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# Elevated *Lactobacillus salivarius* and genus *Akkermansia* in fecal samples of Taiwanese patients with parkinson's disease and diabetes mellitus

Sheng-Ta Tsai[1,2,3,4], Zi-Lun Lai[5], Ming-Kuei Lu[1,2,3], Marina Walther-Antonio[6,7,8], Yi-Yao Hsu[5], Dy-San Chao[5], Chia-Yang Chen[9,10], Sara Vettleson-Trutza[4,11], John S Kuo[3,9,12,13], Chia-Chun Yang[14,15], Ching-Chu Chen[10,16], Po-Ren Hsueh[2,5], Der-Yang Cho[9,12✉] & Chon-Haw Tsai[1,2,3✉]

Recent advancements in non-invasive collection methods and technological innovations have significantly enhanced the analysis of human gut microbiota, which has become a key approach for understanding complex disease pathogenesis. Epidemiological studies and clinical trials have revealed intriguing connections between Parkinson's disease (PD) and diabetes mellitus (DM). In this study, microbial populations from fecal samples of patients with PD and DM were analyzed. The prospective cohorts included four groups: PD only ($n = 32$), DM only ($n = 170$), and concurrent PD and DM ($n = 10$), matched with healthy controls ($n = 98$) by age and comorbidities. Fecal samples underwent full-length (V1-V9) 16 S rRNA sequencing analysis, and clinical and laboratory variables were collected. The results revealed an increased abundance of *Lactobacillus salivarius* in patients with PD (linear discriminant analysis [LDA] = 2.58, *p* value < 0.05) or DM (LDA = 2.20) compared to healthy controls. Similarly, an elevated abundance of the genus *Akkermansia* was observed in patients with PD (LDA = 4.39) or DM (LDA = 3.92). These findings suggest that gut microbiota alterations, particularly involving *L. salivarius* and *Akkermansia* spp., may play a role in the pathogenesis of PD and DM, warranting further investigation into their significance.

**Keywords** Gut microbiota, 16S rRNA full-length sequencing, *Lactobacillus salivarius*, Akkermansia, Parkinson, Diabetes mellitus

Parkinson's disease (PD) is the most common neurodegenerative movement disorder and its incidence is increasing in the aging population[1]. Current treatment modalities only offer symptomatic relief through

[1]Division of Parkinson's Disease and Movement Disorders, Department of Neurology, China Medical University Hospital, Taichung, Taiwan. [2]School of Medicine, College of Medicine, China Medical University, Taichung, Taiwan. [3]Neuroscience and Brain Disease Center, China Medical University, Taichung, Taiwan. [4]Mayo Clinic Neural Engineering and Precision Surgery Laboratories, Rochester, MN, USA. [5]Department of Laboratory Medicine, China Medical University Hospital, China Medical University, Taichung, Taiwan. [6]Department of Surgery, Mayo Clinic, Rochester, MN, USA. [7]Department of Obstetrics & Gynecology, Mayo Clinic, Rochester, MN, USA. [8]Microbiomics Program, Center for Individualized Medicine, Mayo Clinic, Rochester, MN, USA. [9]Graduate Institute of Biomedical Sciences, College of Medicine, China Medical University, Taichung, Taiwan. [10]School of Chinese Medicine, College of Chinese Medicine, China Medical University, Taichung, Taiwan. [11]Graduate School of Biomedical Sciences Clinical and Translational Science, Mayo Clinic, Rochester, MN, USA. [12]Department of Neurosurgery, China Medical University Hospital, China Medical University, No. 2, Yuh-Der Road, Taichung 404327, Taiwan. [13]Department of Neurosurgery, Dell Medical School, The University of Texas at Austin, Austin, TX, USA. [14]Institute of Epidemiology and Preventive Medicine, College of Public Health, National Taiwan University, Taipei, Taiwan. [15]Department of General Psychiatry, Taoyuan Psychiatric Center, Taoyuan, Taiwan. [16]Division of Endocrinology and Metabolism, Department of Internal Medicine, China Medical University Hospital, Taichung, Taiwan. ✉email: d5057@mail.cmuh.org.tw; windymovement@gmail.com

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administration of dopaminergic agents or deep brain stimulation surgery[2]. In recent decades, scientists have been trying to develop disease-modifying agents, including gut microbiota interventions, based on the gut-brain axis theory for PD pathogenesis[3]. Epidemiological studies revealed an increased incidence of PD among patients with type 2 diabetes mellitus (DM)[4]. Some researchers also consider DM as a potential risk factor for PD[5,6], with evidence suggesting it may increase the risk by 1.85 fold[7]. Impairments in systemic and regional glucose metabolism are observed in PD patients throughout the course of disease[8]. Moreover, both diseases may be considered abnormal amyloid aggregate diseases[9]. In PD patients, misfolded α-synuclein accumulates in the brain. In DM patients, islet amyloid polypeptide accumulates in pancreatic cells. Both types of pathologic amyloid accumulation cause cellular and organ dysfunction and manifest as PD and DM diseases.

Meissner et al.. published striking results of a phase 2, double-blind, randomized, placebo-controlled trial[10]. They found that participants with early PD who received lixisenatide therapy experienced less progressive motor dysfunction than the placebo control group at 12 months. Lixisenatide is an anti-diabetic agent that lowers blood glucose levels by activating endogenous incretin glucagon-like peptide 1 (GLP-1) through GLP-1 receptor agonist activity[11]. Glucagon-like peptide-1 receptor (GLP-1R) agonists have recently garnered significant attention for their remarkable anti-obesity effects[12]. Furthermore, GLP-1R agonists can cross the blood-brain barrier and may have beneficial effects on the brain[13]. Preclinical studies have demonstrated their neuroprotective effects in animal models of PD, by restoring dopamine levels, inhibiting dopaminergic loss, and attenuating neuronal degeneration[14].

In this study, we conducted a prospective clinical cohort study to enroll patients with PD and type 2 DM to test the hypothesis that patients with concurrent PD and DM share similar features of gut microbiota dysbiosis and disease pathogenesis.

## Materials and methods
### Study approval
Procedures involving participant sampling and experiments were reviewed and approved by the Institutional Review Board of China Medical University Hospital (CMUH111-REC1-170 for healthy control [HC] subjects, CMUH111-REC2-209 for PD participants, and CMUH111-REC3-204 for DM patients), in accordance with ethical standards for research involving human subjects. All participants signed the informed consent before being enrolled in the study.

### Patient enrollment
We conducted a prospective clinical cohort microbiota study at China Medical University Hospital (CMUH), a major academic healthcare center in Taichung, Taiwan. Neurologists specializing in movement disorders enrolled 40 patients with PD, based on the United Kingdom Parkinson's Disease Society Brain Bank clinical diagnostic criteria[15]. Detailed clinical data, including age, sex, duration of PD, Hoehn and Yahr stage, off-state Unified Parkinson's Disease Rating Scale (UPDRS, recorded by video and analyzed by a nurse specialized in movement disorders), probiotics use and comorbidities, were collected. Additionally, an endocrinologist recruited a prospective cohort of patients with type 2 DM at the same time. A total of 172 participants with DM were enrolled through the endocrinology outpatient clinic. We excluded participants who were younger than 20 or older than 80 years, had end-stage renal disease (estimated glomerular filtration rate < 15 mL/min/1.73 m²), or had a history of hospitalization for heart failure, acute kidney injury, or renal replacement therapy within the 12 weeks prior to enrollment. For DM patients, we also excluded those who had used laxatives, prebiotics, probiotics, or antibiotics within the 8 weeks preceding stool sample collection. Ninety-eight age and sex-matched healthy control subjects were recruited from individuals visiting our hospital for health examinations. We defined the healthy controls as adults aged 18 and above, with no history of chronic diseases (such as PD, DM, hypertension) and no recent use of prescription medications, especially antibiotics. Among patients with concurrent diagnoses of PD and DM, eight were from the PD cohort and two were from the DM cohort, with diagnoses confirmed by both a neurologist and an endocrinologist. Due to the exploratory nature of our study, no statistical methods were used to pre-determine the sample sizes.

### Stool samples collection
The fecal sample collection kit was provided by the Department of Laboratory Medicine at China Medical University Hospital (CMUH). This sterile kit is specifically designed for microbiome preservation. Participants collected their fecal samples at home and returned them to the hospital at room temperature within 48 h. The sampling procedure has been approved by the Ministry of Health and Welfare, Taiwan, under the Laboratory Developed Tests (LDTs) certification, with the approval number 2022LDT0023. Upon receipt, samples were immediately stored at -80 °C until further processing.

### Fecal DNA extraction
Fecal DNA was extracted using the QIAamp PowerFecal Pro DNA Kit (QIAGEN N.V., Hilden, Germany) along with the QIAcube HT (QIAGEN N.V.), an automated nucleic acid extraction system that reduces the risk of contamination. Genomic DNA was isolated from the fecal samples, and the concentration was measured using the Qubit® 3.0 Fluorometer (Qubit™ dsDNA HS Assay Kit, Invitrogen, Life Technologies, Carlsbad, CA, USA). Samples were stored at -80 °C until library construction. Prior to using the commercial kit, a positive control test was performed using the 20 Strain Even Mix Genomic Material (MSA-1002™, American Type Culture Collection, Manassas, Virginia, USA), with an expected concentration range of 2.69 ng/μL to 4.48 ng/μL (https ://www.atcc.org/products/msa-1002). In our laboratory, aseptic water was used as a negative control (Table S1). Effective results were defined as having at least 8,000 to 10,000 reads per stool sample.

### 16s rRNA full-length amplicon production

The 16s rRNA gene was amplified for PacBio (Pacific Biosciences, Menlo Park, CA, USA) barcoding using the primers 27F (5'-AGRGTTYGATYMTGGCTCAG-3') and 1492R (5'-RGYTACCTTGTTACGACTT-3'). The PCR reaction was carried out in a total volume of 25 μL, using KAPA HiFi HotStart 2X ReadyMix PCR Reagent (Kapa Biosystems, Woburn, MA, USA), 0.375 mM of each primer, and 500–1000 ng of nasal sample DNA as the template. The thermal cycling conditions were as follows: initial denaturation at 95 °C for 3 min, followed by 25 cycles of denaturation at 95 °C for 30 s, annealing at 57 °C for 30 s, and extension at 72 °C for 1 min. A final extension was performed at 72 °C for 1 min.

### Quantification, mixing, and purification of PCR products

PCR products were first evaluated *via* 0.8% agarose gel electrophoresis and then quantified using the Qubit® 3.0 Fluorometer (Qubit™ dsDNA HS Assay Kit already appeared). The quantified PCR products were purified using an equal volume of AMPure PB beads (PacBio, Menlo Park, CA, USA).

### 16s rRNA full-length amplicon library construction and sequencing

Purified PCR amplicons (500 ng) were used as templates to construct the SMRTbell library, following the protocol provided in the SMRTbell Express Template Prep Kit 2.0 (PacBio). After end-repair, adapters were ligated to the ends of the sequences. SMRT sequencing was then carried out on the PacBio Sequel® IIe system using the SMRT 8 M Cell v3 (PacBio). Primary filtering analysis was performed on the Sequel system, while secondary analysis followed the standard procedures in SMRT Link 9.0.

### Analysis pipeline

Full-length 16 S rRNA gene analysis was performed using pb-16s-nf (version 0.7, Pacific Biosciences of California, Inc.). The sequenced reads were processed using the quantitative insights into microbial ecology (QIIME 2, ver: 2023.2[16] Taxonomy was then assigned at 99% similarity based on the SILVA taxonomy and reference database (SILVA_138SSURef NR99 full-length[17,18].) and a rooted phylogenetic tree built using the "align-to-treemafft-fasttree" pipeline from QIIME 2[19]. For validation, the dataset was additionally re-analyzed with the most recent QIIME 2 version 2025.7, and the results were consistent with those obtained using version 2023.2. Outputs from the re-analysis are provided in the Supplementary Figures.

### Sequencing outcome

After quality control and processing, we obtained a total of 11,491,321 sequence reads from 310 samples, with an average of 37,069 ± 31,716 reads per sample. The resulting data were visualized using QIIME 2 software.

### α-Diversity and β-diversity analysis

We utilized QIIME2 software to perform both α-diversity (within-sample) and β-diversity (between-sample) analyses. For α-diversity, we calculated various metrics, including observed features, Shannon diversity, evenness, and Faith's phylogenetic diversity. For β-diversity, we employed metrics such as Bray-Curtis, weighted and unweighted UniFrac, and Jaccard distance, along with emperor plots for visualization. Additionally, we applied permutational multivariate analysis of variance (PERMANOVA), a distance-based analysis of variance method based on permutation, to assess associations between groups[20]. Metrics with statistically significant *p*-values were reported following rarefaction.

### Differential abundance analysis

We applied the non-parametric Kruskal-Wallis (KW) sum-rank test[21] to identify features with significantly different abundances across the class of interest. Biological consistency was then assessed using pairwise comparisons among subclasses via the unpaired Wilcoxon rank-sum test[22]. Finally, linear discriminant analysis (LDA) effect size (LEfSe) was used to estimate the effect size of each differentially abundant feature. This approach supports high-dimensional class comparisons, particularly in metagenomic analyses[23]. Class comparison methods typically identify biomarkers as features that deviate from the null hypothesis of no difference between classes. Additionally, we detected a subset of features with abundance patterns consistent with an algorithmically encoded biological hypothesis and estimated the magnitude of significant variations.

### Blood sample analysis

At the time of stool collection, peripheral venous blood samples (20 mL) were concurrently obtained from each participant for biochemical and metabolomic profiling. Blood draws were performed in the morning, between 05:00 and 11:00 h, following an overnight fast of at least 8 h to minimize diurnal and dietary variability in circulating metabolites. Samples were collected in serum-separating and ethylenediamine-tetraacetic acid (EDTA)-containing tubes, promptly transported to the central laboratory of China Medical University Hospital, and processed within 1 h of collection. Serum and plasma were separated by centrifugation at 3,000 × g for 10 min at 4 °C, aliquoted into cryovials, and immediately stored at −80 °C to prevent metabolite degradation. Biochemical parameters, including serum creatinine and glycated hemoglobin (HbA1c), were measured using standard enzymatic and immunoassay methods in the hospital's clinical laboratory, which is accredited by both the Taiwan Accreditation Foundation and the College of American Pathologists (CAP). All analyses were performed strictly in accordance with standardized operating procedures for clinical diagnostics. Quality control samples and internal standards were incorporated to ensure analytical reproducibility, inter-assay consistency, and accuracy across all measurement batches.

## Statistical analysis

We used MATLAB (R2024a, Natick, Massachusetts), Python (v3.11.8) with NumPy (v1.24.0), pandas (v1.5.3), and SciPy (v1.14.1) to calculate $p$-values and confidence intervals. Independence, normality, and homogeneity of variances were assessed for each variable to select the appropriate statistical test. For example, for age across four groups, the Shapiro–Wilk test indicated non-normality in two groups, and Levene's test showed unequal variances; therefore, the non-parametric Kruskal–Wallis test was applied.

## Results

### Metabolic and clinical differences between PD, DM, and concurrent PD and DM

We categorized the enrolled patients into four study groups: HC, PD only, DM only, and concurrent PD and DM (PD + DM), with their characteristics (Table 1). The median age of participants was comparable across the groups ($p = 0.151$), with the highest median age observed in the PD + DM group (69.5 years old). The proportion of female participants varied among groups, with HC having the highest percentage (50%) and PD + DM the lowest (10%), although the difference did not reach statistical significance ($p = 0.062$).

Significant differences were observed in body mass index (BMI), with DM only patients having the highest BMI (median = 26.3 kg/m²), while PD only and PD + DM groups had lower BMI values (23.4 and 23.6 kg/m², respectively) ($p < 0.001$). Similarly, HbA1C levels were significantly higher in the DM only (7.0%) and PD + DM (6.8%) groups than in the PD only group (5.8%) ($p < 0.001$). Regarding Parkinson's disease severity, UPDRS scores were comparable between PD only (median = 34) and PD + DM groups (median = 51) ($p = 0.325$). Similarly, Hoehn & Yahr staging, PD duration, and L-dopa equivalent daily dose (LEDD) did not differ significantly between these two groups ($p = 0.318$, 0.286, and 0.268, respectively). Taking together, significant metabolic differences were observed between the groups, particularly in BMI, HbA1C, and serum creatinine levels, highlighting the metabolic impact of diabetes.

### PCoA analysis reveals distinct compositional patterns among study groups

We utilized Jaccard Emperor principal coordinates analysis (PCoA) plots to illustrate the differences in scattering among the four groups (Fig. 1). The different scatters are represented by red (HC), green (DM only), blue (PD only), and purple (PD + DM) dots, with $n = 310$ samples, with statistical significance observed ($p = 0.001$). The PCoA1 accounts for 1.25% of the variance, while the PCoA2 explains 0.9% of the variation. The scatter plots reveal a distinct clustering pattern among groups, with some overlap observed between PD only and PD + DM groups, whereas HC and DM only groups appear to have more separation. These clustering patterns suggest that DM only patients exhibit more distinct compositional differences compared to the other groups, while PD only and PD + DM groups share more similarities. The dispersion of PD + DM subjects within the plot suggests that these individuals may have overlapping characteristics from both conditions.

We presented the results of α-diversity, including evenness, Faith's phylogenetic diversity, observed features, and Shannon diversity, in Supplementary Figs. 1–4. For β-diversity, we employed multiple metrics, including Bray–Curtis, Jaccard distance, unweighted UniFrac, and weighted UniFrac, and visualized the results using Emperor plots. The β-diversity analyses, along with the corresponding ANOVA tables, are provided in Supplementary Figs. 5–12.

| | HC | PD only | DM only | PD + DM | |
|---|---|---|---|---|---|
| **N** | 98 | 32 | 170 | 10 | ***p*-value [a]** |
| Age | 65 [61-70.8] | 67.5 [59.8–70.3] | 67 [60–72] | 69.5 [67–76] | 0.151 |
| Female (n, %) | 49 (50%) | 12 (37.5%) | 68 (40%) | 1 (10%) | 0.062 |
| BMI (kg/m²) | 23.8 [22-25.4] | 23.4 [22–25.2] | 26.3 [23.3–29.6] | 23.6 [23.3–25.4] | **< 0.001** *** |
| HbA1C (%) | NA[d] | 5.8 [5.5–6.0] | 7.0 [6.6–7.5] | 6.8 [6.4–6.9] | **< 0.001** *** |
| Serum creatinine (mg/dL) | NA | 0.9 [0.7–1.4] | 1.0 [0.8–1.4] | 1.0 [0.8–1.1] | 0.061 |
| UPDRS [b] | NA | 34 [27–57] | NA | 51 [44–60] | 0.325 |
| H&Y scale | NA | 2 [1.5–2.5] | NA | 2.5 [2–3] | 0.318 |
| PD duration [c] (year) | NA | 7.5 [4.8–10.2] | NA | 5.5 [4.0–7.0] | 0.286 |
| LEDD (mg) | NA | 650 [444–1039] | NA | 575 [430–723] | 0.268 |

**Table 1.** Patients' characteristics of the four groups. HC, healthy controls; PD, Parkinson's disease; DM, diabetes mellitus; BMI, body mass index; SD, standard deviation; UPDRS, Unified Parkinson's Disease Rating Scale; H&Y, Hoehn and Yahr; LEDD, L-dopa equivalent daily dose. [a]The above variables are presented as median [Q1–Q3], where Q1 and Q3 indicate the 25th and 75th percentiles, respectively. *P*-values were calculated by MATLAB (R2024a), Python (v3.11.8) using NumPy (v1.24.0), pandas (v1.5.3), and SciPy (v1.14.1). The Kruskal–Wallis test (age, BMI, HbA1C, creatinine) and Chi-squared test (gender) were used for comparisons involving more than two groups, while the Mann–Whitney U test was applied for two-group comparisons. Although disease duration and LEDD met normality assumptions, the Mann–Whitney U test was used for consistency given the small sample size, yielding unchanged results. *$p < 0.05$, ***$p < 0.001$. [b]The UPDRS was measured during off state (drug off, deep brain stimulation off). [c]Defined as the duration since classic motor symptoms (bradykinesia, rigidity, or tremor) onset. [d]NA: not applicable/Not collected.
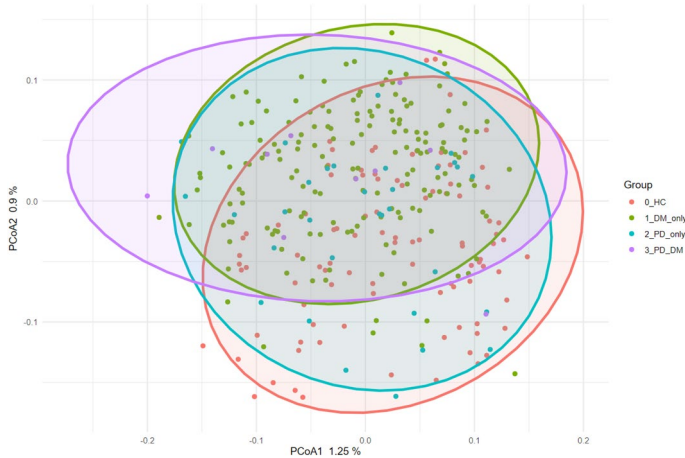
**Fig. 1**. ß-diversity presented by Jaccard Emperor PCoA plots of gut microbiota in four groups. Jaccard Emperor PCoA plots show the maximum dissimilarity between samples from the four groups. Healthy controls are colored red, patients with PD only are colored blue, patients with DM only are colored green, and patients with concurrent PD and DM are colored purple, $n = 310$, $p$-value = 0.001. PD, Parkinson's disease; DM, diabetes mellitus; HC, healthy controls; PCoA, principal coordinates analysis.

| Group 1 | Group 2 | Sample size | Permutations | pseudo-F | *p*-value | q-value |
|---------|---------|-------------|--------------|----------|-----------|---------|
| HC | DM only | 268 (98 + 170) | 999 | 1.417789 | **0.001***** | **0.003**** |
| HC | PD only | 130 (98 + 32) | 999 | 1.101979 | **0.002**** | **0.004**** |
| HC | PD + DM | 108 (98 + 10) | 999 | 1.069052 | **0.021*** | **0.0315*** |
| DM only | PD only | 202 (170 + 32) | 999 | 1.107888 | **0.001***** | **0.003**** |
| DM only | PD + DM | 180 (170 + 10) | 999 | 0.98397 | 0.759 | 0.759 |
| PD only | PD + DM | 42 (32 + 10) | 999 | 0.990276 | 0.629 | 0.7548 |

**Table 2**. PERMANOVA power Estimation of differences between each pair of groups. HC, healthy controls; DM, diabetes mellitus; PD, Parkinson's disease. *$p$ or q < 0.05, **$p$ or q < 0.01, ***$p$ or q < 0.001.

### PERMANOVA analysis reveals group differences between PD and DM

The power estimation for individual groups performed by PERMANOVA (Jaccard distance) is presented in Table 2. The HC vs. DM-only comparison yielded a pseudo-F of 1.42 ($p = 0.001$), and the PD-only vs. DM-only comparison yielded a pseudo-F of 1.11 ($p = 0.001$), both indicating significant differences. Significant differences were also observed between HC and PD-only ($p = 0.002$) and between HC and PD + DM ($p = 0.021$), suggesting that both conditions contribute to the observed variations. However, the DM only vs. PD + DM comparison did not yield significant results ($p = 0.759$), indicating similarity between these two groups. Likewise, no significant difference was found between PD only and PD + DM groups ($p = 0.755$), suggesting that the presence of DM in individuals with PD may not introduce additional variation detectable through PERMANOVA.

### Increased abundance of specific gut microbiota in PD and DM: a cladogram analysis

Taxonomic differences in gut microbiota among the four groups, as illustrated in the cladogram, revealed an increased abundance of several bacterial families, including *Lactobacillaceae* and *Akkermansiaceae*, in patients with concurrent PD and DM (Fig. 2), with LDA scores > 2, indicating statistical significance ($p < 0.05$). Given that findings at the family level can be difficult to interpret, we further examined differences at the genus and species levels. Using LDA scores, we compared bacterial genera and species between PD only vs. HC and DM only vs. HC, with the results detailed in Table 3. To account for potential confounding effects of probiotic use (five users in the PD cohort and two in the concurrent PD and DM cohort), we excluded these patients and repeated the analysis. Notably, both *Lactobacillus salivarius* and the genus *Akkermansia* remained significantly more abundant compared to HC. Additionally, we conducted pairwise comparisons of taxonomic differences in fecal microbiota, including PD only vs. HC (Supplementary Fig. 13), DM only vs. HC (Supplementary Fig. 14), and PD only (excluding probiotic users) vs. HC (Supplementary Fig. 15).

### Discussion

In this prospective cohort clinical study, we compared two groups, PD and DM, and observed an increased abundance of *Lactobacillus salivarius* and the genus *Akkermansia*. To ensure robust results, we carefully reviewed the data and excluded seven participants who were probiotic users (five in the PD group and two in the concurrent PD and DM group). The findings remained consistent after this adjustment.
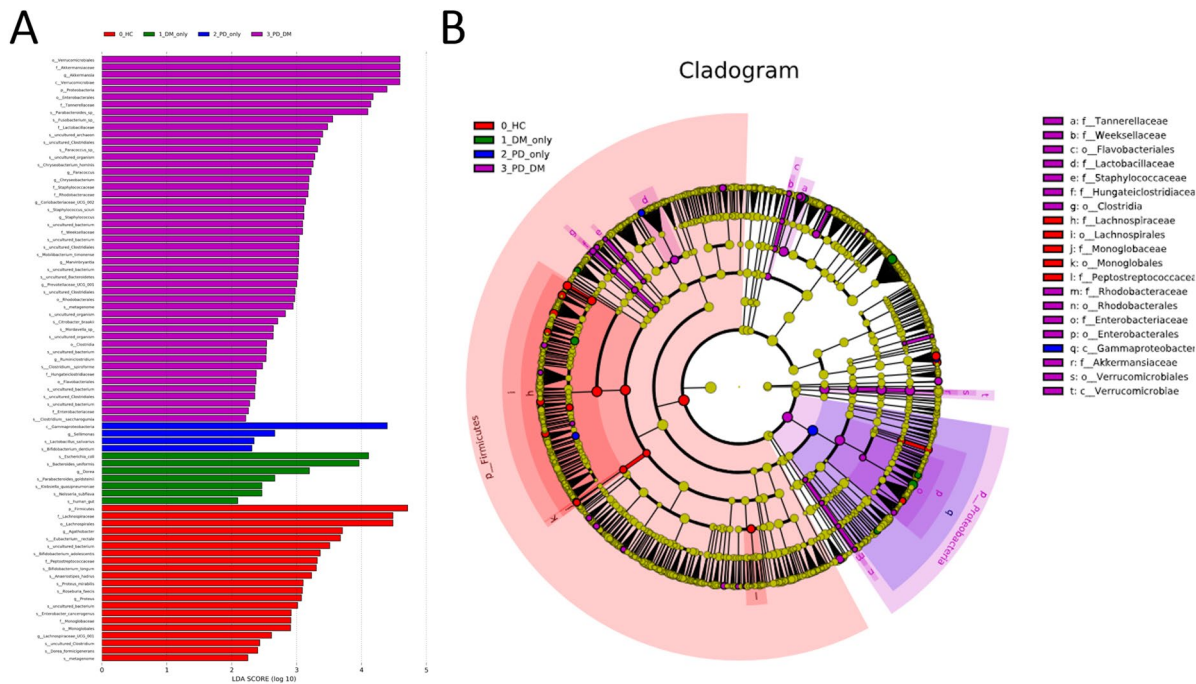
**Fig. 2**. Taxonomic differences of fecal microbiota in four groups. (**A**) Linear discriminant analysis (LDA) effect size (LEfSe) analysis revealed significant bacterial differences in fecal microbiota between the four groups: HC (colored red), DM only (colored green), PD only (colored blue), and concurrent PD and DM (colored purple). LDA scores (log10) > 2 and $p < 0.05$ are shown. (**B**) A cladogram was generated using the LEfSe method, indicating the phylogenetic distribution of fecal microbiota across the four groups. PD, Parkinson's disease; DM, diabetes mellitus; HC, healthy controls.

|  | PD only | DM only | PD + DM |
|---|---|---|---|
| *Lactobacillus salivarius* | 2.58 | 2.20 | 2.31 |
| Genus *Akkermansia* | 4.39 | 3.92 | 4.57 |
| *LDA score after excluding probiotics users* | | | |
| *Lactobacillus salivarius* | 2.59 | 2.20 | 2.33 |
| Genus *Akkermansia* | 4.29 | 3.92 | 4.77 |

**Table 3**. Linear discriminant analysis (LDA) score compared to healthy controls (HC). PD, Parkinson's disease; DM, diabetes mellitus

To the best of our knowledge, no previous studies have directly compared the gut microbiota of two disease cohorts within the same clinical setting. However, numerous investigations have characterized gut microbiome alterations by comparing patients with a single disease to healthy controls. For example, in PD, patients often exhibit a gut microbial profile marked by reduced abundance of short-chain fatty acid (SCFA)-producing genera such as *Butyricicoccus* and *Coprococcus*, alongside an enrichment of pro-inflammatory taxa, including *Akkermansia*[24]. In type 2 diabetes mellitus, findings have been more variable, though a systematic review of 18 studies reported consistent patterns of decreased abundance of *Faecalibacterium prausnitzii*, *Roseburia*, *Dialister*, *Flavonifractor*, *Alistipes*, *Haemophilus*, and *Akkermansia muciniphila*, with increased levels of *Lactobacillus*, *Streptococcus*, *Escherichia*, *Veillonella*, and *Collinsella* in at least two studies. Moreover, *Lactobacillus* abundance was positively correlated with fasting plasma glucose, HbA1c, and/or homeostatic model assessment of insulin resistance in four studies[25].

The genus *Lactobacillus* is estimated to constitute about 6% of the total bacterial cell count in the human duodenum[26] and approximately 0.3% of all bacteria in the colon[27]. *Lactobacillus salivarius* is part of the indigenous microbiota in the human gastrointestinal tract, exhibiting significant genomic and phenotypic diversity[28]. A study demonstrated that when human peripheral blood mononuclear cells were co-cultured with *Lactobacillus*, *L. salivarius* Ls-01 reduced pro-inflammatory cytokines and increased anti-inflammatory cytokines in vitro[29]. Additionally, it does not carry the *TDC* gene (tyrosine decarboxylase), which is known to decrease levodopa bioavailability in PD patients[30].

In a double-blind, randomized controlled study in Denmark, targeting obese adolescents, *L. salivarius* strain Ls-33 intake was associated with an increased ratio of the *Bacteroides-Prevotella-Porphyromonas* group

to *Firmicutes*, suggesting that Ls-33 may modulate the gut microbiota[31]. Conversely, a multicenter randomized controlled study found that probiotic prophylaxis, using a combination of six probiotic strains (including *Lactobacillus acidophilus*, *Lactobacillus casei*, *L. salivarius*, *Lactococcus lactis*, *Bifidobacterium bifidum*, and *Bifidobacterium lactis*) in patients with predicted severe acute pancreatitis, was associated with increased mortality risk[32]. This finding underscores that gut microbial interventions can have both positive and negative effects[33].

Current systemic review and meta-analysis have indicated that *lactobacillus*-based probiotics can alleviate constipation symptoms in PD patients[34]. However, current probiotic interventions are not specifically tailored to the PD gut microbiome and may therefore pose more risks than benefits, given the lack of well-designed trials assessing their efficacy and safety in PD[33].

Regarding diabetes, a cohort study involving Swedish women reported a positive correlation between *Lactobacillus* species and both fasting glucose and HbA1c levels (glycosylated hemoglobin)[35]. In a randomized controlled trial conducted in Saudi Arabia, six months of multi-strain probiotic supplementation (including *Lactobacillus salivarius*) as monotherapy in type 2 DM patients led to a significant decrease in homeostasis model assessment of insulin resistance, though no significant changes were observed in glucose, insulin, or C-peptide levels[36]. Although basic studies suggest a role for *Lactobacillus salivarius* in regulating sugar absorption in gut epithelial cells[37], the impact on patients with DM remains unclear.

Genus *Akkermansia* is a Gram-negative, oval-shaped, non-motile, and oxygen-tolerant anaerobic bacterium with a relatively short history of study, due to its recent isolation[38]. The genus *Akkermansia* is known for degrading the intestinal mucin layer[39], which may increase intestinal permeability—a phenomenon reported in PD[40]. A recent meta-analysis of the human gut microbiota showed consistent alterations, with enrichment of the genera *Lactobacillus*, *Akkermansia*, and *Bifidobacterium*, and a decrease in bacteria belonging to the *Lachnospiraceae* family and the *Faecalibacterium* genus, both of which are important short-chain fatty acid producers[41]. After adjusting for confounding factors such as body mass index, constipation, sex, age, and catechol-O-methyl transferase inhibitor use, increases in the genera *Akkermansia* and *Catabacter*, as well as in the *Akkermansiaceae* family, were observed across a cohort from five countries[42]. In contrast, genera such as *Roseburia*, *Faecalibacterium*, and the *Lachnospiraceae* ND3007 group were found to be reduced in individuals with PD[42]. Additionally, an increase in *Akkermansia* was associated with accelerated disease progression in early PD over two years, as measured by UPDRS-III motor symptom scores[43]. During the progression of PD, *Akkermansia* abundance tends to increase[43]. Supported by research data, the study group suggested that this increase in *Akkermansia* is likely a driver of PD progression rather than a consequence of the disease itself[43].

Interestingly, *Akkermansia* is thought to have protective effects DM and obesity in humans[44,45]. However, while *Akkermansia*-mediated improvements in glucose metabolism are beneficial, it is unclear how this may contribute to PD prevention[43]. One possible explanation is the altered intestinal environment in PD patients. For example, the expression of the tight junction protein occludin is decreased in the intestines of PD patients, leading to increased intestinal permeability through structural changes[46].

Among the *Akkermansia* genus, *Akkermansia muciniphila* is the most well-studied species[47]. In DM, *Akkermansia muciniphila* enhances insulin sensitivity and glucose tolerance through its anti-inflammatory effects[48]. In a randomized, double-blind, placebo-controlled pilot study involving overweight and obese insulin-resistant volunteers, pasteurized *Akkermansia muciniphila* improved insulin sensitivity by 28.62% ± 7.02% ($P$ = 0.002), reduced insulinemia by 34.08% ± 7.12% ($P$ = 0.006), and lowered plasma total cholesterol by 8.68% ± 2.38% ($P$ = 0.02)[49].

The abundance of *Akkermansia muciniphila* is also linked to metformin use[50]. In mouse studies, increased *Akkermansia* levels have been suggested to contribute to the antidiabetic effects of metformin[51,52]. In a Colombian cohort of 28 diabetic and 84 healthy control participants, diabetic participants taking metformin exhibited higher relative abundances of *Akkermansia muciniphila* compared to those without diabetes[53]. In our study population with DM, 139 out of 170 participants (81.8%) were on regular metformin therapy, suggesting that the elevated abundance of genus *Akkermansia* in these individuals is largely attributable to metformin use.

PD has been positively associated with inflammatory bowel diseases, a group of disorders involving T-cell-driven inflammation of the gut mucosa, which strongly depends on the composition of the gut microbiota[54]. T-cell-driven inflammation that mediates dopaminergic neurodegeneration in PD is possibly triggered in the gut mucosa[54]. Interestingly, a cohort study in Spain checked patients' blood cells and observed an increase in type 1 T regulatory (Tr1) cells and CD4 + IL10 + lymphocyte levels in DM[55]. This highlights potential immune dysfunction in DM patients and may also participate in PD pathogenesis.

It is intriguing to note that the functional perturbation of the insulin signaling pathway within the brain is also hypothesized to contribute to PD pathogenesis[56]. Impairment of insulin signaling may increase the activation of glycogen synthase kinase 3β and lead to α-synuclein accumulation (the main pathology of PD) and exacerbate autophagy[56]. The results of these studies may provide a possible linkage between PD and DM pathogenesis.

In addition, we observed sex differences in microbiota composition[57] in this study (Table S2). In the PD only group and the concurrent PD and DM group, the increases in *L. salivarius* and the genus *Akkermansia* were primarily contributed by male patients. In contrast, in the DM only group, the increase was driven by female patients. However, based on our data, the exact mechanism underlying this gender-dependent microbiota composition remains unclear.

Our study has several limitations. First, the sample size of patients with concurrent PD and DM is relatively small, which limits the statistical power and the strength of interpretation for that subgroup. Second, in the DM only group, a slightly higher creatinine level is observed, without significant statistical difference (*p*-value = 0.061), indicating that some patients meet the criteria for diabetic kidney disease (DKD). DKD is associated with diverse, compositional, and functional alterations of the gut microbiota[58]. In the future, with additional advances in PD biomarkers, such as the use of seed amplification assay to detect misfolded α-synuclein[59], these

serum/cerebrospinal fluid markers may be added to gut microbiota analysis for more potential insights. Future studies will further elucidate the mechanisms linking the gut-brain axis, specifically if gut microbiota (especially *L. salivarius* and genus *Akkermansia*) are implicated in the pathogenesis of PD and DM and potentially suggest new therapeutic strategies.

In conclusion, our prospective cohort study showed an elevated abundance of *L. salivarius* and the genus *Akkermansia* in patients with PD and DM. Further research is needed to elucidate the underlying mechanisms of these associations with disease pathogenesis.

## Data availability

The datasets generated and/or analysed during the current study are available in the NCBI Sequence Read Archive under BioProject accession number PRJNA1244045. These data are publicly available. A reviewer link for anonymous access during peer review is available at: https://dataview.ncbi.nlm.nih.gov/object/PRJNA1244045?reviewer=7l3h31aona17308phd3aa0sjbb. Any additional information required to reanalyze the data reported in this paper is available from the corresponding author upon reasonable request.

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## Author contributions

Conceptualization: STT, CHT, CCC, DYC; Data Curation and formal analysis: YYH, ZLL, DSC, CYC, CCY; Funding acquisition: PRH, JSK, DYC; Methodology: YYH, ZLL, DSC, MWA, CCY; Project administration: STT, MKL; Supervision: PRH, JSK, DYC; Writing- original draft: STT, CHT; Writing- review and editing: SVT, MWA, CCC, JSK.

## Declarations

## Competing interests

The authors declare no competing interests.

## Additional information

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**Correspondence** and requests for materials should be addressed to D.-Y.C. or C.-H.T.