The changes of gut microbiota in drug-naïve first-episode MDD patients under treatment by SSRIs

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Abstract

Background: Recently, several studies reported that transplanting stool from depressed patients could induce depression-like behaviors in mice. In addition, antidepressants presented not only antidepressant effects, but also antibacterial effects in those animals. Therefore, this study firstly investigated on the changes of gut microbiota in depressed patients under effective antidepressant treatment.

Methods: We recruited 30 patients with drug-naïve first-episode MDD (Patients group) and 30 healthy controls (Control group), and collected their stool samples to complete 16S rRNA sequencing. Next, Patients group received 15-20 mg/d of escitalopram. After the symptoms improved, the feces of Patients group were collected and marked as Follow-up group to complete sequencing for the second time. We then investigated into the differences of gut microbiota between patients (Patients and Follow-up groups) and controls (Control group), the characteristics of gut microbiota under treatment, and the potential differences in metabolic functions.

Results: A significant difference in gut microbiota abundance was found after escitalopram treatment. The Firmicutes/Bacteroides ratio significantly decreased in the Follow-up group. After treatment, the species diversity of gut microbiota tended to be back to normal state in Follow-up group. The mainly difference of metabolic function were found as follows: Transport and catabolism, Nervous system, Glycan biosynthesis and metabolism.

Conclusions: Under escitalopram treatment, the gut microbiota diversity of MDD patients tended to back to normal state. However, several structures and metabolic pathways in microbes remained differences between patients and controls, which might be related to the relapse of MDD.

Background

As a common mental disorder accompanied by high disability and suicide, [major depressive disorder](#) (MDD) has become a worldwide issue concerned [1]. In recent years, more and more evidences were reported to indicate that gut microbes might take part in the course of MDD [2-4]. In several paralleled researches, MDD patients showed specific features of gut microbes differing from normal controls [5-8]. Several studies have reported that transplantation of stool from MDD patients into rodents could lead to depression-like behaviors, which had been used to establish animal models of MDD [5, 9, 10]. The similar phenomenon was observed in fecal bacteria transplantation between animal models. Moreover, it also observed that fecal bacteria transplantation could lead to the increase of microglial cell density and expression of IL-1 in the ventral hippocampus [11]. Taken together, the change of gut microbiota could be correlated with the occurrence of MDD.

At present, selective serotonin reuptake inhibitors (SSRIs) are widely used in clinic and have therapeutic effects in the treatment of depression [12]. Besides, several SSRI's drugs, including sertraline, fluoxetine, paroxetine and escitalopram, could present antibacterial effects directly [13, 14]. For example, staphylococcus and enterococcus are especially vulnerable to sertraline, fluoxetine and paroxetine [14-

16]. The SSRIs could target on potential enterotoxigenic bacteria as well. Therefore, SSRIs behave both of antidepressant and antimicrobial properties [17]. The mechanism underlying these interactive dual effects remains as an ongoing challenge.

Overall, SSRIs could directly or indirectly influence the changes of gut microbiota which might play the key role in the development of MDD. The purpose of this study was to further exploring the changes and effects of gut microbiota through 6S rRNA gene sequencing analysis on the feces of patients with first episode MDD by SSRIs treatment.

2. Method

2.1 Participants

The 60 local subjects, including 30 depressed patients and 30 normal subjects, were recruited in this study. The inclusion criteria for subjects described as follows: (1) Age was between 18-65 years old; (2) Body mass index (BMI) was between 18-28 Kg / m²; (3) No history of treatment with antipsychotic medication; (4) Duration of symptoms was between 1 to 24 months; (5) No history of treatment with antidepressant medication; (6) Currently in the acute episode with the Hamilton Depression Rating Scale for Depression (HAMD) score ≥ 24 ; (7) Other mental disorders such as axis I, personality disorder and mental retardation were excluded; (8) Psychotropic drugs were never used; (9) Diagnosis of depression in Patients group was made by two psychiatrists according to the Mini-International Neuropsychiatric Interview (MINI) [18]; (10) In Controls group, a diagnosis of mental disorder was excluded by two psychiatrists according to the MINI, and HAMD-17 score was < 7 ; (11) Patients group was defined as Follow-up group after receiving the drug treatment.

In addition, a series of exclusion criteria, based on the previous work, were employed in this study to exclude the factors affecting gut microbiota [19]. Those include: (1) No somatic diseases known to affect the gut microbiota such as inflammatory bowel disease, immune system diseases, diabetes, etc.; (2) Without antibiotics, probiotics or microbiological products used in recent 3 months; (3) Without the history of medical examination or surgery through the gastrointestinal tract in recent 6 months; (4) Without obvious changes in dietary habits or the presence of obvious diarrhea, constipation and other symptoms in recent 1 month.

According to the questionnaire, subjects' life events that may affect the mood, such as examinations, unemployment and bereavement during the last six months and the whole research period, were surveyed and recorded. All of subjects in this study were required to sign an informed consent. According to the Helsinki Declaration, the protocol for sample collection and analysis was approved by the Ethics Committee of Peking University Sixth Hospital and Beijing Hospital of Chinese Traditional and Western Medicine.

2.2 Sample collection

Fecal samples were obtained from subjects enrolled. Subjects were instructed by staff to discharge feces into a clean container. After defecation, the staff collected 2 g fecal sample and quickly placed it into a container containing liquid nitrogen. The samples were then transferred to -80 °C refrigerator within 1 hour and kept for the following experiments.

2.3 Treatment

All patients with depression received standardized treatment with escitalopram. The starting dose of escitalopram was 5 mg / d from day 1- day 7 and increased to 10 mg / d from day 8. According to the individual response, the dose of escitalopram could be adjusted, and the maximum dose was 20 mg / d. After 4-6 weeks of treatment, the patients were evaluated by HAMD scale. When the scale reduction rate of HAMD is $\geq 50\%$ compared with baseline, their fecal sample was collected for the second time and recorded as 'Follow-up group' to be used in the comparison of gut microbiota.

2.4 16S rRNA Amplification of V3-V4 region and Illumina Sequencing

Using a PowerSoil DNA kit (MoBio, USA), DNA extraction was performed from 200 mg fecal samples according to manufacturer's instructions. KAPA HiFi HotStart ReadyMix (KAPA, USA) was used to amplify the 16S rRNA (V3-V4) gene marker. Each DNA sample of the bacterial 16S rRNA gene was amplified with primers 341F (GGACTACHVGGGTWTCTAAT) and 805R (ACTCCTACGGGAGGCAGCAG). The primers include a unique 8-nucleotide barcode and an Illumina adapter. Polymerase chain reaction (PCR) conditions were set as follows: initial denaturation at 95°C for 5 minutes, 98°C denaturation for 20 cycles for 20 seconds, 58°C annealing for 30 seconds, 72°C extension for 30 seconds, and 72°C final extension for 5 minutes. The amplicons obtained by PCR were analyzed on 1.5% agarose gel electrophoresis, and a band of a desired size was purified using a QIAquick gel extraction kit (QIAGEN, Germany). The product was submitted to the second-generation sequencing laboratory of Beijing institute of bioinformatics for sequencing on Illumina HiSeq 2500 platform.

2.5 Bioinformatics analysis

The QIIME software was used to filter and sequence the original sequence to obtain optimized sequences (Tags) [20]. Fragments containing ambiguous characters in the sequence or more than two nucleotide mismatched primers were removed. Usearch software was used to cluster Tags at a similarity level of 97% to obtain OTUs [21]. OTUs were annotated based on the Silva (bacterial) and UNITE (fungi) taxonomy databases. QIIME software was used to generate species richness tables at different taxonomic levels, and R language tools were used to draw community structure maps at each taxonomic level of the sample. The community structure map of each sample was obtained at the level of taxonomy, class, order, family, genus, species.

In order to identify the difference in microbial community richness between the Patients group and Controls group, the Metastats software was used to perform a T test on the species richness data between two groups to obtain the p-value [22]. The q-value was obtained by correcting the p-value.

Species were selected based on p-values or q-values that caused differences in the composition of the two groups of samples. The analysis was performed at the level of phylum, class, order, family, genus, species taxonomy to analyze the significance between groups.

Mothur (version v.1.30) software was used to evaluate the Alpha Diversity Index of the samples [23]. The species diversity within a single sample was studied by Alpha Diversity Analysis, and the Ace, Chao1, Shannon, and Simpson indices of each sample at the 97% similarity level were counted; Beta diversity analysis was performed using QIIME software. Beta diversity analysis mainly used the binary jaccard algorithm to calculate the distance among samples to obtain the β value between samples. Based on the distance matrix obtained from the Beta diversity analysis, PCoA analysis was performed using R language tools to further demonstrate the differences in species diversity among samples [24]. Hierarchical clustering was performed on samples using unweighted paired average method (UPGMA) to determine the similarity of species composition among samples. According to the species abundance table obtained by clustering, spearman correlation coefficient among samples was calculated by Psych package in R language, and then the heatmap was drawn by Pheatmap package in R language. The closer of the calculated Spearman correlation coefficient was to 1, the redder of the color was in the heatmap, thus indicating the stronger correlation of two samples. Then, LEfSe tools were used the Wilcox.test function of the R language STATS package to estimate the impact of the abundance of each component (species) on the effect of the difference between components, so that the comparison of two subgroups can be realized to find the species marker (Biomarker) with significant difference in the abundance.

PICRUSt software was used to compare the species composition information obtained from 16S sequencing data to deduce the functional gene composition in the samples, thereby determining the functional differences between different groups [25]. Using the KEGG orthology database (KOs) in the Kyoto Encyclopedia of Genes and Genomics (KEGG) database, the changes in metabolic pathways of functional genes of microbial communities between different groups were evaluated through differential analysis of KEGG metabolic pathways [26].

2.5 Statistics analysis

Statistical analysis was performed using SPSS19.0 software. Participants' gender, tobacco and alcohol consumption were expressed in terms of proportional or percentages. Independent t-tests, Welch t-tests, and White non-parametric t-tests were used for continuous variables. Pearson chi-square test or Fisher's exact test were used for classification variables. All significance tests were two-sided tests, and $p < 0.05$ or adjusted $p < 0.05$ was considered statistically significant.

3. Results

3.1 Clinical data

In this study, thirty patients with drug-naive first-episode MDD and thirty healthy controls were recruited, respectively. There were no statistically significant differences between patients' group and controls

group in terms of age, height, weight, and tobacco and alcohol consumption (Table 1). Patients group received a standardized treatment and finally got a dose of 20mg/d. The average dose of escitalopram was 16.33 ± 3.46 mg / d. The depressed symptoms of patients improved significantly when the mean time of drugs administration was 34.53 ± 5.18 days and the HAMD score decreased by more than 50%.

Table 1 Demographic characteristics of patients and controls

	Patients (n=30)	Controls (n=30)	p-value
	M±SD	M±SD	
Age	44.83±11.00	43.97±10.57	0.757
Height[m]	1.68±0.07	1.70±0.05	0.171
Weight[Kg]	67.83±6.86	69.21±7.14	0.447
BMI (kg/m2)	23.99±2.05	23.83±2.08	0.761
tobacco(%)*	46.67%	30.00%	0.288
alcohol(%)*	53.33%	33.33%	0.192

*Chi-square test; compared with HCs, $P < 0.05$; BMI: body mass index

3.2 Sequencing data and Bacterial taxonomic composition

Total 4,790,651 original sequences were obtained from 90 samples. After double-end Reads splicing and filtering, a total of 4,444,748 Clean tags were generated. Each sample generated at least 12,039 Clean tags, and an average value were 49,386 Clean tags. Taxonomic annotation of OTUs based on Silva (bacterial) and UNITE (fungi) taxonomic databases.

At phylum level, the dominated gut microbiota were Bacteroidetes, Firmicutes, Proteobacteria and Actinobacteria in three groups. Bacteroidetes and Firmicutes accounted for nearly 90% of the total gut microbiota. At the genus level, the abundance distribution ratio of microbiota was different ($p \leq 0.05$) (Supplementary Table S1). By the calculation, the Firmicutes / Bacteroides ratio of Patients group, Follow-up group and Controls group were 0.64, 0.46, and 0.70, respectively. The ratio in Follow-up group was significantly lower than the other two groups. There were significant differences among the three groups ($p \leq 0.05$) (Figure1).

Between Patients group and Controls group, there were significant differences in the abundance of multiple intestinal flora at the genus level. The relative abundance of Parasutterella, Prevotella_9, Fusobacterium, Prevotella_2, Christensenellaceae_R-7_group, Odoribacter and [Eubacterium]_ruminantium_group in Patients group was significantly lower than that in Controls group. Meanwhile, The relative abundance of Parabacteroides, Lactobacillus, Anaerostipes, Ruminococcaceae_UCG-014 and Dialister was significantly increased in Patients group ($p \leq 0.05$) (Supplementary Table S1).

After escitalopram treatment, the abundance of several gut microbiota, including Christensenellaceae_R-7_group, [Eubacterium] _ruminantium_group and Fusobacterium, in Patients group was lower than that in Controls group and significantly increased in Follow-up group ($p \leq 0.05$). The abundance of gut microbiota Lactobacillus in Patients group was higher than that in Controls group and significantly decreased in the Follow-up group ($p \leq 0.05$). The main change of gut microbiota abundance in Follow-up group was Bacteroides (Supplementary Table S2).

In addition, there were also several differences in the gut microbiota between Follow-up group and Controls group. In Follow-up group, the relative abundance of Parabacteroides, Prevotellaceae, Ruminiclostridium_6, Flavonifractor were significantly increased ($p \leq 0.05$), while that of Prevotella_2, Lachnospira, Collinsella, and Clostridium_sensu_stricto_1 were significantly decreased ($p \leq 0.05$). Moreover, the abundance of Faecalibacterium and Lachnoclostridium in Follow-up group and Patients group was significantly lower than that in Controls group ($p \leq 0.05$) (Supplementary Table S2).

3.3 Diversity analysis

Alpha diversity mainly reflected the richness and diversity of the species in samples. As shown in Figure 3, the Chaos1, Ace, and Shannon indices of Patients group were significantly higher than those of Follow-up group and Controls group, and the Simpson index was significantly lower in Patients group than others. This showed that the number and the diversity of gut microbiota in Patients group were significantly higher than those of Follow-up group and Controls group, and there were statistically differences. Four indices value of Follow-up group was between the values of other two groups, which was significantly different from that of Patients group but not statistically different from that of Controls group. This meant that the Alpha diversity of gut microbiota in patients returned to the normal level. The statistics of Alpha diversity index values of each groups were showed in Table 2.

Table 2 Richness and diversity index values of patients, follow-up and controls

	Patients	Follow-up	Controls
ACE	254.88±2.30	187.39±11.09	173.43±3.80
Chao1	257.38±2.63	188.93±11.07	172.69±4.46
Shannon	3.57±0.07	3.17±0.11	2.99±0.09
Simpson	0.09±0.01	0.12±0.01	0.13±0.01

Mothur (version v.1.30) software was used to calculate the Alpha diversity index for samples. The larger of the index values of Ace and Chao1 showed the greater number of species in the samples. The larger of the Shannon index value and the smaller of the Simpson index value showed more species categories of samples.

Beta diversity was used to compare the similarity of species diversity among different groups. The binary jaccard algorithm was used to calculate β diversity, and there were statistical differences among three groups ($R = 0.273$, $p = 0.001$) (Figure 4). The gut microbiota of Patients group was significantly different from Controls group, and the gut microbiota within Patients group was more similar.

In addition, the gut microbiota profiles of some patients treated with escitalopram were more similar to those of the control group, but the others' profiles remained closer to those of patients. The other analysis methods employed in this study also produced similar results. The unweighted paired average method (UPGMA) was used in the R language tool to perform hierarchical clustering of each groups. It found that the gut microbiota of Patients group was significantly different from that of Controls group, and the gut microbiota of Follow-up group was more like that of Controls group (Supplementary Figure S2).

Spearman correlation coefficient between samples was calculated to draw the heatmap. The closer of the calculated Spearman correlation coefficient was to 1, the redder of the color was in the heat map, thus indicating the stronger correlation between two samples. As shown in Figure 4, the follow-up group could be divided into two subgroups. The gut microbiota profiles of parts of treated patients remained the similar with those of Patients group, while others' profiles were more like those of Controls group. This result suggested that the gut microbiota of parts of patients returned to the normal state during the remission of depression, which might be correlated with the improvement of the disease. We used LEfSe for the quantitative analysis of biomarkers with in two subgroups ($LDA > 4$). We found that several microorganisms could be selected as biomarkers in the two subgroups. Gut microbiota of follow-up group 2, that the subgroup was associated with Patients group, was differently enriched with p_Bacteroidetes, o_Bacteroidales, c_Bacteroidia, g_Prevotella_9, etc. While gut microbiota of follow-up group 1, that the subgroup was associated with Controls group, was differently enriched with p_Firmicutes, p_Actinobacteria, f_Lachnospiraceae, f_Bifidobacteriaceae, o_Bifidobacteriales, c_Actinobacteria, g_Bifidobacterium, etc (Supplementary Figure S3).

3.4 Functional properties predicted by PICRUST

The study considered that the profiles of gut microbiota in Follow-up group could not completely return to the normal state. The PICRUST software was used to compare the species composition information obtained from 16S sequencing data to infer the functional gene composition between patients and controls. Through the annotation of the KEGG metabolic pathway, it found that there were differences in the metabolic pathways of Transport and catabolism, Nervous system, Glycan biosynthesis and metabolism, Cell motility and Membrane transport between Follow-up group and Controls group (Figure 5). In Patients group, the pathways above also had certain differences with those of Control group but not significantly differences with those of Follow-up group. It thus suggested that the gut microbiota of Follow-up group might still involve in the occurrence of depression (Supplementary Table S5).

Discussion

This study demonstrated that the gut microbiota from patients of drug-naïve first-episode MDD was significantly different from that of healthy controls, and the composition and structure of the gut microbiota within patients was more similar. This suggested that the occurrence and development of MDD might be associated with a special and similar group of gut microbiota. In 2011, Manimozhiyan Arumugam *et al.* proposed the concept of enterotypes [27]. In the sequencing results, they reported that the human gut microbiome could be divided into three robust clusters, including Prevotella-enterotype (enterotype P), Bacteroides-enterotype (enterotype B) and Ruminococcus-enterotype (enterotype R). Different enterotypes have characteristic advantages of respective functional states [28]. For example, enterotype P has stronger fermentation ability and can generate more short-chain fatty acids, but enterotype B has more specific enzymes sufficiently to degrade sugars and proteins [29]. Therefore, further research on the enterotypes and corresponding functional changes of gut microbiota would be helpful to understand the impact of gut microbiota on the development of MDD.

Secondly, the gut microbiota tended to return to the normal gut microbiota structure under SSRIs treatment, thus indicating a positive effect of SSRI antidepressants on the change of gut microbiota. However, the gut microbiota was still different from healthy controls in Follow-up group. The results suggested that the recovery of the gut microbiota might take longer time than the improvement of depressive symptoms. The recovered gut microbiota might still partially maintain the pathological state, which would be a trigger for depression recurrence. Currently, there are no effective biomarkers to indicate recurrence in depressed patients. The dynamic changes of gut microbiota under antidepressant treatment provided the potential biomarkers for further investigation [30]. Therefore, it could be determine the duration and degree of recovery through evaluating gut microbiota of patients in the future.

In addition, some studies have found that several antibiotics have antidepressant effects, such as ceftriaxone, minocycline and doxycycline [31-33]. This might further support that the changes of the gut microbiota had a positive effect on the treatment of MDD. Current studies have claimed that minocycline could be used as a synergistic agent for fluvoxamine in the treatment of moderate and severe obsessive-compulsive disorder [34]. This provided a basis for the efficacy and safety of SSRI drugs combined with antibiotics in the treatment of mental illness. Moreover, the transplantation of normal gut microbiota has been reported to be a treatment for MDD [35, 36]. The related treatments were established on the hypothesis of the re-normal of gut microbiota in depression patients. In the future, promotion of healthy gut microbiota could be a new way in the prevention of depression occurrence.

The outcomes of current study were limited to the relatively small sample size and short follow-up time. In this study, we were unable to further observe the correlation between the effects of different doses of antidepressants on gut microbiota. In future studies, the sample size can be further increased and collect stool samples from patients at different doses of antidepressant. On the basis of exploring the changes of gut microbiota, we can further observe the correlation between gut microbiota and clinical phenotypes.

Moreover, metabolomics and proteomics omics technologies are strongly suggested to be employed to elucidate the relationship between gut microbiota and depression.

Conclusion

In conclusion, this study found that the gut microbiota of patients with first-episode depression was similar and less different, and it was significantly different from healthy controls. After escitalopram treatment, gut microbiota diversity of depressive patients tended to transform to the normal state. However, there were still several structures and metabolic pathways difference in the gut microbiota between follow-up patients and healthy controls, which might be relate to the relapse of depression.

Abbreviations

MDD: [major depressive disorder](#)

SSRIs: Selective serotonin reuptake inhibitors

16S rRNA: 16S ribosomal Ribonucleic Acid

HAMD: Hamilton Depression Scale

BMI: Body mass index

OUTs: Operational taxonomic units

KEGG: Kyoto Encyclopedia of Genes and Genomes

UPGMA: unweighted paired average method

PCoA: Principal coordinate analysis

Declarations

Ethics approval and consent to participate

The study was explained to all participants both verbally and in writing, and written informed consent was obtained from each participant. According to the Helsinki Declaration, the protocol for sample collection and analysis was approved by the Ethics Committee of Peking University Sixth Hospital and Beijing Hospital of Chinese Traditional and Western Medicine.

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 on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Author contributions

YS, XY, JYG and YL designed the study and wrote the protocol. XXX and XY, JYG managed the literature searches and analyses. YS, ZYL and YCH undertook the statistical analysis, and YS, XY and GFL wrote the first draft of the manuscript. All authors contributed to and have approved the final manuscript.

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Figures

Figure 1

Histogram of species distribution. QIIME software was used to generate species abundance tables at different taxonomic levels, and R language tool was used to draw community structure charts at different taxonomic levels. (A) relative proportions of species distribution at the phylum level; (B) relative proportions of species distribution at the genus level.

Figure 2

Visualization of Alpha diversity index. (A) Ace index; (B) Chao1 index; (C) Shannon index; (4) Simpson index. ** means the statistical difference between the two groups, $p < 0.05$

Figure 3

PCoA analysis shows the distribution coordinate diagram of samples: In the graph, the distance between the dots represents the similarity of the samples. Samples with high similarity tend to cluster together. The yellow dots represent the patient group, the blue dots represent the follow-up group, and the red dots represent the control group. The results showed a statistically significant difference among the three groups ($R=0.273$, $p=0.001$).

Figure 4

Spearman correlation coefficient heat map between samples. The closer the calculated Spearman correlation coefficient is to 1, the redder the color in the heat map, indicating the stronger correlation between the two samples. Follow-up group 1 had high correlation with Controls group. Follow-up group 2 had high correlation with Patients group.

Figure 5

KEGG metabolic pathway analysis. The left side of the figure shows the abundance ratio between the two groups. The middle section shows the proportional variation in functional abundance within the 95% confidence interval. The p-value is on the right.

Supplementary Files

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