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Full-length Article

Alteration of the fecal microbiota in Chinese patients with Parkinson's disease

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ABSTRACT

Emerging evidences suggest that gut microbiota dysbiosis plays a role in Parkinson's disease (PD). However, the alterations in fecal microbiome in Chinese PD patients remains unknown. This case-control study was conducted to explore fecal microbiota compositions in Chinese PD patients. Microbiota communities in the feces of 45 patients and their healthy spouses were investigated using high-throughput Illumina Miseq sequencing targeting the V3-V4 region of 16S ribosomal RNA (rRNA) gene. The relationships between fecal microbiota and PD clinical characteristics were analyzed. The structure and richness of the fecal microbiota differed between PD patients and healthy controls. Genera *Clostridium IV, Aquabacterium, Holdemania, Sphingomonas, Clostridium XVIII*, *Butyricicoccus* and *Anaerotruncus* were enriched in the feces of PD patients after adjusting for age, gender, body mass index (BMI), and constipation. Furthermore, genera *Escherichia/Shigella* were negatively associated with disease duration. Genera *Dorea* and *Phascolarctobacterium* were negatively associated with levodopa equivalent doses (LED). Among the non-motor symptoms (NMSs), genera *Butyricicoccus* and *Clostridium XlVb* were associated with cognitive impairment. Overall, we confirmed that gut microbiota dysbiosis occurs in Chinese patients with PD. A well-controlled population involved was beneficial for the identification of microbiota associated with diseases. Additionally, the fecal microbiota was closely related to PD clinical characteristics. Elucidating these differences in the fecal microbiome will provide a foundation to improve our understanding the pathogenesis of PD and to support the potentially therapeutic options modifying the gut microbiota.

1. Introduction

Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by motor and non-motor symptoms that currently affects approximately 1.7% of people over 65 years of age in China (Zhang et al., 2005). The cause of PD remains unknown. Both the clinical symptoms (Adams-Carr et al., 2016; Cersosimo et al., 2013) and pathological changes (Braak et al., 2006; Shannon et al., 2012) of PD indicate that intestinal dysfunctions appear even earlier than the typical PD motor symptoms and pathogenesis (α-synuclein (α-syn) forms). Direct evidences of α-syn forms spreading from the gastrointestinal tract to the brain in rats had supported the hypothesis that PD might begin in the gut (Holmqvist et al., 2014).

The gut microbiota, which is also referred to as the second brain, may affect brain activity through the gut-microbiota–brain axis under both physiological and pathological conditions (Wang and Kasper, 2014). The evidence base is growing to discover that gut microbiota may play a key role in the progress of PD. The experiments in α-*syn*-overexpressing mice suggested a role of gut microbiota in promoting microglia activation and α-syn aggregation, as well as motor deficits (Sampson et al., 2016). Additionally, a recent study also demonstrated that gut microbiome dysbiosis might contribute to rotenone-induced toxicity in mice by our team (Yang et al., 2018). While for the human beings, emerging studies from North America and Europe using 16S ribosomal RNA (rRNA) gene sequencing have shown that patients with PD exhibit gut microbiota dysbiosis during the past three years

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(Heintz-Buschart et al., 2017; Hill-Burns et al., 2017; Hopfner et al., 2017; Keshavarzian et al., 2015; Petrov et al., 2017; Scheperjans et al., 2015). However, there are clearly major differences in the bacterial profiles of gut dysbiosis that have been reported to be associated with PD in different world populations. The geographic origin had a greater impact on the composition of the gut microbiota than body mass index (BMI) and gender (Escobar et al., 2014). Meanwhile, the diet in China is quite different from Western diets (Li et al., 2017). Increasing evidence indicates that the key factor in determining gut microbiota composition is diet (David et al., 2014; Graf et al., 2015). Particularly, couples share more of their gut microbiota than individuals from different households (Song et al., 2013), and thus, the spouses of PD patients could serve as controls to minimize variation caused by diets.

In the current study, we analyzed and compared the microbiota communities in the feces of PD patients with those of their healthy spouses using 16S rRNA gene sequencing. Furthermore, the relationships between fecal microbiota and PD clinical characteristics were analyzed.

2. Materials and methods

2.1. Study subjects

Each participant was informed of the purpose of this study, and all measurements and questionnaires were voluntary. All enrolled subjects provided written informed consent. This study protocol was approved by the Research Ethics Committee, Ruijin Hospital, Shanghai Jiaotong University School of Medicine, Shanghai, China.

We recruited 70 idiopathic PD patients and their healthy spouses living in same household from the Movement Disorders Clinic at the Department of Neurology of Ruijin Hospital from June 2015 to February 2016. A total of 45 patients (23 [51.1%] female; mean [standard deviation, SD] age 68.1 [8.0] years) and their healthy spouses (22 [48.9%] female, mean [SD] age 67.9 [8.0] years) were included in the final analysis (recruitment flowchart provided in Fig. 1). All PD patients eligible for this study were diagnosed with PD according to the UK Brain Bank criteria (Daniel and Lees, 1993). Exclusion criterions for patients were serious chronic illnesses (e.g., diabetes, heart failure, liver cirrhosis, malignancy, or hematological or autoimmune diseases). The healthy controls exhibited no disease symptoms. In particular, each participant was assessed using the Rome III Criteria to exclude irritable bowel syndrome (IBS) (Koloski et al., 2015), which has been shown to influence gut microbiota (Kassinen et al., 2007). Individuals currently taking antibiotics or probiotic supplements within the three months prior to sample collection were also excluded.

2.2. Clinical data collection

Clinical data were collected through face-to-face interviews with movement disorder specialists. Each subject's weight and height were measured, and the body mass index (BMI) was calculated. PD clinical characteristics included disease duration, age of onset, motor and non-motor symptoms, medication and motor complications (according to the Unified Parkinson's Disease Rating Scale, UPDRS part IVA and IVB) (Chapuis et al., 2005). The UPDRS and the Hoehn and Yahr stage (H&Y stage) of patients were examined during the "on" state. Levodopa equivalent doses (LED) were calculated using a method reported in a previous study (Tomlinson et al., 2010).

PD-related non-motor symptoms (NMSs) were evaluated using the Non-Motor Symptoms Questionnaire for Parkinson's disease (NMS-Quest), Hamilton Anxiety Scale (HAMA), Hamilton Depression Scale (HAMD), Mini Mental State Examination (MMSE), and Montreal Cognitive Assessment (MoCA). Constipation was assessed using the Rome III Criteria (Longstreth et al., 2006).

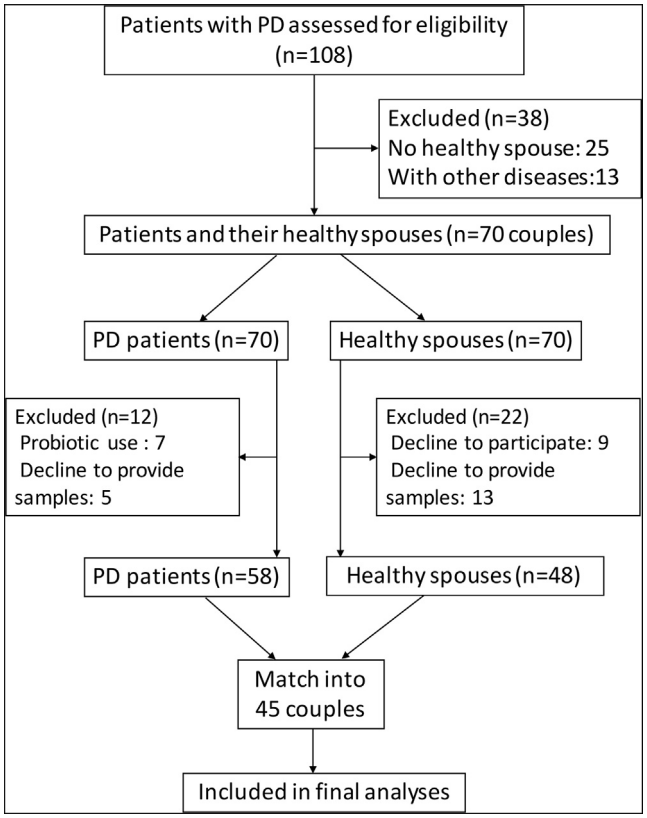

Fig. 1. Flowchart illustrating the recruitment of patients with PD and their healthy spouses based on the exclusion and inclusion criteria.

2.3. Sample collection and DNA extraction

Each study participant was asked to collect a fecal sample in the morning using fecal collection containers. The containers were transferred on ice and stored at -80 °C prior to processing. Total fecal DNA was extracted using a QIAamp DNA Stool Mini Kit (Qiagen, Hilden, Germany). All the procedures of DNA extraction were prepared under a Class II biologic safety cabinet. The concentration of genomic DNA in each fecal sample was quantified using a NanoDrop 2000 spectrophotometer (Thermo Scientific, MA, USA). DNA integrity and sizes were assessed using 1% agarose gel electrophoresis (AGE). The DNA was re-suspended in H_2O and stored at -80 °C prior to use.

2.4. 16S rRNA gene amplicon and sequencing

Universal primers (341F and 806R) linked with indices and sequencing adaptors were used to amplify the V3-V4 regions of the 16S rRNA gene. The amplicons were sequenced on an Illumina Miseq platform to obtain 300-bp paired-end reads. The total samples resulted in 9,161,827 clean reads with an average of 101,798.1 ± 24,891.1 clean tags per sample. Considering the sequencing saturation and integrity of each sample, 34,000 tags were randomly calculated for each sample. Detailed descriptions of the amplicons and the sequencing analysis protocol are provided in the eMethods in the Supplementary data.

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