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Gut microbiota in patients with Parkinson's disease in southern China

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ABSTRACT

Introduction: Accumulating evidence has revealed alterations in the communication between the gut and brain in patients with Parkinson's disease (PD), and previous studies have confirmed that alterations in the gut microbiome play an important role in the pathogenesis of numerous diseases, including PD. The aim of this study was to determine whether the faecal microbiome of PD patients in southern China differs from that of control subjects and whether the gut microbiome composition alters among different PD motor phenotypes.

Methods: We compared the gut microbiota composition of 75 patients with PD and 45 age-matched controls using 16S rRNA next-generation-sequencing.

Results: We observed significant increases in the abundance of four bacterial families and significant decreases in the abundance of seventeen bacterial families in patients with PD compared to those of the controls. In particular, the abundance of Lachnospiraceae was reduced by 42.9% in patients with PD, whereas Bifidobacteriaceae was enriched in patients with PD. We did not identify a significant difference in the overall microbial composition among different PD motor phenotypes, but we identified the association between specific taxas and different PD motor phenotypes.

Conclusions: PD is accompanied by alterations in the abundance of specific gut microbes. The abundance of certain gut microbes was altered depending on clinical motor phenotypes. Based on our findings, the gut microbiome may be a potential PD biomarker.

1. Introduction

Parkinson's disease (PD) is a neurodegenerative disorder characterized by the progressive accumulation and aggregation of misfolded α-synuclein (α-syn) and other proteins, forming the neuropathological hallmark of the disease (Lewy bodies) in the substantia nigra [1,2]. Postmortem studies have also detected Lewy bodies in the enteric nervous system [3–5]. In addition, a wide spectrum of non-motor symptoms, such as hyposmia, gastrointestinal dysfunction, constipation, and cardiovascular and urogenital abnormalities, have been reported to precede the onset of motor symptoms in patients with PD by years [6–8]. As shown in recent studies, gastric motility disorders, particularly constipation, are observed in 70–100% of PD patients [9–11]. These evidences associated with the clinical finding that gastrointestinal tract is involved in PD.

The human gut hosts tens of trillions of microorganisms and may be regarded as an extracorporeal organ system; therefore, a well-balanced gut microbiota is critical for maintaining general health [12,13]. Emerging findings have linked alterations in the composition of the gut microbiota to a range of disorders, including inflammatory, metabolic, and oncological disorders, as well as PD [2,14,15]. Increased intestinal permeability has been observed in clinical patients and a mouse model of PD [2,16,17]. Moreover, gut microbiota may impact neuroinflammation and α-syn aggregation via the gut-brain axis in a mouse model of PD [18]. Thus, a wide spread hypothesis that PD pathogenesis may primarily act via the gut. Furthermore, dysbacteriosis may result in

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increased intestinal permeability, which significantly contributes to the spreading of the gut microbiota and its products from the gut to the brain [19,20].

Based on the early gastrointestinal involvement in PD and the effect of alterations in the gut microbiota on intestinal mucosa, we aimed to explore whether patients with PD display differences in the faecal microbiome compared to that of control subjects. These differences may help us identify the role of the gut microbiome in the pathogenesis of PD.

2. Methods

This study was conducted in accordance with the Human Subjects Policies and Guidance policy. The protocol was approved by the Ethics Committee of Zhujiang Hospital of Southern Medical University. All participants provided informed consent, according to the principles of the Declaration of Helsinki.

2.1. Study subjects and clinical data

One hundred twenty participants were enrolled from the Department of Neurology of Zhujiang Hospital, Southern Medical University, from May 2016 to April 2017.

All patients with PD (n = 75) were diagnosed by a movement disorder specialist according to the 2015 Clinical Diagnostic Criteria for Parkinson's disease, from the International Parkinson and Movement Disorder Society, and were receiving the medications to treat PD at the time of this study. Four patients with PD accepted deep brain stimulation (DBS). Exclusion criteria for subjects with PD were as follows: (1) secondary Parkinsonism; (2) intake of probiotics or antibiotics within the last three months; (3) chronic and inflammatory gastrointestinal diseases; and (4) an unstable medical, neurological, or psychiatric illness.

The inclusion criteria for control subjects (n = 45) were as follows: (1) a spouse of a participant in the PD group; (2) no use of probiotics or antibiotics within the last three months; (3) no neurodegenerative disease; and (4) no history of chronic or acute gastrointestinal disorders.

Disease severity was measured in the “off” state using the Unified PD Rating Scale (UPDRS part III) and Hoehn&Yahr (H&Y) stage. The degree of constipation was assessed with the Wexner constipation score. Clinical data were recorded, such as age, sex, and medications. On the day of stool sample collection, all the participants completed a questionnaire assessing their dietary habits within the last 3 months.

2.2. 16S rRNA amplicon analysis

Native stool samples were collected as described in a previous study [21]. Fresh faecal samples were immediately frozen at −80 °C for subsequent analysis. Genomic DNA was extracted from faecal samples using a Huirui.® DNA kit according to the manufacturer's protocols. We amplified the highly conserved V4 region of the bacterial 16S ribosomal RNA gene using polymerase chain reaction (PCR). PCR was performed with bacterial-specific primers V4T9 (5′-GTGTGYCAGCMG-CCGCGG TAA-3′) and V4R19 (5′-CCGGACTACNVGGGTWTCTAAT-3′). The following reaction conditions used: an incubation at 94 °C for 2 min, followed by 30 cycles of denaturation at 94 °C for 30s, annealing at 52 °C for 30s, and elongation at 72 °C for 45s, and a final elongation step at 72 °C for 5 min. Products were purified using paired-end sequencing on the Illumina HiSeq PE250 platform (Illumina, Inc., USA). Paired-end read pairs were then assembled using SeqPrep, and the resulting consensus sequences were demultiplexed, trimmed of artificial barcodes and primers, and assessed for chimaeras using UCHIME in closed mode, as implemented in Quantitative Insights into Microbial Ecology (QIIME; release v.1.9.1). Quality checked, trimmed sequences were then clustered into Operational Taxonomic Units (OTUs) using SortMeRNA (2.0) with the Greengenes database (V13.8) at 97% similarity. SortMeRNA was used to classify these sequences into specific taxa using the Greengene's database. Rarefaction at 3420 resulted in the exclusion of two samples.

2.3. Statistical analysis

We performed an Adonis analysis of BMI, dietary habits, age, gender, constipation and disease duration, with statistical significance determined at an alpha of 0.05. Alpha diversity metrics, including Chao1, Shannon's index, PD whole tree, and observed OTUs, were calculated using QIIME. Beta diversity metrics, including UniFrac weighted or unweighted metrics, were calculated using QIIME. The Adonis analysis was used to estimate the dissimilarity of the microbial composition between groups. Unweighted-UniFrac distances based on the phylogenetic metric were used for Adnosis analyses. Linear discriminant analysis (LDA) effect size (LEfSe) was the algorithm used for high-dimensional biomarker discovery and an explanation that identifies genomic features (genes, pathways, or taxa) characterizing the differences between two or more biological conditions (or classes). It emphasizes statistical significance, biological consistency and effect relevance, allowing researchers to identify features with different abundances that are also consistent with biological behaviours. The LEfSe analysis includes a nonparametric factorial Kruskal-Wallis rank sum test to identify taxa with differential abundances. A LDA was used to calculate a LDA score and estimate the effect size of each differentially abundant feature and, if desired by the investigator, to perform dimension reduction. Alpha values of 0.05 were used with a threshold on the logarithmic score of LDA of ≥2.0, consistent with reports from other groups in the field [22]. All the results presented here are summarized after applying the tool to 16S rRNA gene and whole genome shotgun datasets to detect bacterial organisms and functional characteristics with different abundances between two or more microbial environments [22]. All P values were adjusted for multiple testing using the false discovery rate (FDR) calculation.

3. Results

3.1. Demographics and clinical data

Detailed demographic and clinical data are presented in Table 1. Most of the studied variables were similar in patients and controls. Only age ($P = 0.033$), BMI ($P = 0.003$), disease duration ($P = 0.032$) had effects on the microbial composition, but FDRadjusted *P*values failed to reveal the effects of age and disease duration on the microbial composition. Although patients with PD had a significantly greater severity of constipation than did controls, constipation was not a significant covariate for the microbial composition (8.81 ± 5.03 versus 2.04 ± 1.02, FDR adjusted P value = 0.753). Dietary habits had no effects on the microbial composition (FDR adjusted P value = 0.960). Only four patients were using COMT inhibitors (Table 1).

3.2. Gut microbiome

Shannon's, PD whole tree and observed OTUs indexes were not significantly different between patients with PD and controls (unadjusted P value = 0.74, 0.162, 0.233, respectively, Wilcoxon Test). Only Chao 1 index (unadjusted P value = 0.022) showed significant difference in community alpha diversity between PD patients and controls. Thus, the samples from patients with PD did no display statistically differences in community structure compared with samples from controls. As shown in Fig. 1B, the samples from patients with PD and controls exhibited a significant difference in the microbial composition (unweighted UniFrac distance, unadjusted P value < 0.001).

Significant differences in abundance were observed at all levels. We only displayed the data on microbial differences at the phylum and