Original Article

Dysbiosis of the Beneficial Gut Bacteria in Patients with Parkinson's Disease from India

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Abstract

Objectives: Recent advancement in understanding neurological disorders has revealed the involvement of dysbiosis of the gut microbiota in the pathophysiology of Parkinson's disease (PD). We sequenced microbial DNA using fecal samples collected from PD cases and healthy controls (HCs) to evaluate the role of gut microbiota. **Methods:** Full-length bacterial 16S rRNA gene sequencing of fecal samples was performed using amplified polymerase chain reaction (PCR) products on the GridION Nanopore sequencer. Sequenced data were analyzed using web-based tools BugSeq and MicrobiomeAnalyst. **Results:** We found that certain bacterial families like Clostridia UCG 014, Cristensenellaceae, and Oscillospiraceae are higher in abundance, and Lachinospiracea, Coriobacteriaceae and genera associated with short-chain fatty acid production, *Faecalibacterium*, *Fusicatenibacter*, *Roseburia* and *Blautia*, are lower in abundance among PD cases when compared with the HC. Genus *Akkermansia*, *Dialister*, *Bacteroides*, and *Lachnospiraceae NK4A136* group positively correlated with constipation in PD. **Conclusion:** Observations from this study support the other global research on the PD gut microbiome background and provide fresh insight into the gut microbial composition of PD patients from a south Indian population. We report a higher abundance of Clostridia UCG 014 group, previously not linked to PD.

Keywords: 16S rRNA gene sequencing, dysbiosis, gut microbiota, neurodegenerative disease, Parkinson's disease, stool

Introduction

The intestinal microbiota and their metabolites have gained significant importance in the pathophysiology and progression of diseases such as gastrointestinal, neurological, and chronic diseases. Over the past few years, research has shown a strong correlation between gut dysbiosis and Parkinson's disease (PD), a chronic, progressive neurological condition. The primary motor characteristics of PD include resting tremors, stiffness, sluggish movement, imbalance, and abnormal gait.[1] Dry mouth, constipation, and defecatory dysfunction are some of the nonmotor prodromal gastrointestinal symptoms that appear years prior to the onset of motor symptoms.[2]

Two subtypes of PD have been hypothesized based on the origin or principal site of alpha-synuclein (α-Syn) accumulation, the body-first PD, and brain-first PD. The brain-first subtype is characterized by dysfunction of the nigrostriatal pathway and is negative for the symptoms of rapid eye movement sleep behavior disorder during the prodromal stage.[3] According to gut-brain pathophysiology, α-Syn aggregation commences in the distal olfactory and enteric nervous systems and then propagates to the brain stem in a prion-like fashion via the glossopharyngeal and vagal nerves in the body-first PD.[4] Exposure to environmental toxins (herbicides and pesticides or insecticides), bacterial lipopolysaccharides, and dysbiosis disrupt the intestinal barrier integrity and trigger the immune response and neuroinflammation.[5,6] These changes eventually lead to α-Syn aggregation in the enteric neuron-plexus and spread to the brain stem, which has been demonstrated and proven using various animal models.[7,8]

Dysbiosis of the gut microbiota can induce α-Syn misfolding and aggregation, resulting in neurodegeneration in PD. It is recognized that gut microbiota and neuroimmunity have a role in the pathogenesis of PD.[5] Since the first report on the association of gut microbiota with PD clinical phenotype, several investigations have been carried out all over the globe.[6,9] This study was made to better understand the comparative shift or dysbiosis dynamics of gut microbiota in

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Indian PD patients residing on the southwest coast of India using full-length 16S rRNA gene sequencing.

Materials and Methods

Ethical consideration

Subjects were enrolled from the neurology clinic of a tertiary care hospital. The institutional ethics committee approved this research, and the study is registered in the clinical trial registry of India (CTRI/2018/04/013333). All subjects consented to participate in the study with written informed consent.

Study subjects and clinical details

This case-control study compared the gut microbial profile of PD patients diagnosed according to the United Kingdom–Brain Bank Society of PD criteria with their healthy spouses above 40 years of age who willingly consented to participate. We opted to enroll spouses as healthy controls (HC) since diet is a significant modifiable factor impacting gut microbial composition. Exclusion criteria for both the study participants include secondary parkinsonism, primary psychiatric illness associated with aging, primary gastrointestinal pathology, or use of antibiotics within 1 month before sample collection. Structured questionnaires were used to obtain thorough demographic information such as age, gender, occupation, and medical history.[10] We used the Unified Parkinson's Disease Rating Scale (UPDRS) and the modified Hoehn and Yahr scale (H and Y) to evaluate the severity of symptoms and know the disease stage, respectively. The frequency and severity of the nonmotor symptoms were graded using the Non-Motor Rating Scale (MDS-NMS). To identify functional constipation, the Rome IV bowel disorder questionnaires were considered. Food frequency questionnaire was used to understand dietary habits.[11] We have calculated the levodopa equivalent dose for all PD patients.[12]

Sample collection and bacterial DNA extraction

Stool samples were collected from 36 subjects and stored at −80°C within 4 h of collection until processing for microbial DNA extraction. Bacterial DNA was extracted from feces using the Translational Health Science and Technology Institute (THSTI) method,[13] an in-house technique. Individual stool samples were homogenized to remove undigested food and mucus adhering to the bacterial cells. Bacterial cells were lysed using a mixture of enzymes (lysozyme, mutanolysin, and lysostaphin) in addition to physical, chemical, and mechanical methods. Bacterial DNA extraction was carried out by organic extraction of nucleic acid followed by precipitation of nucleic acids and purification of genomic DNA by removal of RNA.[13] The quality and quantity of extracted DNA were estimated by measuring absorbance at 260 and 280 nm wavelengths using NanoDrop spectrophotometer 2000, and the quality was also confirmed by 0.8% agarose gel electrophoresis.

16S rRNA gene sequencing

PCR Amplification: DNA from all samples was amplified for the full-length 16S rRNA gene using region-specific primers (16s rRNA barcode primer) and a LongAmp Taq 2X master mix (NEB). Amplicons of each sample were subjected to agarose gel quality control and purified.

Library preparation: Amplicon library was created using the ligation sequencing and PCR barcoding kit, which also involved end preparation, barcoding, and sequencing adapter ligation. Purified amplicon DNA was end-repaired from each sample using the NEBnext ultra II end repair kit and cleaned. NEB blunt/TA was used for the ligation of the barcode adapter. Qubit fluorimeter was used to measure barcode adapter-ligated products and was cleaned up after the PCR process.

Samples with barcodes were quantified and pooled at an equimolar concentration. Pooled barcoded samples were end-prepared using the NEBNext Ultra II End Repair/dA-Tailing Module, and end-repaired DNA was cleaned up. The adaptor was ligated for 15 min using NEB blunt/TA ligase. Prior to eluting with elution buffer, the library mix was cleaned.

Sequencing: On the GridION X5 (Oxford Nanopore Technologies, Oxford, UK), sequencing was done over the course of 48 h utilizing SpotON flow cell R9.4 (FLO-MIN106). Guppy v2.3.4 was used to basecall (in "fastq" format) and demultiplex nanopore raw readings (in "fast5" format).

Bioinformatics analysis

Microbial Community Analysis: FASTQ files were uploaded to BugSeq (version 1.1) to perform quality control and metagenomic classification.[14] Downstream analysis and visualization of taxonomic profiles and amplicon sequence variant (ASV) classification tables were achieved using MicrobiomeAnalyst. MicrobiomeAnalyst, an online platform, was used to evaluate the bacterial composition and statistical comparisons of the metagenomic specimens.[15] To optimize the downstream statistical analysis, data were filtered and normalized. The phylum/family/genus abundance profiling was based on the aggregate counts for each group. Using the method outlined by Rodrguez-Rabassa *et al*.[16] and Zapała *et al*.,[17] the core microbiome assessment of a taxonomic cluster that represented a sizable fraction of the population and a comparative analysis of the microbiome within and between the groups was examined.

Shannon, Simpson metrics, and Mann–Whitney/Kruskal–Wallis tests were used to evaluate α-diversity profiling and significance. Mann–Whitney *U* test statistical approach was used to examine the univariate statistical comparison at the genus level, with a *P* value cutoff of 0.05. Beta diversity (the proximity and difference between bacterial populations) was analyzed using various diversity indices such as the Bray–Curtis index, Jaccard index, and Jensen–Shannon divergence and distance method, and permutational multivariate analysis of variance (MANOVA) was used for statistical analysis.[16] Principal coordinates analysis (PCoA) was performed to reduce dimensions and visualize the correlations between data.[15]

Gut bacterial profiles' distinctive qualities were evaluated using linear discriminant analysis (LDA)

effect size (LEfSe), which characterized the biomarkers with the greatest statistical and practical relevance.[18] Graphical techniques were used to assess the taxonomic classifications and demonstrate the distinctions between the bacterial populations of the two groups. The composite of classification trees was used to execute the random forest analysis, an approach ideal for large dimensional data analysis, to identify which taxa or organisms are associated with groups by randomly selecting features from a bootstrap aggregation of the sample at each branch.[19] Random forest was used to identify which taxa or organisms were associated with groups.

Table 1: Demographic and clinical data of the subjects

Demographic Data of Controls and Cases		HC, *n*=13	PD cases, *n*=23
Age (years)$ (*P*=0.08)		56.38 (±8.24)	60.09 (± 9.1)
Body Mass Index (BMI)$ (*P*=0.06)		20.94 ((±1.41)	21.47 (±2.52)
Gender# (*P*=0.29)	Male	5 (38.9)	13 (56.5)
	Female	8 (61.1)	10 (43.5)
Diet# (*P*=0.92))	Vegetarian	3 (23.1)	5 (21.7)
	Mixed-Diet	10 (76.9)	18 (78.3)
BMI Category# (*P*=0.18)	Underweight	1 (7.7)	2 (8.7)
	Healthy	12 (92.3)	15 (65.2)
	Overweight	-	6 (26.1)
Occupation# (*P*=0.54)	Non-Physical	8 (61.1)	18 (78.3)
	Physical	5 (38.9)	5 (21.7)
Type 2 Diabetes Mellitus# (*P*=0.11)		-	4 (17.4)
Hypertension# (*P*=0.02) *		1 (7.7)	10 (43.5)
Clinical data of the Parkinson's Disease subjects			
Age at onset of disease (years)$ (*P*=0.07)		-	54.60 (± 7.42)
Disease Duration (years)$ (*P*=0.06)		-	2.26 (±1.5)
Family History of PD#		-	2 (8.7)
Cardinal Motor Symptoms	Bradykinesia#		
	Occasionally	-	14 (60.9)
	Frequently	-	9 (39.1)
	Tremor#		
	Occasionally	-	12 (52.2)
	Frequently	-	11 (47.8)
	Rigidity#		
	Occasionally	-	13 (56.5)
	Frequently	-	10 (43.5)
	Balance Impairment#		
	Never	-	13 (56.5)
	Occasionally	-	8 (34.8)
	Frequently	-	2 (8.7)
Other Motor Symptoms	Micrographia#	-	15 (65.2)
	Masked Face#	-	17 (73.9)
	Reduced Eye Blinking#	-	18 (78.3)
	Soft Voice#	-	7 (30.4)
Modified Hoehn and Yahr staging#			
Stage 1		-	3 (13.0)
Stage 1.5		-	6 (26.1)
Stage 2		-	7 (30.4)
Stage 2.5		-	7 (30.4)
Diagnosed as Functional Constipation based on Rome IV Criteria#		-	13 (56.5)
Catechol-O-methyl-transferase inhibitor#		-	18 (78.3)
Anti-Cholinergic#		-	18 (78.3)
Levodopa Equivalent Daily Dose (mg/day)^ (*P*=0.006)		-	520 (330)
UPDRS III -Motor Examination Score$ (*P*=0.07)		-	38.34 (±16.42)
MDS Non-Motor Rating Scale (Total Score)^(*P*=0.032)		-	39 (43)

Note: Variables $Normally distributed data represented as Mean (±SD). ^Data non-normal distribution represented as Media (IQR). #frequency (%) [number of subjects and percentage]. *Statistically Significant at *P*<0.05. Never (0% of the time), Occasionally (≤25% of the time), Frequently (26-75% of the time) and Majority of the time (>75% of the time)

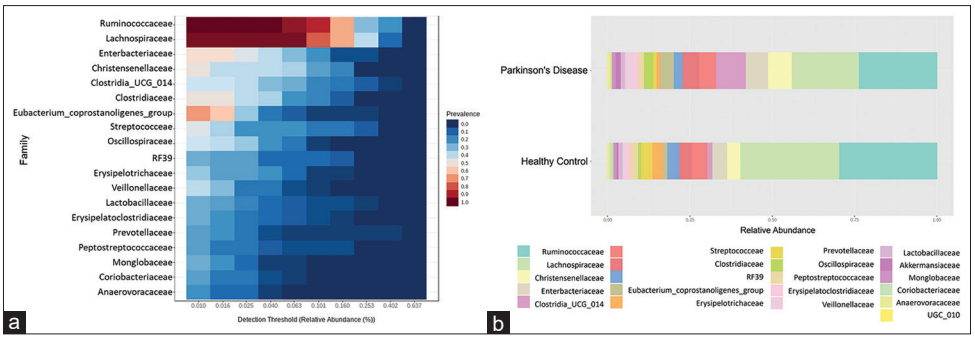

Figure 1: (a). Microbial communities identified at the family level. Heat map of the core microbiome analysis to identify core taxa at the family level. The *y*-axis represents the prevalence level of core features across the detection threshold (relative abundance) range on the *x*-axis. A gradient of color indicates the variation in the prevalence of each family from blue (decreased) to red (increased). (b). Relative abundance of the bacterial families. Stacked bar chart exhibiting the taxonomic composition of Parkinson's disease and healthy controls by direct quantitative comparison of relative abundances

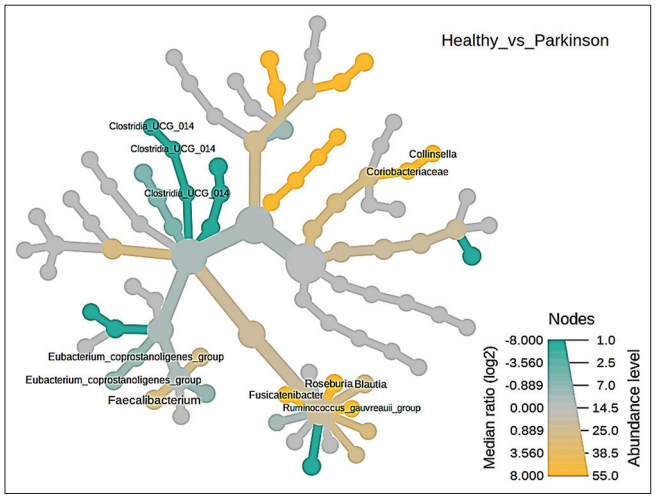

Figure 2: Difference in abundance of core microbiome between PD and HC. Taxonomic differences in intestinal microflora between Parkinson's disease and healthy controls; only the significant taxa are labeled

Results

Demographic and clinical information of the subjects

Thirty-six subjects were enrolled (23 PD patients and 13 HC), considering the criteria for inclusion and exclusion described in the methodology. The mean age of the subjects was 60 (±9.1) years for PD patients and 56 (±8.24) years for HC. The medical history and demographic details of the subjects are listed in Table 1. Hypertension was reported in both groups. In contrast, other medical conditions such as type-2 diabetes mellitus (T2DM) (14.7%), arthritis (4.3%), hypothyroidism (4.3%), and heart ailments (4.3%) were noted only in PD subjects. All were being treated for these ailments with medicines.

According to the modified H and Y staging score, the cases recruited were in various stages of the disease, with a mean of 1.8 (±0.5). Only about 8.7% of the subjects had a family history of PD. In addition, 56.5% of the PD cases were diagnosed with functional constipation using Rome IV criteria. We have examined the frequency and history of the cardinal motor and other motor symptoms reported by PD patients. Mean and standard deviation have been calculated for clinical details such as the NMS score, LEDD, treatment with catechol-O-methyl-transferase inhibitor, and anticholinergics, as represented in Table 1. FFQs were used to understand the dietary pattern of the subjects to rule out the potential confounding factor, as diet significantly influences microbial composition. Most subjects who followed a mixed dietary pattern and occasionally consumed meat mostly only twice a month. Nutritional habits are not anticipated to be a significant confounder in our study since all our subjects were on a carbohydrate-rich diet and consumed dairy and dairy products with no unique dietary habits or restrictions.

Microbiome evaluation

To analyze the microbiota, a total of 1,372,361 high-quality reads were generated, with a mean read count of 42,886 per sample. Figure 1a and 1b, heat map and bar graph, represent the core microbiome analysis identifying core taxa. Graphical depiction of the average taxonomic richness at the family level included all 36 samples, Ruminococcaceae (27% in HC, 20% in PD), Lachnospiraceae (27% in HC, 18% in PD), Enterobacteriaceae (7% in HC, 8% in PD), and Christensenellaceae (4% in HC, 7% in PD) are the top four abundant families. The heat-tree analysis demonstrates the intestinal microflora in all the samples of PD and HC [Figure S1]. Whereas heat-tree-labeled healthy versus Parkinson analysis demonstrates the taxonomic differences in intestinal microflora between those with PD and HC, showing only the significant taxon *Clostridia_UCG_014, Eubacterium coprostanoligenes* group*, Faecalibacterium, Roseburia, Blautia, Fusicatenibacter* and *Ruminococcus gauveraii* group*, Collinsella* and Coriobacteriaceae [Figure 2]. The nonparametric Wilcoxon Rank Sum test with a 0.05 Wilcoxon *P* value cutoff was used to determine statistical significance.

The intestinal microbiome profiles of PD patients and HC differed considerably, as did the α and β diversity in the two groups evaluated. The α-diversity indices, including Shannon's (*P* value = 0.03) and Simpson's (*P* value = 0.02), were significantly different at the family level between PD and HC [Figure 3]. However, we did not observe

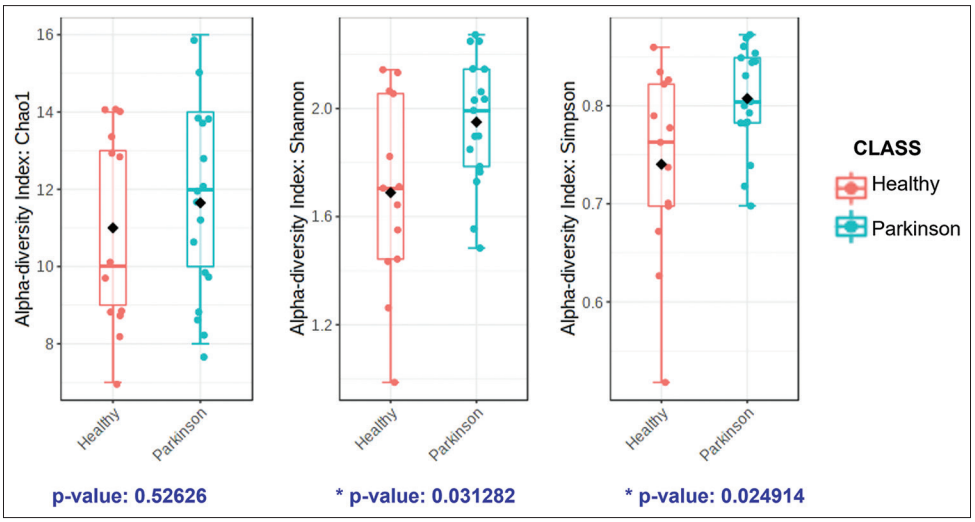

Figure 3: Alpha diversity profiling and significance. Chalo1, Shannon, and Simpson α-diversity analysis were performed in filtered data input by using the Mann–Whitney/Kruskal–Wallis statistical method. The groups were represented on the *x*-axis and their estimated diversity was on the *y*-axis. Each sample or boxplot is colored based on disease status (healthy control = red, Parkinson's disease = blue). *Statistical Significance

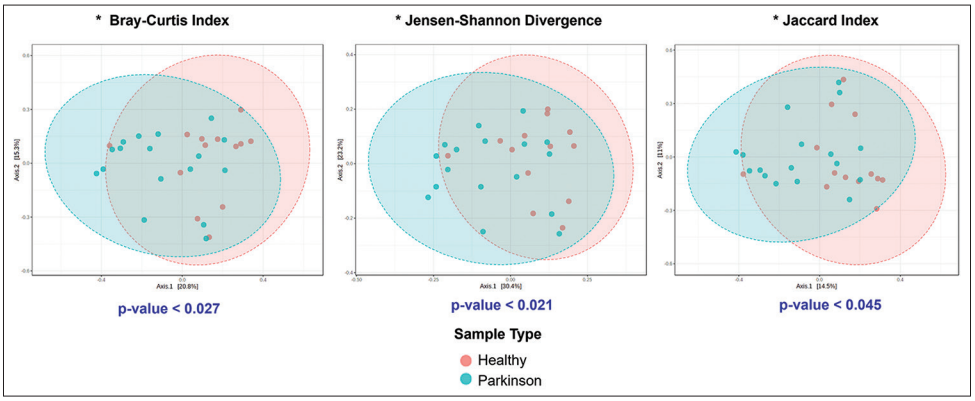

Figure 4: Beta-diversity analysis. Nonphylogenetic Bray–Curtis index, Jensen–Shannon divergence and Jensen–Shannon divergence distance method were used to establish the abundance of taxa present in the dataset. Principle coordinate analysis (PCoA) was used to visualize these matrixes in the plot. Each point in the graph (Healthy = red, Parkinson = blue) represents the entire microbiome analysis of a single sample. The statistical significance of the clustering pattern was evaluated by using Permutational MANOVA. *Statistical Significance

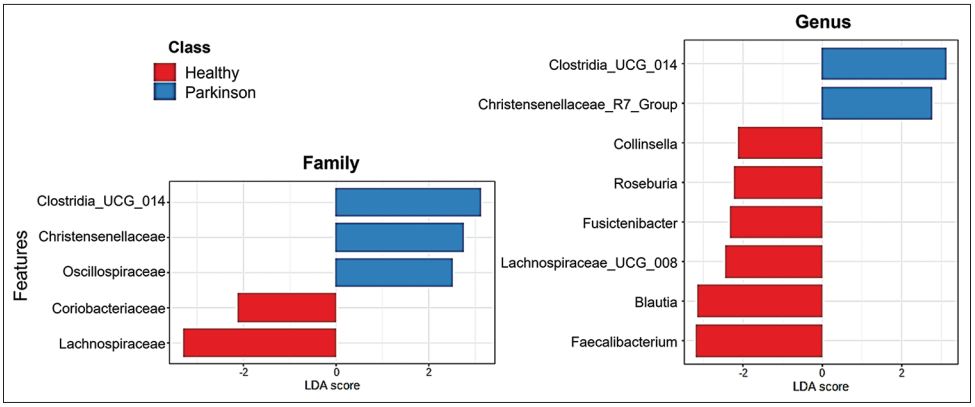

Figure 5: The differences between the groups were evaluated using the linear discrimination analysis effect size (LEfSe). The bar graph shows the LDA scores of significant bacteria. The colors (Healthy = red, Parkinson = blue) represent which group was more abundant compared with the other group at the genus and family level. The *P* value cutoff was 0.1 with an adjusted false discovery rate

any significant difference in richness based on observed Operational Taxonomic Unit (OTU) (P value = 0.5) and Chalo1 (P value = 0.5) between the groups. In Figure 4, β diversity is demonstrated as PCoA based on the Bray-Curtis Index (P-value < 0.02), Jensen-Shannon (P-value < 0.02), and Jaccard (P-value < 0.04), indicating the statistical difference between PD and HC.

The random forest prediction and LEfSe evaluation suggest family [Table S1a and S1b, Figures 5 and 6] Christensenellaceae, Oscillospiraceae, Coriobacteriaceae, Lachinospiraceae, and genus *Clostridia_UCG_014* group, *Christensenellaceae_R7* group, *Balutia, Fusicatenibacter, Collinsella, Faecalibacterium, Roseburia, Lachinospiraceae_UCG_008* group, might be possible biomarkers of PD in the stool. Out of the top 25 families and genera of bacteria, Lachnospiraceae (r = −0.43, P value = 0.01), *Faecalibacterium* (r = −0.4, P value = 0.02), *Fusicatenibacter* (r = −0.38, P value = 0.03), *Ruminococcus gauvreauii* group (r = −0.35, P value = 0.05), negatively correlated with PD, whereas *Clostridia_UCG_014* (r = −0.35, P value = 0.05) positively correlated as presented in Figure 6. Spearman's correlation analysis indicated the association of the gut microbiome with functional constipation. *Lachnospiraceae_NK4A136* group (r_s = 0.5, P value = 0.02), *Dialister* (r_s = 0.49, P value = 0.02), *Akkermansia* (r_s = 0.43, P value = 0.06), and *Bacteroides* (r_s = 0.43, P value = 0.06) correlated with Rome IV criteria for functional constipation and are possible biomarkers for constipation by LEfSe [Tables S2 and S3, Figures S2 and S3].

Discussion

Numerous studies have been conducted to comprehend the role and association of gut microbiota with PD in various populations, including studies from China, Japan, Russia, Finland, Germany, Italy, and the United States.[6,9] The gut microbial profile of PD from the Indian subcontinent has never been examined. Our work provides fresh insight into the gut microbial composition of PD patients from a Coastal South Indian population. Most investigations on the gut microbiome in PD have either used shotgun metagenomics or real-time quantitative PCR to target a particular group of organisms or specific variable regions of the 16S rRNA gene (short reads).[6] To the best of our knowledge, this is the first study on the gut microbiome of PD that has used long-read 16S rRNA gene sequencing on a Nanopore platform. Full-length/long-read 16S sequencing provides higher taxonomic accuracy than target-specific short-read second-generation sequencing.[20]

In our study, a series of statistically significant relative abundance variations between the PD and HC groups were noticeable, which defined a PD-specific intestinal microbial profile. The statistical significance in α diversity revealed the diversity throughout the cohort was distinct though we did not find a significant difference in the richness. Similar to this finding, a study reported a variation in the α diversity,[21] whereas another reported no statistical significance.[22] In concordance with other studies, we noted the statistical significance in β diversity, indicating a microbial community profile specific to PD when bacterial richness between PD and HC groups was compared.[17,21] PD-specific gut bacterial profile was consistent with β diversity differences. At the family level, a higher abundance of Christensenellaceae and a lower abundance of Lachnospiraceae are consistent with reports from several earlier studies.[21,23-25] At the same time, the lower abundance of Coriobacteriaceae was incompatible with Barichella *et al*. study.[21] Furthermore, our PD population reports a higher abundance of Oscillospiraceae and Clostridia UCG-014. At the genus level, a lower abundance of

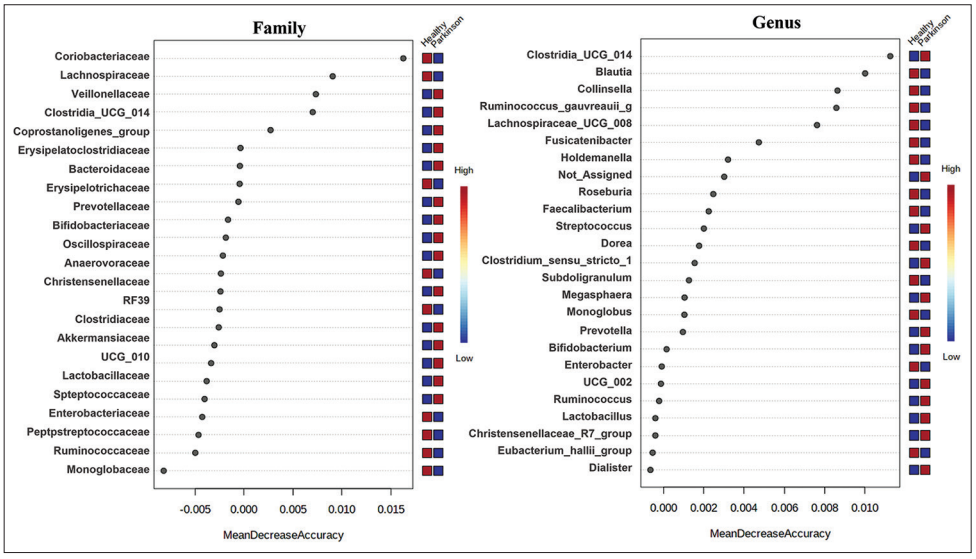

Figure 6: Random forest analysis. The families and genera were ranked by contributions to classification accuracy and Pearson correlation. The blue color represents negative correlations, whereas the red color represents positive correlations. The deep color (blue or red) means a stronger correlation. The mini heat map on the right side of the plot shows the high or low abundance in two groups

Blautia,[22,24-26] *Lachnospiraceae_UCG*,[21,23,27] *Roseburia*,[21-26,28] *Faecalibacterium*[22-24,26,28,29] and *Fusicatenibacter*[22,28,29] identified here, has been constantly linked with PD in previous studies. Although Cirstea *et al*.[23] found *Collinsella* to be more prevalent in PD individuals, it was fewer in our investigation. We report an overrepresentation of the genus *Christensenellaceae R-7group*[27] and *Clostridia UCG_014* in PD subjects.

Genus *Collinsella* of the family Coriobacteriaceae (phylum Acinetobacter) is part of human core fecal flora and a dominant microbiota in the adult gut. Coriobacteriaceae perform significant tasks in the gut, including converting bile salts and steroids and activating dietary polyphenols. A lower abundance of *Collinsella* is linked with a diet rich in protein, low in fat, and carbohydrate weight loss diet.[30] All the subjects followed a conventional diet high in carbohydrates and had no special dietary requirements or restrictions; the decreased abundance of *Collinsella* and Coriobacteriaceae may imply a substantial function and relationship with PD. An abundance of the family Christensenellaceae is positively correlated with PD[21,23,26,27] and advancing age (extreme longevity)[31] and inversely correlated with BMI.[32] Similarly, the genus *Oscillospira* of the family Oscillospiraceae is linked with low BMI and lean subjects.[33] Since most participants in epidemiological studies have reported gradual weight loss in PD, Shen *et al*.[9] hypothesized that Christensenellaceae might have a role in lipid metabolism and that an increase in specific gut bacteria may influence lipid absorption and lead to weight loss.

Considering the Pearson correlation of the top family, we observed an abundance of Lachnospiraceae,[21,23,24,26] which statistically correlated negatively with PD in concordance with other studies. To the best of our knowledge, a stronger positive correlation of *Clostridia UCG 014* has not yet been documented in conjunction with PD microbial profile and should be taken into account in future research. Comparably, at the genus level, the abundance of *Lachnospiraceae NK4A*[34] is positively correlated, *Faecalibacterium*,[22-25,29] *Blautia*,[24-26] and *Fusicatenibacter*[22,29,35] are negatively associated with PD in agreement with other literature.

Bacterial taxa Lachnospiraceae*, Faecalibacterium*, *Blautia*, and *Roseburia,* are associated with the metabolism of complex carbohydrates and the production of short-chain fatty acids (SCFAs) to maintain intestinal mucosal integrity; these bacteria are significantly lower in abundance in PD.[6] SCFAs are signaling compounds with antioxidant and beneficial anti-inflammatory effects.[36] Unger *et al*.[37] verified that a substantial decrease in SCFA in the feces of PD participants is associated with a decreased quantity of microbiota that produces SCFA. SCFA deficiency in the colon has a detrimental effect on gastrointestinal barrier integrity, increases inflammation, increases the likelihood of α-Syn deposition, and induces neuroinflammation.[38] A recent work speculates that the lower abundance of SCFA-producing bacteria and higher abundance of *Akkermensia* could drive the disease progression.[35]

We observed the genus *Akkermensia* to be associated with constipation, as reported by other studies,[22,23] and we found genera *Lachnospiraceae NK4A, Bacteroides*, and *Dialister* significantly abundant in our PD subjects with constipation. Longer gastrointestinal transit time, firmness of stool, and severity of constipation are identified to be associated with overrepresentation of *Akkermansia.*[6] Increased *Bacteroides* and *Akkermansia* abundance impair the gut barrier.[39] According to research, *Bacteroides* was shown to be more numerous in the colonic mucosa of people with persistent constipation.[40] Genus *Bacteroides* has also been associated with Parkinson's disease progression.[35] A higher abundance of *Lachnospiraceae NK4A* has been observed in northeastern Han Chinese PD patients.[34]

Findings from our research work revealed that Indian PD patients exhibit dysbiosis of the gut microbiota. We were unable to determine the precise functions of the intestinal microbiota in the pathophysiology of PD from this cross-sectional case-control research. We had to limit the sample size due to limited monetary aid and the high expense of performing next-generation sequencing (NGS). A few of our PD cases were with T2DM, and we could not recruit healthy spouses with matching comorbidities for the study. However, we were able to come to a conclusion since we had statistically significant data. Approximately, 57% of our PD subjects were diagnosed with functional constipation, and we observed a correlation between the abundance of bacterial flora and functional constipation in PD. Functional constipation is a major gastrointestinal dysfunction that affects the quality of life in PD. Gut microbiota has been identified as a possible modulator of human health. Dietary and microbial intervention are potential disease-modulatory therapeutic strategies.

Although the taxa related to PD have varied among research globally, findings of gut bacterial dysbiosis in PD appear to be robust across investigations. Longitudinal studies that would help understand the gut microbial significance in the disease progression are essential to investigate dysbiosis relevance in body-first and brain-first PD subtypes. It might enable potential cutting-edge therapeutic strategies meant to alter the gut flora in people with PD to ease their motor and nonmotor symptoms.

Conclusion

In this research, we investigated the intestinal microbial profiles of Indian individuals with PD. Patients with PD have a considerably different gut bacterial profile than HC. Our study resonates with the previous research with respect to a lower abundance of beneficial SCFA-producing bacteria; henceforth, the study reflects dysbiosis dynamics in PD. More research should be done on a larger study population recruited from several sites of various geographical locations and dietary habits. In addition, it might be valuable in correlating PD symptoms and subtypes to distinct microbiome profiles in an Indian population to tailor personalized therapy.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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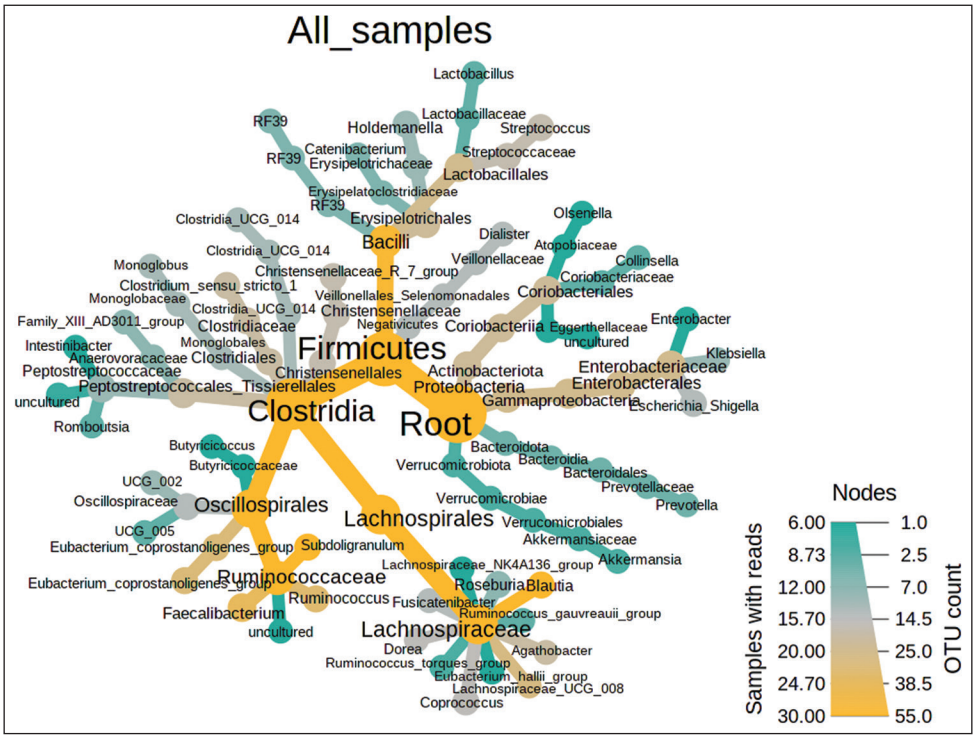

Figure S1: The heat tree analysis demonstrates the intestinal microflora in all the samples of Parkinson's disease and healthy controls

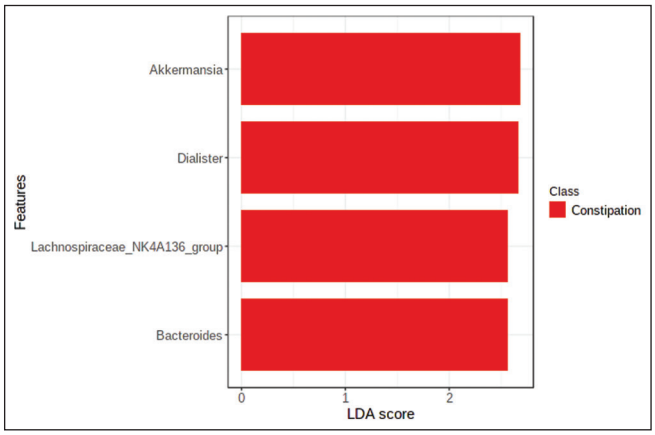

Figure S2: Abundance of genus *Akkermansia, Dialister, Lachnospiraceae* and *Bacteroides* positively correlated with Rome IV functional constipation

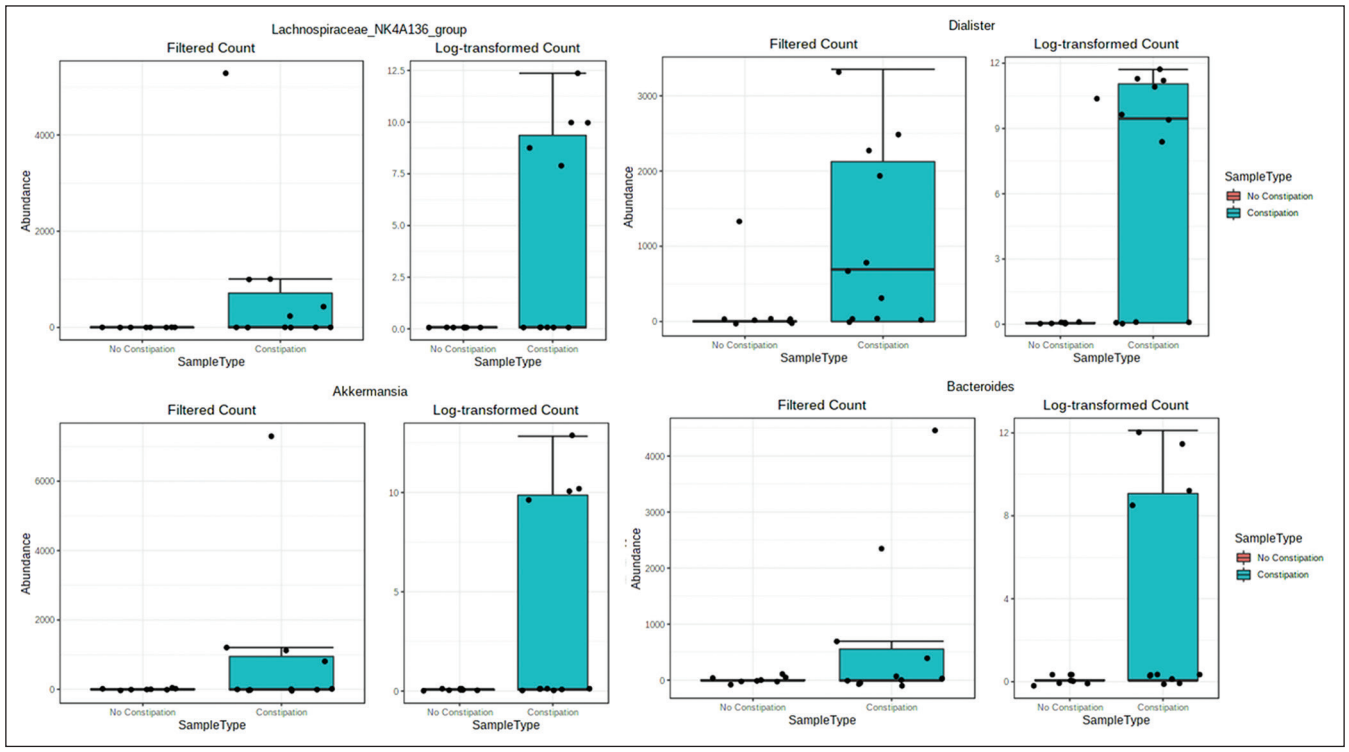

Figure S3: Correlation of differentially abundant taxa in functional constipation PD subjects. We observed genus Akkermasia (pValue: 0.06), Bacteroides (pValue: 0.06), Dialister (pValue: 0.03) and Lachnospiraceae NK4A136 group (pValue: 0.03) significantly abundant by considering a pValue cutoff 0.1 in subject diagnosed with functional constipation

Table S1: Linear discrimination analysis effect size (LEfSe) of statistically significant taxa

Table S1a: Family Level

Name ↑↓	*P* ↑↓	FDR ↑↓	Healthy↑↓	Parkinson↑↓	LDAScore↑↓
Clostridia UGC 014	0.017079	0.40228	606.46	3193.4	3.11
Coriobacteriaceae	0.032182	0.40228	422.54	164.88	-2.11
Lachnospiraceae	0.082414	0.46732	10503.0	6714.6	-3.28
Christensenellaceae	0.084629	0.46732	1448.7	2561.2	2.75
Oscillospiraceae	0.093464	0.46732	401.23	1030.8	2.5

Table S1b: Genus Level

Name ↑↓	*P* ↑↓	FDR ↑↓	Healthy↑↓	Parkinson↑↓	LDAScore↑↓
Clostridia UGC 014	0.017079	0.28691	606.46	3193.4	3.11
Ruminococcus gauvreauii group	0.018844	0.28691	246.31	63.647	-1.97
Blautia	0.028005	0.28691	5486.9	2811.0	-3.13
Collinsella	0.032182	0.28691	422.54	164.88	-2.11
Faecalibacterium	0.034155	0.28691	4571.0	1610.9	-3.17
Fusicatenibacter	0.041826	0.29278	581.46	166.53	-2.32
Lachnospiraceae UCG 008	0.064987	0.28992	1286.0	745.47	-2.43

Table S2: Linear discrimination analysis effect size (LEfSe) of statistically significant taxa associated with constipation

Name ↑↓	*P* ↑↓	FDR ↑↓	No Constipation ↑↓	Constipation↑↓	LDAScore↑↓
Lachnospiraceae NK4A136	0.033134	0.54395	1.0	724.09	2.56
Dialister	0.034509	0.54395	168.88	1084.2	2.66
Akkermansia	0.063994	0.54395	1.0	948.09	2.68
Baccteroides	0.063994	0.54395	1.0	724.91	2.56

LEfSe is a tool developed by the Huttenhower group to find biomarkers between 2 or more groups using relative abundances

Table S3: Correlation of differentially abundant taxa in functional constipation PD subjects

Spearman's Correlation	Correlation coefficient	T-Stat	*P*	FDR
Lachnospiraceae NK4A136	0.50215	567.55	0.02846	0.50836
Dialister	0.49829	571.95	0.029903	0.50836
Akkermansia	0.43657	642.31	0.061648	0.52401
Bacteroides	0.43657	642.31	0.061648	0.52401