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Research paper

Prevotella and Klebsiella proportions in fecal microbial communities are potential characteristic parameters for patients with major depressive disorder

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ABSTRACT

Background: The diagnosis of major depression disorder (MDD) and other mental disorders were depended on some subjective survey scales. There are confirmed relationship between the gut flora and the mental states of MDD patients.
Methods: The V3–V4 region of the 16S rRNA gene was extracted from the fecal microbial communities in MDD patients, PCR amplified and sequenced on the Illumina Miseq platform.
Results: More phylum Firmicutes, less Bacteroidetes, and more genus Prevotella, Klebsiella, Streptococcus and Clostridium XI were found in MDD patients. The changes of the proportion of Prevotella and Klebsiella were consistent with Hamilton depression rating scale.
Limitations: The conclusion was limited by small sample sizes and potential uncontrollable influence factors on fecal microbiota.
Discussion: Prevotella and Klebsiella proportion in fecal microbial communities should be concerned in the diagnosis and therapeutic monitoring of MDD in future.

1. Instruction

Major depressive disorder is one of the major and common psychiatric disorders with high rates of self-harm and suicide attempts. The real causes and pathogenesis of major depressive disorder are not well understood (Hegerl et al., 2013). A diverse contribution of genetic, neurochemical and environmental factors are involved in the onset and progression of depression (Wang et al., 2015). The bidirectional interactions between the central nervous system, the enteric nervous system, and the gastrointestinal tract have illustrated the influences on the emotional behavior, stress and pain modulation systems and brain neurotransmitter systems in several rodent animal experiments (Carabotti et al., 2015; Mayer et al., 2015). That means the composition and changes of gut flora can influence and interference mental states of major depressive disorder patients, even the diet and other factors can influence gut microbiota composition and may influence depressive illness (Dash et al., 2015).

There are about 10^{14} microorganisms cover on human intestine and more than 80% of them are uncultivated. To analyze the gut bacterial diversity, many culture-independent methods, such as clone library, real-time quantitative polymerase chain reaction (qPCR), and denaturing gradient gel electrophoresis, have been applied and investigated in these years where the majority of the gut bacteria are not culturable (Chen et al., 2011).

High-throughput sequencing can detect hundreds of thousands to millions of DNA molecules in one time and it is a cost-effective means of comprehensive analysis of the intestinal microbial community. The reading length is shorter than previous sequencing technique. In these new sequencing techniques, 454 pyrosequencing on 16S rRNA genes has expanded with low-cost, high-throughput sequencing instruments. Now pre-existing 454 pyrosequencing workflows can transfer to Illumina MiSeq sequencing by changing the sequencing adapters of

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the primers (Nelson et al., 2014). In this study, the V3-V4 hypervariable regions of the 16SrRNA gene were detected with Illumina MiSeq sequencers to identify the gut bacterial diversity in patients with Major depressive disorder (Kang et al., 2013).

Hamilton depression rating scale (HAM-D) was used to evaluate the mental states of MDD patients. There are 17 quantitative parameters in this well accepted evaluation criteria. Naseribafroue's and Jiang's studies (Jiang et al., 2015; Naseribafrouei et al., 2014) illustrated the relationship between MDD and fecal flora. Here we reported the dynamic observation of the fecal flora composition and the correlation between the specific bacterial compositions and the HAM-D scores.

2. Methods

2.1. Ethics statement

The institutional Review Board (IRB) at Shanghai Mental Health Center, Shanghai Jiao Tong University School of Medicine approved the study. Patients who expressed interest in joining this study were introduced the details and signed the written consent forms.

2.2. Subject recruitment and study procedure

Sixty MDD patients were recruited in this study, and another 60 people were selected as control. The basic enrollment criteria of the patient recruitment are: (1) age 20–85 years; (2) no usage of any type of antibiotic, antifungal medications or any probiotics and probiotics related drink within the last month; (3) MDD Group: the patients with depression were assessed with the major depressive disorder criteria in the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV-TR) and 17-items HAM-D scales ≥23; (4) Control Group: in good mental and physical health: no stomach/gut problems such as chronic diarrhea, constipation, gas, heartburn, bloating, etc. Unrelated to an individual with MDD. All MDD subjects in this study received a single escitalopram treatment as 10 mg once per day. Patients with change of dose during the study period will be excluded from the study.

In this study there are three visits occurred on day 1, day 15 and day 29. In each visit, besides the vital signs and other regular physical and biochemical tests, a revised HAM-D assessment was used to evaluate the mental states of each patients and fecal samples was selected at the same day. This revised 15-items HAM-D assessment was based on the original 17-items HAM-D Rating Scale removed two potential gut associated “parameters somatic symptoms - gastrointestinal” and “loss of weight”. To evaluate the correlation between the revised HAM-D scores and the fecal bacterial composition, we selected the patients those revised HAM-D scores continuously reducing in the 3 visits and sent their specimen for gene sequencing. After that, the bacteria with most significant differences in the 3 visits will selected to be verified with genus specific real-time PCR.

2.3. Sample collections and DNA extraction

Fecal samples (200 mg) were immediately frozen on collection and stored at −70 °C before analysis. Single fecal sample was selected from each subject in each visit. One aliquot was added to a 2.8 mL ASL lysis buffer from the Tiagen DNA Stool Mini Kit (Tiagen Biotech, Beijing, China). Subsequent steps of DNA extraction followed the Tiagen kit protocol for Stool DNA extraction. The quantity and quality of DNA were assessed by measuring absorbance at 260 and 280 nm using a NanoDrop ND-2000 spectrophotometer (NanoDrop Technology, Rockland, DE). Before we sent genomic DNA samples to the sequencing facility for MiSeq sequencing, we confirmed PCR amplification with universal bacterial primers, and ran agarose gel (1%, w/v) electrophoresis to confirm the efficiency of PCR amplification visually (Kang et al., 2013).

2.4. 16S rRNA Gene sequencing and analysis

The V3–V4 region of the 16S rRNA gene was PCR amplified with primer 343F (TACGGRAGGCAGCAG) and 798R (AGGGTATCTAATCCT) (Nossa et al., 2010). The resulting amplicons were purified, quantified, and barcoded with custom primers and sequenced on the Illumina Miseq platform (paired end, 2×300) according to manufacturer's protocols by Shanghai Yuanxu Biotechnology Co. Ltd. Sequences were analyzed with the mothur software package (v.1.30) (Kozich et al., 2013; Schloss et al., 2009). Briefly, matched paired-end reads were assembled using ‘make.contigs’ command in mothur package, and any sequences with an ambiguous base were removed. Sequences were aligned to a reference database (Silva v.119, provided in mothur), and sequences aligned to incorrect region were culled. After the end trimming of the sequences (Schloss, 2013), the unique sequences were further denoised using a precluster-ing algorithm (Schloss et al., 2011) and their frequency in each sample was calculated. Chimeras in the sequences were removed by use of UCHIME (Edgar et al., 2011) and the resulting sequences were then classified using the Bayesian classifier (Wang et al., 2007). Finally, sequences were clustered into operational taxonomic units (OTUs) at a 3% dissimilarity level. Each OTU was assigned a taxonomic classification at all levels from phylum to genus using the reference Silva database. Species richness and diversity indices were estimated by calculating ACE and Chao, as well as the Shannon and Simpson diversity indices using mothur (Schloss et al., 2009). Data tables were constructed using several custom R scripts and the vegan package. Principal coordinates analysis (PCoA) was applied to the distance matrices for visualization and was plotted against each other to summarize the microbial community compositional differences between samples.

2.5. Verification of the clinical significance of bacterial constitution of MDD by genus specific real-time PCR

The original fecal samples from late excluded MDD patients were selected to verify the clinical significance of these genera by real-time PCR. The corresponding genus specific primers were referred from previous studies (Anbazhagan et al., 2011; Matsuki et al., 2002; Picard et al., 2004; Song et al., 2004): CACRGTAAACGATGGATGCC and GGTCGGGTTGCAGACC, ACGCTACTTGAGGAGGA and GAGC CGTAGCCTTTCACT, GTACAGTTGCTTCAGGACGTATC and ACGTTCGATTTCATCACGTTG, CATCTCGATCTGCTGGCCAA and GCGCGGATCCAGCGATTGGA were used for genus Prevotella, Clostridium cluster XI, Streptococcus and Klebsiella, respectively.

2.6. Statistical analysis

The statistical software SPSS17.0 was used in this study. The significance of differences between the means of bacterial composition in fecal samples was analyzed by the double-sided Student's *t*-test and Wilcoxon's Sign Rank Test for phylum level and genus level comparitions respectively. A P value < 0.01 was considered significant.

3. Results

3.1. Subject characteristics

Ten MDD patients performed the study procedure completely, that means total 30 patients’ stool specimen were collected for metagenomic sequencing in this study. Another 10 control stool samples were randomly selected from the 60 subjects. The common characteristics of 10 MDD patients and control subjects were shown in Table 1.