Journal of Psychiatric Research 113 (2019) 90–99

Contents lists available at ScienceDirect

Journal of Psychiatric Research

journal homepage: www.elsevier.com/locate/jpsychires

Similarly in depression, nuances of gut microbiota: Evidences from a shotgun metagenomics sequencing study on major depressive disorder versus bipolar disorder with current major depressive episode patients

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ARTICLE INFO

Keywords:
Gut microbiota
Major depressive disorder
Bipolar disorder
Shotgun metagenomics sequencing
Diversity
G_m coefficient

ABSTRACT

Background: To probe the differences of gut microbiota among major depressive disorder (MDD), bipolar disorder with current major depressive episode (BPD) and health participants.

Methods: Thirty one MDD patients, thirty BPD patients, and thirty healthy controls (HCs) were recruited. All the faecal samples were analyzed by shotgun metagenomics sequencing. Except for routine analyses of alpha diversity, we specially designed a new indicator, the G_m coefficient, to evaluate the inequality of relative abundances of microbiota for each participant.

Results: The G_m coefficients are significant decreased in both MDD and BPD groups. The relative abundances of increased phyla *Firmicutes* and *Actinobacteria* and decreased *Bacteroidetes* were significantly in the MDD and BPD groups. At genus level, four of top five enriched genera (*Bacteroides, Clostridium, Bifidobacterium, Oscillibacter* and *Streptococcus*) were found increased significantly in the MDD and BPD groups compared with HCs. The genera *Escherichia* and *Klebsiella* showed significant changes in abundances only between the BPD and HC groups. At the species level, compared with BPD patients, MDD patients had a higher abundance of *Prevotellaceae* including *Prevotella denticola F0289*, *Prevotella intermedia 17*, *Prevotella ruminicola*, and *Prevotella intermedia*. Furthermore, the abundance of *Fusobacteriaceae, Escherichia blattae DSM 4481* and *Klebsiella oxytoca* were significantly increased, whereas the *Bifidobacterium longum subsp. infantis ATCC 15697 = JCM 1222* was significantly reduced in BPD group compared with MDD group.

Conclusions: Our study suggested that gut microbiota may be involved in the pathogenesis of both MDD and BPD patients, and the nuances of bacteria may have the potentiality of being the biomarkers of MDD and BPD.

1. Introduction

Major depressive disorder (MDD) and bipolar disorder (BD) are among the leading causes of burden and disability worldwide (Collins et al., 2011). Although the clinical features of bipolar disorder with current major depressive episode (BPD) and MDD are similar, they have different pathologies and distinct treatment protocols (Ghaemi et al., 2001). Misdiagnosis has occurred frequently due to indistinguishable symptoms and a lack of pathological detection tools (Ghaemi et al., 2001). To probe the pathological differences in the depressive

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https://doi.org/10.1016/j.jpsychires.2019.03.017
Received 4 October 2018; Received in revised form 16 March 2019; Accepted 18 March 2019

symptoms connected to BPD and MDD, a large number of studies have been conducted (Batmaz et al., 2013; Carvalho et al., 2015; Cuellar et al., 2005; Deng et al., 2018; Grotegerd et al., 2013; Lan et al., 2014; Moreno et al., 2012; Parikh, 2010; Ren et al., 2017; Tas et al., 2015; Taylor, 2014; Uchida et al., 2015). In this field, the gut microbiota are considered a link between the brain, behaviour and moods (Liu and Zhu, 2018).

Gut microbiota play an important role in the pathologies of both MDD and BPD (Brietzke et al., 2011; Cattaneo et al., 2015; Winter et al., 2018). Gut microbiota studies on MDD clearly demonstrated the bi-directional interactions between the gut and the brain in three major systems, neuroimmune, neuroendocrine and sensory neural pathways (Dantzer, 2009; Evrensel and Ceylan, 2015; Mittal et al., 2017; Ng et al., 2018; Winter et al., 2018). Studies of the gut microbiota in MDD patients revealed significant alterations in the abundance of different genera within the phyla *Bacteroidetes, Firmicutes, Proteobacteria* and *Actinobacteria* (Jiang et al., 2015). Naseribafrouei et al. found an overrepresentation of the order *Bacteroidales* and underrepresentation of the family *Lachnospiraceae* at a high taxonomic level and an over-representation of *Alistipes* and *Oscillibacter* strains at a low taxonomic level in MDD patients (Naseribafrouei et al., 2014). In contrast, gut microbiota studies on BD, especially for BPD patients, are rare. Painold et al. found a negative correlation between gut flora alpha diversity and BD illness duration (Painold et al., 2018), and Evans et al. observed a decreased fractional representation of the phylum *Firmicutes* in the BD group. Furthermore, an increase in the family *Faecalibacterium* was associated with better physical health situations, milder depressive status, and better sleep in the BD group (Evans et al., 2017). Unfortunately, although investigating the subtle differences between MDD and BPD is important, to our knowledge, there are no studies on this topic. Depressive symptoms of MDD and BPD are similar, but due to their different pathologies (Batmaz et al., 2013; Cuellar et al., 2005; Deng et al., 2018; Grotegerd et al., 2013; Lan et al., 2014; Ren et al., 2017; Tas et al., 2015; Taylor, 2014), the clinical practice proposals for each disorder are distinct (Forty et al., 2008; Mitchell et al., 2011; Perlis et al., 2006; Uchida et al., 2015). Based on the evidence mentioned above, we hypothesized that MDD and BPD patients may have a similar gut flora structure in general but that they are significantly different when compared with that of health controls (HCs). Furthermore, we presumed that gut microbiota differences between MDD and BPD would be nuanced due to the indistinguishable clinical features of depression.

Compared with 16S rRNA sequencing (Muyzer et al., 1993), shotgun metagenomics sequencing (SMS) can offer increased resolution, enabling a more specific taxonomic and functional classification of sequences as well as the discovery of new bacterial genes and genomes (Franzosa et al., 2015). Importantly, SMS allows the simultaneous study of archaea, viruses, virophages, and eukaryotes (Norman et al., 2014). As a result, we decided to use SMS to identify the gut microbiota differences among the MDD, BPD and HC groups. We also explored the relationship between gut microbiota and clinical features.

2. Materials and methods

The study was conducted in accordance with the Declaration of Helsinki, and the protocol of this study was approved by the Human Ethic Committee of Shenzhen Kangning Hospital. Informed written consent was obtained from all of the subjects.

2.1. Study population

2.1.1. MDD and BPD patient samples

MDD and BPD patients were recruited from the inpatient and outpatient units of Shenzhen Kangning Hospital (Shenzhen, Guangdong, China). The inclusion criteria for the MDD and BPD patient samples were as follows: 1. age between 18 and 65; 2. diagnosis of MDD or bipolar disorder with current major depressive episode (BPD) according to the DSM-5 criteria; and 3. during a depressive episode with current Hamilton Depression Scale-17 (Hamilton, 1960)(HAMD) score > 17. The exclusion criteria were as follows: 1. other comorbid mental disorders; 2. history of psychoactive substance abuse; 3. history of stroke, epilepsy, hypertension, endocrine disease, diabetes mellitus, fatty liver disease or severe cardiovascular disease; 4. extreme diet, such as a weight loss diet or vegetarianism; 5. history of transcranial magnetic stimulation (TMS) or electroconvulsive therapy (ECT) treatments within the previous 6 months; 6. pregnancy; 7. history of antibiotic, probiotic, prebiotic or synbiotic use within the previous one month; 8. body mass index (BMI) > 24; and 9. unwillingness to provide detailed address or refusal to participate in follow-up.

2.1.2. Clinical measures

The HAMD, Hamilton's Anxiety Scale (Hamilton, 1959) (HAMA), Mood Disorder Questionnaire (Hirschfeld et al., 2000) (MDQ) and Hypomania Checklist (Angst et al., 2005) (HCL-32) were used to assess the severity of anxiety and mania symptoms.

2.1.3. HC sample

HCs from the nearby districts were screened using a semi-structured clinical interview to exclude psychiatric or physical illnesses. The exclusion criteria of the HC group are the same as those listed in section 2.1.1.

2.2. Sample size calculation

The present study is a pilot study, and we set the sample number at 35 participants per group.

2.3. Metagenome sequencing and diversity analysis

Metagenome sequencing of faecal samples was performed as described in a previous study (Zhou et al., 2016).

2.3.1. Fecal sample collection and DNA extraction

Fecal samples were collected and stored at $-80\,^{\circ}C$ until they were shipped to prior to Imunobio Co. Ltd (Shenzhen, China) for DNA extraction. DNA was extracted from stool samples using a StoolGen DNA kit (CWBiotech Co., Beijing, China).

2.3.2. Shotgun metagenomic sequencing

According to the protocol used in a previous study, we performed the shotgun metagenomic sequencing. Shotgun metagenomic libraries were constructed with a TruSeq DNA Sample Preparation kit (Illumina, San Diego, CA, USA).Libraries that passed QC (> 3 ng/μl) were sequenced using an Illumina Hiseq2500 sequencer (Illumina, San Diego, CA, USA) instrument with the paired-end 150-bp sequencing model based on 4Graw data output per sample, the alpha diversity index of each sample was computed using VEGAN's (Dixon, 2003) diversity function.

2.4. G_m, a novel algorithm of relative abundance inequality

The Shannon index is widely used for comparing the alpha diversity of gut microbiota and is influenced by two parameters: the richness measure based on the number of bacterial types and the inequality of the relative abundance of bacteria. The more strains of microbiota there are, the higher the Shannon index is; conversely, the less the inequality of relative abundances is, the higher the Shannon index is. As a result, we cannot examine the heterogeneity of relative abundances by using the Shannon index without the influence of richness, which was calculated from the numbers of species. Therefore, we borrowed a well-known economics index, the Gini coefficient, to evaluate the inequality of relative abundances of microbiota. The Gini coefficient, developed by the Italian statistician and sociologist Corrado Gini, is a popular

measure of inequality of income or wealth (Gini, 1912, 1936, 1997). A higher Gini coefficient means higher inequality. A Gini coefficient value of zero means absolute equality (e.g., everyone has equivalent wealth); in contrast, a Gini coefficient of one represented the maximal inequality (e.g., only one person has the whole wealth, and the others have none). Theoretically, the inequality of wealth is the only factor that can impact the Gini coefficient because it is calculated by the shape of the Lorenz curve, which plots the proportion of the cumulative wealth, and therefore, the Gini coefficient is not related to the number of residents. Thus, we borrowed the Gini coefficient to describe the inequalities of the relative abundances of microbiota (relative abundances as wealth, please see Supplemental file: Fig. S1). In this study, the Gini coefficient of microbiota (G_m coefficient) is calculated by the cumulative curve of species for each participant. That is, the G_m coefficient could be an indicator of the predominance of several dominant bacteria. A high G_m coefficient means the increased predominance of the dominant bacteria, and a lower G_m coefficient represents the declination of the dominant advantage of the dominant gut microbiota.

2.5. Statistical analysis

Because genes with too low an abundance in the MDD, BPD and HC samples might not appropriately reflect the actual situation, genes with a mean abundance less than 1e-7 in the MDD, BPD or HC group were discarded. The differentially expressed genes were identified by the Wilcoxon rank-sum test and were adjusted using false-discovery rate (FDR) multiple testing correction with a threshold of FDR $p < 0.05$. The estimated p values were calculated using the q value R package (Storey and Tibshirani, 2003). We used the Kruskal-Wallis test to test the differences in the G_m coefficient among the three groups and the Steel-Dwass test as post hoc analyses. We also calculated the relationship between G_m coefficients and clinical performance for each group separately. For metabolic function analyses, the metagenomic catalog was annotated to the Kyoto Encyclopedia of Genes and Genomes (KEGG) orthologies (KOs) database (Kanehisa and Goto, 2000). Then we performed the principal components analysis (PCA) to visualize the differences of identified KOs among the three groups, and the detailed significant KOs were selected according to a linear discriminant analysis (LDA) test (LDA > 2.5).

3. Result

3.1. Subject characteristics

Due to contamination of faecal samples, failure to collect clinical information, *etc.*, data from sequencing of 31 MDD patients, 30 BPD patients, and 30 HCs from January 2015 to 12 January 2017 were included in the final analyses. The patients and HCs had comparable demographic characteristics, and no significant demographic differences were found among the three groups. The differences in episode duration, MDQ and HCL-32 were significant between MDD and BPD patients (Table 1).

3.2. The results of alpha diversity measures

The Chao 1, Shannon Index, Inverse Simpson index and G_m coefficient were tested for alpha diversity. Only the MDD group had a significantly lower Chao 1 (Table 2) than the HC group ($p = 0.003$). Furthermore, there were no significant differences in the Shannon and Inverse Simpson indices (Table 2) among the three groups ($p = 0.053$ and $p = 0.316$). However, the G_m coefficient (Table 2) was significantly reduced in both the MDD and BPD groups compared with that in the HC group ($p = 0.008$ and $p = 0.011$) (Fig. 1). The four measures of alpha diversity above were also tested for associations with the clinical features (Supplemental file: Table S1). In the MDD group, a Pearson correlation analysis showed a significant positive correlation of body mass index (BMI) (Fig. 2A) with the Shannon and Inverse Simpson indices ($p = 0.014$ and $p = 0.009$) and a negative correlation of BMI with G_m coefficient ($p = 0.029$). No significant associations were observed in the BPD group. In addition, Pearson correlation showed a significant positive correlation of HAMA (Fig. 2B) with G_m coefficient ($p = 0.028$) and a negative correlation of HAMA with Shannon index ($p = 0.018$) only in the BPD group.

Finally, the Bray-Curtis analysis was used to determine the degree of microbial phylogenetic similarity (β-diversity) in patients' group. The plots of Bray-Curtis analysis showed that there was no difference in the gut microbial community between MDD group and BPD group (Supplemental file: Fig. S2).

Table 1
Descriptive data of included subjects in the study.

Parameter	MDD Mean(SD)	BPD Mean(SD)	HC Mean(SD)	*p*-value
Subject, n	31	30	30	
Age, years	41.58 (10.40)	38.40 (8.33)	39.47 (10.22)	0.43
Gender, F/M	22/9	15/15	16/14	0.20
BMI, kg/m^2	21.46 (2.13)	21.92 (2.22)	21.97 (3.18)	0.69
Clinical characteristics				
Medication, yes/no	23/8	25/5	NA	0.38
SSRIs, yes/no	13/18	11/19	NA	0.79
SNRIs, yes/no	8/23	3/27	NA	0.18
Other antidepressants, yes/no	6/25	1/29	NA	0.10
Atypical antipsychotics, yes/no	0/31	7/23	NA	< 0.01
Age of onset, years	33.29 (9.68)	29.03 (7.46)	NA	0.06
Total disease course, years	8.44 (6.84)	8.52 (5.52)	NA	0.96
This episode duration, days	80.94 (98.36)	37.50 (50.41)	NA	0.03
Episodes of disease	3.19 (2.02)	3.83 (1.66)	NA	0.18
Total treatment time, years	5.63 (7.24)	5.97 (6.62)	NA	0.85
MDQ	1.71 (2.78)	8.83 (2.26)	NA	< 0.01
HCL-32	6.68 (7.15)	20.23 (4.58)	NA	< 0.01
HAMD	20.23 (3.11)	20.37 (3.41)	NA	0.87
HAMA	15.00 (6.68)	14.87 (8.25)	NA	0.95

Table 1 details basic information by diagnosis for all study subjects included in the analyses. *P*-values are given for group comparisons using one-way ANOVA (for age and BMI), chi-square test (for gender and medication) and *t*-test (for the other parameters). Abbreviations: MDD, major depressive disorder; BPD, bipolar disorder; HC, Healthy Control; SD, standard deviation; BMI, body mass index; MDQ, Mood Disorder Questionnaire; HCL-32, Hypomania Check List-32; HAMD, Hamilton's Depression Scale; HAMA, Hamilton Anxiety Scale.

Table 2
Microbial richness and diversity in the fecal microbiota of 31 MDD and 30 BPD patients and 30 HCs.

Alpha diversity	MDD Mean(SD)	BPD Mean(SD)	HC Mean(SD)	*F* value	Steel-Dwass test
Chao 1	2755.52 (117.67)	2798.30 (127.98)	2927.20 (194.76)	11.39**	MDD < HC
Shannon index	3.64 (0.46)	3.62 (0.52)	3.38 (0.51)	5.87	
Inverse Simpson index	15.26 (6.70)	14.87 (7.31)	12.74 (7.00)	2.31	
G_m coefficient	0.94 (0.01)	0.94 (0.01)	0.95 (0.01)	11.58**	MDD < HC, BPD < HC

Significance levels were indicated by asterisks as $*p < 0.05$, $**p < 0.01$.

3.3. The results of bacterial community measures

To understand the differences among groups from a fine level, analyses of phylotypes with a mean relative abundance larger than 1% and 0.01% at the genus and 0.001% at the species levels, indicated that *Bacteroidetes*, *Firmicutes*, *Proteobacteria*, and *Actinobacteria* were the four most common dominant taxa among the three groups (Fig. 3A). The abundance of *Firmicutes* and *Actinobacteria* was significantly increased in the MDD and BPD groups compared with that in the HC group. However, the abundance of *Bacteroidetes* was significantly lower in the MDD and BPD patients than in HCs. The *Proteobacteria* ($p = 0.010$) showed significant changes only between the BPD and HC groups (Fig. 3A).

At the genus level, four of the top five enriched genera (*Bacteroides, Clostridium, Bifidobacterium, Oscillibacter* and *Streptococcus*) were commonly increased in the MDD and BPD groups compared with those in the HC group (Fig. 3B). Concomitantly, *Escherichia* ($p = 0.009$) and *Klebsiella* ($p = 0.026$) showed significant changes only between the BPD and HC groups (Fig. 3B).

At the species level, we identified 306 species and subspecies showing significant changes among the three groups, including 230 species and subspecies between the MDD and HC groups (Supplemental file: Table S2), 264 species and subspecies between the BPD and HC groups (Supplemental file: Table S3) and 8 species between the MDD and BPD groups (Fig. 3E). The top twenty enriched species are shown in Fig. 3 (C and D). Significant increases in 12 species or subspecies were observed, whereas the *Haemophilus parainfluenzae T3T1* was significantly reduced in both the MDD and BPD groups compared with that in the HC group (Fig. 3C and D).

At the species level, we first identified 8 species showing significant changes in abundance between the MDD and BPD groups. Compared with BPD patients, MDD patients had microbial communities that were characterized by a higher abundance of *Prevotellaceae* including *Prevotella denticola F0289* ($p = 0.029$), *Prevotella intermedia 17* ($p = 0.030$, Wilcoxon rank-sum test), *Prevotella ruminicola* ($p = 0.017$) and *Prevotella intermedia* ($p = 0.042$). Furthermore, the abundance of *Fusobacteriaceae* ($p = 0.006$), *Escherichia blattae DSM 4481* ($p = 0.042$) and *Klebsiella oxytoca* ($p = 0.030$) were significantly increased in the BPD group compared with those in the MDD group, whereas the *Bifidobacterium longum subsp. Infantis ATCC 15697 = JCM 1222* ($p = 0.042$) was significantly reduced in the BPD group compared with that in the MDD group (Fig. 3E).

For detailed information of this section (3.3), please see the Supplemental file: Table S4. Because the use of atypical antipsychotics was significantly different between the MDD and BPD groups, we did a sub-group analysis after excluding the 7 atypical antipsychotics users. As presented in Table 3, relative abundance of *Prevotella* and *Bifidobacterium* remained significant.

3.4. Results of functional analyses

1313 KOs were analyzed among the three groups, and we found no significant KOs among the three groups and the PCA and LDA plots were shown in the supplemental file Fig. S3. The detailed results of top 500 KOs were shown in the supplemental file Table S5.

4. Discussion

To our knowledge, we are the first to explore the gut microbiota by using SMS among MDD, BPD, and HC participants and the first to design a novel index, the G_m coefficient, to probe the inequality of relative abundances. Our results demonstrated significant gut flora differences among the three groups. Additionally, we found some uniquely different species or subspecies between MDD and BPD patients, which may reflect the nuanced pathological differences between the two disorders with indistinguishable depressive symptoms.

4.1. G_m coefficients demonstrated the differences in ecology in the both MDD and BPD groups

In the ecological examinations, we found decreased richness in both the MDD and BPD groups by using the Chao 1 index. This result is similar to Kelly et al. (2016). Paradoxically, no significant difference in diversity measured by the Shannon index or inverse Simpson's index of diversity 1-D was observed among the three groups. Regarding the diversity studies, the results were inconsistent: Jiang et al. (2015) found higher Shannon's index value in active MDD patients, and Painold et al. found a negative correlation between gut flora alpha diversity and BD illness durations (Painold et al., 2018); however, Kelly et al. (2016) showed no significant difference in Shannon's index between MDD and HC groups. The gut microbiota is composed of some dominant commensal bacteria and many other non-dominant microbiota (Eckburg et al., 2005). Notably, the alterations in dominant commensal bacteria may play a role in the pathological course of some diseases (Kelly et al., 2005; Macdonald and Monteleone, 2005). The algorithm of Shannon index is affected by two major features, the number of species (as OTUs in the 16S rRNA sequencing method) and the inequality of the relative abundances. These characteristics make Shannon's index difficult to use for investigating inequality without the influence of the unequal species number among each participant. To quantify the dominance level (in other words, the inequality) except the interference of the number of bacteria, we borrowed the Gini coefficient, an indicator of the difference between the rich and the poor in economics, to create the G_m coefficient for the quantitative analysis of microbiota inequality. By using the G_m coefficient, we found that the degree of inequality in the MDD and BPD groups was reduced; that is, the predominance levels of dominant bacteria in the MDD and BPD groups decreased significantly. In further correlation analyses, we found that BMI and G_m were negatively related in the MDD group. This finding suggests that the imbalance of the gut flora may be related to lipid metabolism (Holmes et al., 2011) in MDD patients. Moreover, the G_m coefficient was positively associated with HAMA scores in the BPD group. This result suggests that the inequality of the gut flora may be related to mental symptoms, particularly in BPD subjects, and is worth further study. In short, G_m coefficient is a macroscopic indicator reflecting the inequality of the gut flora. Thus, we conducted more detailed analyses to further explore the specific differences in microbiota between the MDD, BPD and HC groups as discussed below.

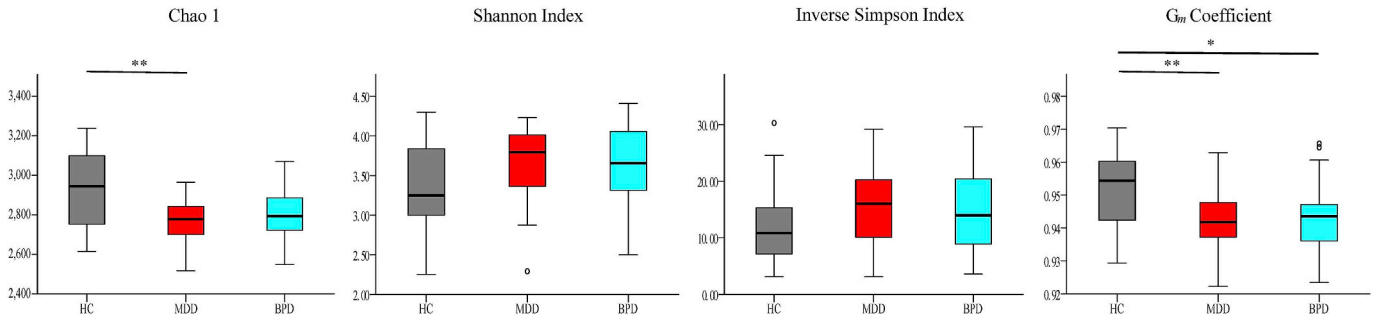

Fig. 1. Microbial richness and diversity in the fecal microbiota of 31 MDD and 30 BPD patients and 30 HCs. Richness was obtained from the observed number of species by extrapolation using estimators such as the Chao 1 index, and diversity was based on Shannon, Inverse Simpson indices and G_m coefficient. Kruskal-Wallis test followed by Steel-Dwass test was used for multiple comparisons. Significance levels were indicated by asterisks as $*p < 0.05$, $**p < 0.01$.

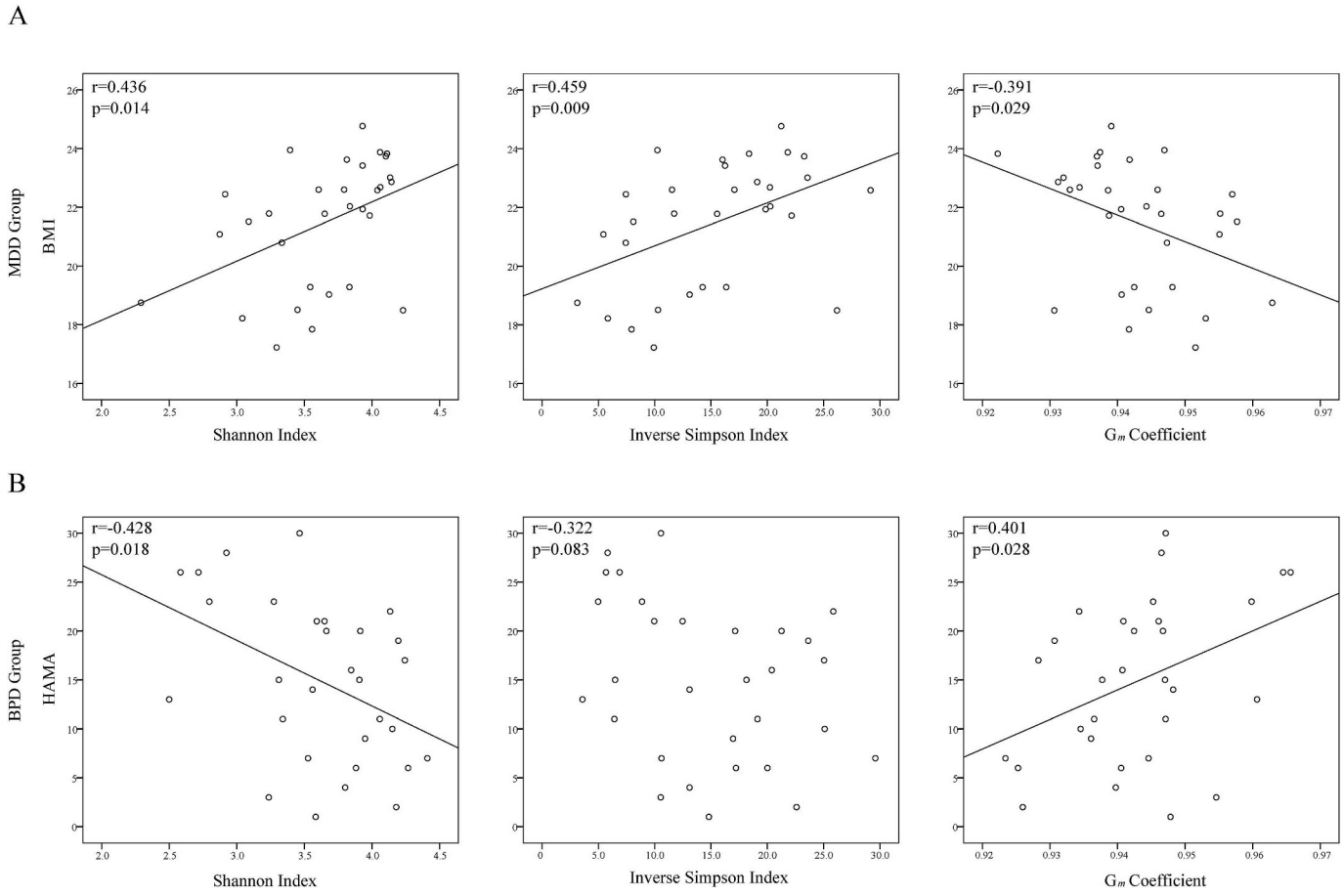

Fig. 2. Alpha diversity was significantly correlated with BMI and HAMA. (A) Plots of Pearson correlation analysis in MDD group, BMI vs Shannon and Inverse Simpson indices, showed positive correlations and BMI vs G_m coefficient, showed negative correlation. (B) Plots of Pearson correlation analysis in BPD group, HAMA vs. Shannon index, showed negative correlations and BMI vs G_m coefficient, showed positive correlation.

4.2. Differences at the phylum level between the MDD, BPD and HC groups

The gut microbiome play a causal role in the development of depressive-like behaviours (Zheng et al., 2016) and may play a key role in depression related pathology (Dinan and Cryan, 2013; Evrensel and Ceylan, 2015; Forsythe et al., 2010; Maes et al., 2008; Winter et al., 2018). At the phylum level, a significantly lower relative abundance of *Bacteroidetes* was detected in both the MDD and BPD groups, similar to previous findings in MDD patients (Chen et al., 2018; Evans et al., 2017; Jiang et al., 2015; Lin et al., 2017; Zheng et al., 2016). *Bacteroidetes* are present at a dominant level (Eckburg et al., 2005). Polysaccharide A, a *Bacteroides* expression product, can induce regulatory T cell growth and related cytokine expression, which has been shown to be effective against colitis (Round et al., 2011; Zhou and Zhi, 2016). A meta-analysis demonstrated that the level of *Bacteroides* was significantly lower in inflammatory bowel disease patients; moreover, the *Bacteroides* level was even lower in the active phase than in the remission phase (Zhou and Zhi, 2016). This finding suggests that *Bacteroides* are associated with intestinal inflammation. Considering the close association between depression and inflammation (Dantzer, 2009; Schiepers et al., 2005), our results suggest that *Bacteroides* may be an indicator of depression-related intestinal inflammation. However, whether there is a linear relationship between *Bacteroides* abundance and the severity of intestinal inflammation needs further exploration. We also found increased Firmicutes in both the MDD and BPD groups. However, the results of previous studies are ambiguous. Evans et al. and Jiang et al.

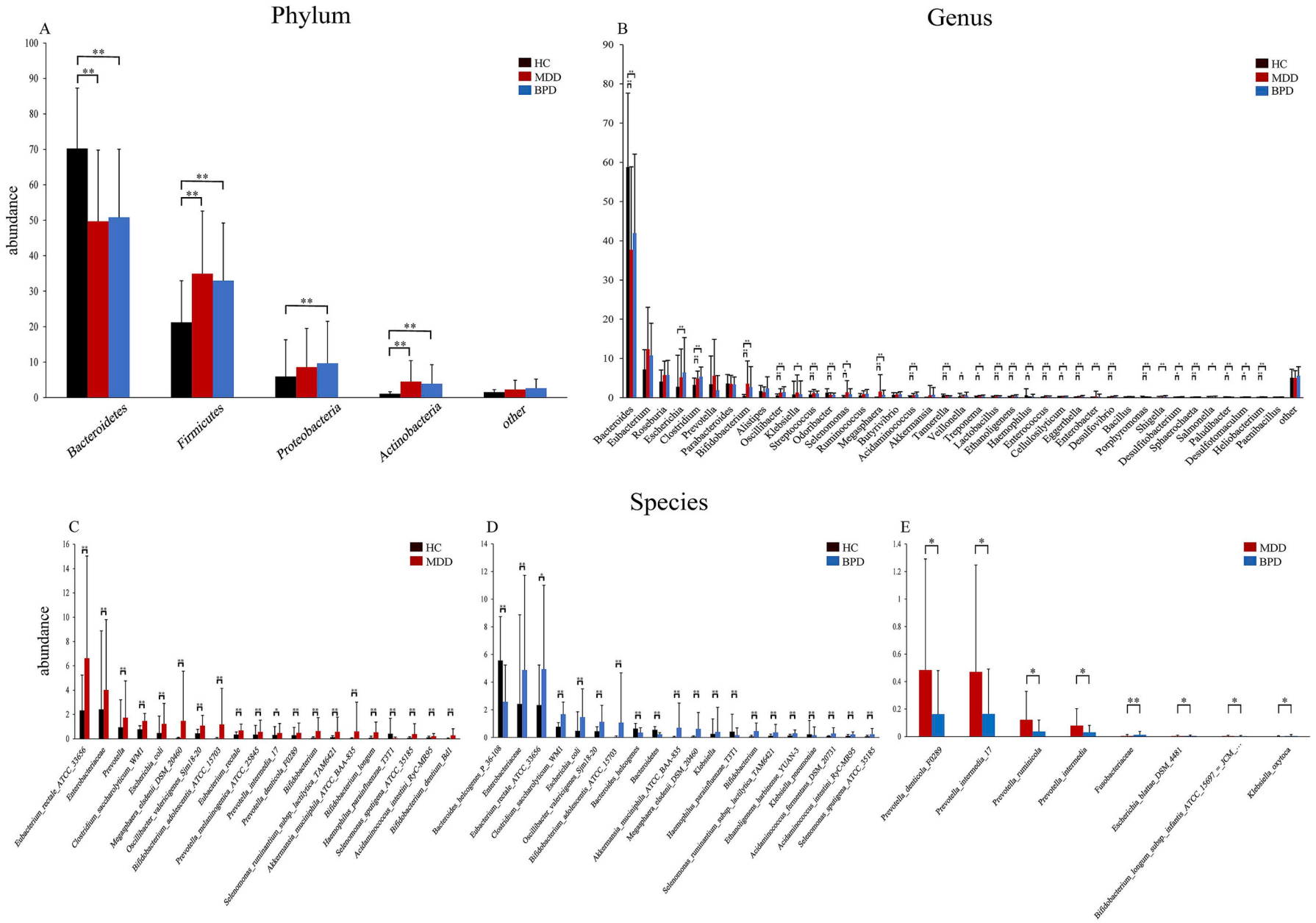

Fig. 3. Comparison of the microbial abundance among the major depressive disorder (MDD), bipolar disorder with current major depressive episode (BPD) and healthy control (HC) groups. Red, blue and black indicate the MDD patients, BPD patients and healthy controls, respectively. The phylogenetic abundance of phyla that had mean values less than 1%, that of genera were less than 0.01% and that of species were less than 0.001% were excluded. After exclusion, Wilcoxon rank-sum tests were applied to identify the differentially abundant phyla, genera, and species. Among these, the highest means of the phylogenetic abundance in the enriched cohort were drawn as barplots. Significance levels were indicated by asterisks as $*p < 0.05$, $**p < 0.01$. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 3
Results of sub-group analysis without atypical antipsychotics users.

Species or subspecies	Relative abundance (%)		*p* value (FDR corrected)
	MDD	BPD	
	Mean (SD)	Mean (SD)	
Prevotella melaninogenica ATCC 25845	0.566 (0.958)	0.195 (0.386)	0.005
Prevotella denticola F0289	0.483 (0.809)	0.178 (0.354))	0.006
Prevotella intermedia 17	0.469 (0.779)	0.179 (0.365)	0.006
Prevotella ruminicola	0.121 (0.207)	0.042 (0.093)	0.006
Prevotella denticola	0.095 (0.169)	0.030 (0.068)	0.039
Prevotella intermedia	0.079 (0.123)	0.033 (0.057)	0.038
Bifidobacterium longum subsp. longum BBMN68	0.025 (0.041)	0.010 (0.009)	0.038
Bifidobacterium longum NCC2705	0.020 (0.026)	0.008 (0.007)	0.015

Detailed results of significant different species or subspecies between MDD and BPD group. Abbreviations: MDD, major depressive disorder; BPD, bipolar disorder with current major depressive episode; SD, standard deviation.

showed reduced *Firmicutes* in depressed participants (Evans et al., 2017; Jiang et al., 2015), but Lin et al. got the opposite results (Lin et al., 2017). Additionally, Zheng et al. suggested that the results of phylum *Firmicutes* are complex, in which some OTUs of the *Firmicutes* were found to be increased in MDD, while others were decreased (Zheng et al., 2016). In our study, we identified several species belonging to phylum *Firmicutes*, and detailed information is shown in Table 2. In other words, by using SMS, we were the first to explore the differences in *Firmicutes* among the three groups.

4.3. Multiple differences at the genus level

At the genus level, four genera (*Clostridium, Bifidobacterium, Oscillibacter* and *Streptococcus*) were increased in the MDD group compared with HC group. *Bifidobacterium*, usually believed to be a probiotics, is considered beneficial for the human body (Collins and Gibson, 1999). In contrast, in the present study, we found that the relative abundance of *Bifidobacterium* was significantly higher in the MDD and BPD groups. The results of *Bifidobacterium* among patients are ambiguous. Romijn et al. investigated whether probiotics could improve mood, stress or anxiety but found no significant difference between the probiotic and placebo groups (Romijn et al., 2017). Furthermore, another study concluded that the genus *Bifidobacterium* accumulated in ankylosing spondylitis patients (Wen et al., 2017). These studies and our results demonstrated that accumulated internal probiotics may not always mean a good health situation. We speculated that in some situations, a high probiotic level may reflect an intestinal environment change signal. In brief, the acute relationship between probiotics and diseases should be studied further. *Oscillibacter* is another increased genus in both the MDD and BPD groups in our study. *Oscillibacter* was thought to have a significant connection with depression (Naseribafrouei et al., 2014), and Yu et al. demonstrated that increased *Oscillibacter* were also found in a rat model of depression (Yu et al., 2017). Since valeric acid, a main metabolic end product of *Oscillibacter* (Katano et al., 2012), structurally resembles GABA, and can bind the GABAa receptor (Naseribafrouei et al., 2014), it may be the reason that increased *Oscillibacter* was found in both MDD and BPD patients.

Similar to Lina et al. (Lin et al., 2017), we also found that the relative abundances of *Prevotella* and *Bifidobacterium* were increased in the MDD group. The two genera are known to be short-chain fatty acid (SCFA) producers (Koh et al., 2016), and SCFAs play a protective role against inflammation in the gut (De Filippo et al., 2010; Scheppach and Weiler, 2004). The increase in SCFA-related bacteria in our study may indicate similar inflammatory pathological pathways in both the MDD and BPD groups. Furthermore, the relative abundance of *Prevotella* was lower in the medicated MDD group than in the nonmedicated MDD group, and the *Prevotella* level was also negatively correlated with medication in MDD patients. Low-grade inflammation plays a key role in the pathophysiology of depression (Chrobak et al., 2016; Dantzer, 2009; Schiepers et al., 2005), and antidepressants have anti-inflammatory effects (Abdel-Salam et al., 2003; Hannestad et al., 2011; Horowitz et al., 2014; Kappelmann et al., 2018; Kohler et al., 2014; Liu et al., 2011; Michelson, 1976; Rosenblat et al., 2016; Tynan et al., 2012). As a result, this evidence implies that *Prevotella* are sensitive to antidepressants use. In addition, because Macedo et al. found both antidepressants and antimicrobials presenting neuroprotective/antidepressant and antimicrobial effects (Macedo et al., 2017), we speculated that a possible anti-depressant mechanism of antidepressants is restoring the balance of gut microbiota by their anti-inflammatory actions. Furthermore, the difference in *Prevotella* between BPD and HC wa not significant, and the relative abundance of *Prevotella* was significantly higher in the MDD group than in the BPD group. Although, robust evidence of increased proinflammatory cytokines was found in both MDD and BD patients (Brietzke et al., 2011; Duffy et al., 2014; Modabbernia et al., 2013; Noto et al., 2014) and BD is widely considered to be associated with the inflammatory system (Goldstein et al., 2009), our results demonstrated that the gut inflammatory status was milder in BPD tan in MDD. In fact, our results support the inflammatory hypothesis of depression (Schiepers et al., 2005) and may provide additional evidence.

4.4. Unique differences at the species level between MDD and BPD

Compared with 16S rRNA sequencing, SMS has multiple advantages, such as enhanced power to detect bacterial species and diversity, and increased ability to predict genes (Ranjan et al., 2016). Generally, by using SMS, we could study the bacteria at a more elaborate taxonomic scale. At the phylum and genus level, the differences between the MDD or BPD groups versus the HC group are similar. As mentioned above, in our study, decreased abundance of the genus *Prevotella* was found in the BPD group versus increased abundance of *Prevotella* in the MDD group, which was similar to Lin et al. (2017). However, by the power of SMS, we found significant differences in eight species or subspecies between the MDD and BPD groups. Among them, four belong to the *Prevotella*, which is considered a key genus in determining the gut microbiome profile (Arumugam et al., 2011); they are *Prevotella denticola F0289*, *Prevotella intermedia 17*, *Prevotella ruminicola*, and *Prevotella intermedia*. The relative abundances of these four taxons were significantly higher in the MDD group than in the BPD group. Misdiagnosis of MDD and BPD is the clinical focus and difficulty. Often BPD patients are misdiagnosed with MDD in clinical practice (Menezes et al., 2018), but in our study, species and subspecies of the genus *Prevotella* showed potential as biomarkers for distinguishing MDD and BPD.

Klebsiella oxytoca, a gram-negative opportunistic pathogen, is found

with higher relative abundance in the BPD group. Unfortunately, related studies of this topic are rare and inconsistent. Maes et al. demonstrated that the lipopolysaccharide (LPS) of *Klebsiella* may play a role in MDD pathophysiology (Maes et al., 2008). However, cytotoxin production in animal isolates of *Klebsiella oxytoca* and its pathogenic properties have not been characterized (Zollner-Schwetz et al., 2015). Undoubtedly, the topic of gram-negative bacteria induced inflammatory dysregulation (inflammatory cytokines and LPS) is important (Rios et al., 2017), and we look forward more investigations.

4.5. The significance of the present study

Correct classification of BPD and MDD is very difficult but critical (Culpepper, 2014; Daigneault et al., 2015; Hantouche et al., 1998). Mood disorders, as a group of diseases with different pathologies, are often recognized in inflammatory conditions. In recent years, researchers tried to identify MDD and BPD by using many different biological methods, such as behavioural measurements, cytokine detection, gene sequencing, and brain imaging techniques (Maffioletti et al., 2016; Marchand et al., 2013; Menezes et al., 2018; Vazquez et al., 2018). To our knowledge, our study is the first gut microbiota study to compare MDD, BPD, and HC participants by using the SMS method. First, we found ecological differences among the three groups. Specifically, we borrowed an economic index, the Gini coefficient, to construct the G_m coefficient, which may measure inequality among the gut microbiota. As a result, we found that the dominance levels of dominant bacteria in the MDD and BPD groups were decreased significantly. Second, we identified hundreds of different bacteria species or subspecies among the three groups, which were allocated in the genera *Prevotella*, *Fusobacterium*, *Escherichia*, *Bifidobacterium* and *Klebsiella*. In particular, several of these species or subspecies were first discovered. Third, we studied the relationships between ecological indicators, relative abundance of bacteria and clinical features and added new knowledge to the inflammatory hypothesis of mood disorders (Schiepers et al., 2005; Spalletta et al., 2006) including that the inflammatory status associated with intestinal flora may be a key factor in understanding the pathological differences between MDD and BPD. Furthermore, eight bacteria species or subspecies showed potential as biomarkers to distinguish MDD and BPD patients.

4.6. Limitations

Our study has several limitations. First, because the present research is an exploratory study, we analyzed only 91 participants, and the limited sample size makes it impossible to explore more related factors. Second, this study is a case control trial, and the nature of case control studies limits the ability to draw conclusions about the causal relationship between the gut microbiota and depressive symptoms. Third, only 13 patients were treatment-naïve; as a result, we could not further explore the effects of medication on the gut microbiota, and we hope we can further discuss these effects in future trials. Forth, dietary habits and lifestyle behaviour are potential confounders, which strongly influence the composition of gut microbiota. As such behaviors differ between certain populations, the generalization of our findings to other patient populations require further validation. Future research should take such effects into account.

5. Conclusions

By using SMS, our study provides novel evidence suggesting that gut microbiota may be involved in the intestinal pathogenesis of both MDD and BPD patients. Furthermore, the different faecal bacteria may have the potential to be biomarkers that can differentiate MDD and BPD patients.

Authors' contributions

HR conducted the study, XHX designed the G_m coefficient algorithm. HR and XHX supervised the whole study. DX, YYG, JZ and YHL collected the data, WTL, MBW, FSH, LX and WFD analyzed the data. JZ, WTL and SXX drafted the manuscript. SXX, XHX, WFD, QFY, MBW, FSW, LX, SW, YLZ and TBL revised the manuscript. All authors read and approved the final manuscript.

Declaration of interest

None.

Role of the funding sources

The funding sources had no role in the design of this study and will not have any role during its execution, analyses, interpretation of the data, or decision to submit results.

Acknowledgements

This work was supported by the Sanming Project of Medicine in Shenzhen (Grant Number: SZSM201812052), Science and Technology Program of Huizhou (Grant Number: 2018Y128), Chinese National Natural Science Foundation (Grant Number: 81201047), and CAS Pioneer Hundred Talents Program (Grant Number: 2017-074).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jpsychires.2019.03.017.

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