95% CI 0.556-0.771]; $p=0.004$; not shown. Logistic regression classifier including abundances of Prevotellaceae, Lactobacillaceae, Bradyrhizobiaceae and Clostridiales Incertae Sedis IV: AUC = 0.722 [95% CI 0.641-0.804], $p<0.001$; optimal probability cut-off: 0.54. Logistic regression classifier as above, but including also Wexner constipation score: AUC = 0.832 [95% CI 0.766-0.897], $p<0.001$; optimal probability cut-off: 0.55. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

from Table 2 as covariates retained Prevotellaceae, Lactobacillaceae, Bradyrhizobiaceae, and Clostridiales Incertae Sedis IV as predictors of study group (Supplemental Data eTable 6). This classifier (Fig. 2) achieved a higher area under the curve than Prevotellaceae alone ($P = 0.020$ one-sided⁴⁵) and provided 90.3% specificity and 47.2% sensitivity ($LR+ = 4.86$, $LR- = 0.58$). When Wexner total score as a clinical measure of constipation was added to the model (Supplemental Data eTable 7), the discriminative power was further increased ($P = 0.022$ one-sided⁴⁵) because of better sensitivity (66.7%) and preserved 90.3% specificity ($LR+ = 6.86$, $LR- = 0.37$; Fig. 2).

Prevotellaceae abundance showed a significant association with UPDRS-III total score (Supplemental Data

eTable 8). However, patients were allowed to use their normal medication or DBS before and during the UPDRS examination. Thus, we cannot exclude that in patients with motor fluctuations being ON or under DBS treatment at time of examination, UPDRS scores were giving a falsely optimistic rating of disease severity. The same analysis restricted to those patients without motor fluctuations or DBS ($n = 43$; Supplemental Data eTable 8) did not show any significant associations with bacterial abundances. We did not find significant associations of bacterial abundances with age, body-mass-index, total UPDRS, and time from motor or non-motor symptom onset (GLM, data not shown).

Enterobacteriaceae were significantly more abundant in patients with a PIGD phenotype than in TD patients (Supplemental Data eFigure 2, eTable 9). Potential confounders such as sex ratio, body-mass-index, comorbidities, medication, Wexner score, or time from motor symptom or NMS onset did not differ significantly between TD and PIGD subgroups. However, PIGD subjects tended to be older (66.3 ± 5.7 vs. 63.4 ± 5.2 years; $P = 0.051$) and to have higher NMSS total scores (42.0 [32.0-69.5] vs. 33.0 [19.0-51.0]; $P = 0.053$) than TD subjects. The only antiparkinsonian drug class significantly associated with Enterobacteriaceae abundance was COMT-inhibitors (users: 0.56 [0.24-7.40]; non-users: 0.09 [0.02-0.62]; $P = 0.043$). We used a GLM to quantify effects of these possible confounders versus the effect of PD phenotype on Enterobacteriaceae abundance. In this model, PIGD and akinetic-rigid subscores, were positively associated with Enterobacteriaceae abundance, whereas the negative association with tremor subscore slightly missed the level of significance (Table 3). The association with PIGD subscore remained significant when patients with motor fluctuations or DBS treatment were excluded from the analysis ($n = 43$; Table 3).

Discussion

Subject Characteristics

The reported accuracy of clinical PD diagnosis is 26% to 92%, improving with longer disease duration

TABLE 3. GLM Results (Motor Subscores)

Covariate/Factor	Enterobacteriaceae	Enterobacteriaceae
	(All Patients Included; $n = 72$)	(Motor Fluctuations and DBS Excluded; $n = 43$)
Tremor subscore	-0.254 [-0.517- -0.009], 3.572, 0.059	-0.143 [-0.619-0.332], 0.349, 0.555
PIGD subscore	0.931 [0.500-1.362], 17.955, <0.001	2.643 [1.698-3.588], 30.075, <0.001
Akinetic-rigid subscore	0.445 [0.134-0.757], 7.857, 0.005	-0.210 [-0.695-0.274], 0.724, 0.395
NMSS total score	-0.237 [-0.638-0.164], 1.340, 0.247	-0.344 [-1.065-0.377], 0.876, 0.349
Age	0.156 [-0.150-0.462], 0.996, 0.318	-0.366 [-0.920-0.188], 1.676, 0.196
COMT-inhibitor No vs. Yes	-0.712 [-1.495-0.071], 3.173, 0.075	-3.502 [-5.836- -1.167], 8.643, 0.003

Results of the GLMs for Enterobacteriaceae abundance (sequence counts) based on phenotype subscores and possible confounders. Negative binomial distribution with log link. Based on z-transformed values. Results shown as: B [95% CI], Wald chi-square, P -value. DBS, deep brain stimulation; PIGD, postural instability and gait difficulty; NMSS, Non-Motor Symptoms Scale; COMT: catechol-O-methyl transferase.

and response to dopaminergic medication.^{46,47} In the present study, the median time from motor symptom onset was 5 years, and all but two patients were using antiparkinsonian medication. Furthermore, we aimed at maximizing the accuracy of PD diagnosis using strict inclusion and exclusion criteria and verification procedures. Also the distribution of the three motor phenotypes in our sample is in line with previous reports demonstrating the validity of our clinical assessments.^{36,48} Most comorbidities and medications were evenly distributed in both groups, and we adjusted our analyses for those few parameters that were not.

Parkinson's Disease, the Gut, and Microbiota

No previous study has compared the composition of the whole fecal microbiome between PD patients and control subjects.⁴⁹⁻⁵² The theoretical basis for suspecting involvement of intestinal bacteria in PD is intriguing. Alpha-synuclein-related neurodegeneration in the ENS is a frequent and likely premotor manifestation of PD.^{3,6} It is associated with chronic constipation and pathophysiological changes in the intestinal wall.⁵³⁻⁵⁵ Gut microbiota influence the activity of enteric neurons, possibly affecting cellular alpha-synuclein secretion.^{26,29,56,57} Within the central nervous system (CNS), early alpha-synuclein pathology is often found in structures supplying parasympathetic innervation to the gut.^{2,4} Thus, the vagal nerve could be a route for spreading of alpha-synuclein-related neuropathology from the ENS to the CNS.⁵⁸ The vagal nerve is also crucial for the communication between gut microbiota and the brain, and microbiota influence the activity of vagal afferents.^{59,60} These observations demonstrate a pathway through which gut microbiota could be related to ENS and CNS neuropathology in early PD.

The main findings of our study are the reduced abundance of Prevotellaceae in PD patients and the positive association of Enterobacteriaceae abundance with PIGD symptoms. Decreased levels of *Prevotella* and increased abundance of Enterobacteriaceae in feces of autistic children support the relevance of these bacteria in CNS disorders.^{61,62} *Prevotella* is a commensal microbe in the colon that not only can degrade a broad spectrum of plant polysaccharides and mucin glycoproteins in the mucosal layer of the gut, but also may interact with the immune system.^{61,63-65} *Prevotella* is the main contributor of one of the recently suggested gut microbiome enterotypes.⁶³ Our findings indicate that the *Prevotella* associated gut microbiome enterotype could be underrepresented among PD patients. This enterotype is related to higher levels of health-promoting neuroactive short chain fatty acids and a high capacity for biosynthesis of thiamine and folate.^{26,63,66} Thus, a reduced prevalence of this enterotype would be in line with decreased levels of these

vitamins in PD patients.^{67,68} Supplementation of these vitamins and short chain fatty acids may have therapeutic potential in PD.⁶⁸⁻⁷⁰ Decreased Prevotellaceae abundance also fits well with observations of increased gut permeability in PD because low *Prevotella* levels may indicate decreased mucin synthesis, which is associated with increased gut permeability.^{53,71} Increased mucosal permeability could lead to local and systemic exposure to bacterial endotoxin, which has been suggested as an environmental trigger of PD and can lead to increased alpha-synuclein expression in the colon.^{53,72,73} Recently a small pilot study reported decreased Prevotellaceae abundance in constipated patients.⁷⁴ However, the study was performed on obese pediatric patients, and the degree of constipation was not quantified. Furthermore, the nonconstipated controls tended to have higher dietary fiber intake than constipated subjects, which may have influenced the results.^{65,74} Therefore, although this finding is interesting, a direct comparison with our study is difficult.³⁴ In the present study, we aimed to control for the more severe constipation in PD patients, using a validated questionnaire, and found abundance of Verrucomicrobiaceae and Bradyrhizobiaceae, but not Prevotellaceae, to be associated with degree of constipation (Table 2).³⁸ Therefore, more severe constipation is unlikely to explain the lower Prevotellaceae levels in our PD subjects. Future studies using objective measures of intestinal transit time to assess gut motility and severity of constipation may give more insight into this matter.

Also, the abundances of Lactobacillaceae, Verrucomicrobiaceae, Bradyrhizobiaceae, and Clostridiales Incertae Sedis IV were independently associated with PD. Decreased abundance of Prevotellaceae and increased abundance of Lactobacillaceae have been associated with decreased levels of the gut hormone ghrelin.⁷⁵ Gut hormones such as ghrelin regulate nigrostriatal dopamine function and may restrict neurodegeneration in PD.⁷⁶ Accordingly, impaired ghrelin secretion has been reported in PD patients.⁷⁷ *Lactobacilli* further modulate activity of ENS neurons and vagal afferents, which may affect cellular alpha-synuclein secretion.^{56,57,59}

Further possible mechanisms for microbiome-host interactions in PD could involve neurotoxins, neurotrophic factors, and neurotransmitters.^{26,29,30,78,79} However, the relevance of the above-mentioned pathways with regard to PD remains unclear.

The association of Enterobacteriaceae with motor phenotype extends the scope of our findings from a pure association of microbiota with PD diagnosis to an actual correlation with motor symptoms. Parkinson's disease is a clinically heterogenic disorder, and different pathophysiological mechanisms may underlie the different expressions of tremor and nontremor

symptoms between patients. In comparison with TD patients, patients with a non-TD phenotype progress faster, have a worse prognosis, and show more severe alpha-synuclein pathology in the colonic ENS.^{55,80} Our results suggest that this may be associated with higher abundance of Enterobacteriaceae in the fecal microbiome of non-TD patients. A recent study suggested abnormalities related to Enterobacteriaceae in PD, namely, translocation of *Escherichia coli* into the colonic mucosa, supporting the relevance of these bacteria in PD.⁵³ Future studies may reveal whether this association is mediated via an endotoxin-induced cascade or other mechanisms.⁷²

The association of microbiota abundances with COMT-inhibitors is interesting because this drug class frequently causes gastrointestinal side effects.⁸¹

A Potential Biomarker

Biomarkers for PD are urgently needed.⁸² In our study, a person with a high abundance of Prevotellaceae was very unlikely to have PD. However, low Prevotellaceae levels do not seem to be specific for PD, because they have been reported in patients with autism and type 1 diabetes.^{61,71} Based on these observations, high fecal abundance of Prevotellaceae could be a useful biomarker to exclude PD. A broader analysis of microbiome composition may increase accuracy. Because bowel dysfunction occurs in the premotor phase of PD and the microbiome of an individual is remarkably stable over time, investigating whether microbiota analysis could be used as a biomarker for premotor PD seems worthwhile.^{10,12,83}

Limitations

This pilot study addressed a rather uncharted aspect of PD with limited resources. Therefore, we chose a case-control design, acknowledging that it would not allow definite conclusions about the temporal and causal relationship between microbiome alterations and PD. The fact that we did not find an association between bacterial abundances and time from symptom onset argues against microbiome alterations being a consequence of PD. Likewise, the association of Enterobacteriaceae abundance with motor phenotype is rather difficult to explain by an uncontrolled confounder. However, although we controlled many potential confounders, we cannot completely exclude that an uncontrolled confounder may have affected our results. Furthermore, the studied population came from a geographically restricted region, limiting the scope of our conclusions at this point.³⁴

The main limitation of this study is the lack of information about the dietary habits of our subjects. Major long-term dietary differences between PD patients and controls could cause differences in the

microbiome.^{25,65,66} However, previous studies of dietary effects on PD risk are inconclusive and reported small effect sizes.⁸⁴ Furthermore, in our study, no significant difference was found in body-mass-index between study groups, arguing against major dietary differences. Higher consumption of vegetables and carbohydrates has been reported in PD patients, but this does not explain decreased Prevotellaceae levels, because these correlate positively with carbohydrate and fiber consumption and negatively with protein intake.^{25,65,66,85,86} Possibly different dietary habits related to the higher prevalence of transient ischemic attack (TIA)/ischemic stroke in the control group unlikely explain the microbiome differences between the PD and control groups. First, TIA/ischemic stroke was included as a covariate in our GLM analysis (Table 2). Second, in a separate Metastats analysis contrasting subjects with and without previous TIA/ischemic stroke, of those bacterial families from Table 2, only Lactobacillaceae showed a difference, with $P < 0.05$ (Supplemental Data eTable 10) supporting the GLM results. Third, TIA/ischemic stroke would most likely be associated with higher fat and protein and lower fiber consumption and is therefore unlikely explaining higher Prevotellaceae levels in control subjects.^{65,66,86} A corresponding analysis regarding atrial fibrillation (Supplemental Data eTable 11) did not show significant differences, which corroborates the GLM results (Table 2). Importantly, even if dietary differences between our PD patients and control subjects existed, gut microbiota still may play a crucial role in translating health effects of food compounds on the host organism.²²

Although the taxonomic composition of the gut microbiome has been associated with several diseases, functional aspects related to, for example, metabolic and immunological interactions may be even more relevant and should be addressed in future studies.^{17,87}

Conclusions

Our findings shed new light on previous reports regarding gastrointestinal involvement in PD. Investigating whether high abundance of Prevotellaceae has protective effects against PD or whether low abundance is rather an indicator of disturbed mucosal barrier function will be important. Although very sensitive, low Prevotellaceae levels alone are not specific for PD. Inclusion of other bacterial families may increase accuracy, and further exploring the potential of fecal microbiome analysis as a biomarker for PD seems worthwhile. Further studies may elucidate the temporal and causal relationships between gut microbiota and PD and the mechanisms involved. ■

Acknowledgment: We thank all referring physicians for their efforts in finding suitable subjects for our study. We thank Prof. Turgut

Tatlisumak, Saija Eirola, Marja Metso, and Päivi Leimuvaara for support related to administration and sample storage. We also thank Marja Patrikainen for her assistance with subject recruitment. Eeva-Marja Turkki, Kirsi Lipponen, and Ursula Lönnqvist are acknowledged for skillful technical assistance in the laboratory.

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