Metagenomic analysis of gut microbiota in Parkinson's disease patients from Central China

Fan Zhang
Hubei University of Medicine

Qiang Zhao
Hubei University of Medicine

Xing Fang
Hubei University of Medicine

Meiling Xu
Hubei University of Medicine

Jie Tang
Hubei University of Medicine

Yu Zhang
Hubei University of Medicine

Ping Gao
Hubei University of Medicine

Xiaodong Sun
Hubei University of Medicine

Ming Sang
Hubei University of Medicine

Puqing Wang (✉ wpq20110328@qq.com)
Hubei University of Medicine

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Abstract

Background: Parkinson's disease (PD) is one of the most common neurodegenerative diseases, pathologic and epidemiologic studies suggest that gut microbiota may play important roles in the occurrence and progression of Parkinson's disease. However, the alterations in fecal microbiome in PD patients from Central China has not been investigated. Therefore, in this case-control study, we characterised the gut microbial community of 46 PD patients and compared it to those of healthy spouses by using metagenomic shotgun sequencing. Correlation between altered microbiota and clinical features were examined, functional pathways of gut microbiota were estimated, and potential biomarker were explored for further understaning of gut microbiota in PD.

Results: Microbial communities in the feces of PD patients were notably different from those of healthy spouses at species level. Gut microbiota of patients was characterized by depletion of Prevotella_copri and Bacteroides_fragilis, while the Bacteroides_stercoris and Escherichia_coli were markedly elevated. Correlation analysis found that most identified species were negatively correlated with disease clinical features. In particular, Prevotella_copri was negatively correlated with age and UPDRS III score. Random forest model indicated that 6 species including Prevotella_copri had good predictive value for disease. Functional analyses of the metagenomes revealed differences in microbiota metabolism. Pathways associated with superpathway of thiamin diphosphate biosynthesis, 4-aminobutanoate degradation, glucose-1-phosphate degradation and methylphosphonate degradation were significant increase in patients, while pathways associated with aromatic amino acid biosynthesis, chorismate biosynthesis, thiamin formation and pyrimidine deoxyribonucleosides salvage were significantly decrease. Functional pathways of Prevotella_copri were mainly concentrated in UMP biosynthesis, S-adenosyl-L-methionine cycle and guanosine ribonucleotides de novo biosynthesis.

Conclusion: Our findings confirmed changes of gut microbiota in Chinese patients with PD. Altered microbiota had correlation with the clinical characteristics of disease, which may used as potential biomarkers. Different functional pathways of gut microbiota in PD patients will help to improve our understanding of the mechanism in disease, and targeting on gut microbiota may be one of the new therapeutic choices of PD in the future.

Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disease with pathological hallmark of abnormally aggregated proteins (Lewy bodies) in the central nervous system (CNS)[1], affecting an estimated 1.7% of the Chinese population over 65 years of age[2]. Both motor symptoms and non-motor symptoms of PD present substantial physical and economic burden to patients and society. Unfortunately, mechanism of the disease remains unclear, and vailable therapies can only relieve the symptoms. Gastrointestinal dysfunction, particular constipation, are observed in 80-90% of PD patients[3], often precedes the onset of classical motor symptoms by years[4]. Lewy bodies and a-
synuclein (α-Syn) may originate in the gut, and spread from enteric nervous system (ENS) to CNS via vagal [5-7]. These observations supported the hypothesis that etiology of some PD may begin in the gut.

Emerging evidence suggests well-balanced gut microbiota is critical for maintaining general health. The gut-brain axis (GBA) connects gut microbiota and CNS by means of nerves, hormones and immunization, which has constructed a bidirectional communication system [8, 9]. Altered composition of the gut microbiota may link to a range of disorders including inflammatory, metabolic, and oncological disorders, as well as PD [10-12]. In the mouse model of α-Syn overexpression, gut microbiota can promote neuronal degeneration and motor dysfunction through microglial activation [13]. Moreover, fecal microbiota transplantation could suppress neuroinflammation, resulted to alleviated physical impairment, increased striatal dopamine (DA) and serotonin (5-HT) in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) treated mice [14]. Clinical researchs also had reported altered composition [15, 16] and metabolites [17, 18] of gut microbiota in patients with PD. These consistent results revealed that gut microbiota may play a key role in the progress of PD.

Due to the variable geographic origins and ethnic backgrounds of PD patients [16, 17, 19], gut bacterial profiles of studies in different world populations are heterogeneous. Considering the important influence of diets on gut microbiota [20], and the dietary habits of Chinese population are significantly different from those in Western population [21]. We hypothesize Chinese patients with PD may have different gut microbiota from Western PD patients. In additionally, quantitative PCR (qPCR) and 16S rRNA sequencing have limitations for detecting more detailed information about microbiota on species level and functional analysis [17]. Therefore, more studies using shotgun metagenomic sequencing should be performed to improve resolution and examine distant microbiota’s function.

In our study, we only focused on the Chinese patients with PD and their healthy spouses, in order to minimize the variation of gut microbiota caused by diets. Characteristic of gut microbiotal composition and functional pathways of PD patients were analyzed by using shotgun metagenomic sequencing method. Furthermore, we also evaluated the correlation between gut microbiota and PD clinical features, and explored potential biomarkers for disease diagnosis.

**Results**

**Demographics and Clinical Data**

46 PD patients and their paired healthy spouses were recruited in this study (Table 1). There was no significant difference in sex ratio and age between PD and Spouse groups. PD patients had average age of onset of 60.0 ± 6.5 years, disease duration of 3.0 (1.0-5.0) years, Hoehn and Yahr (H&Y) stage of 1.8 (1.0-2.5), and Unified Parkinson's Disease Rating Scale (UPDRS) total scores of 30.5 (20.8-43.0). According to clinical phenotype, patients were divided into 3 subgroups: tremor dominant (TD, n = 20), postural instability and gait difficulty (PIGD, n = 20) and indeterminate (n = 6). Hereditary factors play a role in PD, and 4 (9.7%) patients had family history in our study. Pesticides exposure is a risk of PD, and 4
(9.7%) patients had ever suffered pesticides exposure (Additional file 1). Meanwhile, prevalence of constipation between two groups were significant different (63.0% vs 10.7%, P < 0.001).

**Sequencing data and taxonomic composition of all samples**

Total of 5812686124 raw reads were obtained from 92 samples. The average number (mean ± SD) of raw reads per sample in PD and Spouse groups were 62387159 ± 17744069, 63975582 ± 24281624, respectively. Filtered clean reads of per sample were 59484557 ± 16536993 and 62907844 ± 24719821 in PD and Spouse groups (Additional file 2). GraPhlAn was used to construct classification tree (Fig. 1), and gut microbiota annotation to Archaea, Bacteria, Eukaryota and Viruses. The average relative abundance of these kingdoms were 0.108%, 99.762%, 0.005%, 0.125% in PD patients, and 0.036%, 99.184%, 0.002%, 0.778% in spouses, respectively. Meanwhile, the relative abundance of Viruses in patients was significantly lower than that in spouse (P = 0.002). *Bacteroidetes, Firmicutes, Proteobacteria* and *Actinobacteria* were the dominant Bacteria at phylum level, with the relative abundance accounting for more than 98%. In the remaining phylum with the relative abundance less than 1%, *Viruses_noname* in Viruses accounted for 0.45% and *Euryarchaeota* in Archaea accounted for 0.07%. At species level, relative abundance of *Prevotella_copri, Bacteroides_stercoris, Faecalibacterium_prausnitzii, Escherichia coli* and *Bacteroides_uniformis* were more than 3% in both two groups.

**Composition of gut microbiota between PD and Spouse groups**

Relative abundance of gut microbiota at the level of phylum, family and species were compared, respectively. The results showed no significant difference in the top 5 phyla of two groups. 70 families were annotationed and *Bacteroidaceae* was significantly increased in PD, while *Prevotellaceae* was significantly decreased. In the top 10 of all 484 species, *Prevotella_copri* and *Bacteroides_fragilis* was significantly decreased in patients, while *Bacteroides_stercoris* and *Escherichia coli* were significantly increased. Heatmap (Fig. 2a) showed the composition of top 50 species in all samples, and boxplot (Fig. 2b) showed different abundance of top 15 species in two groups. 23 species with significant differences between two groups were screened out, and principal coordinates analysis (PCoA) (Fig. 2c) based on the Bray-Curtis distance matrix showed significantly different beta diversity (analysis of similarities ANOSIM: R = 0.035, P = 0.036).

**Correlation between gut microbiota and clinical features of PD**

All samples were divided into < 60, 60-70 and > 70 years subgroups to further analyze the differences of *Prevotella_copri* (Fig. 3a). The results indicated *Prevotella_copri* decreased significantly in patients of 60-70 and > 70 subgroups compared with those in paired spouse groups. Moreover, the relative abundance of *Prevotella_copri* in PD was decreased significantly as the increase of age, but this phenomenon didn't occur in spouse subgroups.

Structure of gut microbiota in patients were analyzed, and significant difference were found between 3 age subgroups (Fig. 3b). Then filtered 22 species that had significant correlation with group factors
(Spearman’s test, $P < 0.05$), and used generalized linear model (GLM) to calculate the correlation coefficient between microbiota and clinical features of disease, such as age, disease duration and severity (H&Y stage, UPDRS score). Most of the identified species in gut microbiota were negatively correlated with disease clinical features (Table 2). In 7 species with average relative abundance of more than 0.1%, *Prevotella_copri* had significant negative correlation with age and UPDRS $\alpha$ score. *Parabacteroides_merdae* had negative correlation with disease duration, UPDRS total score, UPDRS $\beta$ and $\gamma$ score. *Alistipes_onderdonkii* had negative correlation with age, H&Y stage, UPDRS $\alpha$ and $\gamma$ score.

Gut microbiota in patients with family-history, dysosmia, constipation, pesticide expose and sleep disorder were further studied. The result indicated altered microbiota were significantly correlate with these non-motor symptoms (Additional file 3).

**Prediction models for PD based on gut microbiota biomarkers**

In order to evaluate the predictive value of gut microbiota for disease, we first searched out 23 species that had significantly different abundance between PD and Spouse groups by Wilcoxon rank-sum test. Then filtered out 6 important species by Boruta(Fig. 4a) and constructed random forest (RF) classification model. Relative abundance of *Prevotella_copri, Parabacterid_merdae, Alistipes_onderdonkii, Bacteroides_fragilis, Lachaceae__3_1_57* and *Providencia_rettgeri* were involved to predict the disease status. The results (Fig. 4b) showed that the area under curve (AUC) of random forest model was 0.772 (95% CI: 0.559-0.985; Sensitivity: 0.875; Specificity: 0.500).

**Altered functional pathways of gut microbiota in PD patient**

Statistical Analysis of Metagenomic Profiles softwares (STAMP) and LDA Effect Size (LefSe) were used to compare the different microbiotal function pathways between PD and Spouse groups, and the overlapped functional pathways from two methods were focused on. By mapping to the MetaCyc databases, 15 and 42 PWY pathways (Fig. 5a,b) significantly changed between PD and Spouse groups were found by STAMP and LefSe, respectively (Additional file 4). The results showed that the pathways associated with aromatic amino acid degradation/chorismate metabolism were significantly increased, while the biosynthesis-related pathways were significantly decreased. Another significant change in patient was significant increase in $\gamma$-aminobutyric acid (GABA) degradation, carbohydrate metabolism, and methylphosphonate degradation pathways. In the aspect of vitamin metabolism, pathways in vitamin B1 synthesis were increased in both two groups, while the synthetic pathway in patients was mainly from *Escherichia_coli*. In additionally, vitamin B6 synthesis pathway in the patients was significantly increased. Functional annotation can be precise to species level as the advantages of metagenomic sequencing. 7 functional pathways of *Prevotella_copri* were investigated, and the pathways which involve UMP biosynthesis I, S-adenosyl-L-methionine cycle I and guanosine ribonucleotides de novo biosynthesis were significant differences between PD and Spouse groups.

Clean data were also mapped to Kyoto Encyclopedia of Genes and Genomes (KEGG) orthology database, and KEGG orthology (KOs) of top 50 relative abundance are shown in heatmap (Fig. 5c). STAMP and
LefSe screened 61 and 200 KOs, respectively, and 30 overlapped KOs were summarized and annotationed (Additional file 5). There were 163 overlapped Clusters of Orthologous Groups of proteins (COGs) and 32 overlapped gene ontology (GOs) (Additional file 6,7), Bray-Curtis distance matrix based on these genes showed different composition of genes between two groups (Fig. 5d,e).

Relationship between clinical features of PD and differentially functional pathways were also analyzed, and clinical features were found to be related to many of functional pathways (Fig. 6). For example, aerobactin biosynthesis was positively associated with UPDRS II, gluconeogenesis I and L-methionine biosynthesis III were negatively associated with H&Y stage.

Discussion

Gut microbiota interact extensively with the host health, and research on human disease and the gut microbiota is a relatively hot field. The compositional features of the gut microbiota of PD patients have been described previously using quantitative PCR (qPCR), and 16S rRNA sequencing as well as metagenomic sequencing. In our study, PD patients and healthy spouses were recruited, and metagenomic sequencing was used to analyze the composition and function of gut microbiota at the species level. Such study design was highly feasible and innovative in microbiotal studies of Chinese population, which extended these earlier study results and was helpful to reveal the changes of gut microbiota and its important role in the diseases.

In our results, significantly decreased Viruses in patients were observed, while no significant difference between patients and spouses in the relative abundance of Bacteria. Ratio of Firmicutes/Bacteroidetes varied with age, and in infants, adults and elderly individuals were 0.4, 10.9 and 0.6[22], respectively. The average ratio of Firmicutes/Bacteroidetes in our samples was 0.69, which was consistent with previous studies. It was reported that the imbalance of Firmicutes/Bacteroidetes may be associated with amyotrophic lateral sclerosis (ALS)[23] and multiple system atrophy (MSA)[24], but this phenomenon has not been reported in PD patients yet. At family and species levels, Prevotellaceae and Prevotella_copri in PD patients were significantly reduced, which was consistent with the results of metagenomic study in Germany patients[18]. Scheperjans et al[16] first reported significant decrease of Prevotellaceae in PD patients from Finland, and Unger et al[25] confirmed this phenomenon in patients from Germany. While the alterations of Prevotellaceae and Prevotella_copri in Chinese patients are still controversial. Studies from northeast China[26] and Taiwan[27] have revealed the decreased Prevotellaceae and Prevotella in PD patients, while no significant difference in the abundance of Prevotellaceae between PD patients and healthy control in researches from Beijing[28] and Guangzhou[10]. And it was not mentioned in study from Shanghai[17] which recruited healthy spouses as controls. The reason may be related to the differences in sample size, region and dietary habits. Therefore, it still needs to be further verified by large sample and multi-region studies.

There are several theories about the mechanism of Prevotellaceae in PD. First, low Prevotella levels may indicate decreased mucin synthesis, which is associated with increased gut permeability[29], and cause
to bacterial endotoxin exposure. Endotoxins stimulate the enteric immune response either directly or via glial cells to promote local oxidative stress, which will lead to α-synuclein misfolding, aggregation, and subsequent neuronal damage in the enteric nervous system (ENS) in individuals genetically susceptible for PD[30]. Second, decreased *Prevotellaceae* led to a decrease in short chain fatty acids (SCFAs) synthesis. As neuromodulators, SCFAs can regulate inflammatory responses through leukocytes and endothelial cells[31], which may have a protective effect on PD. Decreased synthesis of SCFAs leads to weakened immune regulation and anti-inflammatory effects on the enteric nervous system, thus playing an important role in occurrence of neuroinflammatory and PD[32]. Studies reported that the SCFAs[25] or SCFAs-producing bacteria[33] in PD are decreased, which can support this hypothesis to some extent. Sampson reported that SCFAs may be the trigger of microglia activation and α-Syn aggregation[13], so the role of SCFAs in neurodegenerative diseases remains controversial. The third hypothesis links *Provotella* and hydrogen sulfide to explore the protective effect of *Provotella*. Hydrogen sulfide is a gaseous gut neurotransmitter that is secreted by *Provotella* and that exerts a protective effect on dopaminergic neurons in rat PD models[34, 35]. Considering that hydrogen sulfide is a gaseous neurotransmitter that exerts protective effects on dopaminergic neurons, it may be theorized that the human body increases gut permeability to enhance the benefits of a decreased level of gut hydrogen sulfide under the conditions of a reduced *Provotella* population[36]. Constipation may represent a conjoint mechanism underlying the increase in gut permeability to increase hydrogen sulfide absorption. This hypothesis could explain the non-motor symptoms of PD, while more clinical studies are needed for further confirmation.

The results indicated increased *Escherichia coli* (*E.coli*) in PD patients, which has not been reported in studies by 16S rRNA analysis. As metagenomic sequencing can detect species and strain levels, which is more suitable for the study of gut microbiota and human diseases[29]. Study had reported *E.coli* translocation in the colonic mucosa of PD. Invasive *E.coli* can lead to stress response, and the release of pro-inflammatory cytokines IL-β and IL-6, which cause increased intestinal permeability[37]. Since human gut may be regarded as a complex organ system, microbial balance and interaction may provide a new view to reveal its mechanism.

Many studies have focused on the correlation between gut microbiota and disease characteristics. This study showed significant differences in the composition of gut microbiota in patients with different age subgroups. Correlation analysis also found out microbiota that had significant correlation with age, disease duration, H&Y stage and UPDRS score. Most microbiota were negatively correlated with disease clinical characteristics, which was consistent with another Chinese study[17]. These results suggest that microbiota may changes with disease progression. A longitudinal study revealed that low counts of *Bifidobacterium* and *Bacteroides fragilis* at year 0 were associated with worsening of UPDRS I scores in 2 years. In addition, low counts of *Bifidobacterium* at year 0 were associated with worsening of hallucinations/delusions in 2 years[37]. Correlation analysis and longitudinal study will help to find the microbiota playing key roles in disease and reveal the mechanism of disease progression.
Random forest prediction model revealed 6 species including *Prevotella copri* had good predictive value for PD. Scheperjans[16] found that the AUC of predictive model based on *Prevotellaceae* was 0.591, which was 0.722 after including *Lactobacillaceae, Bradyrhizobiaceae and Losteridiales Incertae Sedis IV*. Bedarf[18] reported the AUC of *Eubacterium* for disease prediction was 0.63, which could reach 0.84 when involved other 5 genera. And Qian[17] reported the AUC prediction model of 18 genera could reach 0.807. These results suggested that gut microbiota may play as potential biomarker for PD in the future.

Function of gut microbiota in PD patients and spouses was compared, and the different functional pathways mainly focus on the metabolism of aromatic amino acid/chorismate, GABA, carbohydrate and methylphosphonate. The decreased synthesis or increased degradation of aromatic amino acids will lead to decrease of tyrosine in the body, which cause decreased dopamine and plays an important role in the occurrence of PD. GABA is important inhibitory neurotransmitter in the central nervous system. Normal levels of GABA help to maintain the tightness and function of the blood-brain barrier[38]. Animal experiments have shown that the levels of GABA in the striatum of PD model are decreased, while high-frequency stimulation can significantly increase the levels of GABA in the striatum[39]. Clinical studies have found reduced levels of GABA are associated with visual hallucinations in PD[40]. Pathways related to energy and inorganic metabolism were significantly increased in patients, which is similar to another Chinese study[17].

We also found that the effect of *Prevotella copri* may involve UMP biosynthesis, S-adenosyl-L-methionine cycle and guanosine ribonucleotides de novo biosynthesis. Studies have shown that oral administration of uridine can increases synaptic membranes and dendritic spines in rodents, significantly elevated striatal dopamine, tyrosine hydroxylase (TH) activity in 6-OHDA model[41]. S-adenosine methionine can reduce microglial aggregation and neuroinflammatory by increasing plasma hydrogen sulphide[42], which play neuroprotective role in central nervous system. Therefore, the decrease of *Prevotella copri* will participate in the occurrence and progression of PD by leading loss of dopaminergic neurotransmission and neuroprotective effect.

There were several limitations in the study that should be considered. Firstly, all the samples came from local area, which had weaknesses in wide applicability. Secondly, more detailed of the dietary habits of PD patients is needed for further research. Finally, the study was conducted in single period, and longitudinal studies of different stages of patients will be attempted in the future.

**Conclusion**

This study further validated the significant changes in gut microbiota of Chinese PD patients, explored the correlation and predictive value of altered microbiota to disease. These results will help to improve our understanding of the mechanism in Parkinson's disease. For clinical perspective, finding microbiota markers may provide new point for diseases diagnose, and modulation of gut microbiota and supplementation of specific metabolites may lead to new ideas for the treatment of diseases.
Methods

The study was approved by the ethics committee of Xiangyang No.1 People's Hospital and all participants gave informed consent. The study was registered at chictr.gov (ChiCTR1800016749).

Subjects

All patients (PD, n=46) were diagnosed as primary Parkinson's disease by two movement disorder specialists according to the Movement Disorder Society Clinical Diagnostic Criteria for Parkinson's disease (MDS-PD Criteria, 2015)[43] in Xiangyang No.1 People's Hospital, Hubei University of Medicine, Hubei, China. Exclusion criteria for patients: (1) Parkinsonism-Plus syndrome or secondary Parkinsonism; (2) psychiatric illness; (3) diabetes, gastrointestinal disease, surgical history or infectious diseases; (4) the use of antibiotics/probiotics for nearly three months. Healthy spouses (Spouse, n=46) living in same household with PD patients. Spouses had no obvious symptoms of the disease and use of antibiotics/probiotics for nearly three months. Samples were collected in outpatient and inpatient department of Xiangyang No.1 People's Hospital from June 2017 to January 2018.

Gender, age and diet habits were collected in all subjects. Clinical data such as onset age, duration years, subtype, Hoehn and Yahr (H&Y) stage, Unified Parkinson's Disease Rating Scale (UPDRS) score, first-side, initial-symptoms, dysosmia, constipation, sleep disorder, family history, pesticides exposure and medication were collected by neurologist through face-to-face interviews.

Fecal sample collection and DNA extraction

Participants were asked to collect a fecal sample with specialized fecal collection containers (Tiny Gene, China). Fecal samples were quickly placed in a refrigerator at -80 °C after shaking and mixing. Weighed 250 mg feces for DNA extraction, process was accorded to the QIAamp Fast DNA Stool Mini Kit operating manual (Qiagen, Germany). All the operations were processed on ice. The concentration and purity of DNA were measured by spectrophotometer (Implen, Germany). Quality of extracted DNA was detected by 1% agarose gel electrophoresis to ensure that it could be used for downstream experiments. Genomic DNA was cryopreserved in environment of -80°C prior to use.

Metagenomic shotgun sequencing

The DNA was randomly interrupted by the ultrasonic high performance sample processing system (Covaris) and selected to obtain a fragment of about 350bp. Repaired fragment ends with the 'A' base at the 3 'end and the library junction at both ends, then ligation mediated PCR was performed. Appropriate amplified product was taken for rolling circle amplification single-chain separation to generate DNA Nano Ball, and qualified product could be sequenced by BGISEQ-500 platform. The original image data obtained by sequencing was transformed into raw reads by BGISEQ-500 Base Calling and stored in FASTQ file format, called raw data.

Bioinformatics analysis
Raw data was first evaluated by FastQC (version 0.11.8) and mapped against the human genome (hg38) from University of California Santa Cruz (UCSC) with Bowtie2 (version 2.3.5.1), reads aligned to the human genome were removed, and filtered clean reads were used for further analysis. Subsequent analysis was performed as previous study described[44]. Gut microbiotal composition were predicted using MetaPhlan2.0[45] (version 2.7.7), GraPhlAn (version 0.97) was used to construct the Phylogenetic tree. Gene family profiles and pathway profiles were predicted using HUMANN2 [46](version 0.99) with default parameters. The gene family profile was normalized by reads per kilobase, annotated to the UniProt Reference Cluster (UniRef90). Further pathway mapping and regrouping were performed using the MetaCyc metabolic pathway database. The gene family profile was regrouped to the orthologous groups using the KEGG, EggNOG and GO databases. Gene pathways were calculated from the constituent gene family abundance for each individual. Statistical analysis was performed using the R (version 3.5.1) and Statistical Analysis of Metagenomic Profiles softwares [47](STAMP, version 2.1.3) were used for the statistical analysis. Welch's t test, Wilcoxon and Krukal-Wallis rank sum test were applied for continuous variables, and Pearson chi-square test was used for categorical variables between groups. All tests of significance were two sided, and Benjamini-Hochberg method was used to control false positive rates (FDR). P<0.05 or corrected P<0.05 was considered statistically significant. Linear discriminant analysis (LDA) effect size (LEfSe)[48] was used the identification of the different biomarkers of each group, and the log value for the LDA analysis was set to be 2.0. Spearman's correlation analysis was used to find gut microbiota related with group factors. The relative abundance was transformed by additive log ratio (ALR), and be used to calculate the correlation coefficient with clinical features of disease by generalized linear model (GLM).

**Additional File Information**

Additional file 1: Related characteristics of PD subjects. (XLSX 13 kb)

Additional file 2: Sequence data of all subjects. (XLSX 12 kb)

Additional file 3: Correlation between gut microbiota and non-motor symptoms. (DOCX 15kb)

Additional file 4: Functional pathways mapped based on MetaCyc database. (XLSX 432kb)

Additional file 5: Functional pathways mapped based on KO database. (XLSX 4.7mb)

Additional file 6: Functional pathways mapped based on EggNOG database. (XLSX 34mb)

Additional file 7: Functional pathways mapped based on GO database. (XLSX 4.7mb)

**Abbreviations**

PD: Parkinson's disease; α-Syn: alpha-synuclein; CNS: central nervous system; GBA: gut-brain axis; H&Y: Hoehn and Yahr; UPDRS: Unified Parkinson's Disease Rating Scale; PcoA: principal coordinates analysis;
ANOSIM: analysis of similarities; GLM: generalized linear model; AUC: area under curve; STAMP: Statistical Analysis of Metagenomic Profiles softwares; LefSe: LDA Effect Size; GABA: γ-aminobutyric acid; KEGG: Kyoto Encyclopedia of Genes and Genomes; KO: KEGG orthology; COG: Clusters of Orthologous Groups of proteins; GO: gene ontology; ALS: amyotrophic lateral sclerosis; MSA: multiple system atrophy; ENS: enteric nervous system; SCFAs: short chain fatty acids

## Declarations

### Ethics approval and consent to participate

The study was approved by the Ethics Committee of the Xiangyang No.1 People’s Hospital (2017GCP0531). All participants received information concerning their participation in the study and gave written informed consent.

### Consent for publication

Not applicable.

### Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

### Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

All authors participated in the conception and design of the study; FZ and QZ conceived and drafted the manuscript; XF, MLX and JT collected fecal and extracted DNA from all samples; YZ and PG collected the basic patient information and imaging data; FZ, QZ, XDS analyzed the data; YZ and MS reviewed the manuscript; PQW supervised the project and revised the manuscript. All authors read and approved the final manuscript.

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Authors' Information

1Department of Neurology, Xiangyang No.1 People's Hospital, Hubei University of Medicine, No.15 Jiefang Road, Xiangyang 441000, China

2Hubei Clinical Medicine Research Center for Parkinson's Diseases, No.15 Jiefang Road, Xiangyang 441000, China

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Tables

Table 1. Characteristics of the study subjects
|                        | PD group (n=46) | Spouse group (n=46) | P value |
|------------------------|-----------------|----------------------|---------|
| Female, n(%)           | 20 (43.5%)      | 26 (56.5%)           | 0.211   |
| Age (years)\(^a\)     | 63.6±6.9        | 63.8±7.0             | 0.869   |
| Constipation, n(%)     | 29 (63.0%)      | 2 (10.7%)            | < 0.001 |
| Onset age (years)\(^a\)| 60.0±6.5        | —                    |         |
| Disease duration (years)\(^b\) | 3.0 (1.0-5.0) | —                    |         |
|                        | 12 (26.1%)      | —                    |         |
|                        | 26 (56.5%)      | —                    |         |
|                        | 8 (17.4%)       | —                    |         |
| H&Y stage\(^b\)       | 1.8 (1.0-2.5)   | —                    |         |
| UPDRS total score\(^b\) | 30.5 (20.8-43.0) | —                |         |
| UPDRS \(\alpha\) score\(^b\) | 2.0 (0-4.0)   | —                    |         |
| UPDRS \(\beta\) score\(^b\) | 8.0 (5.0-12.3) | —                    |         |
| UPDRS \(\gamma\) score\(^b\) | 18.5 (11.0-28.0) | —            |         |
| Family history, n(%)   | 4 (9.7%)        | —                    |         |
| Pesticide, n(%)        | 4 (9.7%)        | —                    |         |
| Dysosmia, n(%)         | 19 (41.3%)      | —                    |         |
| Sleep-disorder, n(%)   | 25 (54.3%)      | —                    |         |
| Subtype                |                 |                      |         |
| TD                     | 20 (43.5%)      | —                    |         |
| PIGD                   | 20 (43.5%)      | —                    |         |
| indeterminate          | 6 (13.0%)       | —                    |         |

Data are shown as (mean±SD)\(^a\) or (median [IQR])\(^b\)

PD, Parkinson’s disease; H&Y stage, Hoehn and Yahr stage; UPDRS, Unified Parkinson's Disease Rating Scale; TD, Tremor dominant; PIGD, postural instability and gait difficulty

Table 2. Correlations between species and clinical characteristics.
| Species                | Age b   | Age P  | Duration b | Duration P | H&Y stage b | H&Y stage P | UPDRS total score b | UPDRS total score P |
|------------------------|---------|--------|------------|------------|-------------|--------------|----------------------|---------------------|
| *Prevotella_copri*     | -0.224  | 0.024  | -0.036     | 0.503      | -0.025      | 0.151        | -0.028               | 0.947               |
| *Bacteroides_stercoris* | 0.059   | 0.832  | 0.041      | 0.017      | 0.011       | 0.776        | 0.263                | 0.744               |
| *Parabacteroides_merdae* | -0.094  | 0.688  | -0.170     | 0.016      | -0.013      | 0.686        | -0.772               | 0.037               |
| *Alistipes_onderdonkii* | -0.754  | 0.036  | -0.068     | 0.379      | -0.069      | 0.002        | -0.809               | 0.179               |
| *Bacteroides_fragilis* | 0.310   | 0.307  | 0.031      | 0.593      | -0.008      | 0.684        | 0.184                | 0.682               |
| *Escherichia_coli*     | 0.003   | 0.996  | -0.087     | 0.569      | 0.067       | 0.166        | 0.689                | 0.562               |
| *Butyrivibrio_crossotus* | 0.090   | 0.842  | -0.097     | 0.187      | -0.033      | 0.295        | -0.025               | 0.971               |

7 significant species (average relative abundance more than 0.01%) were screened by Spearman's test based on the group factors (PD and Spouse group, *P* < 0.05). Results of the GLMs show the correlations between the fecal microbiota (transformed by ALR) and PD clinical characteristics.

**Figures**
Figure 1

Phylogenetic tree analysis of all samples
Different composition of gut microbiota between PD and Spouse groups. a Clustered heatmap based on top 50 species relative abundance. Horizontal showed sample information; longitudinal showed species information. The value showed the Z score obtained after the relative abundance of species was standardized. b Top 15 highly abundant species of PD and Spouse groups. *P < 0.05. c PCoA analysis of the microbiota composition among PD and Spouse groups.
Figure 3

Different composition of gut microbiota between different age subgroup of PD patients. a Relative abundance of Prevotella_copri between patients and spouses of different age subgroups. *P < 0.05 when compared to PD aged “< 60” ; #: P<0.05 when compared to PD of paired age. b PCoA analysis of the microbiota composition among PD of different age subgroups

Figure 4
Disease status predictive model based on species. a 6 important species filtered from 23 species by the Boruta feature selection algorithm. b ROC curves for distinguishing PD patients from spouses using 6 species from Boruta feature selection algorithm

Figure 5

Functional pathways of gut microbiome between PD and Spouse groups. a STAMP analyze of pathways mapped to MetaCyc databases. b LefSe analyze of pathways mapped to MetaCyc databases. c Heatmap based on the relative abundance of top 50 KOs mapped to KEGG orthology database. The value showed the Z score obtained after the relative abundance of KOs was standardized. d PCoA analysis of the COGs mapped to EggNOG database among PD and Spouse groups. e PCoA analysis of the GOs mapped to GO database among PD and Spouse groups
Figure 6

Correlations between functional pathways and clinical features. Colors indicate the Spearman correlation coefficients. *P < 0.05

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- EggNOGdatabasemappedresult.xlsx
- KOdatabasemappedresult.xlsx
- Sequencedataofallsubjects.xlsx
- MetaCycdatabasemappedresult.xlsx
- GOdatabasemappedresult.xlsx
- Correlationbetweenengutmicrobiotaandnonmotorsymptoms.docx
- CharacteristicsofPDsubjects.xlsx