Original Article

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# Intestinal microbiota distribution and changes in different stages of Parkinson's disease: A meta-analysis, bioinformatics analysis and *in vivo* simulation

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**SUMMARY**: Parkinson's disease (PD) is a progressive disease that requires effective staging management. The role of intestinal microbiota in PD has been studied, but its changes at different stages are not clear. In this study, meta-analysis, bioinformatics analysis and *in vivo* simulation were used to explore the intestinal microbiota distribution of PD patients and models at different stages. Two PD models at different stages were established in rotenone-treated rats and MPTP-induced mice. The differences in the intestinal microbiota among the different stages of PD patients or models were compared and analyzed. There were significant differences between PD patients and controls, including *Actinobacteriota*, *Deltaproteobacteria*, *Clostridiales*, *Lachnospiraceae*, *Parabacteroides*, *etc*. Through bioinformatics analysis, we revealed significant differences between PD patients at different stages and controls, including *Actinobacteriota*, *Methanobacteria*, *Erysipelotrichales*, *Prevotellaceae*, *Parabacteroides*, *Parabacteroides gordonii*, *etc*. Through meta-analysis, we found that *Actinobacteriota* and *Erysipelotrichaceae* had significantly increased in the chronic MPTP model, while *Prevotellaceae* had significantly decreased. PD rats and mice presented significant damage to motor function, coordination, autonomous activity ability and gastrointestinal function, and the damage in the late group was greater than that in the early group. There were significant differences in intestinal microbiota between PD patients or models at different stages and the control groups. In the early stage, the dominant microbiota are *Akkermansia*, *Alistipes*, *Anaerotruncus*, *Bilophila*, *Rikenellaceae*, *Verrucomicrobia* and *Verrucomicrobiae*, whereas in the late stage, the dominant microbiota are *Actinobacteriota* and *Erysipelotrichaceae*. These differences can lay a foundation for subsequent research on the treatment and mechanism of PD at different stages.

***Keywords***: Parkinson's disease (PD), intestinal microbiota, meta-analysis, bioinformatics analysis, different stages, Staging simulation of PD

## 1. Introduction

Parkinson's disease (PD) is a common neurodegenerative disease. The symptoms and disease burden of PD patients gradually increase, which requires timely and effective stage management. Margaret Hoehn and Melvin Yahr developed the first PD scale, called the Hoehn-Yahr (HY) scale, which divides PD into five stages (*1*). Researchers have proposed a modified HY scale based on this, adding 0.5 grades to the original scale (*2*). Clinically, PD patients with HY scores between 1.0-2.5 are in the early stage, while PD patients with HY scores between 3-5 are in the middle to late stages of PD (*3*). However, the motor function scale is generally used for the diagnosis of PD in different stages, and more objective biomarkers are lacking. The identification of biomarkers for PD patients and models at different stages has positive significance for the staging treatment and diagnosis of PD.

In recent years, many studies have clarified the role of the gut microbiome in communication between the gut and the brain, called the microbiota-gut-brain axis (*4*). Changes in the balance of gut microbes are closely associated with the progression of neurodegenerative diseases such as PD (*5,6*). As longitudinal studies have increased, some studies on gut microbiota distribution in PD patients or models have reported conflicting results (*7*). There is no consensus on which intestinal microbiota is closely related to PD patients or models, and there is a lack of systematic studies on the distribution of intestinal microbiota in different stages of PD patients or models. Clarifying these has positive significance for the treatment of different stages of PD based on

intestinal microbiota (*8*). In this study, meta-analysis, bioinformatics analysis and *in vivo* simulation were combined to explore the distribution of intestinal microbiota in different stages of PD patients or models, providing a basis for subsequent studies on the staging treatment and diagnosis of PD based on intestinal microbiota.

## 2. Materials and Methods

### 2.1. Search strategy and data extraction

We searched 7 databases including PubMed, Web of Science, the Cochrane Library, Embase, CNKI, Wanfang and VIP, from the establishment of the database to April 8, 2023. Subject words combined with free words were used for retrieval. The subject words were Parkinson Disease and Gastrointestinal Microbiome. The free word consists of their synonyms. First, we removed duplicate studies and then preliminarily screened the literature according to the title and abstract. Secondary screening was performed according to the inclusion and exclusion criteria. Basic information and the relative abundance of gut microbiota at different taxonomic levels were extracted from the included studies. The included studies with PD patients were assessed by two researchers using the Newcastle-Ottawa Scale (NOS), and studies with PD models were assessed by two researchers using the Systematic Review Centre for Laboratory Animal Experimentation (SYRCLE), with the average of the two researchers' scores calculated as NOS or SYRCLE scores. As for the staging definition of PD model, the 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP) model with staging was selected. The acute model was intraperitoneal injection of MPTP 4 times a day, the subchronic model was intraperitoneal injection of MPTP for 5 consecutive days, and the chronic model was intraperitoneal injection of MPTP for 5 weeks, twice a week (*9*). The rest of the search strategies and evaluation scales can be found in Supplemental data 1 (*https://www.biosciencetrends.com/action/getSupplementalData.php?ID=234*).

### 2.2. Inclusion and exclusion criteria

We used the following inclusion criteria: Relevant results were published in Chinese or English. For the study of PD patients, case-control trials were selected, and the study objects were PD patients with definite diagnosis or healthy controls. For PD models, the research objects were models with different stages and controls. There was no statistical significance in the general data between the PD patient group or model group and the control group, which was comparable. The study focused on the distribution of the intestinal microbiota, which describes the relative abundance of at least one intestinal microbe. Sufficient data can be obtained for meta-analysis.

We used the following exclusion criteria: The subjects were PD models without staging; The study was a review, meta-analysis or comment; The research data were incomplete or too few to be applied (the number of articles including gut microbes was at least ≥ 3); It was not clear whether products such as probiotics that affect the distribution of the intestinal microbiota were taken within three months; The results could not be converted into data, using the form of results such as images, fan charts that could not be converted into data.

### 2.3. Meta-analysis

Stata 17.0 was used for meta-analysis. A combination of a random effects model and fixed effects model was used to test the data. The standard mean difference (*SMD*) was used as the effect index, and the 95% confidence interval (*CI*) was calculated. We generated forest and funnel plots and performed sensitivity analysis and Egger bias analysis.

### 2.4. Bioinformatic analysis

We identified two studies in the National Center for Biotechnology Information (NCBI, https://www.ncbi.nlm.nih.gov/) database, study numbers PRJEB30615 and PRJNA588035. These were the only two studies that identified each patient's HY score and uploaded the original sequence of the gut microbiota. Quantitative Insights Into Microbial Ecology version 2 (QIIME2) software (Version QiiME2-202202) was used to analyze the raw data of intestinal microbiota. The feature table and representative table of the intestinal microbiota were obtained. These were used to construct evolutionary trees for species composition analysis and differential abundance analysis.

### 2.5. Animals and Study Design

Specific Pathogen Free (SPF) C57BL/6 male mice and SPF male Sprague Dawley (SD) rats were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. The experimental animal license number is SCXK (Beijing) 2021–0006. All the animals were kept at the SPF Animal Laboratory Center of Beijing University of Chinese Medicine at ambient temperature (22 ± 2), relative humidity (50 ± 10) %, and a light and dark cycle of 12 h. All procedures conformed to the requirements of international ethics for laboratory animals, and the Animal Ethics Review Committee of Beijing University of Chinese Medicine approved these experiments (BUCM-2023090604-3121).

After 1 week of adaptation, the rats were randomly divided into control group, early PD group and late PD group ($n$ = 10) according to random number table classification. All rats in the PD group were injected subcutaneously with rotenone sunflower oil solvent

(1.5 mg/kg) once a day in the neck or back of the early group for 7 days (*10,11*), and in the late group for 28 days (*12*). The rats in the control group were injected subcutaneously with 1 mL/kg sunflower oil in the neck or back for 28 days. After 5 days of adaptation, the mice were randomly divided into control group, early PD group and late PD group ($n$=10) according to random number table classification. Mice in all PD groups were intraperitoneally injected with MPTP[25 mg/(kg·3.5 d)], and the modeling duration was 7 days in the early group and 35 days in the late group. Mice in the control group were intraperitoneally injected with 10 mL/(kg·3.5 d) normal saline for 35 days (*13*). The participants were equipped with protective clothing, gas mask and goggles to prepare MPTP hydrochloride saline solution in the fume hood. After each experiment, the related equipment and liquid were treated to be harmless with 1% disinfectant 84.

2.6. Behavioral and gastrointestinal function tests

We used the pole test, inclined plate test and open field to investigate the behavioral function of rats and mice, and fecal water content to test the gastrointestinal function of rats and mice (*14-18*). The rat or mouse was placed on the top of a pole, and the time from placement to landing of the hind legs was recorded three times a week. The rat or mouse was placed vertically on the rubber pad of the inclined board. If the rat could stay on the inclined plate for 5 s and the mouse could stay on the inclined plate for 15 s, the angle of the inclined plate was increased until the stay time was less than 5 s or 15 s. The rats or mice were placed in an open field of their respective sizes. The bottom of the open field was divided into 16 (4×4) squares, the middle 4 (2×2) squares constituted the center area of the open field, and the remaining squares constituted the edge area. Video tracking technology was used to record the moving distance, moving speed and resting time of the rats in different areas of the open field within 10 min, and the above indices were recorded within 5 min for mice. Fresh feces were collected from each group every week, and the weight at the time of collection was measured as the wet weight of the feces. After drying at 65°C in a vacuum oven for 2 days, the weight was measured again as the dry weight of the feces. Fecal water content = (fecal wet weight - fecal dry weight)/fecal wet weight ×100%.

2.7. Sample collection and tissue pretreatment

After all the behavioral tests, the fresh feces of each group were collected, frozen in liquid nitrogen, and stored at -80 °C for future use. Rats were anesthetized intraperitoneally with 20% urethane and underwent cardiac perfusion. After fixation, the whole brain was quickly separated on ice. The obtained tissues were soaked in paraformaldehyde solution and then used for immunohistochemistry.

2.8. 16S rRNA sequencing analysis

Total genomic DNA was extracted *via* the Cetyltrimethylammonium Bromide (CTAB) method. The diluted genomic DNA was amplified *via* Polymerase Chain Reaction (PCR) in the 16S V3–V4 region (primer sequences CCTAYGGGRBGCASCAG, GGACTACNNGGGTATCTAAT). Quantitative libraries were collected on the Illumina platform for sequencing. Paired-end reads were assigned according to the unique barcode of the sample and were truncated by cutting the barcode and primer sequence. QIIME2 software was used for species annotation and fast multiple sequence alignment, and the Silva 138.1 database was used for species annotation. The data of each sample were homogenized, and a phylogenetic tree was constructed. On this basis, alpha diversity indices were calculated, and beta diversity indices were compared. Linear discriminant analysis Effect Size (LEfSe) was used to reveal the differentiation of community structure, and Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt2, V2.3.0) was used to predict the functional composition and metabolic potential of the microbiome.

2.9. Immunohistochemistry

Tissue sections were embedded in paraffin wax and dewaxed to water. Tissue sections were placed in citric acid antigen repair buffer for antigen repair in the microwave oven. Slices were placed in 3% hydrogen peroxide solution and incubated for 25 min in the dark. Samples were incubated with 3% Bovine Serum Albumin (BSA) for 30 min. Overnight incubation with anti-tyrosine hydroxylase (TH) was then performed at 4°C. Tissue was then incubated with the corresponding secondary antibodies for 50 min in the dark. Diaminobenzidine (DAB) chromogenic agent was added, and the nucleus was counterstained with hematoxylin. The images were observed using a fluorescence microscope (Nikon Eclipse C1, Tokyo, Japan). Image-pro plus 6.0 (Media Cybernetics, Inc., Rockville, MD, USA) was used for mean density analysis. Mean density was obtained from the cumulative optical density value/pixel area of the tissue.

2.10. Statistical analysis

SPSS 25.0 software was used for statistical analysis. Shapiro-Wilk was used for normality test before statistical analysis. Results matching normal distribution were compared between groups using the LSD test of one-way ANOVA. The Kruskal–Wallis test was used to compare species abundance differences and predict their functions. GraphPad Prism 8.0.2 software was used to

draw the correlation histogram and other results.

## 3. Results

### 3.1. Study selection

2540 studies were retrieved. We removed 1063 duplicate and retracted articles and included 32 studies (*19-50*) in PD patients and 17 studies (*51-67*) in PD models for meta-analysis. The screening process is shown in Figure 1. Chinese and English studies in 9 different countries were included. 3356 samples were included, including 1718 PD patients, 1352 healthy controls, 145 PD models, and 141 control groups. All studies were matched for baseline data such as age and sex. NOS and SYRCLE scores of the included studies were all higher than 6.5. The basic information of the research subjects included in the studies is shown in Table 1 and Table 2.

### 3.2. Intestinal microbiota with differential abundance between PD patients and healthy controls

Through meta-analysis, we found that there were generally significant differences between PD patients and healthy controls in 8 phyla of bacteria (*Actinobacteriota*, *Bacteroidetes*, *Verrucomicrobia*, *etc.*), 5 classes of bacteria (*Deltaproteobacteria*, *Methanobacteria*, *Verrucomicrobiae*, *etc.*), 4 orders of bacteria (*Clostridiales*, *Methanobacteriales*, *Verrucomicrobiales*, *etc.*), 25 families of bacteria (*Fusobacteriaceae*, *Lachnospiraceae*, *Lactobacillaceae*, *etc.*), 32 genera of bacteria (*Lachnospira*, *Parabacteroides*, *Prevotella*, *etc.*) and 19 species of bacteria (*Akkermansia muciniphila*, *Faecalibacterium prausnitzii*, *Parabacteroides merdae*, *etc.*). These microbiomes and the trends of the differences are shown in Table 3. Forest plots and funnel plots of all the microbiota are shown in Supplemental data 2 (*https://www.biosciencetrends.com/action/getSupplementalData.php?ID=234*). The funnel plots of the vast majority of bacteria were symmetrical, and Egger bias was not present. However, owing to the literature limitations, the specific microbiome distribution and HY score of each patient could not be obtained.

### 3.3. Intestinal microbiota with differential abundance between PD patients at different stages and healthy controls

Through bioinformatics analysis, we explored the microbiota with differential abundance between PD patients at different stages and healthy controls in PRJEB30615 (*68*) and PRJNA588035 (*33*). The two studies involved 47 patients with early PD, 26 patients with middle to late PD, and 64 healthy controls. There were significant differences in the relative abundance of 4 phyla of bacteria (*Actinobacteriota*, *Proteobacteria*, *Verrucomicrobia*, *etc.*), 7 classes of bacteria (*Actinobacteriota*, *Erysipelotrichia*, *Verrucomicrobiae*,

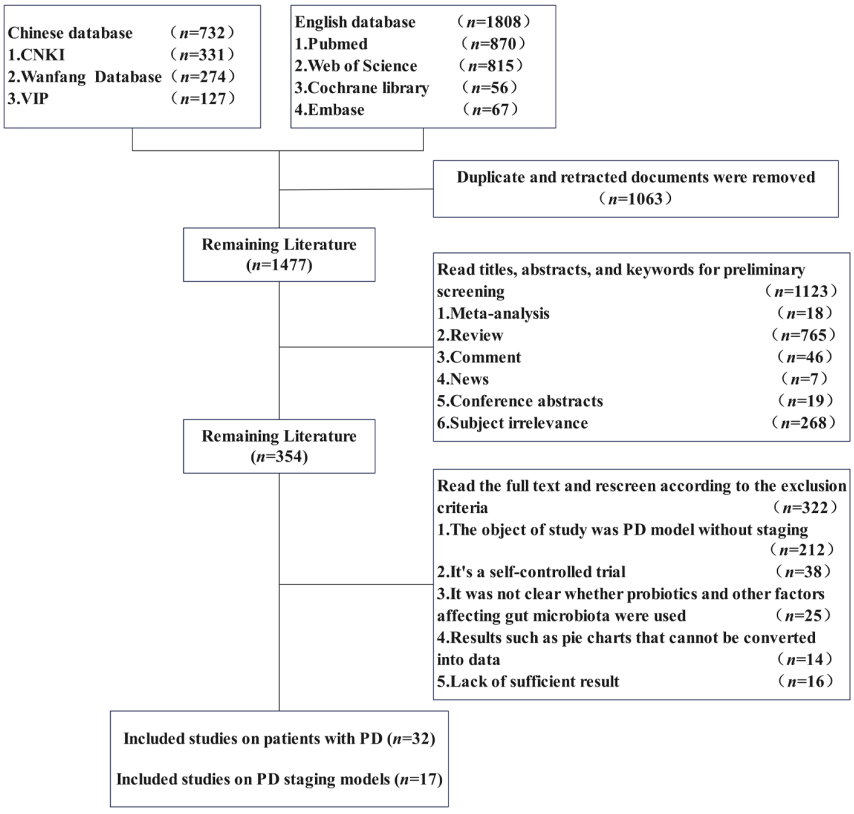


**Figure 1. Literature selection process for the meta-analysis.**

**Table 1. Basic information of the research subjects included in the literature**

| Included study | Country and region | Sample size | | Ages | | Detection method | Average HY score of PD patients | NOS score |
|---|---|---|---|---|---|---|---|---|
| | | PD group | Control group | PD group | Control group | | | |
| Aho VTE, 2019 (*19*) | Finland | 64 | 64 | 65.2 ± 5.52 | 64.45 ± 6.9 | 16S rRNA | 2.5 (2-3) | 6.5 |
| Babacan Y G, 2023 (*20*) | Istanbul, Turkey | 42 | 42 | 60.62 ± 9.31 | 58.33 ± 9.61 | 16S rRNA | 2 (1-3) | 8 |
| Barichella M, 2018 (*21*) | Milan, Italy | 193 | 113 | 67.6 ± 9.7 | 65.9 ± 9.9 | 16S rRNA | 2.0 ± 0.8 | 7.5 |
| Bi ZA, 2018 (*22*) | Nanjing, China | 14 | 15 | 65.14 ± 9.11 | 60.80 ± 7.33 | 16S rRNA | 2.32 ± 0.70 | 8 |
| Bolliri C, 2022 (*23*) | Italy | 20 | 20 | 67.8 ± 9.6 | 67.8 ± 9.6 | Metagenomic sequencing | 2.0 ± 1.0 | 8 |
| Cerroni R, 2022 (*24*) | Italy | 18 | 13 | 63.5 ± 8.1 | 62.8 ± 7.8 | 16S rRNA | 2.15 ± 0.5 | 7.5 |
| Chen H, 2017 (*25*) | China | 20 | 20 | 63.60 ± 11.75 | 65.00 ± 11.09 | 16S rDNA | 2.35 ± 0.95 | 7.5 |
| Cirstea MS, 2020 (*26*) | Canada | 75 | 50 | 66 (57.5, 69) | 64.5 (57, 70) | 16S rDNA | / | 7.5 |
| Hill-Burns EM, 2017 (*27*) | America | 197 | 130 | 68.4 ± 9.2 | 70.3 ± 8.6 | 16S rRNA | / | 6.5 |
| Li F, 2019 (*28*) | Jinzhou, China | 10 | 10 | 79.5 ± 8.0 | 76.5 ± 7.5 | 16S rRNA | 2.4 ± 1.1 | 7.5 |
| Li KS, 2020 (*29*) | Shanghai, China | 26 | 26 | 67.00 ± 3.89<br>69.08 ± 4.70 | 69.08 ± 4.70 | 16S rRNA | 2.50 (1.50, 2.50)<br>1.75 (1.00, 2.50) | 8 |
| Li T, 2020 (*30*) | Beijing, China | 25 | 25 | 68.89 ± 7.79 | 69.17 ± 7.17 | 16S rRNA | / | 8 |
| Li Y, 2020 (*31*) | Beijing, China | 30 | 30 | 67.0 ± 6.0 | 65.0 ± 8.0 | 16S rRNA | 2 | 8 |
| Lin AQ, 2018 (*32*) | Guangzhou, China | 75 | 45 | 60.48 ± 10.72 | 63.20 ± 6.00 | 16S rRNA | / | 7 |
| MAO LW, 2021 (*33*) | Xiangyang, China | 39 | 39 | 63.95 ± 6.92 | 64.82 ± 6.86 | Metagenomic sequencing | 1.94 ± 0.91 | 8 |
| Nakahara K, 2023 (*34*) | Japan | 5 | 5 | 70.0 (67.0, 71.0) | 69.0 (59.0, 71.5) | 16S rRNA | 2.0 (1.5, 2.5) | 7 |
| Pietrucci D, 2019 (*35*) | Italy | 80 | 72 | 66.2 ± 8.7 | 62.6 ± 8.7 | 16S rRNA | 2.5 ± 0.7 | 7.5 |
| Qian YW, 2020 (*36*) | Shanghai, China | 40 | 40 | 66.6 ± 7.1 | 66.3 ± 8.1 | Metagenomic sequencing | 2.3 ± 0.8 | 7.5 |
| Ren T, 2020 (*37*) | China | 13 | 14 | 60.00 ± 9.20 | 63.00 ± 8.76 | 16S rRNA | 1.89 ± 0.49 | 7.5 |
| Scheperjans F, 2015 (*38*) | Finland | 72 | 72 | 65.3 ± 5.5 | 64.5 ± 6.9 | 16S rRNA | / | 7 |
| Tan AH, 2021 (*39*) | Malaysia | 104 | 96 | 65.4 ± 8.4 | 62.4 ± 9.0 | 16S rRNA | 2.2 ± 0.5 | 7 |
| Tetz G, 2018 (*40*) | New York, USA | 31 | 28 | 64.8 ± 9.5 | 65.6 ± 10.4 | Metagenomic sequencing | / | 7 |
| Tong QW, 2021 (*41*) | Wenzhou, China | 30 | 30 | 66.1 ± 7.2 | 64.8 ± 5.0 | 16S rDNA | 2.11 ± 0.65 | 7.5 |
| Vascellari S, 2020 (*42*) | Italy | 64 | 51 | 71.39 ± 10.99 | 51.67 ± 12.42 | 16S rRNA | / | 7.5 |
| Wallen ZD, 2022 (*43*) | America | 158 | 51 | 68.7 ± 8.5 | 65.8 ± 8.8 | Metagenomic sequencing | / | 6.5 |
| Wang YJ, 2022 (*44*) | China | 30 | 30 | 59.64 ± 5.7 | 61.28 ± 6.2 | 16S rRNA | / | 7.5 |
| Zhang F, 2020 (*45*) | Xiangyang, China | 63 | 74 | 64.0 ± 7.4 | 63.4 ± 6.6 | 16S rRNA | 2.1 ± 0.8 | 8 |
| Zhang F, 2020 (*46*) | Xiangyang, China | 46 | 46 | 63.6 ± 6.9 | 63.8 ± 7.0 | Metagenomic sequencing | / | 8 |
| Zhang LN, 2021 (*47*) | China | 20 | 20 | 67.80 ± 7.84 | 66.25 ± 7.04 | 16S rRNA | / | 7.5 |
| Zhang TQ, 2019 (*48*) | Beijing, China | 38 | 15 | 68.76 ± 7.343 | 69.80 ± 7.253 | 16S rRNA | / | 8 |
| Zhao C, 2018 (*49*) | China | 24 | 14 | 73.75 ± 6.26 | 74.64 ± 5.57 | 16S rRNA | / | 8 |
| Zhuo WY, 2018 (*50*) | China | 52 | 52 | 66.57 ± 11.82 | 65.33 ± 10.19 | 16S rDNA | 2.53 ± 0.89 | 7.5 |

**Table 2. Basic information of the studies included in MPTP staging model**

| Included study | Sample size | | Detection method | Staging type | SYRCLE score |
|---|---|---|---|---|---|
| | PD group | Control group | | | |
| Aktas B, 2023 (*51*) | 10 | 10 | 16S rRNA | acute/subchronic | 8 |
| An YY, 2019 (*52*) | 6 | 6 | 16S rRNA | chronic | 8 |
| An YY, 2019 (*53*) | 6 | 6 | 16S rRNA | chronic | 8.5 |
| Chen XX, 2022 (*54*) | 6 | 6 | 16S rDNA | chronic | 8.5 |
| Chen XX, 2022 (*55*) | 6 | 6 | 16S rDNA | chronic | 8.5 |
| Dong XL, 2020 (*56*) | 8 | 8 | 16S rRNA | acute | 8.5 |
| Jang JH, 2020 (*57*) | 10 | 6 | 16S rRNA | subchronic | 7 |
| Jeon H, 2021 (*58*) | 5 | 5 | 16S rRNA | subchronic | 6.5 |
| Liao JF, 2020 (*59*) | 12 | 12 | 16S rRNA | subchronic | 7.5 |
| Liu MM, 2022 (*60*) | 3 | 3 | 16S rRNA | subchronic | 7 |
| Liu X, 2021 (*61*) | 8 | 8 | 16S rRNA | subchronic | 7.5 |
| Liu X, 2021 (*62*) | 8 | 8 | 16S rRNA | chronic | 8 |
| Liu X, 2022 (*63*) | 8 | 8 | 16S rRNA | chronic | 8.5 |
| Shi Y, 2021 (*64*) | 10 | 10 | 16S rDNA | acute | 7.5 |
| Sun MF, 2018 (*65*) | 15 | 15 | 16S rRNA | subchronic | 7.5 |
| Sun Z, 2022 (*66*) | 12 | 12 | 16S rRNA | subchronic | 8.5 |
| Zhang LY, 2020 (*67*) | 12 | 12 | 16S rDNA | subchronic | 8.5 |

*etc*.), 9 orders of bacteria (*Actinomycetales*, *Erysipelotrichales*, *Lactobacillales*, *etc*.), 21 families of bacteria (*Bacteroidaceae*, *Lactobacillaceae*, *Prevotellaceae*, *etc*.), 25 genera of bacteria (*Bacteroides*, *Lachnospira*, *Parabacteroides*, *etc*.) and 52 species of bacteria (*Akkermansia muciniphila*, *Faecalibacterium prausnitzii*, *Parabacteroides gordonii*, *etc*.) between PD patients at different stages and healthy controls. In the two studies, the microbiomes with significant differences in expression between PD patients with different stages and healthy people are shown in the Table 4 (Onlin Table: *https://www.biosciencetrends.com/action/getSupplementalData.php?ID=234*).

### 3.4. Differences in intestinal microbiota abundance of MPTP models at different stages through meta-analysis

Through meta-analysis, we found that the MPTP models of different stages had different relative abundance of intestinal microbiota at different classification levels. We found that *Actinobacteriota*, *Bacteroidetes* and *Bacteroidales* increased significantly in the subchronic MPTP model. *Actinobacteriota*, *Firmicutes*, *Deferribacteraceae*, *Erysipelotrichaceae*, *Ruminococcaceae*, *Allobaculum* and *Oscillibacter* were significantly increased in the chronic MPTP model. *Bacteroidetes*, *Prevotellaceae*, *Blautia* and *Prevotellaceae UCG-001* decreased significantly in the chronic MPTP model. Forest plots and funnel plots of all the microbiota are shown in Supplemental data 2 (*https://www.biosciencetrends.com/action/getSupplementalData.php?ID=234*).

### 3.5. Rotenone induced motor and gastrointestinal dysfunction in PD rats at different stages

The rotenone-treated group presented yellow hair, reduced activity, and slow movement at 7 days. The rotenone-treated rats presented dirtier hair, almost no activity, and unsteady gait at 28 days. The pole climbing time of rotenone-treated rats in the early group was significantly greater than that of the control group ($P < 0.01$), and that of the late group was significantly lower than that of the control group ($P < 0.01$). The inclined plate angle of the model rats gradually decreased, and the angle of the late group was lower than that of the early group. Compared with the control group, the movement distance of the rats in the early group was significantly lower ($P < 0.01$), the movement distance and average movement speed of the rats in the late group were significantly lower ($P < 0.001$, $P < 0.001$), and the rest time of the rats in the late group was significantly increased ($P < 0.001$). The distribution of different groups of rats in the central and peripheral areas of the open field also differed. The fecal water content of the late group was significantly lower than that of the control group ($P < 0.001$). TH in the late group was significantly lower than that in the control group ($P < 0.001$). These results are shown in Figure 2.

### 3.6. MPTP induced motor and gastrointestinal dysfunction in PD mice at different stages

Compared with the control group, slow movement was observed on the 7th day after MPTP induction. Compared with the control group, the pole climbing time of the MPTP-induced mice in the late group was significantly lower ($P < 0.001$). The inclined plate angle of the late group was lower than that of the early group. Compared with the control group, the moving distance and average speed of the mice in the early and late groups were significantly lower ($P < 0.05$). The distribution of different groups of mice in the central and peripheral areas of the open field also differed. The fecal

**Table 3. Intestinal microbiota with differential abundance between PD patients and healthy people**

| Phylum | | Class | Order | | Family | | Genus | | Species | |
|---|---|---|---|---|---|---|---|---|---|---|
| Dominant bacteria in PD patient group | Dominant bacteria in health control group | Dominant bacteria in PD patient group | Dominant bacteria in PD patient group | Dominant bacteria in health control group | Dominant bacteria in PD patient group | Dominant bacteria in health control group | Dominant bacteria in PD patient group | Dominant bacteria in health control group | Dominant bacteria in PD patient group | Dominant bacteria in health control group |
| *Actinobacteriota* | *Bacteroidetes* | *Bacilli* | *Clostridiales* | *Pasteurellales* | *Bifidobacteriaceae* | *Prevotellaceae* | *Actinomyces* | *Blautia* | *Akkermansia muciniphila* | *Faecalibacterium prausnitzii* |
| *Euryarchaeota* | *Fusobacteria* | *Deltaproteobacteria* | *Methanobacteriales* | | *Christensenellaceae* | *Micrococcaceae* | *Akkermansia* | *Faecalibacterium* | *Anaerotruncus colihominis* | |
| *Lentisphaerae* | | *Methanobacteria* | *Verrucomicrobiales* | | *Coriobacteriaceae* | *Lachnospiraceae* | *Alistipes* | *Fusicatenibacter* | *Bacteroides stercoris* | |
| *Proteobacteria* | | *Synergistia* | | | *Corynebacteriaceae* | *Fusobacteriaceae* | *Anaerotruncus* | *Fusobacterium* | *Bifidobacterium adolescentis* | |
| *Synergistetes* | | *Verrucomicrobiae* | | | *Dehalobacteriaceae* | *Comamonadaceae* | *Barnesiella* | *Lachnospira* | *Bifidobacterium bifidum* | |
| *Verrucomicrobia* | | | | | *Desulfovibrionaceae* | *Alcaligenaceae* | *Bifidobacterium* | *Paraprevotella* | *Bifidobacterium breve* | |
| | | | | | *Enterobacteriaceae* | | *Bilophila* | *Prevotella* | *Bifidobacterium longum* | |
| | | | | | *Enterococcaceae* | | *Christensenella* | *Roseburia* | *Clostridium asparagiforme* | |
| | | | | | *Eubacteriaceae* | | *Collinsella* | | *Clostridium saccharolyticum* | |
| | | | | | *Lactobacillaceae* | | *Corynebacterium* | | *Escherichia coli* | |
| | | | | | *Methanobacteriaceae* | | *Desulfovibrio* | | *Eubacterium dolichum* | |
| | | | | | *Mogibacteriaceae* | | *Enterobacter* | | *Gordonibacter pamelaeae* | |
| | | | | | *Oxalobacteraceae* | | *Enterococcus* | | *Lactobacillus salivarius* | |
| | | | | | *Peptococcaceae* | | *Escherichia* | | *Megasphaera elsdenii* | |
| | | | | | *Porphyromonadaceae* | | *Gordonibacter* | | *Parabacteroides distasonis* | |
| | | | | | *Rikenellaceae* | | *Lactobacillus* | | *Parabacteroides merdae* | |
| | | | | | *Ruminococcaceae* | | *Megasphaera* | | *Streptococcus anginosus* | |
| | | | | | *Synergistaceae* | | *Methanobrevibacter* | | *Streptococcus thermophilus* | |
| | | | | | *Verrucomicrobiaceae* | | *Parabacteroides* | | | |
| | | | | | | | *Peptoniphilus* | | | |
| | | | | | | | *Porphyromonas* | | | |
| | | | | | | | *Scardovia* | | | |
| | | | | | | | *Slackia* | | | |
| | | | | | | | *Varibaculum* | | | |

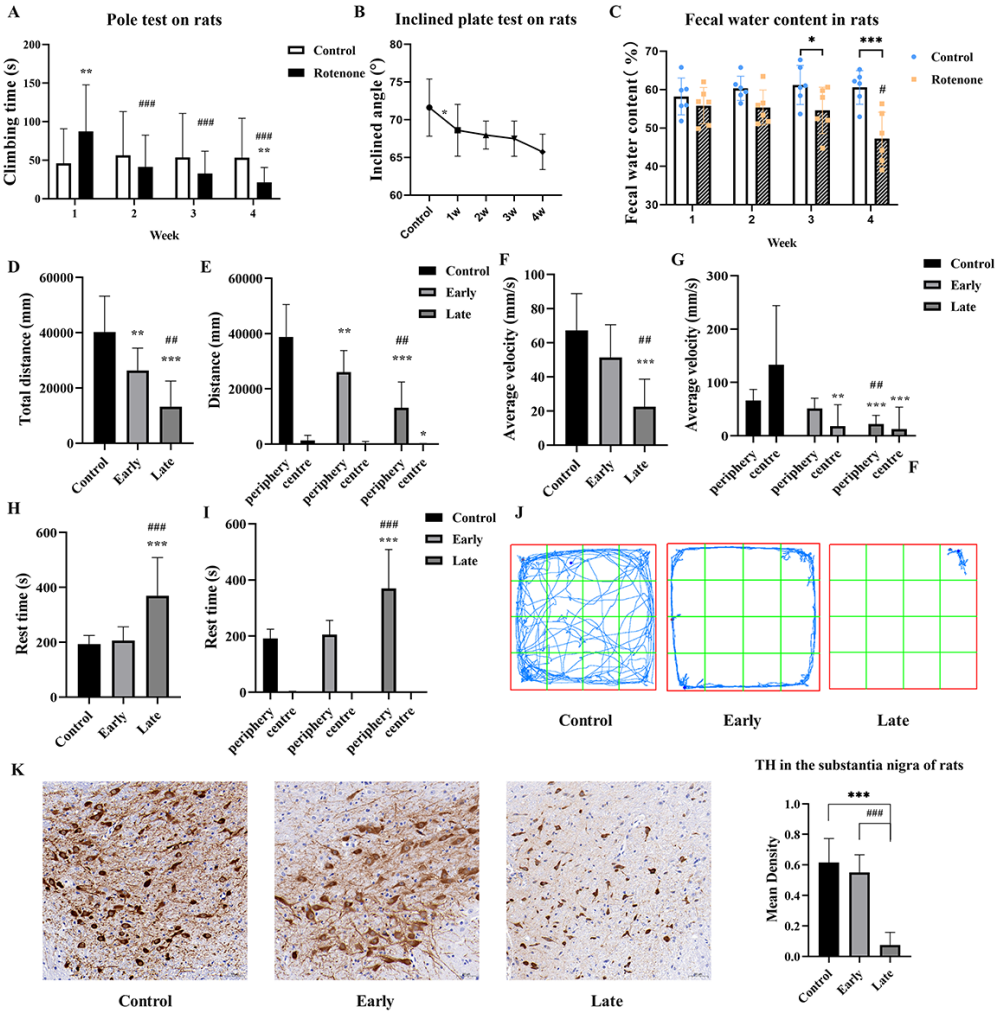


**Figure 2. Motor and gastrointestinal dysfunction in PD rats induced by rotenone. (A)** Pole climbing time of rats in different groups ($n = 10$). **(B)** Angle of the inclined plate of rats in different groups ($n = 10$). **(C)** Changes in the fecal water content of rats in different groups over time ($n = 6$). **(D, E)** Total distance and distance distributions of rats in the open field in different groups ($n = 10$). **(F, G)** Average velocity and distribution of different groups of rats in the open field ($n = 10$). **(H, I)** The resting time and distribution of different groups of rats in the open field ($n = 10$). **(J)** Movement tracks of rats in different groups in the open field. **(K)** TH immunohistochemistry results of the substantia nigra in different groups of rats ($n = 3$). Compared with the control group, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. Compared with the early group, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$.

water content of mice in the late group was significantly lower than that of the control group ($P < 0.001$). These results are shown in Figure 3.

### 3.7. The intestinal microbiota of rats and mice with PD at different stages changed significantly

We obtained the Amplicon Sequence Variants (ASVs) sequences and species abundance tables for each group of samples at different taxonomic levels. LEfSe analysis showed significant differences in potential biomarkers among different groups, as shown in Figure 4. The Kruskal–Wallis test showed that 9 phyla, 12 classes, 17 orders, 23 families, 40 genera and 24 species of bacteria were significantly differentially expressed among the different groups of rats. There were 6 phyla, 6 classes, 8 orders, 14 families, 35 genera and 10 species of bacteria whose expression significantly differed among the different groups of mice, as shown in the Supplemental data 3 (*https://www.biosciencetrends.com/action/getSupplementalData.php?ID=235*). There were significant differences in the diversity of the intestinal microbiota in PD rats and mice at different stages. Compared with the control group, the α diversity indices of rats and mice in the early group showed significant differences. There were significant differences in β diversity among different groups of rats and mice. These results are shown in Supplemental data 4 (*https://www.biosciencetrends.com/action/getSupplementalData.php?ID=234*). The prediction results of intestinal microbial function in different groups showed that 156 pathways were significantly different between rats and 159 pathways were significantly different between mice, as shown in Figure 5.

### 3.8. Changes in the intestinal microbiota in PD patients

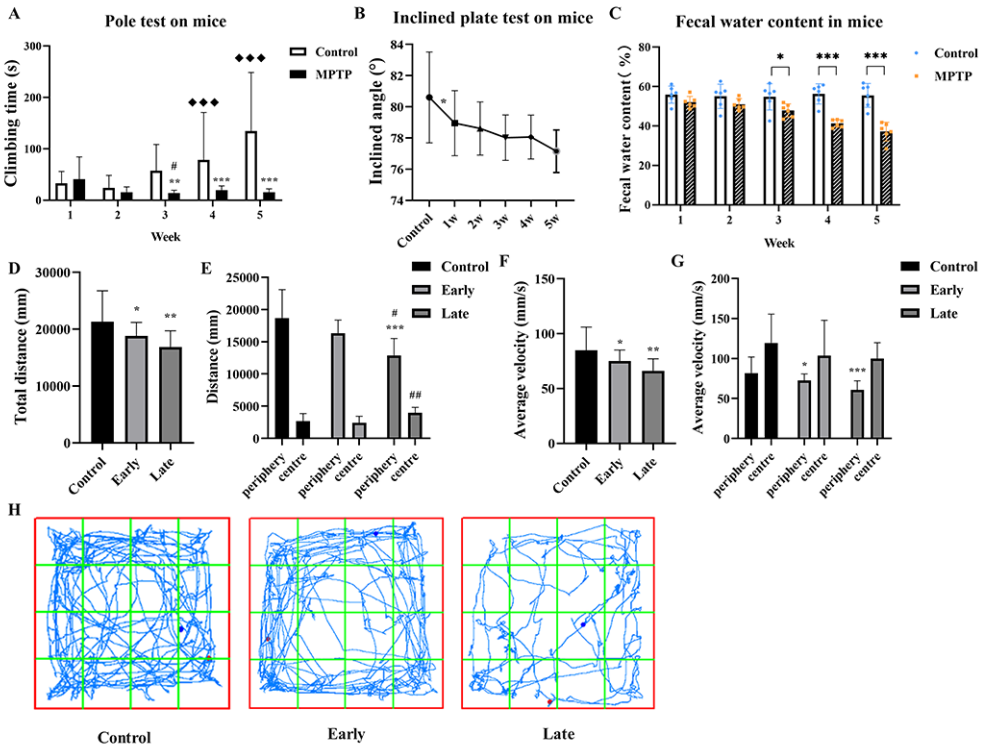


**Figure 3. MPTP induced motor and gastrointestinal dysfunction in PD mice at different stages. (A)** Pole climbing time of the mice in different groups ($n$ = 10). **(B)** Angle of the inclined plate of the mice in different groups ($n$ = 10). **(C)** Changes in the fecal water content of the mice in different groups over time ($n$ = 6). **(D, E)** Total distance and distance distributions of the mice in the open field in different groups ($n$ = 10). **(F, G)** Average velocity and distribution of the different groups of mice in the open field ($n$ = 10). **(H)** Movement tracks of the mice in different groups in the open field ($n$ = 10). Compared with the control group, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. Compared with the early group, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$. Compared with the 1-week control group, $^{\blacklozenge\blacklozenge\blacklozenge}P < 0.001$.

and models at different stages

The results of the meta-analysis, bioinformatics analysis, and the rats and mice simulations revealed significant changes in the intestinal microbes at different stages of PD. Ultimately, we found that in the early PD, *Akkermansia, Alistipes, Anaerotruncus, Bilophila, Rikenellaceae, Verrucomicrobia and Verrucomicrobiae* were predominant. In the late PD, *Actinobacteriota* and *Erysipelotrichaceae* were predominant. The significant changes in these microbiomes across the different results are shown in Table 5.

## 4. Discussion

Parkinson's disease is a chronic and progressive neurodegenerative disease. Clinically, PD patients are divided into different stages according to the degree of motor function impairment. However, the typical biomarkers of PD patients or models at different stages are not known, which presents a great challenge for the treatment and accurate diagnosis of PD at different stages. Many studies have focused on the role and changes in the intestinal microbiota in the course of PD, and the intestinal microbiota is considered one of the important factors regulating gut–brain interactions in the course of PD (*69*). In this study, the intestinal microbiota distribution of PD patients or models at different stages was taken as the core object of study. We used meta-analysis to explore the differences in intestinal microbiota between PD patients and healthy people, PD staging model and control group, and bioinformatic analysis to explore the distribution characteristics of intestinal flora between PD patients with different stages and healthy people. PD models of different stages were established in rotenone-treated rats and MPTP-induced mice, and the intestinal flora of PD model rats or PD model mice at different stages were investigated.

We first used meta-analysis to screen case–control studies on PD patients and the gut microbiota. We found that there were significant differences between PD patients and healthy controls. On this basis, we screened the studies that could obtain the HY score and original sequence of the microbiota of each PD patient. We performed bioinformatics analysis in PRJEB30615 and PRJNA588035. The two studies included 47 patients with early PD, 26 patients with middle to late stage PD, and 64 healthy controls. In two studies, there were significant differences between PD patients at different stages and healthy controls. We then screened studies with closely related gut microbiota in PD models with different stages. We found significant differences in

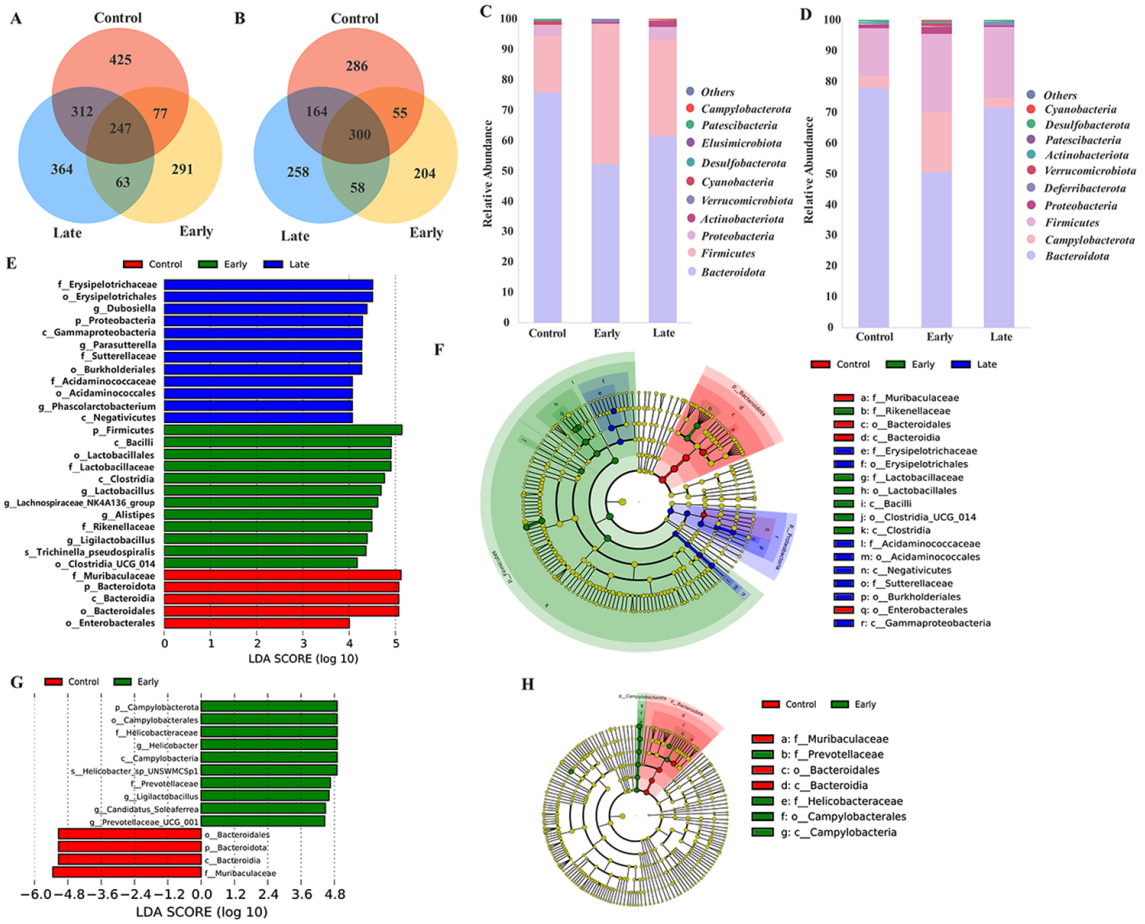


**Figure 4. Intestinal microbiota characteristics of rats and mice in different groups. (A)** Venn diagram of the number of ASVs in different groups of rats ($n$ = 6). **(B)** Venn diagram of the number of ASVs in different groups of mice ($n$ = 6). **(C)** Species abundance histogram of rats in different groups under phylum classification (top 10). **(D)** Species abundance histogram of mice in different groups under phylum classification (top 10). **(E)** LDA histogram of different microbiota in different groups of rats (red represents the control group, green represents the early group, and blue represents the late group, as are figures F, G, and H). **(F)** Evolutionary cladistics of different microbiota in different groups of rats. **(G)** LDA histogram of different microbiota in different groups of mice. **(H)** Evolutionary cladistics of different microbiota in different groups of mice.

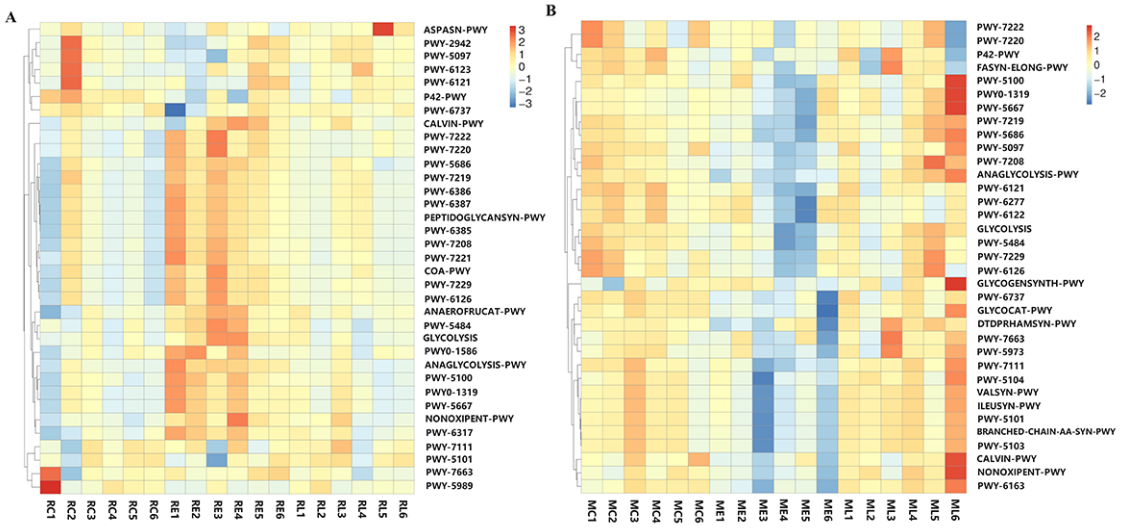


**Figure 5. Predictive functional clustering heatmaps of the intestinal microbiota in different groups of rats or mice. (A)** Predictive functional cluster heatmaps of samples from different groups of rats (control group of rats, RC; early group of rats, RE; late group of rats, RL). **(B)** Predictive functional cluster heatmaps of samples from different groups of mice (control group of mice, MC; early group of mice, ME; late group of mice, ML).

**Table 5. Intestinal microbiota with common changes between PD patients and models at different stages**

| Treatments | Meta 1 | Meta 2 | PRJEB30615 | PRJNA588035 | Rat | Mice |
|---|---|---|---|---|---|---|
| *Akkermansia* | PD | / | Early | / | / | Early |
| *Alistipes* | PD | / | / | Early | Early | / |
| *Anaerotruncus* | PD | / | / | Early | / | Early |
| *Bilophila* | PD | / | / | Early | Control | Early |
| *Rikenellaceae* | PD | / | / | Early | Early | / |
| *Verrucomicrobia* | PD | / | Early | / | / | Early |
| *Verrucomicrobiae* | PD | / | Early | / | / | Early |
| *Actinobacteriota* | PD | Late | Late | Late | Late | / |
| *Erysipelotrichaceae* | / | Late | Late | / | Late | / |

Meta 1 represents the meta-analysis between PD patients and healthy controls. Meta 2 represents the meta-analysis of MPTP models at different stages. PD indicates that the bacteria are dominant in PD patients. Control indicates that the bacteria have a dominant expression in the control group. Early indicates that the bacteria have a dominant expression in the early stage of PD patients or models. Late indicates that the bacteria have a dominant expression in the late stage of PD patients or models. / indicates that no significant changes in the bacteria have been observed in this study.

the distribution of intestinal flora in acute, subchronic, chronic MPTP models and control mice.

At present, there is no model that can fully simulate PD, and each model has its own advantages and disadvantages (*70*). Rotenone can cause apoptosis of dopamine neurons and induce pathological features similar to PD in rats, but there is no staging model (*71*). It has the characteristics of short half-life, fast degradation and strong self-healing ability (*72*). Previous studies have shown that the change of the rotenone subcutaneous injection model first occurred at about 7 days, and extremely significant motor function loss occurred at about 28 days (*73*). Therefore, different stages of the PD rat model were simulated for 7 and 28 days. 1-Methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP) is a neurotoxin that easily crosses the blood–brain barrier (*74*). MPTP-induced PD models can be divided into acute, subchronic and chronic models, among which the chronic models develop gradually and are more consistent with the clinical symptoms of PD patients (*75,76*). In this study, the changes in the intestinal microbiota of rats and mice were integrated, which was helpful for more comprehensively discovering the changes in PD models at different stages.

The results showed that the motor and gastrointestinal functions of PD rats and mice were significantly damaged, and the damage in the late group was more serious than that in the early group, which was consistent with the evolution of PD at different stages. There are still some problems that need to be considered; for example, the pole climbing time of early PD rats is significantly longer than that of the control group, and this may be related to damage to cognitive function, as these rats need more time to adapt to and understand pole climbing. In addition, as more tests were conducted, the control mice took significantly longer to climb the pole, even though they had learned how to leave the pole. In contrast, the climbing time of PD mice was significantly shorter, indicating that their motor function was impaired. Compared with the PD mice, the damage of PD rats was more obvious with the increase of stage, and the simulation effect was better. Therefore, we investigated the TH expression of the substantia nigra in rats, and the TH expression in the late group showed a significant decrease.

The intestinal microbiota of the different groups of rats and mice significantly differed. The α diversity indices of intestinal microbiota in the early PD was significantly affected. The results of β diversity analysis also revealed significant changes in the intestinal microbiota distribution among the different groups. In late PD, the damage was manifested by significant changes in the abundance of dominant species. Combined with all the results of this study, we found that *Akkermansia*, *Alistipes*, *Anaerotruncus*, *Bilophila*, *Rikenellaceae*, *Verrucomicrobia* and *Verrucomicrobiae* were significantly increased in early PD patients and models. *Actinobacteriota* and *Erysipelotrichaceae* significantly increased in late PD patients and models.

*Akkermansia* is the only representative member of *Verrucomicrobia* found in mammalian gastrointestinal samples (*77*). Previous studies and this meta-analysis have both confirmed the increase in the relative abundance of *Akkermansia* in PD patients (*78*). However, whether an increase in the relative abundance of *Akkermansia* is beneficial or harmful remains a matter of debate (*79*). Studies have shown that oral administration of *Akkermansia* can improve motor function and relieve neuroinflammation in PD mice (*80*). It has also been suggested that excessive enrichment of *Akkermansia* may alter mucin degradation processes, thereby impacting the intestinal barrier and inducing the secretion of inflammatory factors (*81*). This study suggests that *Akkermansia* is significantly increased in early PD. Its beneficial or harmful effects may be related to its expression, and it is likely to play different roles in different stages of PD, which is closely related to the regulation of neuroinflammation.

*Alistipes*, which belongs to *Rikenellaceae*, is found in the gut microbiota of healthy people and plays a role in inflammation and many diseases (*82,83*). Previous

studies have shown that *Alistipes* may be beneficial or harmful (*84*). In this study, the abundance of *Alistipes* significantly increased in early PD; coincidentally, it was also related to inflammation, similar to *Akkermansia*. In addition, previous studies have found an increase in *Rikenellaceae* in PD patients and models, but its increase in the early stage was the first to be found and requires further exploration (*85-87*). There have been few studies on *Anaerotruncus* and *Bilophila* in PD, but they have been found to be significantly increased in PD patients, consistent with the results of this study (*31,88*), and it is worthwhile to investigate their role in the early PD.

In this study, *Actinobacteriota* increased significantly in the late PD patients and models, and the changes were consistent with previous studies (*89,90*). Previous studies explored the role of five candidate bacterial biomarkers of the *Actinobacteriota* in PD patients and reported that they were associated with abnormal inflammation (*91*). These indicate that the increase in *Actinobacteriota* may be related to the severity of PD and can be considered as a biomarker for late PD. The expression of *Erysipelotrichaceae* has been reported to change over time in PD. In a longitudinal study, *Erysipelotrichaceae UCG-003* was found to be differentially expressed in PD patients at 0, 6 and 12 months (*92*). Furthermore, the abundance of *Erysipelotrichaceae* in mice induced by MPTP for 3 weeks was significantly increased compared with that in those induced for 2 days (*93*). These are consistent with this study, indicating that *Erysipelotrichaceae* has great potential as a biomarker of late PD models.

This study also has some limitations. Although the included studies excluded factors such as probiotics and antibiotics, the vast majority of patients included in clinical studies had PD treatment history, which could also affect the intestinal microbiota. In addition, there is a great gap between the pathologically and pathophysiologically of PD patients and PD animal models, so the two cannot be simply confused, and ultimately there is no microbiota with absolutely consistent trends in all studies. But we screened for the more common ones, and at least these flora showed similar changes in both. It is worth taking them as follow-up research points and conducting research based on PD models, so as to provide some microbiota related treatment references for relieving the pain of PD patients.

In summary, this study explored the differences between PD patients at different stages and healthy people, and between PD models at different stages in terms of changes in the intestinal microbes at different classification levels. Subsequent research on early PD patients and models can be based on *Akkermansia, Alistipes, Anaerotruncus, Bilophila, Rikenellaceae, Verrucomicrobia and Verrucomicrobia*. Research on late PD patients and models may be based on the *Actinobacteriota* and *Erysipelotrichaceae*.

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